

## REVIEW ARTICLE

# Advancing wound healing with three-dimensional bioprinted hyaluronic acid-based tissue constructs: From mechanistic insights to clinical translation

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## Abstract

Skin functions as a primary protective barrier against mechanical injury, microbial invasion, and dehydration. Extensive trauma and chronic diseases pose significant clinical challenges to wound healing. Conventional wound dressings and skin substitutes often lack the structural and biochemical sophistication to dynamically interact with the wound. Hyaluronic acid (HA), a key glycosaminoglycan in the extracellular matrix, has emerged as a versatile biomaterial for wound repair due to its biocompatibility, hydration capacity, and intrinsic bioactivity. However, native HA suffers from limitations such as rapid degradation and poor mechanical strength, necessitating advanced engineering strategies, including chemical modification, biofunctionalization, and compositing, to enhance its versatility. Three-dimensional bioprinting has recently emerged as a transformative technology, enabling the precise deposition of HA-based biomaterials to form biomimetic constructs with spatial heterogeneity. In this review, we first elucidate the mechanism by which HA orchestrates the wound healing cascade, followed by a list of engineered approaches to enhance HA's functionality. This review then focuses on the capabilities of mainstream bioprinting technologies for fabricating HA-based wound dressings and stratified skin substitutes. Finally, we discuss prevailing challenges and outline future perspectives, emphasizing innovations in dynamic biomaterials, hybrid bioprinting strategies, and the integration of artificial intelligence to advance the clinical translation of HA products for wound healing.

**Keywords:** Wound healing; Hyaluronic acid; Three-dimensional bioprinting; Wound dressing; Skin substitutes

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## 1. Introduction

Skin, the largest organ of the human body, serves as the primary protective barrier against mechanical injury, microbial invasion, and dehydration.<sup>1</sup> Upon breaching this barrier, a sophisticated, tightly regulated biological program, the wound healing

cascade, is activated to restore homeostasis.<sup>2</sup> The wound healing cascade is a highly coordinated yet dynamic sequence of biological events, classically delineated into four interdependent phases: hemostasis, inflammation, proliferation, and remodeling. This integrated process is ultimately orchestrated to swiftly re-establish tissue integrity and functional barrier capacity.<sup>3,4</sup> However, in cases of extensive trauma, burns, chronic diseases (e.g., diabetes), or compromised host conditions, the healing process can be significantly delayed or disrupted, leading to chronic wounds, severe infections, scarring, and substantial morbidity.<sup>5,6</sup> The management of such challenging wounds, particularly hard-to-heal ulcers, remains a major clinical and socioeconomic burden, necessitating advanced therapeutic strategies.<sup>7</sup>

This clinical imperative has driven the evolution of wound dressings from passive covers to active, biomaterial-based therapeutic systems. An ideal wound dressing should not only provide a protective barrier but also actively modulate the local microenvironment to promote healing.<sup>2</sup> This includes maintaining optimal moisture, facilitating gas exchange, absorbing exudate, preventing infection, and delivering bioactive cues to guide cellular behavior.<sup>8,9</sup> Given the complexity of the wound microenvironment, there is a compelling scientific rationale for developing biomaterials that recapitulate key features of the native extracellular matrix (ECM). Hyaluronic acid (HA), a major non-sulfated glycosaminoglycan (GAG) component of the skin ECM, stands out due to its unique physicochemical and biological properties.<sup>10–12</sup>

Hyaluronic acid is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine. Its high density of carboxyl and hydroxyl groups confers exceptional hydrophilicity, enabling it to retain water up to 1,000 times its own weight, a property crucial for maintaining a moist wound environment and establishing the hydrated matrix essential for all subsequent cellular repair processes.<sup>13,14</sup> Beyond providing hydration, HA exhibits bioactivity that appears to be influenced by its molecular weight (MW). This emerging understanding of MW-dependent functionality suggests its potential multifaceted roles throughout the wound healing cascade, positioning HA as a promising platform for regenerative medicine.<sup>13</sup> Consequently, HA-based formulations, including gels, films, sponges, and scaffolds, have shown extensive clinical application in the management of burns, chronic ulcers (e.g., diabetic foot ulcers, venous leg ulcers), and various surgical settings.<sup>12,15</sup> Their clinical utility is attributed to well-documented biocompatibility, biodegradability, and the ability to support key healing processes such as re-epithelialization

and granulation tissue formation.<sup>16–18</sup>

However, native HA possesses inherent limitations that constrain its therapeutic potential. These include rapid enzymatic degradation *in vivo*, poor mechanical strength, and a lack of structural and biochemical complexity necessary for the precise, spatiotemporal orchestration of healing.<sup>19,20</sup> These drawbacks significantly hinder the efficacy of native HA in treating complex, non-healing wounds that require sustained, stage-specific intervention. To overcome these constraints, two principal engineering paradigms have been established: molecular modification and multicomponent compositing.<sup>21</sup> Molecular modification serves as the foundational engineering approach. It involves the chemical derivatization of the HA backbone, primarily to introduce crosslinkable groups (e.g., methacrylation, tyramine conjugation) that directly enhance structural integrity and processing versatility—thereby addressing the inherent mechanical deficiencies of native HA. A key extension of this approach is biofunctionalization, whereby motifs with inherent antimicrobial, antioxidant, or cell-instructive properties are grafted onto the HA chain to directly combat infection, modulate inflammation, or promote specific cellular responses.<sup>13</sup> In parallel, compositing HA with other natural or synthetic polymers (e.g., collagen, chitosan [CS], alginate, polycaprolactone [PCL]) creates hybrid materials that synergistically combine the unique advantages of each component, resulting in superior mechanical performance, controlled degradation, and augmented bioactivity.<sup>12,22</sup> Collectively, these strategies transform native HA into a versatile class of advanced biomaterials with tailored physicochemical properties and advanced therapeutic functions, capable of meeting the complex demands of modern wound care.

When considering HA as a biomaterial alongside other widely used biopolymers such as gelatin (a denatured collagen derivative) or polyvinyl alcohol (PVA; a synthetic polymer), its relative merits become apparent. Compared to gelatin, which offers excellent cell adhesiveness but suffers from poor mechanical stability and rapid degradation, engineered HA hydrogels can achieve superior structural integrity and tunable degradation kinetics while retaining crucial bioactivity.<sup>23</sup> In contrast to the high mechanical strength and stability of PVA, which is largely bio-inert, HA provides intrinsic, context-dependent biological signaling.<sup>24</sup> This signaling capacity, primarily mediated by its MW-dependent interactions with cells, is indispensable for actively guiding the wound healing cascade. Thus, the unique value proposition of HA lies not in surpassing all materials in every property, but in its unparalleled combination of native bioactivity, high hydrating capacity,

and versatile chemical modifiability. Together, these properties provide an ideal foundation for designing intelligent, stage-specific wound therapies.

The transformation of HA into a bioactive, multifunctional scaffolding material demands advanced manufacturing strategies to fully exploit its therapeutic potential with high spatial and compositional fidelity. Three-dimensional (3D) bioprinting has emerged as a preeminent fabrication strategy by enabling the precise, digitally guided assembly of complex, cell-instructive biomaterials.<sup>25</sup> By transcending the limitations of conventional scaffold fabrication, this technology affords unparalleled control over macro- and microarchitecture, mechanical heterogeneity, and the deliberate, 3D patterning of multiple cell types and biochemical signals.<sup>26</sup> Such capabilities are indispensable for engineering patient-specific wound interfaces and stratified skin equivalents that can dynamically interact with and direct the healing cascade.<sup>8</sup> 3D bioprinting proceeds via distinct modalities, defined by the fundamental unit of deposition (Figure 1): droplet-based (zero-dimensional [0D]) processes (e.g., inkjet bioprinting) utilize discrete droplets; filament-based (one-dimensional [1D]) techniques (e.g., extrusion-based bioprinting [EBB]) rely on the continuous deposition of viscoelastic filaments; and layer-based (two-dimensional [2D]) strategies (e.g., digital light processing [DLP]) employ vat photopolymerization to solidify entire layers at once. Collectively, these complementary bioprinting modalities establish a versatile fabrication framework, enabling the transformation of rationally engineered HA-based biomaterials into structurally defined and biologically active constructs for advanced wound therapy.<sup>1,26</sup> In practice, the choice of bioprinting modality imposes distinct material constraints: 0D techniques such as inkjet favor low-viscosity bioinks, 1D extrusion-based methods demand shear-thinning inks with yield stress behavior, and 2D vat photopolymerization requires cytocompatible photochemistry and precise control of light dose.<sup>27–29</sup>

This review aims to provide a comprehensive overview of the convergence of advanced HA-based biomaterials and 3D bioprinting for wound healing and tissue regeneration. Following a detailed exposition of the MW-dependent mechanisms by which HA orchestrates the wound healing cascade, the review systematically examines material engineering strategies to enhance its functionality, covering chemical modification, biofunctionalization, and composite-material approaches. An overview of existing commercial HA-based wound products is subsequently presented to contextualize clinical translation. The core of this review focuses on the capabilities of mainstream 3D bioprinting technologies, categorized and illustrated

in Figure 1, for fabricating these advanced HA-based biomaterials into precise, biomimetic constructs. Finally, this review concludes by addressing the prevailing challenges in the field and delineating forward-looking strategies to advance the field. Emphasis is placed on pioneering innovations in biomaterial design, enhancing printing fidelity, and implementing personalized therapeutic approaches. These developments are pivotal for translating functional, bioprinted HA scaffolds from bench to bedside, ultimately enabling effective management of complex and chronic wounds.

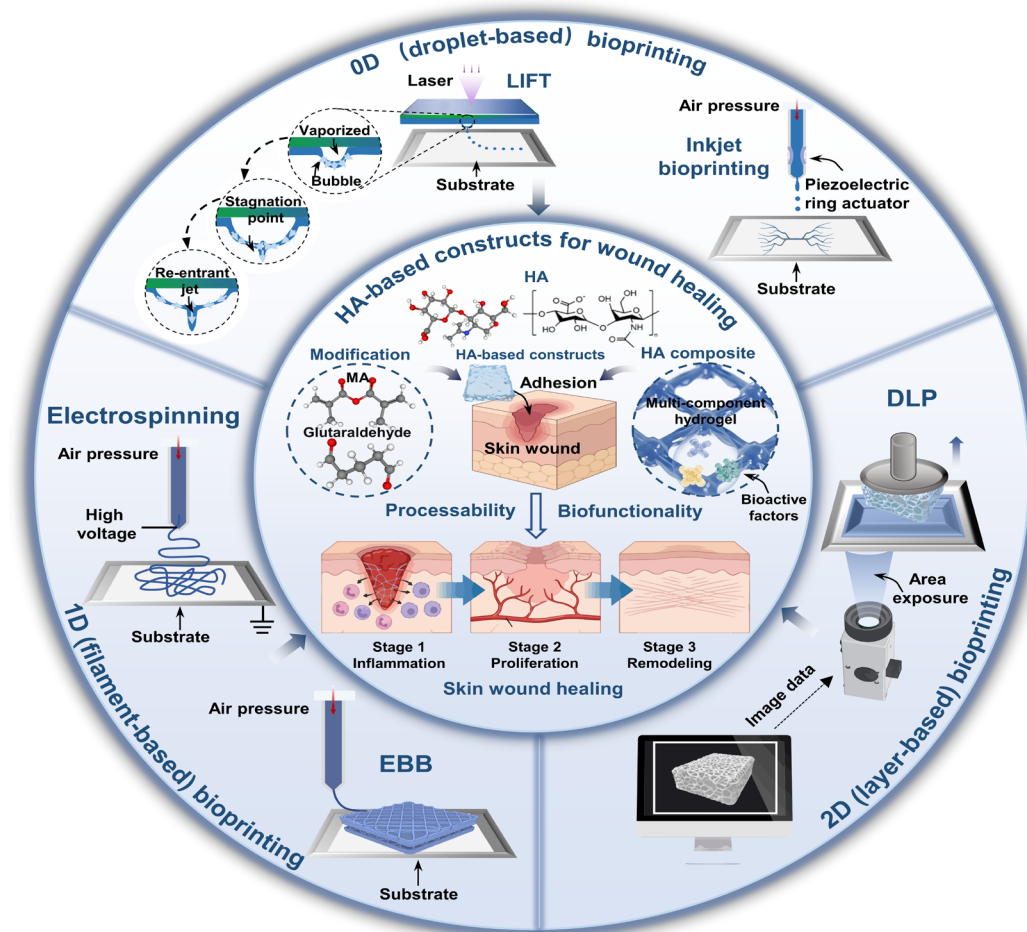
## 2. Hyaluronic acid in wound healing: Characteristics and mechanistic roles

Hyaluronic acid is not merely a passive structural element or a hydrating molecule in the ECM. It is a dynamic, information-rich biopolymer whose diverse and highly context-dependent biological functions are encoded in its molecular structure and metabolism. Therefore, a thorough understanding of its biosynthesis, MW spectrum, and context-dependent interactions is essential to elucidate its multifaceted role in the tightly regulated process of wound healing. This section establishes the necessary biological foundation regarding HA. It first describes the molecular machinery governing HA synthesis and degradation, a dynamic lifecycle that dictates its functional presence in tissues. Subsequently, the central paradigm of HA biology is examined: the profound influence of MW on its biological activity. This paradigm is applied to analyze HA's stage-specific functions across the sequential phases of wound healing. Finally, a critical assessment of the inherent limitations of native, unmodified HA in meeting the complex demands of advanced wound care is presented.

### 2.1. Introduction to hyaluronic acid: Structure, biosynthesis, and turnover

The multifaceted roles of HA in wound healing are rooted in its dynamic molecular lifecycle. This section details the foundational elements of the lifecycle: the defining chemical structure of HA, its biosynthesis, which establishes the initial MW landscape, and its rapid turnover that dynamically reshapes this distribution to generate diverse biological signals.

Structurally, HA is a linear, non-sulfated GAG composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine, linked by alternating  $\beta$ -1,4 and  $\beta$ -1,3 glycosidic bonds.<sup>30,31</sup> This deceptively simple primary structure is the basis for polymers of exceptional length and polydispersity. HA exists across a broad spectrum of MWs, ranging from oligosaccharides to multimillion-Dalton polymers. This inherent polydispersity provides



**Figure 1.** Graphical overview of the review. Integrating HA biology, material engineering, and 3D bioprinting for wound healing. These technologies enable the precise fabrication of customized HA scaffolds for advanced wound healing and skin regeneration applications.

Abbreviations: 0D: Zero-dimensional; 1D: One-dimensional; 2D: Two-dimensional; 3D: Three-dimensional; DLP: Digital light processing; EBB: Extrusion-based bioprinting; HA: Hyaluronic acid; LIFT: Laser-induced forward transfer.

the physical foundation for its wide-ranging, context-dependent biological activities.

The biosynthesis of this polydisperse pool is uniquely catalyzed at the plasma membrane by a family of integral membrane enzymes, the hyaluronan synthases (HAS). In mammals, three isoforms, namely HAS1, HAS2, and HAS3, orchestrate this process.<sup>32–34</sup> These enzymes utilize cytoplasmic uridine diphosphate sugar precursors to polymerize the disaccharide chain, concurrently extruding it into the extracellular space. Critically, the three HAS isoforms are not functionally redundant. They exhibit distinct enzymatic kinetics, synthesizing HA chains with characteristically different average MWs: HAS3 predominantly produces shorter chains, HAS1 generates polymers of intermediate length, and HAS2 is responsible for high-MW HA (HMW-HA; often >1 MDa).<sup>33,35</sup>

Consequently, the differential expression and activity of these isoforms, regulated by growth factors, cytokines, and the local tissue microenvironment, serve as the primary determinant of the initial HA's MW profile in any given tissue.

Following its synthesis, the HA pool undergoes constant and rapid turnover, with an estimated one-third of the total bodily pool (approximately 15 g in adults) degraded and replenished daily.<sup>36,37</sup> This high flux, essential for tissue homeostasis and remodeling, is driven by specific enzymatic and non-specific oxidative degradation pathways. The primary catabolic route involves hyaluronidases (HYALs), notably HYAL1 and HYAL2. HYAL2 cleaves HMW-HA at the cell surface, generating intermediate-sized fragments (~20 kDa) that are internalized and further processed by HYAL1 in lysosomes to small oligosaccharides.<sup>33,38,39</sup> In



pathological or inflammatory contexts, such as wounds, reactive oxygen species (ROS) become a major contributor to HA fragmentation.<sup>37,40</sup> ROS, including hydroxyl radicals and peroxyxynitrite, attack glycosidic bonds in an unregulated manner, thereby significantly increasing the local concentration of lower MW fragments. The resulting degradation products are cleared systemically via lymphatic drainage to lymph nodes or through the circulation for ultimate hepatic clearance.<sup>35,41</sup>

Importantly, these degradation products, particularly small oligosaccharides, are not mere metabolic waste. They possess distinct and often potent bioactivity compared to their HMW precursors and can participate in feedback signaling.<sup>33</sup> Therefore, the dynamic interplay between synthesis (governed by HAS isoforms) and degradation (mediated by HYALs and ROS) functions as an integrated system. This system continuously generates and maintains a broad spectrum of HA's MWs within tissues. This spectrum, from native high polymers to bioactive fragments, constitutes a versatile array of molecular signals. By engaging differentially with cell surface receptors such as cluster of differentiation 44 (CD44) and the receptor for hyaluronan-mediated motility (RHAMM), specific HA sizes within this spectrum can trigger opposing cellular responses, promoting or inhibiting inflammation, proliferation, and migration. This fundamental principle of size-dependent functionality, established by the molecular lifecycle detailed here, provides a cornerstone for understanding HA's stage-specific actions in wound healing. However, it is crucial to acknowledge that HA's functional outcomes are not solely determined by MW. Factors such as endotoxin contamination (particularly relevant in studies of low-MW HA [LMW-HA]), HA source and polydispersity, cellular receptor expression (e.g., CD44, RHAMM, toll-like receptors [TLRs]), and the specific injury context significantly modulate its biological effects. Therefore, while the MW framework is highly informative, it should be applied with consideration of these contextual variables.

## 2.2. The molecular weight-dependent functional paradigms of hyaluronic acid

The biological activity of HA is profoundly influenced by its MW, serving as a key framework for understanding its functional duality. This parameter determines its 3D architecture, binding affinity for specific cell surface receptors, and the activation of divergent intracellular signaling pathways, thereby enabling HA to perform context-dependent and often opposing functions.<sup>42,43</sup> For conceptual clarity, the vast MW continuum of HA is categorized into two primary functional classes: HMW-HA

(>1 MDa), which predominates in stable tissues, and LMW-HA, along with its oligosaccharides (o-HA; <250 kDa), which are generated during tissue turnover and injury.<sup>44,45</sup>

### 2.2.1. High-molecular-weight hyaluronic acid: The maintenance of tissue homeostasis

In healthy skin, HMW-HA serves as a critical structural and regulatory component of the ECM. Its exceptionally long, polyanionic chains bind vast amounts of water, forming a highly hydrated, viscoelastic pericellular gel that maintains tissue turgor, facilitates molecular diffusion, and provides mechanical resilience.<sup>46</sup> Beyond this physicochemical role, HMW-HA exerts potent anti-inflammatory and immunosuppressive effects. Its high degree of polymerization allows multivalent interactions with receptors such as CD44, leading to receptor clustering and the initiation of signaling cascades that suppress inflammatory responses.<sup>47,48</sup> Specifically, HMW-HA can inhibit the recruitment and activation of innate immune cells, downregulate the expression of pro-inflammatory cytokines (e.g., interleukin-1 beta [IL-1 $\beta$ ], tumor necrosis factor-alpha [TNF- $\alpha$ ]), and antagonize signaling through TLR4).<sup>49,50</sup> Concurrently, HMW-HA exhibits anti-angiogenic and anti-proliferative properties, effectively inhibiting endothelial cell migration, proliferation, and capillary tube formation, thereby preventing unnecessary vascularization in quiescent tissues.<sup>51,52</sup> These combined functions establish HMW-HA as a primary regulator of tissue homeostasis and stability.

### 2.2.2. Low-molecular-weight hyaluronic acid/oligosaccharides: Pro-inflammatory and pro-regenerative roles

The fragmentation of HMW-HA, whether through enzymatic cleavage by HYALs or via oxidative stress from ROS, generates LMW-HA and o-HA fragments.<sup>42,53</sup> These are not mere metabolic byproducts but are potent bioactive molecules that function as endogenous danger signals, or damage-associated molecular patterns. In stark contrast to HMW-HA, LMW-HA and o-HA are powerfully pro-inflammatory. They act as ligands for pattern recognition receptors, most notably TLR2 and TLR4, on macrophages and dendritic cells.<sup>15,54</sup> This binding triggers a myeloid differentiation primary response 88-dependent nuclear factor kappa B signaling cascade, resulting in a robust production of key pro-inflammatory cytokines (e.g., TNF- $\alpha$ , IL-1 $\beta$ , IL-8) and chemokines essential for pathogen clearance and debridement.<sup>15</sup> Furthermore, these fragments are strong inducers of angiogenesis and cellular proliferation. They stimulate vascular endothelial cell activities crucial for neovascularization and promote

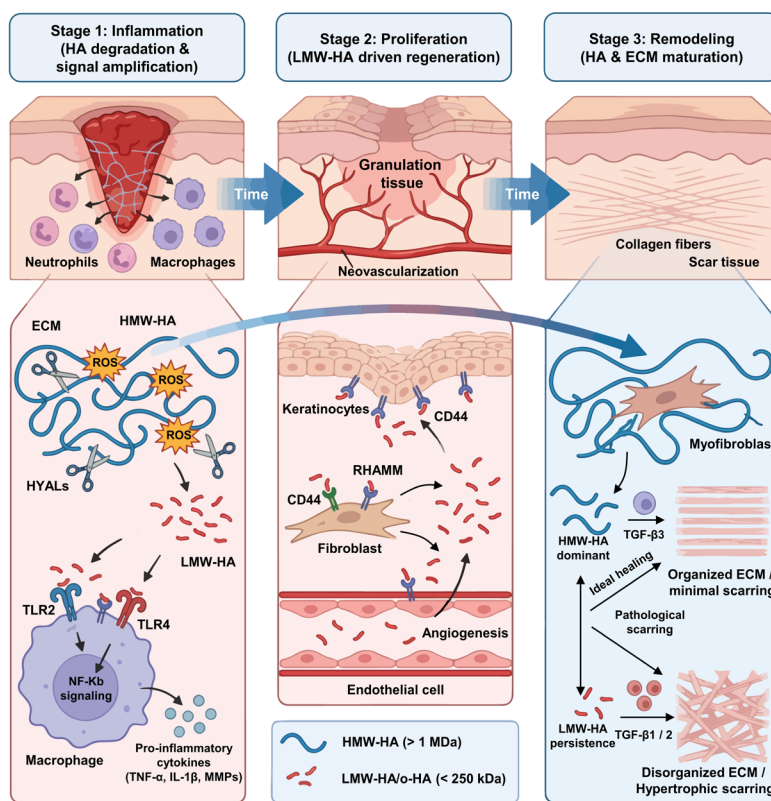
the migration and proliferation of dermal fibroblasts and keratinocytes, the key effectors of tissue reconstruction.<sup>51,55</sup> This bioactivity is frequently mediated through interactions with receptors, including CD44 and RHAMM.<sup>56,57</sup>

Critically, the generation and functional impact of these fragments are governed by the dynamic equilibrium between HA synthesis and catabolism. The MW profile of local hyaluronan is continuously sculpted by the relative activities of biosynthetic and degrading agents. Specifically, it depends on the balance between HASs (HAS1, HAS2, HAS3), which polymerize chains of differing lengths, and the primary degrading agents, HYALs and ROS.<sup>34,42</sup> A shift in this balance has profound biological consequences. In fact, the wound healing cascade can be conceptualized as a precisely orchestrated temporal reprogramming of this equilibrium: an initial shift from a HMW-HA-dominated state that favors hemostasis and provisional matrix formation, to a LMW-HA/o-HA-enriched environment that drives inflammation and proliferation, followed by a

programmed return toward higher MW species to facilitate tissue resolution and orderly remodeling.

### 2.3. Stage-specific mechanisms of action of hyaluronic acid across the phases of wound healing

As schematically summarized in Figure 2, the wound healing process can be conceptualized as a dynamic reprogramming of the local HA's MW profile and its associated biological functions across the four phases of healing: hemostasis, inflammation, proliferation, and remodeling. HMW-HA (>1 MDa; blue chains) predominates in the early stages, providing a scaffold and modulating initial responses. Its fragmentation into LMW-HA/o-HA (<250 kDa; red chains) drives inflammation and subsequent regeneration. A return to higher MW species influences late-stage remodeling and scar outcome. Key cellular events and receptor interactions (CD44, TLRs, RHAMM) are highlighted, providing a visual summary of the conceptual framework detailed in this section.



**Figure 2.** Schematic overview of the stage-specific roles of HA in wound healing. HMW-HA (>1 MDa) and LMW-HA (<250 kDa) play distinct roles in wound healing.

Abbreviations: CD44: Cluster of differentiation 44; ECM: Extracellular matrix; HA: Hyaluronic acid; HMW: High-molecular-weight; HYALs: Hyaluronidases; IL-1β: Interleukin-1 beta; LMW: Low-molecular-weight; MMPs: Matrix metalloproteinases; NF-κB: Nuclear factor kappa B; RHAMM: Receptor for hyaluronan-mediated motility; ROS: Reactive oxygen species; TGF: Transforming growth factor; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor-alpha.

As depicted in Figure 2, HA is not a passive component but a dynamic signaling molecule that actively participates in and guides the progression through each of these phases. Its functional role is guided by the temporal evolution of its MW profile within the wound microenvironment, providing stage-specific instructions that regulate cellular behavior and tissue responses.<sup>15,44,58</sup>

### **2.3.1. The inflammatory phase: Hyaluronic acid degradation and signal amplification**

The healing cascade initiates with hemostasis and the establishment of a provisional matrix. During this initial phase, activated platelets release stored HMW-HA into the wound site. Here, it assembles with fibrin and fibronectin, forming a highly hydrated, viscoelastic scaffold that facilitates early cell migration and modulates immune responses (Figure 2).<sup>59</sup> Subsequently, infiltrating neutrophils release HYALs and ROS, which collectively catalyze the rapid cleavage of the HMW-HA scaffold. This degradation leads to a precipitous rise in local concentrations of LMW-HA and o-HA.<sup>15</sup> These fragments serve as pivotal pro-inflammatory amplifiers. Specifically, their engagement with TLR2 and TLR4 on immune cells has been shown to activate sustained nuclear factor kappa B signaling, which in turn upregulates the production of key mediators such as TNF- $\alpha$ , IL-1 $\beta$ , and matrix metalloproteinases (MMPs), which are essential for wound sterilization and debridement.<sup>54,60</sup> Therefore, the catabolic conversion of HMW-HA to LMW-HA represents a crucial biochemical switch that potentiates and prolongs the inflammatory phase.

### **2.3.2. The proliferative phase: Hyaluronic acid-mediated cellular recruitment and angiogenesis**

The LMW-HA/o-HA fragments that fueled inflammation pivot to direct the constructive phase of healing, orchestrating three parallel regenerative processes (Figure 2). Firstly, LMW-HA acts as a potent chemoattractant and mitogen for dermal fibroblasts. Through interactions with receptors such as CD44 and RHAMM, it guides fibroblast migration into the wound bed, stimulates their proliferation, and promotes their differentiation into contractile myofibroblasts under the influence of transforming growth factor-beta (TGF- $\beta$ ).<sup>57,59</sup> Secondly, the pro-angiogenic properties of these fragments become paramount. They stimulate endothelial cell proliferation, migration, and organization into new capillary networks, a process vital for supplying oxygen and nutrients to the nascent granulation tissue.<sup>51,55</sup> This activity is frequently reported to be mediated via CD44 and RHAMM in experimental models.<sup>51</sup> Thirdly, at the wound edges, LMW-HA interacts with CD44 receptors on keratinocytes,

regulating their proliferation, directional migration over the wound bed, and terminal differentiation, thereby restoring the critical epidermal barrier.<sup>61</sup>

Collectively, the pro-regenerative functions of LMW-HA fragments during the proliferative phase exemplify its role as a context-dependent orchestrator rather than a solitary conductor. It is critical to appreciate that these effects are integrated within a broader network of healing determinants. For instance, successful angiogenesis is equally governed by hypoxia gradients (hypoxia-inducible factor 1 $\alpha$ /vascular endothelial growth factor [VEGF] axis), ECM mechanics, and the absence of pathological barriers such as infection—factors that HA can influence but does not control. Thus, the therapeutic impact of HA-based matrices arises from a combination of mechanisms: (i) direct, fragment size-dependent receptor signaling (e.g., via CD44, RHAMM) that guides specific cell behaviors; (ii) the provision of a hydrated, viscoelastic provisional matrix that physically supports cell migration and tissue ingrowth; and (iii) the ability to serve as a delivery platform for other therapeutic agents (e.g., growth factors, antimicrobials). This multifunctionality positions HA not merely as a bioactive molecule but as a versatile biomaterial scaffold that simultaneously modulates the physical, chemical, and biological microenvironment of the wound.

### **2.3.3. The remodeling phase: Hyaluronic acid and extracellular matrix maturation**

During the prolonged remodeling phase, the role of HA becomes subtler and is again influenced by its MW profile (Figure 2). Myofibroblasts, responsible for wound contraction and ECM deposition, are active producers of HA.<sup>62</sup> The balance between HA sizes during this phase can significantly influence scar architecture. Evidence suggests that LMW-HA may promote the expression of the profibrotic isoforms TGF- $\beta$ 1 and TGF- $\beta$ 2, driving excessive and disorganized collagen deposition associated with hypertrophic scarring.<sup>63,64</sup> Conversely, HMW-HA is linked to the upregulation of TGF- $\beta$ 3, an isoform implicated in anti-fibrotic responses and regenerative healing that minimizes scar formation.<sup>63,64</sup> Therefore, optimal healing likely involves a timely shift back toward an HMW-HA-predominant microenvironment. This shift could help terminate myofibroblast activity, promote their apoptosis, and guide the deposition of a more organized, functionally competent ECM, culminating in a mature scar with improved tensile strength. Applied to HA, this requires engineering HA derivatives with built-in, stimuli-responsive triggers (e.g., MMP-cleavable linkers, ROS-sensitive motifs) that are compatible with bioprinting processes.

While the model of MW-dependent HA signaling provides a compelling and useful framework for understanding its roles in wound healing, it is important to acknowledge current limitations in mechanistic understanding, particularly for translational applications. Much of the evidence supporting these stage-specific mechanisms derives from controlled *in vitro* studies or preclinical murine models. In these models, HA metabolism, receptor expression profiles (e.g., the relative contributions of CD44, RHAMM, and TLRs), and immune responses may differ from the complex pathophysiology of human chronic wounds. The precise spatiotemporal dynamics of receptor activation and the crosstalk between signaling pathways in human wound healing remain incompletely mapped. Furthermore, translating this knowledge into therapy requires careful consideration of the therapeutic window for exogenously applied HA fragments. A key challenge is balancing their pro-regenerative signaling against potential unintended pro-inflammatory or profibrotic effects, which necessitates further delineation in more clinically relevant wound models.

In summary, HA functions as a master regulator of wound healing through its dynamically changing MW. The precisely timed transition from HMW-HA-mediated stabilization, through LMW-HA-driven cellular activation, and back toward a stabilizing HMW-HA environment provides the contextual cues necessary to coordinate the complex cellular and biochemical events required for efficient and orderly tissue restoration. The narrative of HA as a master regulator via dynamic MW changes provides a powerful explanatory model for its role in wound healing. It is important to remember, however, that this model operates within and is modulated by the specific biological context, including the factors previously noted. This inherent versatility stems from three core biofunctional advantages encoded within its molecular lifecycle: (i) its synthesis by differentially regulated HAS isoforms establishes a dynamic and context-specific initial MW spectrum; (ii) its continuous, high-flux turnover via HYALs and ROS actively generates a diverse array of bioactive fragments, transforming degradation into potent signaling; and (iii) its MW-dependent duality enables a single biopolymer to deliver opposing, stage-specific biological instructions, thereby coordinating the sequential phases of wound healing.

#### **2.3.4. Dynamic modulation of hyaluronic acid bioactivity by the wound microenvironment**

The biological activity of HA is not an intrinsic, fixed

property but is dynamically programmed and reshaped by the biochemical and physical cues of the surrounding wound microenvironment. This context-dependency is central to its stage-specific functions and presents both challenges and opportunities for therapeutic design.<sup>2</sup>

Oxidative stress is a dominant environmental factor that actively reprograms HA signaling. Elevated levels of ROS, characteristic of the inflammatory phase, directly fragment HMW-HA into LMW-HA and o-HA via non-enzymatic cleavage of glycosidic bonds. This ROS-mediated degradation serves as a feed-forward loop: it depletes anti-inflammatory HMW-HA while simultaneously generating pro-inflammatory and pro-angiogenic LMW-HA fragments, thereby amplifying and perpetuating the inflammatory and proliferative signals necessary for early healing. Consequently, the redox state of the wound acts as a master switch, directly controlling the local HA's MW profile and its resultant biological instructions.<sup>10</sup>

Similarly, shifts in pH exert a profound influence. The wound bed, particularly in chronic or infected states, often becomes acidic (pH 6.0–6.5). This acidic microenvironment can alter the charge state and conformation of HA, potentially affecting its hydration, viscosity, and binding affinity for receptors like CD44. More importantly, pH influences the activity of key enzymes in the HA lifecycle.<sup>65</sup> For instance, the activity of HYALs is pH-dependent, with optimal activity often in acidic ranges. Therefore, wound acidosis can accelerate the enzymatic turnover of HA, further shifting the MW balance toward bioactive fragments. This pH sensitivity also informs the design of “smart” HA-based biomaterials, where crosslinks (e.g., hydrazone bonds) or drug release mechanisms can be engineered to respond specifically to the acidic wound milieu.<sup>2</sup>

Beyond ROS and pH, other microenvironmental variables contribute to this dynamic interplay. Enzyme profiles (e.g., MMPs that cleave HA-binding proteins), ionic strength, and mechanical forces within the granulation tissue can all modulate HA assembly, presentation, and cellular perception. For example, mechanical tension can align HA chains and influence receptor clustering, thereby modulating downstream signaling pathways related to fibroblast activation and differentiation.<sup>11</sup>

In summary, the wound microenvironment functions as an integrated processing unit that continuously modifies HA's physical form and biochemical output. This dynamic relationship underscores that the therapeutic efficacy of exogenous HA depends critically on its interaction with and resilience to these local conditions. It also provides a

compelling rationale for engineering next-generation HA biomaterials that are not merely passive carriers but active participants, designed to sense and respond adaptively to these microenvironmental cues to deliver precisely timed therapeutic actions.

#### **2.4. Inherent limitations of native hyaluronic acid for advanced therapeutic applications**

Despite its central physiological role and demonstrated therapeutic promise in wound care, the direct clinical application of unmodified, native HA remains constrained by inherent limitations. While its high hydrophilicity and biocompatibility are advantageous, native HA is insufficient to meet the stringent mechanical, temporal, and functional demands of managing complex, non-healing wounds. These intrinsic shortcomings highlight the need for advanced HA engineering to transform HA into a robust, versatile platform for next-generation wound therapies.

##### **2.4.1. Poor mechanical strength and structural instability**

Native HA exists in aqueous environments as a viscous solution or a weak physical gel, stabilized primarily by chain entanglements and transient hydrogen bonds. These non-covalent interactions impart low mechanical strength, limited elasticity, and poor structural integrity to HA-based constructs such as sponges or films.<sup>66,67</sup> Under physiological conditions, especially within exudative wound beds, such materials lack the necessary toughness and resilience to provide a durable barrier against infection and mechanical trauma, or to withstand external shear and compressive forces. This mechanical fragility restricts their utility as standalone scaffolds in load-bearing or dynamically stressed wound environments, where sustained structural support is essential for guided tissue regeneration. Critically, these inherent mechanical weaknesses highlight a broader engineering challenge: the very properties that enable HA's potent hydration and bioactivity, including high water content and chain flexibility, often fundamentally conflict with the material performance required for durable, multifunctional wound scaffolds.<sup>68</sup> Reconciling its native biological functions with enhanced mechanical and structural stability remains a central dilemma in HA-based biomaterial design.

##### **2.4.2. Rapid *in vivo* degradation and short residence time**

Hyaluronic acid undergoes exceptionally rapid turnover *in vivo*, with a half-life ranging from less than one day in skin to a few days in other tissues.<sup>69,70</sup> This rapid clearance is attributed primarily to the ubiquitous presence of

HYALs, ROS, and other hydrolytic enzymes, which are abundant in inflammatory wound microenvironments.<sup>71,72</sup> Consequently, topically applied or implanted native HA is swiftly degraded into small fragments, leading to a precipitous loss of its hydrating capacity and bioactivity. Such short functional residence times necessitate frequent reapplication, which is clinically impractical and compromises patient compliance. Moreover, it fails to deliver the sustained, stage-specific biological signaling required to orchestrate the prolonged healing cascade in chronic wounds.<sup>15</sup>

##### **2.4.3. Lack of inherent antimicrobial and multifunctional properties**

Despite its important immunomodulatory roles, HA nonetheless lacks intrinsic broad-spectrum antimicrobial activity. Chronic wounds are frequently complicated by bacterial colonization and biofilm formation, which perpetuate inflammation and impede healing.<sup>73</sup> Native HA does not actively combat infection, and its highly hydrated matrix may even provide a favorable environment for microbial proliferation in the absence of appropriate functionalization.<sup>74</sup> Furthermore, the management of complex wounds often demands integrated functionalities beyond hydration and generic bioactivity, such as controlled drug release, antioxidant defense against oxidative stress, precise cell-instructive cues, and conductive properties for electroactive tissue repair.<sup>75</sup> As a monospecific polysaccharide, native HA lacks the chemical versatility required to intrinsically deliver such a multifunctional repertoire.

##### **2.4.4. Structural and functional homogeneity versus healing complexity**

Wound healing is a highly orchestrated, spatiotemporally dynamic process that requires sequential and often opposing biological signals from initial inflammation and antimicrobial activity to subsequent proliferation, angiogenesis, and regulated remodeling.<sup>2,61</sup> Native HA possesses a relatively homogeneous structure that cannot intrinsically replicate this complexity. It is unable to simultaneously provide robust mechanical support, tunable degradation kinetics, and the controlled release of multiple bioactive agents (e.g., growth factors, antibiotics, antioxidants) in a phase-specific manner.<sup>21</sup> This discrepancy underscores a fundamental biomaterial design challenge: the very properties that endow HA with its potent biological signaling capacity (e.g., rapid degradation, chain flexibility) often conflict with the engineering requirements for a stable, multifunctional scaffold.

In summary, while native HA is indispensable in physiological wound healing, its intrinsic properties are



insufficient to address the multifaceted demands of clinical wound management, particularly in chronic, infected, or large-scale defects. These limitations directly motivate the HA engineering strategies discussed in the subsequent section. Key among these are poor mechanical performance, rapid degradation, a lack of multifunctionality, and the inability to provide spatiotemporally controlled cues. Through chemical modification, biofunctionalization, and compositing, HA can be transformed into a tailor-made, performance-enhanced platform capable of meeting the complex needs of modern regenerative wound therapy.

### 3. Engineering hyaluronic acid: From molecular modification to advanced functionality

Hyaluronic acid is a central orchestrator of the physiological wound healing cascade. However, the native form of HA has intrinsic limitations, including poor mechanical strength, rapid enzymatic degradation, a lack of inherent antimicrobial activity, and functional homogeneity. These limitations restrict its utility as a standalone advanced therapeutic. To bridge the gap between the innate biological signaling capacity of HA and the rigorous demands of clinical wound management, deliberate engineering of HA is essential. This section systematically reviews convergent strategies that transform HA from a simple polysaccharide into a versatile, multifunctional platform for next-generation wound repair. Molecular modification is presented as the foundational engineering approach, which enables two critical, parallel design objectives: enhancing material processability to ensure structural integrity and fabrication compatibility, and conferring direct therapeutic biofunctionality to overcome pathophysiological barriers. Following an exploration of these core modification strategies, the synergistic potential of hybrid and composite systems that integrate HA with other polymers and bioactive components is examined. Several representative applications of engineered HA-based biomaterials are listed in [Table 1](#). Collectively, these engineering pathways provide a robust toolkit for developing HA-based biomaterials that not only protect the wound but also actively and intelligently guide the complex healing process toward regeneration.

#### 3.1. Molecular modification of hyaluronic acid

The transformative potential of HA in advanced wound therapy cannot be realized using its native form alone. As outlined in Section 2.4, the inherent material limitations of natural HA, including its mechanical weakness, rapid degradation, and functional passivity, conflict directly with the requirements for managing complex wounds. To address this disparity, strategic chemical modification of

the HA backbone serves as the fundamental engineering paradigm. This process represents a strategic molecular redesign, a deliberate modification that grafts new chemical functionalities onto the polysaccharide chain. This, in turn, imparts tailored properties beyond the reach of the pristine polymer.

Conceptually, these modifications pursue two primary, complementary objectives that guide all subsequent biomaterial design. The first is a processability-oriented modification that introduces reactive handles, such as methacrylate, thiol, or aldehyde groups, to enable the formation of stable 3D networks via crosslinking. This objective addresses the material deficiencies of native HA, providing structural integrity, controlled degradability, and the rheological properties necessary for fabrication techniques such as 3D bioprinting. The second is biofunction-oriented modification, which involves covalently grafting bioactive molecules or motifs, such as antimicrobial peptides (AMPs), antioxidant agents, or cell-adhesive ligands, directly onto the HA chain. These modifications embed therapeutic functions directly into the biomaterial, enabling it to actively participate in the healing process beyond a purely structural role.

The chemical feasibility of this paradigm is rooted in the accessible functional groups presented by the repeating disaccharide unit of HA: the primary and secondary hydroxyl groups, the carboxyl group, and the N-acetyl group. Each site offers distinct reactivity profiles for covalent conjugation. Hydroxyl groups are commonly modified through esterification to form HA methacryloyl (HAMA) or oxidation to yield dialdehyde HA (oxidized HA [OHA]). The carboxyl group is most frequently functionalized via high-efficiency amidation reactions, often mediated by carbodiimide chemistry, such as using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS), to attach a wide variety of amine-containing compounds. This approach is used to synthesize key derivatives, such as tyramine-modified HA (HA-Tyr) and thiolated HA (HA-SH). Modification of the N-acetyl group, typically through deacetylation to expose a free amine, provides another viable, though less common, route for conjugation.

This versatile chemical toolkit enables the precise engineering of HA derivatives with predefined capabilities. The resulting library of functionalized polymers, whether designed for crosslinking or bioactivity, constitutes the essential building blocks for the next generation of HA-based wound care products. While molecular modification of HA provides tailored functionality, it is crucial to acknowledge that such alterations may also influence its immunogenic profile. Chemical derivatization (e.g., methacrylation,

Table 1. Classification and characteristics of representative hyaluronic acid-based biomaterial formulations for three-dimensional bioprinting and wound healing

Primary strategy	Formulation strategy	Formulation type	Advantages	Limitations	Key performance metrics	Reference
Molecular modification of HA	Processability-oriented	HAMA	Excellent for high-resolution vat photopolymerization (e.g., DLP); enables fabrication of complex 3D architectures with precise spatial control	Requires cytocompatible photoinitiator; UV exposure may compromise bioactivity of encapsulated cells/factors	Compressive modulus: 1–10 kPa; cell viability (encapsulated): 70–90%	15,21
		HA-Tyr	Gentle, cell-friendly crosslinking suitable for cell-laden bioinks; gelation kinetics easily tuned for extrusion bioprinting	Enzyme and hydrogen peroxide concentrations require precise optimization to balance printability and cell viability	Cell viability: > 90%; enzymatic degradation (HYAL, 24 h): 20–80%	305
		OHA	Enables shear-thinning and self-healing behavior, ideal for extrusion printing and injectable delivery; pH-responsive for smart drug release	Periodate oxidation reduces HA MW, which may diminish its native high-MW anti-inflammatory signaling	Swelling ratio: 1,000–3,000%; viscosity: 10–100 Pa·s	21,99
	Biofunction-oriented	RGD peptide-conjugated HA	Crucial for promoting cell adhesion and migration within bioprinted constructs, preventing cell anoikis	Bioactivity is peptide-sequence specific; optimal surface density for 3D culture may differ from 2D	Fibroblast adhesion: 2–5-fold increase vs. unmodified HA	118
		Antimicrobial agent-conjugated HA	Provides intrinsic, localized antimicrobial activity in bioprinted scaffolds, combating infection in chronic wound models	Grafting chemistry must not interfere with bioink printability (e.g., rheology, crosslinking)	Biofilm inhibition: 50–80% reduction	107
	Dual-function	HA-CA	Multifunctional: provides tissue adhesion for printed construct integration, antioxidant activity, and enables gentle metal-ion crosslinking for bioprinting	Catechol oxidation over time may alter hydrogel mechanics and color in long-term implants	Adhesion strength (to skin): 10–30 kPa; compressive strength: 5–50 kPa	91,115

(Cont'd...)

Table 1. Continued

Primary strategy	Formulation strategy	Formulation type	Advantages	Limitations	Key performance metrics	Reference
Blends with natural polymers	Chitosan/HA PECs	Combines HA bioactivity with chitosan's antimicrobial properties; rapid ionic gelation can serve as a support bath for embedded bioprinting		Polyelectrolyte complexation can be too rapid, leading to inhomogeneous filament formation during extrusion	Antibacterial rate: 85–99%; compressive modulus: 2–20 kPa	129,130
		Collagen/gelatin-HA composites	Synergizes HA signaling with essential ECM motifs; improves cell-material interactions in bioprinted dermal layers	Thermo-reversible gels (gelatin) may lack shape fidelity at 37 °C without secondary crosslinking	Pore size: 50–200 µm; storage modulus: 0.5–5 kPa	125,135
	Composites with synthetic polymers	HA-PEG networks	PEG spacer allows modular tuning of mesh size and mechanical properties, enhancing print fidelity and stability	Non-degradable PEG segments may hinder long-term ECM remodeling; can be bio-inert, limiting cell adhesion unless functionalized	Mesh size: 10–50 nm	82,146
		HA-PCL nanofibers	PCL provides superior mechanical strength for load-bearing applications; electrospun mats can serve as stable substrates for subsequent bioprinting	Potential phase separation in composite bioinks; HA bioactivity may be confined to the surface, limiting 3D cellular sensing	Fiber diameter: 200–800 nm	150,151
Hybrid & composite systems	Integration of conductive components	HA-rGO hydrogels	Imparts electrical conductivity for electroactive wound therapy; enhances the mechanical robustness of printed scaffolds	Achieving stable, homogeneous dispersion of rGO in viscous bioinks is challenging; long-term biopersistence needs evaluation	Compressive strength: 10–100 kPa	162,170
		HA-PANI conductive hydrogels	Provides stable electroactivity to guide cell migration (electrotaxis) under electrical stimulation	Often requires acidic doping conditions for processing, which are incompatible with cell-laden bioprinting	Conductivity: $10^{-5}$ – $10^{-3}$ S/cm; antibacterial: 70–95%	155,176,177
	Matrices for bioactive cargo	HA hydrogels loaded with growth factors (e.g., VEGF)	Enables localized, sustained presentation of morphogens within the 3D bioprinted matrix to guide tissue maturation	Burst release is common; achieving spatiotemporal release profiles matching healing phases is a key challenge for bioprinted constructs	Bioactivity retention: >70% after seven days of release	122,123,182
		HA-based sponges with AgNPs	Confers potent, broad-spectrum antimicrobial activity to bioprinted dressings, ideal for infected wound models	Potential cytotoxicity at high doses/near cells; nanoparticle aggregation can clog printing nozzles	Antibacterial: >99.9% kill rate	164,186
		HA hydrogels encapsulating cells (e.g., ADSCs)	Serves as a bioactive 3D delivery vehicle for cell therapy via bioprinting, maintaining stem cell potency	Crosslinking chemistry must be exceptionally cytocompatible; limited nutrient diffusion in thick, cell-dense constructs remains a hurdle	Post-print viability: 75–90%	66,192

Abbreviations: ADSCs: Adipose-derived stem cells; AgNPs: Silver nanoparticles; CA: Catechol; DLP: Digital light processing; ECM: Extracellular matrix; HA: Hyaluronic acid; HAMA: Hyaluronic acid methacryloyl; HA-Tyr: Tyramine-modified hyaluronic acid; HYAL: Hyaluronidase; MW: Molecular weight; OHA: Oxidized hyaluronic acid; PANi: Polyaniline; PCL: Polycaprolactone; PECs: Polyelectrolyte complexes; PEG: Polyethylene glycol; RGD: Arginine-glycine-aspartic acid; rGO: Reduced graphene oxide; UV: Ultraviolet; VEGF: Vascular endothelial growth factor.

grafting of foreign peptides) can introduce neoepitopes or alter the polymer's recognition by endogenous enzymes and receptors, raising the risk of unintended immune activation. Therefore, the biocompatibility and immunological safety of engineered HA derivatives must be rigorously evaluated in relevant preclinical models, particularly for chronic wound applications where immune dysregulation is common. Future designs should aim to balance functional enhancement with the preservation of HA's intrinsic biocompatibility. Furthermore, while chemical modifications are designed to enhance HA's functionality, they may also impact its intrinsic biocompatibility beyond immunological concerns. Alterations such as grafting hydrophobic moieties, introducing synthetic polymer segments, or employing certain crosslinking agents can affect HA's hydration capacity, enzymatic degradation kinetics, and interactions with host cells. These changes could affect critical processes in wound healing, including cell adhesion, proliferation, and migration. Therefore, the biocompatibility of each engineered HA derivative should be evaluated not only in terms of cytotoxicity and immunogenicity, but also in its ability to support physiologically relevant cell behavior and tissue integration. The following two sections examine these parallel engineering streams in detail. Section 3.2 delves into the specific chemistries and crosslinking strategies that transform HA into a robust, processable hydrogel matrix. Subsequently, Section 3.3 explores how targeted molecular grafting equips HA with sophisticated therapeutic functionalities to combat infection, modulate inflammation, and direct cellular behavior.

### 3.2. Processability-oriented modification: Enabling structural integrity and printability

The transformation of native HA from a soluble polysaccharide with inherent bioactivity but limited mechanical robustness into a structurally stable system requires precise engineering of its processability. As established in Section 3.1, modifications aimed at enhancing processability involve introducing specific reactive moieties onto the HA backbone. These functional groups serve as molecular anchors for crosslinking, the critical process that converts discrete polymer chains into 3D hydrogel networks. The selection of both derivatization chemistry and crosslinking mechanism governs key hydrogel properties, including gelation kinetics, rheological characteristics, mechanical strength, degradation behavior, and compatibility with advanced fabrication methods such as 3D bioprinting. The sequential engineering workflow, beginning with chemical derivatization and proceeding to the formation of diverse crosslinked networks, is conceptually summarized in Figure 3. The

following sections will dissect this transformation chain, analyzing the chemical strategies that facilitate the design of HA-based hydrogels with tailored structural properties for challenging wound healing applications.

The selection of derivatization chemistry and crosslinking mechanism is therefore paramount. This choice dictates not only the final hydrogel's mechanical and degradation properties but also, critically, its printability. Printability here encompasses rheological behavior, gelation kinetics, and shape fidelity, all of which are essential for advanced fabrication techniques like 3D bioprinting.

#### 3.2.1. Chemical derivatization of hyaluronic acid: Installing crosslinkable handles

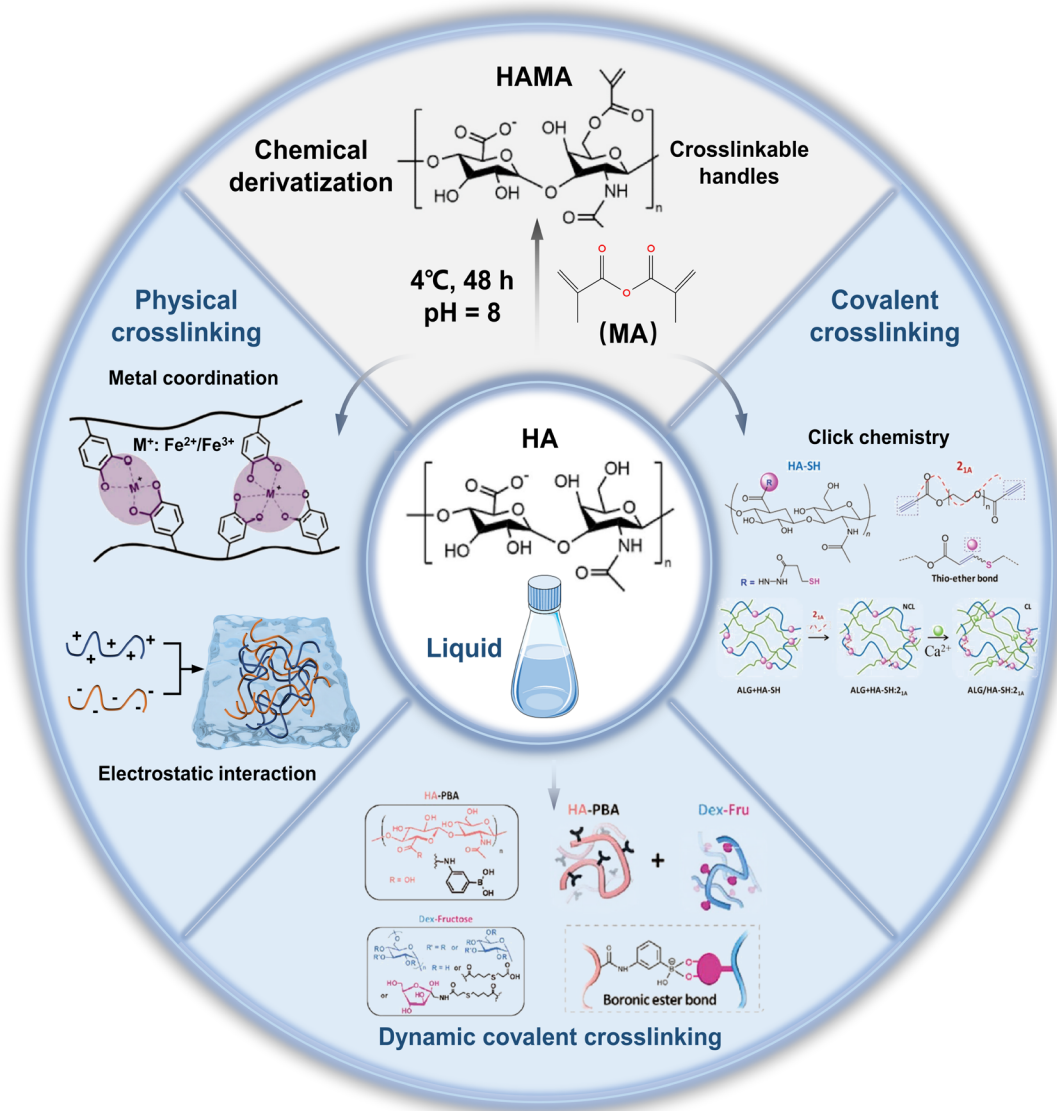
The development of a crosslinkable HA-based material begins with precise chemical derivatization. The linear HA polymer offers three primary sites for modification: the hydroxyl groups ( $-OH$ ), the carboxyl group ( $-COOH$ ), and the N-acetyl group ( $-NHCOCH_3$ ).<sup>75</sup> Each site presents distinct chemical reactivity, enabling the grafting of functionalities that predetermine the subsequent crosslinking mechanism and the final hydrogel's properties.<sup>76</sup>

Hydroxyl group modification is predominantly achieved through esterification or oxidation. Esterification with methacrylic anhydride is a quintessential reaction, yielding HAMA.<sup>15,77</sup> This modification installs photopolymerizable vinyl groups onto the HA backbone, creating a precursor ideally suited for light-initiated radical polymerization. The degree of methacrylation (DM) serves as a critical parameter, offering a direct lever to tune the crosslinking density and the resultant hydrogel's mechanical modulus and swelling ratio.<sup>21</sup> Furthermore, the DM profoundly influences biological interactions. A higher DM leads to a more densely crosslinked network upon polymerization, which significantly retards enzymatic degradation by HYALs due to hindered enzyme diffusion and substrate accessibility. Conversely, this increased crosslinking density can impede cell migration and infiltration, as cells require localized matrix remodeling to move through the hydrogel. Therefore, identifying an optimal DM is crucial to balance the conflicting demands of scaffold stability *in vivo* and the provision of a permissive microenvironment for cell-driven tissue integration. In contrast, periodate oxidation selectively cleaves the vicinal diols on the HA sugar ring, converting them into reactive aldehyde functionalities and generating OHA.<sup>21,75</sup> This transformation not only reduces the HA's MW but also introduces dynamic covalent bonding sites. The aldehydes in OHA can undergo reversible Schiff base reactions with amines or hydrazides,

forming hydrogels that are inherently pH-responsive and often self-healing.<sup>21</sup>

Carboxyl group modification most commonly proceeds via high-efficiency amidation reactions, typically mediated by carbodiimide chemistry such as the EDC/NHS coupling system.<sup>75,76</sup> This robust, aqueous-compatible conjugation strategy enables the attachment of a wide array of amine-bearing molecules, yielding several cornerstone HA derivatives. For instance, the conjugation of tyramine yields HA-Tyr, which introduces phenol groups that enable

gentle, cell-friendly gelation with precise temporal control via enzymatic oxidation using horseradish peroxidase and hydrogen peroxide ( $H_2O_2$ ).<sup>76</sup> Another key derivative, HA-SH, is created by grafting thiol ( $-SH$ ) groups onto the HA backbone. This multifunctional precursor can form irreversible disulfide bonds upon oxidation or engage in rapid thiol-Michael addition reactions with electron-deficient alkenes, providing versatile pathways to both stable and stimuli-responsive networks.<sup>78,79</sup> Furthermore, functionalization with hydrazide groups yields hydrazide-



**Figure 3.** Engineering process for HA-based hydrogels; from chemical derivatization to diverse crosslinking networks. Part of the image was adopted with permission from Abdi *et al.*<sup>304</sup> Copyright © 2020, WILEY-VCH Verlag GmbH & Co.

Abbreviations: HA: Hyaluronic acid; HAMA: Hyaluronic acid methacryloyl; HA-PBA: Phenylboronic acid-modified hyaluronic acid; HA-SH: Thiolated hyaluronic acid.



modified HA, which allows for the formation of hydrazone bonds with aldehydes or ketones. These bonds are stable at physiological pH but hydrolyze under acidic conditions, a property that enables pH-triggered drug release and is highly relevant to the acidic microenvironment of chronic wounds.<sup>76,80</sup> Beyond these, the carboxyl group can also be modified with “click” chemistry handles, such as azides or alkynes, paving the way for highly selective and efficient cycloaddition-based crosslinking strategies.<sup>81,82</sup>

N-acetyl group modification, primarily through deacetylation (e.g., via hydrazinolysis), is a less common but valuable route.<sup>83,84</sup> This process exposes free primary amine groups on the HA chain, providing an alternative conjugation site distinct from the carboxyl group. These amines can be used for further chemical grafting or can participate in auto-crosslinking reactions, contributing to network formation.

In summary, chemical derivatization goes beyond a simple functionalization step to represent a fundamental design decision. The resulting library of HA derivatives, including HAMA, HA-SH, HA-Tyr, and OHA, constitutes a versatile molecular toolkit. Each tool is specialized, defining the subsequent crosslinking language (photopolymerization, enzyme catalysis, dynamic covalent chemistry, etc.) and setting the stage for the hydrogel's final performance profile in terms of gelation control, mechanical resilience, and environmental responsiveness.<sup>85</sup> Crucially, this choice predetermines the biomaterial's compatibility with specific bioprinting modalities: photopolymerizable derivatives such as HAMA are suited for vat photopolymerization, while dynamic systems such as OHA or HA-SH favor extrusion-based printing due to their shear-thinning and self-healing properties.

### 3.2.2. Physical crosslinking mechanisms

Physical crosslinking constructs hydrogel networks through transient, non-covalent interactions. These reversible bonds form under mild, often physiological conditions, frequently without the need for chemical initiators or catalysts, thereby offering superior biocompatibility and minimal risk of cytotoxicity.<sup>21,86</sup> The resulting hydrogels typically exhibit shear-thinning and rapid self-recovery behavior, making them exceptionally suitable for injectable therapeutics and EBB, where the biomaterial inks must flow under shear stress and then maintain the deposited structure.<sup>78,87</sup>

Electrostatic complexation capitalizes on the inherent polyanionic character of HA. When mixed with cationic biopolymers such as CS, instantaneous hydrogel formation occurs through the formation of polyelectrolyte complexes.<sup>78,84</sup> This simple yet effective strategy combines

the unique biological properties of both components. However, the strong, often diffusion-limited electrostatic attraction can lead to the formation of inhomogeneous aggregates with a macroporous, sometimes brittle structure. Precise control over parameters like polymer concentration, charge density, mixing ratio, and ionic strength is crucial to achieve homogeneous and mechanically coherent networks.<sup>21</sup>

Hydrophobic association and self-assembly transform hydrophilic HA into an amphiphilic polymer by conjugating hydrophobic moieties (e.g., cholesterol, alkyl chains, or bile acids).<sup>88</sup> In aqueous environments, these polymers self-assemble into micellar or fibrillar nanostructures, with the hydrophobic domains acting as physical crosslinks. This mechanism confers thermo-responsiveness to the hydrogel, whereby increasing temperature strengthens hydrophobic interactions, thereby inducing gelation. A significant advantage of this strategy is its ability to efficiently encapsulate hydrophobic drugs within the micellar cores, enabling combination therapy within a wound dressing matrix.<sup>88</sup>

Host-guest interactions represent a sophisticated supramolecular approach to physical crosslinking. It relies on the specific molecular recognition between complementary pairs, such as  $\beta$ -cyclodextrin (host) and adamantane (guest). By conjugating these moieties onto separate HA chains or onto HA and a partner polymer, crosslinking is induced via the formation of inclusion complexes.<sup>89,90</sup> The beauty of this system lies in its dynamic reversibility: the host-guest bonds constantly dissociate and re-associate. This endows the hydrogel with remarkable self-healing properties and perfect shear-thinning behavior, as applied shear force breaks the temporary complexes, which rapidly re-form once the stress is removed. This makes host-guest HA hydrogels exemplary biomaterials for EBB and ideal for minimally invasive injection into irregular wound beds.<sup>21</sup>

Metal ion coordination utilizes multivalent cations to crosslink specific ligands grafted onto HA. A prominent example is catechol-modified HA, inspired by mussel adhesive proteins. Catechol groups can form reversible, pH-dependent coordination complexes with ions such as  $\text{Fe}^{3+}$ , leading to rapid gelation.<sup>91,92</sup> By varying the ion concentration or pH, the crosslinking density and thus the hydrogel's mechanical properties can be finely tuned *in situ*. This strategy not only facilitates injectable gel formation but also can impart strong tissue-adhesive properties due to the catechol group's ability to bind to various organic surfaces.

While offering unparalleled advantages for biofabrication and injectability, physically crosslinked

hydrogels are subject to an intrinsic trade-off: the very reversibility that enables their attractive processing features also limits their long-term mechanical stability and resilience under constant physiological stress. They may undergo gradual dissolution or deformation in dynamic wound environments, necessitating careful matching of their degradation kinetics with the healing timeline.<sup>76</sup> Nevertheless, their reversible nature and shear-thinning behavior make them exceptionally suitable for EBB and minimally invasive injection into complex wound beds.

### 3.2.3. Covalent crosslinking strategies

In contrast to the reversible nature of physical gels, covalent crosslinking establishes permanent networks through stable chemical bonds. This results in hydrogels with significantly enhanced mechanical strength, structural integrity, and resistance to dissolution, making them indispensable for applications requiring long-term structural support, shape fidelity after printing, or a durable barrier function in exuding wounds.<sup>85</sup>

Condensation crosslinking employs small, bifunctional molecules to directly link HA chains. For hydroxyl group crosslinking, agents like divinyl sulfone or 1,4-butanediol diglycidyl ether are used under alkaline conditions to form ether linkages.<sup>85</sup> 1,4-butanediol diglycidyl ether-crosslinked HA hydrogels are the foundation of many commercial dermal fillers, valued for their stability. For carboxyl group crosslinking, carbodiimides like EDC are standard, activating the carboxylate for amide bond formation with diamines or hydrazides.<sup>21</sup> While effective, the potential cytotoxicity of residual small-molecule crosslinkers (e.g., glutaraldehyde) or reaction byproducts necessitates stringent purification protocols. EDC-based chemistry, where the activating agent is not incorporated into the final bond, generally offers improved biocompatibility profiles.<sup>21</sup>

Photoinitiated radical polymerization is one of the most powerful and controllable covalent crosslinking methods, with direct relevance to vat photopolymerization bioprinting technologies such as stereolithography and DLP. The process involves irradiating a solution containing vinyl-functionalized HA (e.g., HAMA) and a photoinitiator with light of a specific wavelength. The initiator generates free radicals that propagate the chain reaction between methacrylate groups, leading to rapid network formation within seconds to minutes.<sup>93</sup> The degree of spatial and temporal control is exceptional, enabling the fabrication of complex, high-resolution architectures. Similarly, thiol-ene reactions between HA-SH and multi-vinyl crosslinkers like poly(ethylene glycol) diacrylate (PEGDA) can be photo-triggered, offering alternative network structures

and properties.<sup>66</sup> The critical considerations for biomedical use include the photoinitiator's cytotoxicity and the potential for radical-induced damage to encapsulated cells or bioactive molecules. This cytocompatibility challenge is cell-type-dependent, with sensitive cells such as stem cells and endothelial cells being particularly vulnerable to oxidative stress and DNA damage from photo-generated radicals. These inherent risks constitute a primary rationale for the widespread development of acellular HA-based bioinks, in which the design focus shifts to supporting host cell recruitment rather than sustaining encapsulated cells. Additionally, regulatory pathways require stringent evaluation of photoinitiator residuals and potential leachables, further driving innovation toward biocompatible photoinitiating systems (e.g., visible-light initiators such as lithium phenyl-2,4,6-trimethylbenzoylphosphine [LAP]) and acellular scaffold strategies.<sup>28</sup> Advances in visible-light initiators (e.g., LAP) and ruthenium-based systems have significantly mitigated these concerns, expanding the utility of photopolymerization for cell-laden bioinks.<sup>93</sup>

Click chemistry encompasses a suite of bioorthogonal reactions characterized by high efficiency, selectivity, and mild reaction conditions. The copper(I)-catalyzed azide-alkyne cycloaddition was an early “click” method applied to crosslink azide- and alkyne-modified HA, forming stable triazole linkages.<sup>81</sup> However, concerns over copper cytotoxicity spurred the development of metal-free alternatives. Strain-promoted azide-alkyne cycloaddition utilizes cyclooctyne derivatives that react with azides without a catalyst, enabling the formation of biocompatible hydrogels *in situ*.<sup>21</sup> Another powerful variant is the thiol-Michael addition reaction between HA-SH and electron-deficient alkenes (e.g., vinyl sulfones, acrylates), which proceeds rapidly at physiological pH and is ideal for creating cell-compatible networks.<sup>94,95</sup>

Enzymatic crosslinking offers a uniquely biocompatible route that operates under physiological conditions. As mentioned, HA-Tyr crosslinks in the presence of horseradish peroxidase (HRP) and H<sub>2</sub>O<sub>2</sub>. HRP oxidizes the tyramine's phenol groups to form phenoxy radicals, which subsequently couple to form C-C or C-O bonds between chains.<sup>85,96</sup> The gelation rate and final stiffness can be precisely modulated by varying the concentrations of HRP and H<sub>2</sub>O<sub>2</sub>, allowing the process to be tailored to the sensitivity of encapsulated cells. This gentle, cytocompatible method is exceptionally well-suited for creating cell-laden matrices for tissue engineering and for *in situ* gelation at the wound site.<sup>75</sup>

From a bioprinting perspective, the covalent strategies discussed herein offer distinct pathways to achieve the shape fidelity and structural integrity required for

complex 3D architectures. Photoinitiated polymerization provides unparalleled spatial control and speed, which is foundational for high-resolution vat photopolymerization techniques like DLP. Click chemistry and enzymatic crosslinking, operating under mild conditions, enable the formulation of bioinks that gel predictably upon deposition, a critical requirement for extrusion-based printing. However, a fundamental trade-off exists: the very permanence and high strength that make covalent networks excellent at maintaining printed shape can simultaneously hinder their extrusion (due to a lack of shear-thinning) and their adaptive remodeling in response to cells.

While the strength of covalent networks lies in their permanence and stability, these same properties render them suboptimal for tissue regeneration. Their irreversible, static nature can impede cell migration, proliferation, and tissue remodeling, as cells lack the ability to dynamically remodel their microenvironment. Furthermore, pursuing high mechanical strength can sometimes conflict with the need for degradability and biocompatibility. This inherent dichotomy has driven the exploration of a hybrid approach: dynamic covalent chemistry.

#### 3.2.4. Dynamic covalent crosslinking for adaptable networks

Dynamic covalent crosslinking represents a paradigm-shifting advancement, elegantly bridging the gap between the strength of covalent bonds and the adaptability of reversible interactions.<sup>97,98</sup> In dynamic covalent crosslinking, the crosslinks are covalent but designed to reversibly break and reform in response to specific physiological or applied stimuli. This endows the hydrogels with a suite of advanced properties: self-healing after damage, shear-thinning for injectability/printability, stress relaxation to accommodate cell-generated forces, and stimuli-responsiveness to microenvironmental cues. For wound healing, this translates to materials that are not only robust enough to protect the wound but also intelligent enough to adapt, respond, and potentially participate in the healing cascade.

Schiff base and hydrazone linkages are quintessential dynamic bonds formed between carbonyls (aldehydes or ketones) and amines or hydrazides. OHA, with its abundant aldehyde groups, is a prime substrate for this chemistry. When mixed with amine-rich polymers like glycol CS or hydrazone-modified gelatin, hydrogels form instantaneously via imine or hydrazone bond formation.<sup>99</sup> The dynamic nature of these bonds lies in their pH-dependent equilibrium, where stability is maintained at physiological pH, while hydrolysis occurs under acidic

conditions. This allows the network to undergo continual bond exchange, enabling self-healing (e.g., two separated gel pieces can fuse) and making the gels readily injectable through a fine needle. Furthermore, this acidity-triggered bond cleavage can be harnessed for controlled drug release in the typically acidic milieu of chronic or infected wounds.<sup>21</sup>

Disulfide exchange leverages the intrinsic redox activity of thiol/disulfide chemistry, which is ubiquitous in biological systems. HA-SH can be crosslinked into a hydrogel via oxidation, forming disulfide bridges. In the presence of other thiols such as endogenous glutathione, these disulfide bonds undergo continuous thiol-disulfide interchange reactions.<sup>94,100</sup> This dynamic exchange enables the network to remodel over time, thereby reducing stress and facilitating cellular infiltration, a property that static covalent networks lack. Building on this principle, advanced formulations use sulfur-protected thiols that remain stable until exposed to physiological reducing environments, thus enabling precise crosslinking control *in vivo*.<sup>101</sup>

Diels-Alder reactions offer a unique thermally reversible dynamic covalent system. The [4 + 2] cycloaddition between a diene (e.g., furan-modified HA) and a dienophile (e.g., maleimide-modified polymer) forms a stable covalent adduct at lower temperatures. Upon heating, the retro-Diels-Alder reaction occurs, reverting to the starting materials.<sup>81,101</sup> This thermo-responsiveness enables temperature-triggered gelation or dissolution. More importantly, when combined with a second, permanent crosslinking mechanism (e.g., photopolymerization), it enables the creation of dual-network hydrogels. These materials can first be printed or molded using the dynamic bond's reversibility and then locked into a permanent, high-strength shape via the second crosslink, achieving an optimal balance of processability and final mechanical performance.<sup>102,103</sup>

Boronic ester formation introduces a dynamic bond system with exquisite multi-stimuli-responsiveness. Phenylboronic acid-modified HA reacts with *cis*-diol-containing compounds (e.g., PVA, polysaccharides) to form boronic esters.<sup>104</sup> This bond is reversible and sensitive to pH, competing diols (e.g., glucose), and ROS (e.g., H<sub>2</sub>O<sub>2</sub>). This makes boronic ester-based hydrogels potential sensors and actuators within the complex wound microenvironment. For instance, a hydrogel could be designed to swell and release an antibiotic in response to elevated glucose levels in a diabetic wound or to degrade and deliver antioxidants in response to oxidative stress.<sup>105</sup>

In the context of 3D bioprinting, dynamic covalent chemistry provides a foundational material strategy to

resolve the classic conflict between printability and long-term functionality. Overall, this approach transcends the traditional limitations of hydrogel design. It empowers the creation of HA-based materials that are no longer passive scaffolds but active, adaptive partners in regeneration. By mimicking the dynamic and responsive nature of native ECM, these next-generation hydrogels hold immense promise for developing intelligent wound dressings that can conform to injury, respond to pathological signals, and guide the healing process with unprecedented sophistication. This evolution from static to dynamic materials seamlessly leads to the next frontier: endowing these networks with specific, targeted biofunctions, as explored in Section 3.3.

### 3.3. Biofunction-oriented modification: Conferring targeted therapeutic capabilities

While modifications aimed at enhancing processability (Section 3.2) provide HA with the structural integrity and fabrication compatibility required for scaffold construction, they do not inherently overcome the complex pathophysiological barriers that impair healing in chronic or infected wounds. To advance HA from a passive, albeit bioactive, matrix component to an active therapeutic agent capable of precise intervention, a complementary engineering strategy is implemented: biofunction-oriented modification. This paradigm shift focuses on equipping HA with molecular tools to directly intervene in the dysregulated biology of chronic wounds. This approach involves covalent conjugation of specific bioactive molecules or functional motifs directly onto the HA backbone, leveraging its abundant carboxyl and hydroxyl groups as chemical handles through high-efficiency reactions such as carbodiimide-mediated amidation or esterification.<sup>78</sup> This molecular-level functionalization integrates therapeutic capabilities directly into the biopolymer chain, generating smart biomaterials capable of interacting with the wound microenvironment in a predefined, stage-specific manner to deliver targeted actions beyond mere hydration and passive barrier functions.<sup>106</sup> The principal strategies of this biofunctionalization approach, each designed to address a distinct challenge in the healing cascade, are summarized in Figure 4. This section examines three principal biofunctionalization strategies, each designed to address a distinct challenge in the healing cascade: (i) imparting antimicrobial and anti-biofilm activity to combat infection; (ii) endowing antioxidant and anti-inflammatory capacity to resolve chronic inflammation and oxidative stress; and (iii) incorporating specific cell-targeting and regulatory functions to actively direct cellular behaviors essential for regeneration.

#### 3.3.1. Antimicrobial and anti-biofilm functions

Bacterial colonization and resilient biofilm formation are hallmarks of chronic wounds, perpetuating chronic inflammation and physically impeding cellular repair processes. Although valuable as a scaffold, native HA lacks inherent antimicrobial properties, and its highly hydrated network may provide a favorable environment for microbial proliferation.<sup>13</sup> To transform HA from a potential microbial niche into a defensive barrier, chemical modification strategies have been developed to endow it with intrinsic antimicrobial activity.

One primary strategy involves conjugating cationic antimicrobial agents to the anionic HA backbone. This includes grafting AMPs, such as LL-37 or their synthetic mimics, or quaternary ammonium compounds.<sup>107,108</sup> The resulting HA derivatives can disrupt negatively charged bacterial membranes, thereby conferring localized, broad-spectrum bactericidal activity while frequently maintaining biocompatibility with mammalian cells. Covalent conjugation helps reduce the potential cytotoxicity associated with free small-molecule biocides and can enable a sustained effect as the HA scaffold undergoes degradation.<sup>85,109</sup> Beyond direct bactericidal activity, a more sophisticated strategy involves modifying HA to neutralize bacterial HYALs. This approach not only inhibits these key virulence enzymes used by pathogens to degrade tissues and spread, but also contributes to a bacteriostatic outcome.<sup>110</sup>

To address the significant challenge of established biofilms, HA can be functionalized with biofilm-dispersing agents. Conjugating enzymes such as dispersin B or DNase I to HA enhances their local stability and concentration, promoting the enzymatic degradation of the protective extracellular polymeric substance matrix.<sup>111</sup> This dispersal can re-sensitize embedded bacteria to antimicrobial agents or host immune defenses, potentially disrupting the cycle of chronic infection.<sup>107</sup>

#### 3.3.2. Antioxidant and anti-inflammatory functions

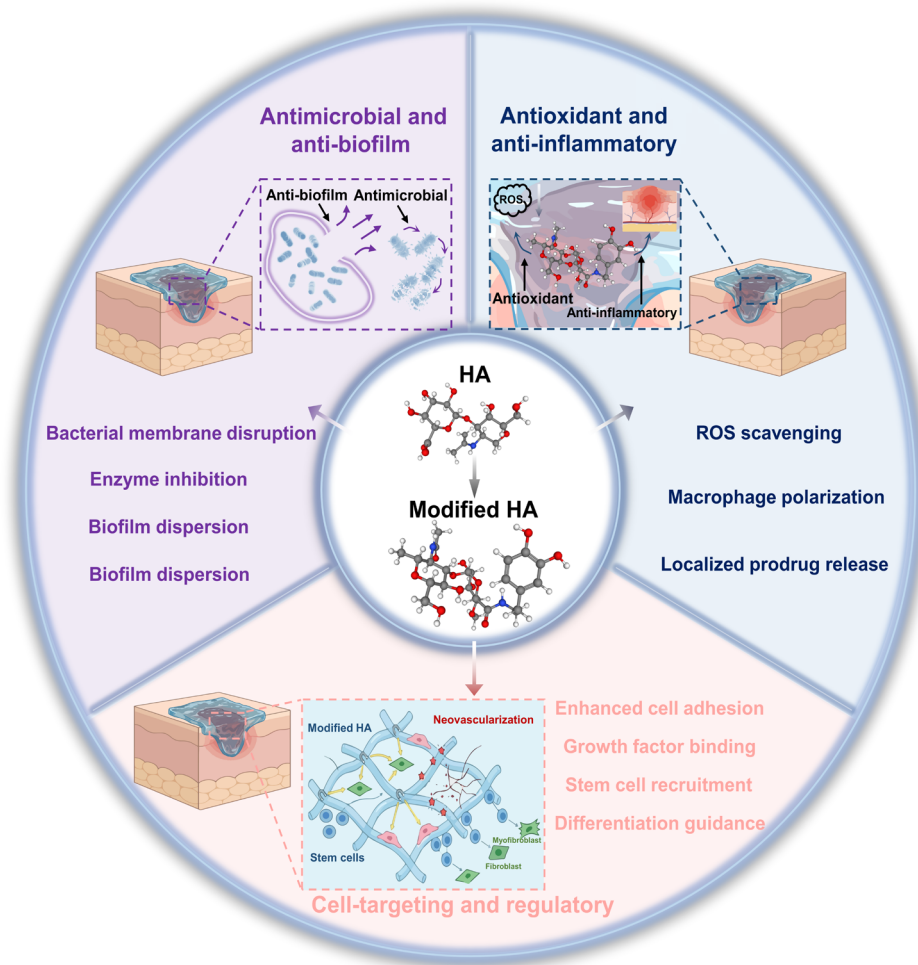
Beyond combating infection, the successful resolution of the inflammatory phase is critical for the progression of healing. To this end, biofunctionalizing HA with motifs that directly counteract oxidative stress and chronic inflammation can help re-establish a regenerative milieu. The chronic wound microenvironment is often characterized by excessive and prolonged production of ROS and pro-inflammatory cytokines, leading to oxidative damage to cells and biomolecules, as well as a failure to progress beyond the inflammatory phase.<sup>112,113</sup> Biofunctionalizing HA with motifs that directly counteract

these pathologies can help re-establish a regenerative milieu.

A direct strategy is the covalent grafting of antioxidant molecules onto HA chains.<sup>114</sup> Molecules such as catechol (inspired by mussel adhesive proteins), gallic acid, various polyphenols, or tocopherol (vitamin E) have been successfully conjugated with HA. These conjugates function as potent radical scavengers, neutralizing destructive ROS such as hydroxyl radicals and superoxide anions. For example, catechol-modified HA not only provides sites for metal-ion coordination crosslinking but also confers significant antioxidant activity, thereby protecting cells from oxidative damage.<sup>115</sup>

Concurrently, HA can be modified to actively modulate inflammatory signaling. While the MW of HA itself

influences inflammatory responses (HMW-HA, typically >1 MDa, generally exhibiting anti-inflammatory properties), chemical modification can precisely tune or enhance this effect. One approach involves conjugating molecules that bind and neutralize specific pro-inflammatory mediators, such as TNF- $\alpha$ .<sup>116</sup> A more advanced strategy is grafting peptides that promote the polarization of macrophages from a pro-inflammatory (M1) phenotype to a pro-healing (M2) phenotype, thereby actively mitigating chronic inflammation.<sup>116</sup> Furthermore, the HA backbone can serve as a carrier for covalently conjugated anti-inflammatory drugs, such as diclofenac, creating a prodrug system that enables localized, sustained release as HA degrades, offering prolonged anti-inflammatory action at the wound site.<sup>117</sup>



**Figure 4.** Multifunctional integration via biofunction-oriented HA modification: antimicrobial/anti-biofilm properties, antioxidant/anti-inflammatory activities, and cell-targeting/regulatory mechanisms

Abbreviations: HA: Hyaluronic acid; ROS: Reactive oxygen species.



### 3.3.3. Cell-targeting and regulatory functions

In addition to countering pathological factors, advanced wound healing requires the precise recruitment, activation, and coordinated behavior of specific cell types. Biofunctionalization can endow HA with the capacity to directly communicate with cells, providing essential adhesive cues, growth factor-mimicking signals, and fate-determining instructions.

Enhancing cell adhesion represents a fundamental initial step, as native HA is typically non-adhesive for most cells due to its high hydrophilicity and negative charge. The classic and highly effective approach involves grafting arginine-glycine-aspartic acid (RGD) peptides onto HA. RGD is a minimal cell-binding sequence found in ECM proteins, such as fibronectin, and primarily interacts with integrin receptors. RGD-functionalized HA hydrogels significantly enhance the adhesion, spreading, proliferation, and migration of fibroblasts, endothelial cells, and keratinocytes, all of which are critical for granulation tissue formation and re-epithelialization.<sup>118,119</sup> To better mimic native ECM complexity, HA can be conjugated to specific peptides, such as those derived from laminin (e.g., YIGSR, IKVAV) or designed to target integrins, thereby guiding specialized cellular responses.<sup>120,121</sup>

Growth factor binding and presentation constitute another potent strategy to enhance regenerative signaling. HA can be modified with heparin-mimicking peptides or sulfated to generate a polyanionic surface that electrostatically binds and stabilizes heparin-binding growth factors, such as VEGF, basic fibroblast growth factor, or platelet-derived growth factor.<sup>122–124</sup> This strategy protects growth factors from rapid degradation and presents them to cells in a biomimetic, matrix-bound fashion, significantly improving their bioavailability and signaling efficacy to promote processes such as angiogenesis.<sup>125</sup>

Finally, for advanced regenerative applications, HA can be engineered to direct stem cell homing and differentiation. Conjugation of chemokine-mimicking peptides can facilitate the recruitment of endogenous stem cells to the wound site. For instance, analogs of stromal cell-derived factor-1 alpha have been used for this purpose.<sup>126</sup> Moreover, presenting specific differentiation cues on HA scaffolds, such as bone morphogenetic protein-mimetic peptides, can steer the fate of encapsulated or infiltrating mesenchymal stem cells toward desired lineages (e.g., osteoblasts, chondrocytes), a concept applicable to specialized wound healing scenarios, including craniofacial defects.<sup>127,128</sup>

In summary, biofunction-oriented modification represents a significant advancement in HA engineering.

By covalently conjugating antimicrobial, antioxidant, anti-inflammatory, and cell-instructive motifs, HA is transformed from a biocompatible scaffold into a multifunctional, signaling-active platform. Such engineered HA can simultaneously address key healing impediments (infection, oxidative stress, chronic inflammation) and actively deliver precise biological instructions to recruit, engage, and guide resident cells, thereby facilitating the complex wound healing cascade toward efficient and regenerative tissue restoration.

### 3.4. Composite hyaluronic acid-based biomaterials

While molecular modification enables precise tailoring of HA's intrinsic properties, an equally powerful and complementary engineering paradigm involves its combination with other materials to form hybrid and composite systems. This approach capitalizes on synergistic interactions to generate biomaterials with superior or multifunctional properties beyond those achievable by any single component. By blending HA with other polymers or incorporating functional fillers, it is possible to create composites that not only overcome the inherent limitations of native HA but also introduce new functionalities essential for advanced wound therapy. This section includes four principal composite strategies: blending with other natural polymers, compositing with synthetic polymers, integrating conductive components, and loading bioactive cargos. Each strategy is designed to address specific material and biological requirements for constructing effective wound dressings and regenerative scaffolds, paving the way for their subsequent processing via 3D bioprinting technologies, as illustrated in Figure 5.

#### 3.4.1. Blends with natural polymers

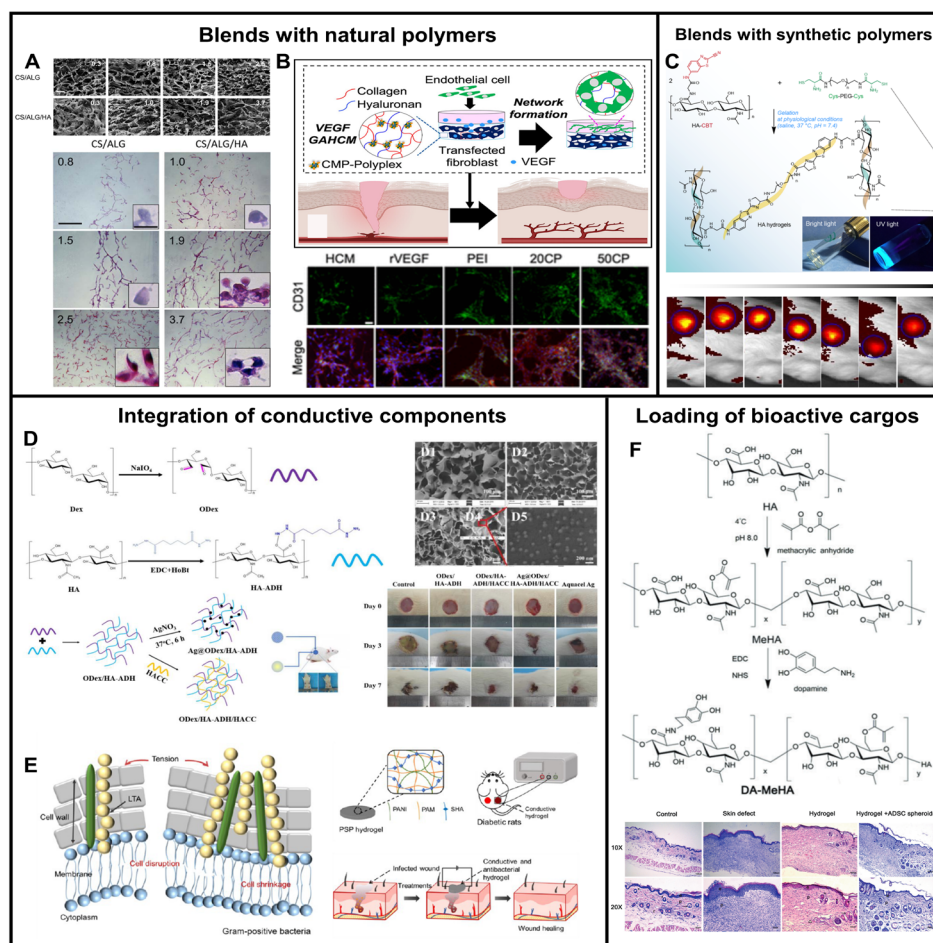
Combining HA with other natural polymers represents a fundamental strategy to create biomimetic composites that more closely recapitulate the compositional and biological complexity of the native ECM.<sup>92</sup> These blends leverage the complementary properties of each component, often resulting in improved mechanical integrity, controlled degradation, and enhanced bioactivity compared to pure HA constructs.

A prevalent combination is HA with CS, a cationic polysaccharide known for its inherent antimicrobial and hemostatic properties. Electrostatic interactions between anionic HA and cationic CS lead to the formation of stable polyelectrolyte complexes.<sup>129</sup> For instance, CS/HA composite sponges produced via freeze-drying exhibit superior porosity, which enhances oxygen and nutrient diffusion, thereby promoting fibroblast proliferation and wound healing<sup>130</sup>, as illustrated in Figure 5A. Furthermore, the incorporation of silver nanoparticles (AgNPs) into CS/

HA sponges confers potent, broad-spectrum antimicrobial activity against pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and methicillin-resistant *S. aureus*, demonstrating significant potential for treating infected diabetic wounds.<sup>131</sup> Beyond sponges, CS/HA composite hydrogels, often formed using Schiff base chemistry between aldehyde-modified HA and amine-rich CS, have been developed to support cell adhesion and accelerate

skin regeneration.<sup>132–134</sup>

Hyaluronic acid is also frequently blended with collagen or gelatin, key structural proteins of the ECM. These combinations synergize HA's hydrating and signaling functions with the robust mechanical support and cell-adhesive properties of collagen/gelatin. For example, a crosslinked gelatin–HA hydrogel created using EDC as a



**Figure 5.** Hybrid and composite HA-based biomaterials: (A, B) blends with natural polymers; (C) blends with synthetic polymers; (D, E) integration of conductive components; and (F) loading of bioactive cargos. (A) A composite sponge combining CS with HA exhibits an optimized microstructure and significantly promotes fibroblast growth, highlighting its potential as a bioactive scaffold for enhanced wound repair. Reprinted with permission from Orellana *et al.*<sup>130</sup> Copyright © 2016, Wiley. (B) A VEGF-encoding, gene-activated collagen-based matrix was developed. It enhanced VEGF expression, promoted angiogenesis, and accelerated wound healing. Scale bar: 50  $\mu$ m. Reprinted with permission from Hwang *et al.*<sup>136</sup> Copyright © 2023, American Chemical Society. (C) Injectable HA hydrogels encapsulating camptothecin nanocrystals are developed for localized intra-articular drug delivery. Reprinted from Gao *et al.*<sup>146</sup> (D) A synergistic antibacterial hydrogel composed of quaternized chitosan and AgNPs is developed, which promotes infected burn wound healing through enhanced antibacterial and anti-inflammatory activities. Reprinted from Chen *et al.*<sup>165</sup> (E) The conductive PAM–SHA–PAni hydrogel demonstrates intrinsic antibacterial activity against Gram-positive bacteria and enhances wound healing under electrical stimulation, offering a promising strategy for treating infected chronic wounds. Reprinted with permission from Wu *et al.*<sup>176</sup> Copyright © 2021, American Chemical Society. (F) A DA–MeHA hydrogel serves as an effective stem cell carrier, enhancing their survival and regenerative functions to accelerate skin wound healing and tissue repair. Reprinted from Gong *et al.*<sup>192</sup>

Abbreviations: AgNP: Silver nanoparticle; ALG: Alginate; CS: Chitosan; DA: Dopamine; HA: Hyaluronic acid; HA–ADH: Adipic dihydrazide hyaluronic acid; MeHA: Methacrylated hyaluronic acid; PAA: Poly(acrylic acid); PAM: Polyacrylamide; PAni: Polyaniline; PDA: Polydopamine; ROS: Reactive oxygen species; VEGF: Vascular endothelial growth factor.

crosslinker maintained a porous structure conducive to cell infiltration and demonstrated a significant reduction (~95%) in wound area *in vivo*, highlighting its efficacy in providing a moist, pro-regenerative environment.<sup>135</sup> Similarly, collagen-HA sponges functionalized with growth factors showed a synergistic enhancement of wound closure and re-epithelialization in full-thickness rat wounds (Figure 5B).<sup>125,136</sup> Building on this synergistic approach, the chemical functionalization of collagen and HA polymers has emerged as a powerful strategy to intrinsically equip wound dressings with multifunctional properties.<sup>137,138</sup> For instance, collagen grafted with gallic acid and HA grafted with dopamine can form an injectable and self-healing hydrogel network via dynamic boronate ester bonds. This design not only provides robust tissue adhesion and conformal coverage but also actively scavenges excess ROS.<sup>139</sup> These inherent capabilities work in concert to effectively promote fibroblast migration, angiogenesis, and collagen deposition, thereby accelerating the healing of chronic wounds.<sup>5,139</sup>

Other natural polymer blends expand the functional repertoire. Alginate, valued for its hemostatic properties and ionic gelation, is combined with HA to form sponges with improved structural porosity.<sup>130,134</sup> Composite alginate/HA sponges loaded with tranexamic acid have been designed specifically to control post-operative bleeding.<sup>140</sup> Silk fibroin imparts remarkable mechanical strength and slow degradation. Silk fibroin/HA composite scaffolds or films, fabricated via water-based methods or casting, exhibit tunable mechanical properties, enhanced stability, and have been used as platforms for the sustained release of growth factors like VEGF.<sup>141,142</sup> Blends with plant-derived polymers, such as cornstarch and HA incorporated with ethanolic propolis extract, yielded films with sustained antibacterial release and proven efficacy in accelerating wound closure in animal models.<sup>143</sup>

In summary, blending HA with natural polymers is a versatile strategy to engineer composites that integrate hydration, structural support, inherent bioactivity (e.g., antimicrobial, hemostatic), and cell-instructive properties, making them highly suitable for a wide range of wound healing applications.

### 3.4.2. Composites with synthetic polymers

To further augment the mechanical performance, processing versatility, and functional durability of HA-based materials, compositing with synthetic polymers is a highly effective strategy. Synthetic polymers offer tunable and often superior mechanical strength, controlled degradation profiles, and the ability to introduce specific chemical functionalities that are not readily available in natural polymers.<sup>144</sup>

A common approach involves combining HA with poly(ethyleneglycol) (PEG) and its derivatives. PEG is widely used to enhance biocompatibility, control hydrophilicity, and serve as a crosslinker or spacer.<sup>145</sup> HA-PEG hydrogels, synthesized via copper-free click chemistry between cyclooctyne-modified HA and azide-functionalized PEG, exhibit excellent mechanical properties and stability, as illustrated in Figure 5C.<sup>146</sup> Thermo-responsive composites, such as poloxamer 407-HA hydrogels, have been used to encapsulate and deliver keratinocyte growth factor 2 for cartilage repair, demonstrating the potential for controlled bioactive factor release.<sup>147</sup> PEGDA is frequently used in photopolymerizable networks with HAMA or HA-SH (e.g., via thiol-ene chemistry) to form high-resolution structures suitable for DLP bioprinting.<sup>148</sup>

For applications requiring robust, long-term structural support, HA is composited with biodegradable polyesters. Poly(lactic-co-glycolic acid) and PCL are frequently employed. HA-coated poly(lactic-co-glycolic acid) porous scaffolds have shown enhanced cell viability within microfluidic channels, indicating improved biocompatibility.<sup>149</sup> HA-modified PCL nanoparticles have been explored for drug delivery, such as encapsulating naringenin for anticancer therapy, showcasing improved cellular uptake.<sup>150</sup> In electrospinning, PCL/HA nanofibrous scaffolds loaded with epidermal growth factor (EGF) have been developed to promote keratinocyte proliferation and infiltration, combining the structural benefits of nanofibers with the bioactivity of HA.<sup>151</sup> Similarly, composites with poly(lactic acid) have been used to create microfibers that regulate vascular endothelial cell behavior.<sup>152</sup>

Other synthetic polymers are used to introduce specific properties. Poly(acrylic acid) is blended with HA to form adhesive hydrogels beneficial for wound dressings that require strong tissue adhesion.<sup>153</sup> PVA forms hydrogen bonds with HA, thereby enhancing the structural integrity and flexibility of hydrogels.<sup>154</sup> Furthermore, the integration of nanoscale reinforcements, such as graphene, into polymer/HA matrices (e.g., CS/graphene/HA scaffolds) has been shown to significantly improve mechanical strength and porosity, creating highly suitable 3D environments for tissue engineering.<sup>22,155</sup> Similarly, zeolitic imidazolate framework-8 nanoparticles incorporated into HA films enhanced both Young's modulus and tensile stress, while also imparting concentration-dependent antibacterial properties.<sup>156</sup>

In essence, compositing HA with synthetic polymers enables the precise engineering of materials that balance bioactivity with enhanced mechanical robustness, tailored degradation, and advanced functionalities, thereby expanding their applicability to more demanding wound



scenarios and to sophisticated fabrication techniques such as 3D bioprinting.

### 3.4.3. Integration with conductive components

Following the blending of HA with natural polymers and compositing with synthetic polymers to enhance structural and biological properties, the integration of conductive components constitutes a specialized composite strategy. This approach focuses on incorporating extrinsic electroactive materials into HA matrices to endow them with electrical conductivity, a property absent in both native HA and most polymer blends. The rationale stems from the electrically sensitive nature of skin and the pivotal role of endogenous electric fields in guiding directional cell migration during wound healing.<sup>157,158</sup> Conductive HA composites are therefore engineered to either deliver exogenous electrical stimulation or modulate endogenous bioelectric cues, presenting a promising therapeutic avenue for accelerating the repair of challenging wounds, particularly chronic, non-healing ulcers such as diabetic foot ulcers.<sup>159–161</sup> The primary conductive materials integrated with HA can be categorized into three classes: metal/metal oxide nanoparticles, carbon-based materials, and conducting polymers.<sup>162,163</sup> Each class imparts distinct electrical, mechanical, and biological properties to the final composite.

Among metal/metal oxide nanoparticles, AgNPs are commonly incorporated into HA hydrogels not only for their well-documented antibacterial properties but also to establish conductive percolation networks. For instance, gallol-modified HA microgels were used to reduce Ag<sup>+</sup> ions *in situ*, forming an injectable system with an electroconductivity of approximately 0.05 S/cm, suitable for 3D bioprinting of electroactive patterns for tissue engineering, as illustrated in Figure 5D.<sup>164,165</sup> Other systems have combined Ag nanoclusters with hollow mesoporous manganese dioxide nanoparticles within HA-based click hydrogels, leveraging the metal components for ROS scavenging and oxygen generation, as well as potential conductive functions.<sup>166</sup> While zinc oxide nanoparticles are frequently added to HA formulations for their antibacterial and anti-inflammatory effects, as seen in HA-based nanofiber scaffolds with cinnamon essential oil<sup>167</sup>, reports explicitly focusing on their role in enhancing electrical conductivity are less common. Similarly, gold-platinum alloy nanoparticles have been embedded in HA/CS hydrogels primarily to introduce biofunctions, such as glucose regulation and oxidative stress alleviation.<sup>168</sup>

Carbon-based fillers, such as graphene oxide (GO) and reduced GO (rGO), are attractive due to their excellent electrical conductivity and mechanical robustness.<sup>162,169</sup>

A notable example is an injectable conductive hydrogel dressing based on HA-graft-dopamine and rGO, crosslinked via a H<sub>2</sub>O<sub>2</sub>/HRP system. This nanocomposite exhibited multifunctionality, including conductivity, antioxidant activity, tissue adhesiveness, and photothermal antibacterial capacity.<sup>170</sup> GO has also been loaded into natural polymer networks containing HA and gelatin to enhance mechanical properties and impart conductivity and immune-regulatory capabilities.<sup>155</sup> Furthermore, carbon dots with unique photodynamic properties have been embedded in soft HA hydrogels to create platforms for combating bacterial infections.<sup>171</sup> Emerging materials like Ti<sub>3</sub>C<sub>2</sub> MXene nanosheets, known for high electrical conductivity and biocompatibility, have been incorporated into HA/alginate biomaterial inks for EBB 3D bioprinting of electroconductive cell-laden constructs.<sup>172</sup>

Conducting polymers such as polypyrrole, polyaniline (PAni), and poly(3,4-ethylenedioxythiophene) (PEDOT) are widely used to render HA hydrogels electroactive, offering advantages in stability and biocompatibility over some metal or carbon materials.<sup>163,173</sup> Polypyrrole has been integrated into HA-based hydrogels to enhance both mechanical and conductive properties.<sup>174</sup> PAni, including its oligomers, has been employed to produce conductive HA hydrogels with additional antibacterial and antioxidant functionalities.<sup>175–178</sup> For example, a hydrogel dressing composed of polyacrylamide, sulfonated HA, and PAni (PAM-SHA-PAni) demonstrated potent bactericidal effects against Gram-positive bacteria and enhanced diabetic wound healing under electrical stimulation<sup>176</sup>, as illustrated in Figure 5E. PEDOT, often used in its poly(styrene sulfonate)-doped form (PEDOT:PSS), has been incorporated into aldehyde-modified HA hydrogels, forming dual crosslinked networks with self-healing, shear-thinning, and adhesive properties suitable for bioprinting.<sup>179</sup> Moreover, functionalization of HA with ionic liquids has been explored to create conductive platforms with inherent antibacterial properties, which, when coupled with exogenous electrical stimulation, significantly improved healing in diabetic wound models.<sup>180</sup>

The integration of conductive components often synergizes with other functionalities. For instance, bioadhesiveness inspired by mussel proteins can be combined with conductivity, as demonstrated in borax-crosslinked, mussel-inspired conductive HA hydrogels designed for electronic skin applications, which also exhibited excellent stretchability and self-healing.<sup>181</sup> Additionally, systems where conductive polymers like tetra-aniline are crosslinked with HA-SH have been designed to create injectable hydrogels capable of inducing a sustained hypoxic microenvironment, beneficial for diabetic wound

repair.<sup>177</sup> More complex composites, such as hydrogels incorporating a polydopamine@polypyrrole (PDA@PPy) nanocomposite within a cystamine-modified HA network, have been developed to simultaneously provide ultraviolet (UV)-blocking ability, photothermal anti-infection, and on-demand removability.<sup>174</sup>

In summary, integrating conductive components (e.g., spanning metal nanoparticles, carbon-based materials, and conducting polymers) transforms HA-based composites into electroactive platforms. These materials can leverage electrical stimulation to promote key healing processes, including cell migration, angiogenesis, and antibacterial activity, while often integrating additional multifunctional attributes, such as self-healing, adhesiveness, and stimuli-responsiveness. This strategy significantly expands the therapeutic arsenal of HA-based biomaterials, making them promising candidates for advanced, interactive wound dressings and skin regeneration scaffolds.

#### 3.4.4. Loading of bioactive cargos

The ability of HA-based matrices to serve as carriers for a diverse range of bioactive molecules is a cornerstone of their therapeutic application. By loading these cargoes, HA scaffolds evolve from structural templates into active drug delivery systems capable of exerting precise temporal and spatial control over the wound microenvironment. This strategy addresses key impediments to healing, including infection, excessive inflammation, and insufficient cellular recruitment or differentiation.

Hyaluronic acid's polyanionic nature enables electrostatic interactions with positively charged growth factors, thereby protecting them and promoting sustained release. This is frequently enhanced by chemical modification; for instance, sulfated HA or HA conjugated to heparin-mimicking peptides can strongly bind and stabilize heparin-binding growth factors such as VEGF, basic fibroblast growth factor, or TGF- $\beta$ 3.<sup>126,182,183</sup> HA-based hydrogels and sponges have been successfully loaded with EGF to accelerate re-epithelialization, with VEGF to promote angiogenesis, and with stromal cell-derived factor-1  $\alpha$  to recruit endogenous stem cells.<sup>122,126,184</sup> Composite systems, such as HA/ $\alpha$ -elastin hydrogels, have been specifically designed for the controlled delivery of VEGF, retaining a significant portion of the factor over several days to stimulate endothelial cell proliferation.<sup>185</sup>

Moreover, to combat infection, HA matrices are loaded with a variety of antimicrobials. AgNPs are widely incorporated, as seen in CS/HA/AgNP composite sponges, which showed dose-dependent antibacterial efficacy against drug-resistant bacteria.<sup>186</sup> Other inorganic agents include iodine (e.g., in HA–povidone–iodine complexes)

and zinc, both of which have shown clinical benefits in promoting healing and reducing scar formation in diabetic foot ulcers and post-surgical wounds, respectively.<sup>187,188</sup> Organic antibiotics, such as sulfadiazine silver in commercial formulations like Connettivina Plus<sup>®</sup>, are also effectively delivered via HA-based creams for treating pressure ulcers.<sup>15</sup>

Small-molecule drugs and natural extracts with anti-inflammatory or antioxidant properties are commonly used to mitigate chronic inflammation and oxidative stress. HA has been used to deliver diclofenac in topical formulations for localized anti-inflammatory action.<sup>189</sup> Natural compounds like propolis extract incorporated into cornstarch/HA films provide sustained antibacterial and healing benefits.<sup>143</sup> Resveratrol, loaded into collagen/gelatin/HA scaffolds, has been shown to accelerate diabetic wound healing and reduce oxidative stress.<sup>190</sup> Furthermore, amino acids (e.g., arginine) and polynucleotides combined with HA have demonstrated enhanced trophic effects, promoting cell proliferation and tissue repair in chronic ulcers.<sup>191</sup>

Advanced regenerative strategies involve loading HA hydrogels with living cells or their secreted products. Adipose-derived stem cells encapsulated in methacrylated HA (MeHA) hydrogels promote vascularization and skin regeneration, as illustrated in [Figure 5F](#).<sup>66,192</sup> Similarly, mesenchymal stem cell-derived small extracellular vesicles (MSC-sEVs) embedded in MeHA dressings have been shown to reduce inflammation, promote M2 macrophage polarization, and accelerate repair in diabetic ulcer models.<sup>193</sup> This approach leverages HA's biocompatible matrix to house and deliver potent cellular therapeutics directly to the wound bed.

Hyaluronic acid itself can functionalize or form part of sophisticated nanocarriers for enhanced delivery. HA-decorated liposomes and ethanosomes improve skin penetration, retention, and targeted delivery of drugs to CD44-overexpressing cells.<sup>194–196</sup> HA-based polymeric micelles and nanostructured lipid carriers offer high drug loading and controlled release for topical applications.<sup>197,198</sup> In cancer therapy, which informs advanced drug delivery concepts, HA-decorated metal–organic frameworks and HA–drug conjugates (e.g., HA-methotrexate) exemplify targeted delivery systems<sup>196</sup>, principles that can be adapted for localized wound therapy.

In summary, the loading of bioactive cargoes, ranging from small molecules to proteins, into cells and advanced nanocarriers empowers HA-based biomaterials to function as multifaceted therapeutic systems. By providing controlled, localized delivery of therapeutic agents, these composites can sequentially address the multifaceted



challenges of hemostasis, infection, inflammation, and regeneration, thereby offering a powerful toolkit for engineering intelligent, stage-specific wound healing interventions.

### 3.5. Clinical translation: Current commercial hyaluronic acid-based products and their applications

The extensive body of research dedicated to modifying and compositing HA has successfully translated fundamental biomaterial principles into clinical practice through commercially available wound care products. These engineered solutions address the inherent limitations of native HA, notably its rapid enzymatic degradation and insufficient mechanical strength, via deliberate strategies such as chemical stabilization and multifunctional integration.<sup>199,200</sup> This section systematically reviews established HA-based wound dressings, analyzing their formulation, functional rationale, and clinical indications, as summarized in Table 2. While representing the current state of the art in HA-based wound management, these

products also reveal, through their inherent design constraints, the persistent need for more sophisticated and patient-specific therapeutic solutions.

Commercially available HA-based wound care products are primarily classified by their physical form, a characteristic that directly dictates their clinical function and application. Common categories include films, hydrogels, sponges, and advanced scaffolds. Transparent HA films, exemplified by Hyaloseal<sup>®</sup>, are designed to create a moist, occlusive barrier over superficial wounds. Fabricated from a fully esterified HA derivative (HYAFF<sup>®</sup>), these films exhibit enhanced resistance to enzymatic degradation. This property provides a durable protective layer while permitting continuous visual monitoring of the wound bed.<sup>201,202</sup> Such films are indicated for the management of moderately exuding superficial wounds, including first- and second-degree burns and post-surgical sites.<sup>202,203</sup> For deeper wounds or those with higher exudate levels, HA-based hydrogels and sponges are preferred. For instance, Hylase<sup>®</sup> wound gel, containing 2.5% sodium

**Table 2. Overview of clinically available hyaluronic acid-based wound care products**

Product name	Product form	Core HA material	Key functional composition	Primary mechanism	Primary clinical indications	Reference
Connettivina <sup>®</sup> Plus	Topical cream	Sodium hyaluronate	Silver sulfadiazine	Moisture retention and sustained local antimicrobial release	Infected or high-risk chronic wounds (e.g., pressure ulcers)	199
Hyalofill <sup>®</sup>	Non-woven fleece/filler	Benzyl ester of HA (HYAFF <sup>®</sup> )	–	Formation of a hydrated, HA-rich gel interface upon fluid absorption to facilitate cell migration	Moderately to heavily exuding cavity or flat chronic wounds (e.g., diabetic foot ulcers)	200,206
Hyalomatrix <sup>®</sup>	Bilayered dermal substitute	Benzyl ester of HA (HYAFF <sup>®</sup> ) fibrous matrix	Semi-permeable silicon membrane (top layer)	A bilayered scaffold for dermal regeneration and moisture management, a moisture-controlling cover	Full-thickness wounds, burns, and surgical defects requiring dermal regeneration	200,202,206
Hyaloseal <sup>®</sup>	Transparent film	Benzyl ester of HA (HYAFF <sup>®</sup> )	–	Provides a transparent, occlusive barrier that maintains a moist wound environment	Superficial, moderately exuding wounds (e.g., superficial burns, donor sites)	201,202
Hylase <sup>®</sup> wound gel	Amorphous hydrogel	Sodium hyaluronate (2.5%)	Emollient base	Provides moisture to prevent tissue desiccation and support autolytic debridement	Dry to lightly exuding wounds (e.g., leg ulcers, pressure ulcers)	199,204
HylaSponge <sup>®</sup>	Porous sponge	Crosslinked HA network	(Often used with secondary dressing)	Absorption of excess exudate while maintaining localized hydration at the wound interface	Acute and chronic exuding wounds	188,205
Laserskin <sup>®</sup>	Engineered cell-carrier membrane	Microperforated benzyl ester of HA (HYAFF <sup>®</sup> )	–	Acts as a biodegradable carrier for autologous keratinocyte delivery and attachment	Deep burns and chronic ulcers treated with autologous keratinocyte transplantation	18

Abbreviation: HA: Hyaluronic acid.

hyaluronate, functions primarily as a moisture-providing agent. It prevents tissue desiccation and supports autolytic debridement in dry to lightly exuding wounds.<sup>199,204</sup> In contrast, more structurally defined sponges, such as HylaSponge<sup>®</sup>, utilize a crosslinked HA network to absorb excess exudate while maintaining a hydrated local environment. These typically require a secondary dressing for securement and are used to manage both acute and chronic wounds.<sup>188,205</sup>

To augment functionality beyond passive hydration, HA is frequently combined with active therapeutic agents. A prominent example is Connettivina Plus<sup>®</sup>, a cream formulation that integrates sodium hyaluronate with silver sulfadiazine.<sup>199,203</sup> This product synergistically combines the pro-regenerative, moist microenvironment facilitated by HA with the broad-spectrum antimicrobial activity of silver, rendering it suitable for contaminated or infected chronic wounds such as pressure ulcers.<sup>199</sup> Similarly, Hyalofil<sup>®</sup> is a fleece dressing composed of the benzyl ester of HA (HYAFF<sup>®</sup>). Upon contact with wound exudate, it forms a soft, hydrophilic gel that establishes an HA-rich interface at the wound bed. This interface is hypothesized to facilitate cellular migration, and the dressing is used in cavity wounds and chronic ulcers, including diabetic foot ulcers.<sup>200,206</sup>

The most technologically advanced category encompasses HA engineered into instructive 3D scaffolds for tissue regeneration. Hyalomatrix<sup>®</sup> is a bilayered dermal substitute consisting of a biodegradable HYAFF<sup>®</sup> fibrous matrix bonded to a semi-permeable silicon sheet.<sup>202,206</sup> The HYAFF<sup>®</sup> matrix facilitates fibroblast infiltration and vascular ingrowth, while the silicon layer controls moisture vapor transmission. This construct served as a temporary, guiding matrix for dermal regeneration in full-thickness wounds.<sup>200,206</sup> For applications requiring cellular delivery, products such as Laserskin<sup>®</sup>, a microperforated membrane made from HYAFF<sup>®</sup>, serve as vehicles for autologous keratinocyte transplantation in the treatment of extensive burns and chronic ulcers.<sup>18</sup> Clinical evidence supports the efficacy of these advanced systems. For example, a two-step therapy employing an autologous fibroblast-seeded HYAFF scaffold (Hyalograft-3D) followed by Laserskin application demonstrated significantly accelerated wound closure in diabetic foot ulcers compared with standard care, validating the scaffold's role in actively guiding tissue regeneration.<sup>207</sup>

Collectively, clinical studies across these product categories substantiate their therapeutic efficacy. Notably, the use of HA-impregnated gauze was shown to significantly reduce wound area and alleviate pain intensity in venous leg ulcers when compared to non-medicated gauze.<sup>201,204</sup>

The success of these commercial products validates core HA engineering approaches: chemical modification, exemplified by esterification, to prolong functional residence time; and strategic compositing to introduce essential multifunctionality, such as antimicrobial activity or cell-instructive properties. However, these commercially available solutions are inherently limited by conventional manufacturing constraints. This is evident when comparing their performance against the idealized requirements for healing complex wounds. For instance, clinical studies confirm that bilayered scaffolds, such as Hyalomatrix<sup>®</sup>, improve healing outcomes compared with standard care. However, their homogeneous, non-porous structure often results in suboptimal tissue integration. Comparative histology further reveals that these scaffolds fail to guide coordinated regeneration across dermal and epidermal layers, as evidenced by disorganized ECM deposition compared to native skin architecture.<sup>2</sup> Similarly, while HA films provide excellent hydration, their static design cannot adapt to dynamic wound contraction or provide spatially graded cues. They exhibit structurally homogeneous designs that fail to replicate the critical anatomical gradients of native skin, such as the gradual epidermal-to-dermal transition.<sup>2</sup> Furthermore, any incorporated bioactive agents are typically released via passive diffusion, lacking the temporal precision required to align with the distinct phases of wound healing. Finally, their standardized shapes and sizes often result in poor conformity to complex wound geometries, potentially leading to gaps, dead spaces, or improper mechanical loading, which can compromise healing outcomes.<sup>62,208</sup>

These limitations collectively underscore the need for a next-generation of HA-based wound therapies. The ideal solutions must offer spatial customization, dynamic and phase-specific bioactivity, and truly patient-specific design. It is precisely these challenges that advanced fabrication technologies, particularly 3D bioprinting, are poised to address. Conventional manufacturing is inherently unable to produce constructs that are structurally anisotropic, mechanically graded, spatially programmable, and endowed with on-demand bioactivity. In contrast, 3D bioprinting enables the creation of complex, biomimetic HA constructs with precisely tailored architecture and biochemistry.

#### 4. Three-dimensional bioprinting strategies for processing hyaluronic acid-based bioinks

Despite the advanced material engineering strategies mentioned above, effectively translating these material advancements into wound therapies necessitates

fabrication technologies capable of imposing precise spatial and architectural order.<sup>5</sup> Traditional scaffold fabrication methods, such as solvent casting, freeze-drying, and electrospinning, often yield constructs with homogeneous structures. These conventional techniques have limited capacity to encode spatial gradients of biochemical cues, controlled variations in porosity, or patient-specific geometries, all of which are essential for guiding the complex, phased progression of tissue regeneration.

To overcome these limitations, 3D bioprinting has emerged as a revolutionary digital fabrication paradigm. This additive manufacturing technology translates digital designs into physical constructs through the controlled, layer-by-layer deposition of bioinks. It allows for precise control over scaffold architecture across various scales, from macroscopic shapes to microscopic pore networks, and facilitates the deliberate spatial patterning of cells, growth factors, and other bioactive signals within a volumetric construct.<sup>1</sup> For HA-based wound management, such capabilities are crucial for engineering biomimetic and instructive interfaces that can actively participate in the healing cascade.

As summarized in [Figure 1](#), prevalent bioprinting technologies are classified by their fundamental deposition unit into three categories: droplet-based (0D), filament-based (1D), and layer-based (2D) approaches. Each category operates on distinct physical principles, imposes specific requirements on bioink properties, and offers unique advantages in terms of resolution, structural integrity, and fabrication throughput.

This section systematically analyzes these core bioprinting modalities in the context of processing HA-based biomaterials. The discussion begins with droplet-based techniques, such as inkjet bioprinting and laser-induced transfer forward (LIFT), which are recognized for their high-resolution, non-contact patterning capabilities. These features make them particularly well-suited for surface functionalization and the creation of precisely defined biochemical landscapes. Next, filament-based methods, particularly EBB, are examined for their efficacy in fabricating volumetric, self-supporting scaffolds using viscoelastic HA hydrogels and composites, thereby establishing a foundational technology for bulk wound dressings and cell-laden grafts. Lastly, layer-based vat photopolymerization techniques, embodied by DLP, are reviewed for their exceptional feature resolution in producing intricate, photocrosslinked HA constructs with smooth surfaces and complex internal microarchitectures.

By delineating the operational principles, material compatibility, and inherent constraints of each bioprinting strategy, this section establishes a rational framework

for selecting and integrating these approaches. The ultimate objective is to effectively translate the advanced functionalities of engineered HA materials into next-generation, personalized constructs that meet the complex demands of wound repair and skin regeneration.

#### 4.1. Zero-dimensional voxel droplet-based bioprinting

Zero-dimensional voxel bioprinting employs discrete droplets as fundamental building blocks, which are deposited in a patterned, layer-by-layer manner to assemble 3D structures. This approach excels in achieving high planar resolution and enables the non-contact, programmable deposition of low-viscosity bioinks, making it particularly suitable for patterning cells and biomolecules with micrometer-scale spatial control.<sup>209,210</sup> For HA-based systems, droplet-based techniques are advantageous for depositing low-concentration or chemically modified HA solutions prior to crosslinking, facilitating the high-resolution patterning of biochemical cues such as growth factors, AMPs, or cell-adhesive motifs. However, inherent limitations of droplet stacking, such as weak interlayer cohesion and structural instability, often restrict the ability to fabricate self-supporting, volumetric scaffolds capable of filling wound cavities or providing sustained mechanical support. Inkjet bioprinting and LIFT represent the two predominant modalities in this category.

##### 4.1.1. Inkjet bioprinting

Inkjet bioprinting, adapted from conventional graphic bioprinting technology, operates on a drop-on-demand principle wherein discrete picoliter- to nanoliter-volume droplets are generated via thermal, piezoelectric, or electrostatic actuation.<sup>27,211</sup> Its non-contact nature, coupled with high deposition frequency (up to 10 kHz), enables rapid and high-resolution patterning, which is particularly beneficial for creating intricate biochemical patterns within wound dressings, such as spatial gradients of VEGF for angiogenesis or localized zones of antimicrobial agents for infection control.<sup>212</sup>

For HA-based bioinks, inkjet bioprinting is primarily compatible with low-viscosity formulations, typically in the range of 3–30 mPa·s.<sup>27</sup> This encompasses many native HA solutions and lightly modified HA derivatives prior to gelation. A key application lies in the precise spatial patterning of multiple HA-based solutions, each loaded with distinct bioactive factors (e.g., VEGF, platelet-derived growth factor, or fibroblast growth factor), onto a substrate or into a crosslinking bath. Post-deposition crosslinking via UV exposure, enzymatic reaction, or ionic interaction can then solidify the patterned droplets into a cohesive hydrogel film or a surface coating with defined biochemical

compartments.<sup>213,214</sup> This capability aligns well with the design of advanced wound dressings that deliver spatially compartmentalized therapies to address heterogeneous wound microenvironments.

However, several constraints limit the broader application of inkjet bioprinting for HA-based wound constructs. The stringent viscosity window excludes most shear-thinning HA hydrogels and mechanically robust composites described in Sections 3.2 and 3.4, such as those crosslinked via hydrophobic associations or dynamic covalent networks. Furthermore, nozzle clogging remains a persistent risk when processing HA bioinks containing cells or particulate fillers (e.g., nanoparticles, microspheres).<sup>27</sup> A critical limitation for wound healing applications is that the droplet-based paradigm struggles to fabricate thick, 3D scaffolds with sufficient structural integrity. The weak coalescence of deposited droplets often results in porous, mechanically fragile structures that may not withstand the dynamic stresses of a wound bed or provide adequate barrier protection.<sup>213</sup> Therefore, while inkjet bioprinting holds significant promise for creating sophisticated surface-functionalized HA films or for high-precision cell patterning, its role in fabricating bulk wound dressings or full-thickness skin substitutes is often ancillary, typically integrated into hybrid bioprinting workflows that complement other fabrication modalities.

#### 4.1.2. Laser-induced transfer forward

Laser-induced transfer forward is a nozzle-free, droplet-based bioprinting technique that circumvents the stringent viscosity and particle-size constraints inherent to inkjet bioprinting.<sup>215</sup> The process operates on a fundamentally different physical principle (Figure 1). A pulsed laser beam is focused through a transparent donor substrate (e.g., quartz) onto a thin, laser-absorbing layer, termed a dynamic release layer (DRL), typically a metal (e.g., gold, titanium) or an explosive polymer (e.g., triazene polymer).<sup>216</sup> Upon absorption of the laser pulse, rapid vaporization or decomposition of the DRL generates a confined, high-pressure vapor bubble, or blister. This sudden pressure impulse propels a discrete volume of the bioink, which is coated atop the DRL, toward a receiving substrate positioned in proximity. The ejected material is deposited as a well-defined microdroplet, forming the fundamental 0D voxel.<sup>216</sup>

The key advantage of LIFT lies in its exceptionally broad operational window for bioink rheology. It has been demonstrated to successfully transfer materials ranging from low-viscosity aqueous solutions (<10 mPa·s) to highly viscous pastes exceeding 100 Pa·s.<sup>215</sup> This makes it uniquely suited for processing the diverse spectrum of

engineered HA materials discussed in Section 3, from native HA solutions to viscous, crosslinkable HA precursor formulations (e.g., MeHA, HA-Tyr) and even composite HA pastes loaded with functional fillers.<sup>217</sup> Furthermore, as a nozzle-free technique, LIFT eliminates the risk of clogging, enabling the deposition of bioinks containing large particles, aggregates, or high cell densities, which is a significant limitation for inkjet bioprinting.<sup>215</sup>

For wound healing applications, this versatility translates into several promising capabilities. LIFT can be used to create sophisticated biochemical patterns with high spatial resolution. For instance, different HA-based bioinks, each functionalized with specific bioactive cues (e.g., an AMP-conjugated HA, a VEGF-loaded HA, or an RGD-grafted HA), can be sequentially or simultaneously deposited onto a pre-formed scaffold or directly onto a wound bed model.<sup>218</sup> This allows for the creation of smart wound interfaces with spatially defined zones designed to combat infection, promote angiogenesis, and enhance cell adhesion in a compartmentalized manner. The technique has also shown efficacy in patterning sensitive primary cells relevant to skin regeneration, such as keratinocytes and fibroblasts, with reported post-transfer viabilities often exceeding 95%.<sup>219</sup>

However, LIFT presents specific challenges that must be considered. The transfer process involves transient localized heating and high-pressure gradients, which, despite being mitigated by optimized DRLs (e.g., triazene polymers that decompose into volatile byproducts or blister-actuated configurations that minimize thermal coupling), could potentially compromise the integrity of exceptionally thermolabile biomolecules.<sup>215</sup> A more fundamental limitation for constructing volumetric wound dressings is shared with other droplet-based methods: the difficulty in building stable, self-supporting 3D structures through droplet stacking. Issues such as gravitational deformation of unsupported droplets, weak inter-droplet cohesion, and relatively slow volumetric build rates constrain its use for fabricating thick, mechanically robust scaffolds needed to fill deep wound cavities.<sup>2,5</sup>

Consequently, droplet-based (0D) bioprinting techniques occupy a distinct niche within the fabrication of HA-based wound constructs. Their principal strength lies in enabling high-resolution, non-contact patterning, a capability crucial for spatially organizing biochemical signals and cells to create functionally graded interfaces. However, the intrinsic difficulty in assembling stable, volumetric structures from discrete droplets fundamentally limits their utility for engineering bulk wound dressings or load-bearing skin substitutes that require continuous mechanical support. Thus, the most impactful application

of inkjet bioprinting and LIFT lies not in standalone scaffold fabrication, but in their use as high-precision tools for imparting controlled heterogeneity. This includes precisely defining cellular composition or orchestrating complex biochemical landscapes on pre-formed substrates.

To transcend this dimensional limitation and fabricate coherent, 3D HA architectures capable of withstanding physiological stresses, a shift in the fundamental deposition unit is required. This necessitates adopting bioprinting modalities that use continuous filaments (1D voxels) or photopolymerized layers (2D voxels) as primary building blocks. These approaches are engineered to provide the structural integrity and design freedom needed to translate the advanced biofunctionality of engineered HA materials into clinically relevant, volumetric constructs for wound repair.

#### 4.2. One-dimensional voxel filament-based bioprinting

In contrast to the precision patterning yet limited structural formability of droplet-based (0D) methods, 1D voxel bioprinting utilizes continuous filaments as the foundational deposition unit. This approach is most prominently embodied by EBB, which has become the most widely adopted and versatile modality in tissue engineering due to its capacity for fabricating volumetric, self-supporting constructs.<sup>220</sup> Additionally, electrospinning, while distinct in its driving mechanism, is another quintessential filament-forming technique that produces nanofibrous mats highly relevant as biomimetic substrates for wound healing. The continuous nature of such filaments enables the direct creation of interconnected, mechanically coherent 3D architectures with controlled porosity.<sup>221</sup> This capability is critical for engineering HA-based wound constructs that must serve not only as a protective barrier but also as a provisional, 3D matrix to manage wound exudate, withstand physiological stresses, and guide organized cellular infiltration and tissue regeneration.<sup>29,222</sup> Filament-based techniques are particularly well-suited for processing the broad range of engineered HA materials described in Section 3, especially those formulated as viscoelastic hydrogels with tailored rheological properties for shape retention after deposition.<sup>223</sup>

##### 4.2.1. Extrusion-based bioprinting

Extrusion-based bioprinting operates by mechanically dispensing bioinks through a micronozzle, typically driven by pneumatic, piston, or screw mechanisms, to deposit continuous filaments along digitally defined paths in a layer-by-layer manner (Figure 1).<sup>29,224</sup> The successful application of EBB with HA-based bioinks is

fundamentally governed by their rheological properties.<sup>225</sup> Optimal formulations exhibit pronounced shear-thinning behavior, where viscosity decreases under the high shear stress experienced within the nozzle, coupled with rapid structural recovery or a sufficient yield stress upon deposition.<sup>20,221</sup> This characteristic enables the bioink to flow during extrusion and maintain its filament geometry on the print bed, which is essential for achieving high shape fidelity and constructing multi-layered structures without deformation or collapse.<sup>225,226</sup> These tailored rheological profiles are achieved through the crosslinking strategies detailed in Section 3.2, including physical interactions (e.g., host-guest complexes, hydrophobic associations), dynamic covalent chemistry (e.g., Schiff bases, disulfide bonds), and post-deposition covalent crosslinking (e.g., ionic, photopolymerization).<sup>21</sup>

The material compatibility of EBB is exceptionally broad, making it the predominant technique for fabricating functional HA-based wound constructs. It can process a diverse spectrum of formulations, ranging from pure, chemically modified HA hydrogels (e.g., HAMA, HA-Tyr, HA-SH) to sophisticated multicomponent composites.<sup>221</sup> These composites synergistically combine HA with natural polymers (e.g., CS, collagen, gelatin) or synthetic polymers (e.g., PEG, PCL) to enhance mechanical robustness and biological functionality, as outlined in Section 3.4.<sup>22,195</sup> Furthermore, EBB readily facilitates the integration of functional fillers directly into the bioink.<sup>227,228</sup> This allows for the single-step fabrication of multifunctional dressings incorporating antimicrobial agents (e.g., AgNPs), conductive components (e.g., GO, polypyrrole for electroactive therapy), or drug-loaded microspheres for controlled release.<sup>131,160,170</sup> A critical advantage of EBB is its compatibility with high cell densities, enabling the bioprinting of living, cell-laden constructs such as full-thickness skin equivalents with spatially organized dermal fibroblasts and epidermal keratinocytes.<sup>1,229</sup>

Nevertheless, EBB is subject to certain inherent constraints. The printing resolution, primarily determined by the extruded filament diameter, is practically limited by the nozzle inner diameter, which is typically chosen ( $\geq 100 \mu\text{m}$ ) to avoid generating excessive shear stress that can compromise cell viability.<sup>221,225</sup> This resolution limit restricts the ability to replicate the finest capillary-scale features ( $< 50 \mu\text{m}$ ) of native microvasculature using standard single-nozzle systems. Additionally, cells within a homogeneous bioink are uniformly distributed throughout the extruded filament, offering limited intrinsic capacity for creating the highly organized, heterogeneous cellular patterning characteristic of native skin.<sup>230</sup> To overcome



these challenges, advanced EBB configurations have been developed. Coaxial extrusion, which employs concentric nozzles to simultaneously extrude different bioinks, enables the fabrication of hollow, vessel-like filaments. This technique serves as a direct method for creating channel structures essential for vascularization.<sup>231</sup> Similarly, the strategy of using sacrificial bioinks, which are bioprinted as a temporary lattice and subsequently removed, permits the creation of interconnected, perfusable networks within a primary hydrogel matrix.<sup>232</sup> This represents a significant advancement for engineering thick, metabolically active tissue grafts.<sup>227</sup> For applications where structural functionality, compositional complexity, and scalability are prioritized over ultra-high resolution in cellular patterning, EBB remains the most practical and extensively utilized fabrication technology.

#### 4.2.2. Electrospinning

While electrospinning is not a conventional bioprinting technique involving programmed 3D deposition, it represents a fundamental filament-based fabrication method highly relevant for wound healing constructs.<sup>233,234</sup> In the voxel-based classification scheme outlined in [Figure 1](#), electrospinning is categorized as a 1D (filament-based) process, as its fundamental deposition unit is a continuous, 1D fiber. This process generates continuous nanofibers by subjecting a polymer solution or melt to a high-voltage electric field, yielding non-woven mats whose structural characteristics closely mimic those of the native ECM. The resulting architectures exhibit high porosity, extensive surface area, and interconnected fibrillar networks, which collectively facilitate essential cellular processes for tissue regeneration, such as attachment, proliferation, and guided migration.<sup>235,236</sup>

Electrospinning native HA poses specific challenges due to its intrinsic physicochemical properties, including high hydrophilicity, polyelectrolyte nature, and limited chain entanglements in solution, all of which impede stable jet formation and uniform fiber deposition.<sup>21</sup> These limitations are addressed through the material engineering strategies detailed in Section 3. A primary method involves blending HA with complementary natural or synthetic polymers that are inherently electrospinnable. For instance, HA has been successfully combined with natural polymers such as collagen, gelatin, and silk fibroin to produce composite nanofibers that merge the bioactivity of HA with the structural and processing benefits of the supporting polymer.<sup>151,237</sup> Similarly, blending with synthetic polymers like PCL or PLA improves mechanical robustness and spinnability while allowing precise control over degradation kinetics.<sup>235,237</sup> Alternatively, chemical modification of the HA backbone, through hydrophobic

grafting or the introduction of crosslinkable functional groups, enables tuning of rheological properties and facilitates electrospinning from biocompatible solvent systems.<sup>75,85</sup>

In wound management, electrospun HA-based nanofibrous mats function as multifunctional interfaces. The nanofibrous architecture acts as a semi-permeable barrier against pathogens, manages wound exudate via capillary action, and maintains an optimal moist microenvironment.<sup>19,238</sup> The high surface area of the fibers allows efficient loading and sustained release of bioactive agents incorporated during spinning. This supports localized delivery of therapeutics such as EGF to enhance re-epithelialization, antimicrobial agents (e.g., AgNPs, essential oils) to prevent infection, and anti-inflammatory molecules to modulate the wound microenvironment.<sup>167,239,240</sup>

Despite these advantages, conventional electrospinning presents inherent limitations for engineering volumetric, cell-instructive constructs required for complex wound healing. Specifically, it predominantly produces 2D mats of densely packed fibers, with insufficient control over pore interconnectivity along the z-axis. This structural constraint restricts deep cellular infiltration and vascular ingrowth, thereby compromising the regeneration of full-thickness wounds.<sup>5,234</sup> Moreover, traditional electrospinning offers minimal capability to fabricate patient-specific 3D geometries that conform to irregular wound cavities. Processing constraints, including the use of high voltage and often organic solvents, also hinder the direct and viable encapsulation of cells during fabrication.<sup>235</sup>

Consequently, electrospinning is also increasingly integrated into hybrid manufacturing workflows rather than used in isolation. One representative strategy combines electrospun nanofibrous substrates with EBB. Here, the electrospun mat serves as a biomimetic basal layer to direct cell behavior, while EBB deposits subsequent layers, introduces spatial heterogeneity, or builds volumetric complexity.<sup>241</sup> This integrated approach leverages the complementary strengths of each technique: the ECM-mimetic nanotopography and high surface area from electrospinning, alongside the design flexibility, structural precision, and cell patterning capabilities inherent to 3D bioprinting.

Although electrospinning and filament-based EBB enable the fabrication of structurally coherent 3D constructs, they remain limited in achieving the ultra-high resolution and smooth surface finish required to replicate fine tissue microarchitectures. To overcome this limitation, layer-based bioprinting technologies utilizing photopolymerization have emerged as a complementary



approach. These methods, which employ 2D layers as foundational voxels, offer distinct advantages in resolution and architectural fidelity, as discussed in the following section.

### 4.3. Two-dimensional voxel layer-based bioprinting

In contrast to the filament-by-filament assembly of 1D-based methods, which can impose a practical limit on feature resolution and surface smoothness, 2D voxel bioprinting adopts a fundamentally different strategy. This approach utilizes light to selectively solidify entire cross-sectional layers of a photosensitive bioink in a single, mask-projection step. The fundamental building block is thus a 2D layer, which is sequentially stacked to form a 3D object.<sup>93</sup> This paradigm shift enables superior printing speed and exceptional spatial resolution, typically 10–50  $\mu\text{m}$ , facilitating the fabrication of constructs with intricate internal microarchitectures, smooth surfaces, and complex geometries that are challenging to achieve with EBB methods alone.<sup>93,242</sup> For HA-based wound healing applications, this capability is crucial for engineering scaffolds with precisely designed porosity for exudate management, biomimetic topographical cues to guide cellular alignment and migration, and patient-specific shapes derived from medical imaging of complex wound cavities.<sup>242,243</sup>

Digital light processing is a prominent vat photopolymerization technology that exemplifies the layer-based bioprinting approach.<sup>28</sup> In a DLP system, a digital micromirror device dynamically projects UV or visible light onto the surface of a vat containing a liquid, photoactive bioink. The irradiated areas undergo rapid photopolymerization, solidifying an entire 2D cross-section in a single exposure.<sup>244</sup> Following each layer's curing, the build platform moves relative to the light source (typically in a bottom-up or top-down configuration), a fresh layer of resin is presented, and the process repeats sequentially. This mask-projection methodology bypasses the time-consuming point-by-point or line-by-line scanning, thereby offering significantly faster build speeds for a given layer complexity.<sup>245</sup>

The successful application of DLP for processing HA-based materials is intrinsically governed by the photochemistry principles detailed in Section 3.2.3. HA modification with photoreactive groups is a prerequisite. HAMA serves as the quintessential bioink for DLP, as the pendant methacrylate moieties readily participate in free radical polymerization upon photoinitiation.<sup>246,247</sup> By formulating HAMA with a cytocompatible photoinitiator (such as LAP) for visible light or UV light, high-fidelity HA hydrogel scaffolds can be fabricated. This allows for precise

control over critical architectural parameters, including pore size, shape, interconnectivity, and even mechanical stiffness gradients within a single construct.<sup>248,249</sup> Such design freedom is invaluable for creating advanced wound dressings with optimized porosity to balance fluid handling and barrier function, or for engineering dermal scaffolds with embedded, perfusable channel networks intended to promote rapid vascularization.<sup>250</sup>

Beyond pure HAMA, DLP is compatible with other photocrosslinkable HA derivatives (e.g., those functionalized with acrylate or thiol-ene groups) and with composite resins where HA is blended with other photopolymerizable polymers, such as PEGDA, to fine-tune mechanical and degradation properties.<sup>148</sup> The high resolution of DLP enables the replication of microscale surface topographies reminiscent of the native skin's basement membrane, which can be used to direct keratinocyte alignment and stratification.<sup>5,242</sup> However, the practical application of DLP with HA-based resins faces key constraints. Optical properties govern light penetration and curing uniformity, requiring adjusted parameters for composites with opaque fillers. Oxygen inhibition at the resin surface leads to incomplete curing, necessitating either optimized light dosage or inert-atmosphere control. Limited light penetration depth restricts single-layer thickness, challenging the fabrication of thick, vascularized constructs. Strategies such as near-infrared initiators or multi-layer approaches are being explored. Moreover, cumulative radical damage in thick, cell-laden prints can deeply compromise viability, prompting the need for graded exposure or antioxidant incorporation.<sup>93</sup> Furthermore, the ability to translate digital designs directly into complex 3D geometries supports the fabrication of patient-specific implants or conformable dressings tailored to the unique topography of an individual's wound, based on 3D scans or medical imaging data.<sup>242</sup>

The application of DLP to HA-based wound constructs faces three interrelated limitations regarding material versatility, structural complexity, and biological fidelity. Firstly, its single-vat configuration inherently restricts spatial control over material composition, limiting the fabrication of biochemical gradients needed to mimic native skin architecture.<sup>28</sup> While emerging multi-vat and hybrid systems address this, they introduce substantial technical challenges in material switching and interfacial adhesion.<sup>28</sup> Secondly, DLP provides minimal control over microscale cell distribution, as cells remain uniformly dispersed in photoresins, precluding the heterogeneous cell patterning achievable with droplet-based methods. Thirdly, the photopolymerization process imposes two concurrent material-level constraints. On the one hand, bioinks

require chemical functionalization with photoreactive groups that may alter HA's native bioactivity. On the other hand, the curing reaction introduces inherent cytotoxic risks originating from residual photoinitiators, unreacted monomers, and transient reactive species.<sup>21,85</sup> Therefore, DLP remains fundamentally limited to photocurable systems and continues to face challenges in scaling toward clinically relevant, functionally graded wound scaffolds.

Selecting an optimal 3D bioprinting technology for fabricating HA-based wound constructs is a strategic decision guided by material formulation, structural complexity, required resolution, and clinical application (Table 3). Droplet-based methods, such as inkjet bioprinting and LIFT, provide high-resolution, non-contact patterning of cells and biochemical signals onto surfaces, yet are limited in their ability to fabricate self-supporting, volumetric scaffolds. In contrast, EBB bioprinting offers broad versatility in processing a wide range of engineered HA bioinks, from pure modified hydrogels to composite formulations, into robust, shape-stable 3D structures. This capability establishes it as the predominant technique for producing volumetric wound dressings and cell-laden grafts. Photopolymerization-based techniques, including DLP, achieve exceptional resolution and architectural control when fabricating intricate scaffolds from photoreactive HA derivatives, though their applicable material scope is narrower. Consequently, the strategic integration of complementary bioprinting modalities, selected for their strengths to meet specific structural or functional requirements, represents a rational approach to engineering biomimetic, multifunctional, and patient-specific HA constructs for advanced wound therapy.

## 5. Three-dimensional bioprinting of hyaluronic acid-based constructs for wound healing

The convergence of advanced engineered HA, as detailed in Sections 2 and 3, with the digital fabrication capabilities of 3D bioprinting technologies (Section 4) establishes a transformative pathway for wound care. This section transitions from foundational principles to applied outcomes, examining the fabrication and therapeutic potential of 3D bioprinted HA constructs. The effective translation of engineered HA bioinks into therapeutic devices is governed not only by their biochemical composition but also by the manufacturing paradigm used to structure them. The choice of bioprinting strategy is, in turn, dictated by the specific clinical objective, which imposes distinct requirements on construct architecture, structural integrity, and biological complexity.

Consequently, applications of bioprinted HA constructs

can be functionally and technically segmented into two primary domains. Wound dressings (Section 5.1) prioritize creating a functional interface that performs critical macroscopic roles: providing a physical barrier, managing exudate through controlled porosity, and enabling the localized release of antimicrobial or pro-healing agents. The manufacturing focus for dressings is therefore on achieving reproducible microarchitectures (e.g., nanofibrous mats, porous hydrogels) that deliver these bulk properties, often without the need to replicate the full histoarchitecture of native skin. In contrast, skin substitutes (Section 5.2) aim to serve as regenerative templates that directly facilitate the reconstruction of damaged tissue. This objective demands higher-order biomimicry, requiring the fabrication of stratified, multicellular constructs with spatially organized biochemical and topographical cues that guide specific cell behaviors, including keratinocyte stratification, fibroblast-driven dermal matrix deposition, and vascular network formation. The fabrication of such substitutes thus pushes the limits of bioprinting technologies, requiring high-resolution patterning, multi-material deposition, and often the integration of viable cells within a biologically permissive HA-based matrix. The following sections dissect the current state of research within these two domains, analyzing how the material strategies from Section 3 and the bioprinting modalities from Section 4 are specifically leveraged and integrated to meet these divergent sets of clinical and engineering challenges.

### 5.1. Three-dimensional bioprinted hyaluronic acid-based wound dressings: From barrier function to active healing

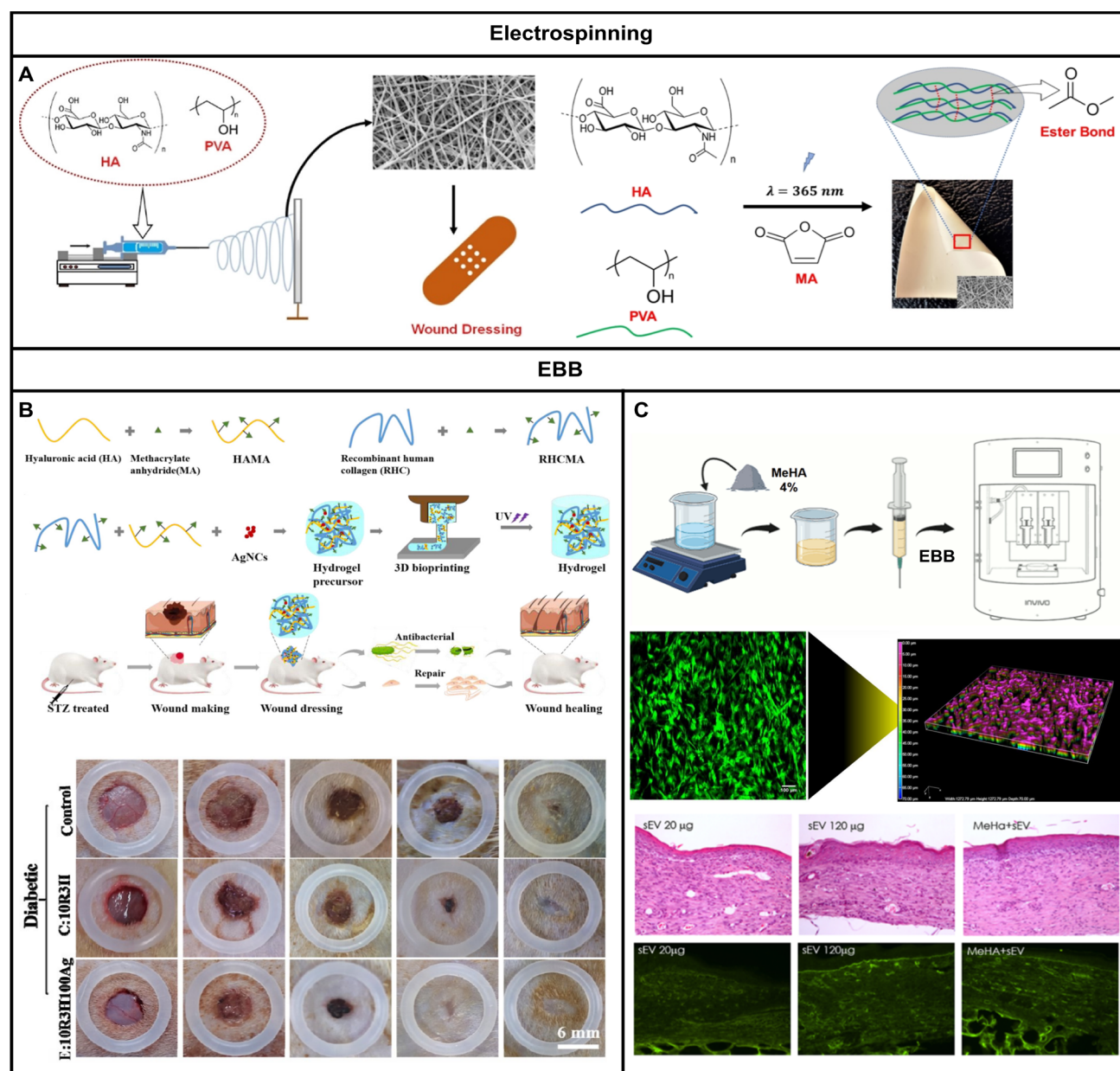
The clinical success of existing HA-based wound care products (Table 2) is tempered by the limitations of their conventional manufacturing processes, such as solvent casting and freeze-drying. These methods typically yield constructs with homogeneous, isotropic structures that lack the programmed spatial heterogeneity in porosity, composition, or mechanical property, which may be critical for managing complex, non-healing wounds.

Advanced biomanufacturing techniques that offer digital control over microstructure and form present a solution. Electrospinning (Section 5.1.1) is a mature, highly effective method for fabricating nanofibrous dressings. Its capacity to generate non-woven mats with high surface area, tunable fiber diameter, and ECM-mimetic topography makes it ideal for creating functional wound interfaces. Beyond electrospinning, a suite of alternative bioprinting techniques (Section 5.1.2) provides a complementary toolkit, enabling the fabrication of dressings with more complex 3D geometries, integrated multi-material compositions, and embedded cellular

Table 3. Comparative analysis of three-dimensional bioprinting techniques

Voxel type	Technology	Mechanism	Typical resolution	Bioink viscosity	Cell viability	Advantages	Limitations	Suitability for HA-based constructs
Zero-dimensional (Droplet)	Inkjet bioprinting	Thermal, piezoelectric, or electrostatic droplet ejection	10–100 $\mu\text{m}$ <sup>209,306</sup>	3–12 mPa·s <sup>27</sup>	>70% <sup>27,213</sup>	High-resolution patterning of low-viscosity HA solutions; non-contact deposition; suitable for biochemical gradient fabrication <sup>307,308</sup>	Narrow viscosity range; prone to nozzle clogging; poor structural integrity for 3D scaffolds <sup>27</sup>	Surface functionalization; patterned biochemical cue deposition; single-cell resolution deposition <sup>20,27</sup>
	Laser-induced transfer forward	Laser pulse-induced droplet transfer via dynamic release layer	20–300 $\mu\text{m}$ <sup>210,215</sup>	1–300 mPa·s <sup>215,217</sup>	>80% <sup>215,219</sup>	Nozzle-free; broad viscosity tolerance; capable of transferring high-cell density and composite HA bioinks <sup>215,217</sup>	Potential thermal/mechanical stress on biomolecules; limited 3D stacking stability <sup>215</sup>	High-resolution cell/biomolecule patterning; multi-material surface engineering; functionalization of pre-formed scaffolds <sup>215</sup>
One-dimensional (Filament)	Extrusion-based bioprinting	Pneumatic, piston, or screw-driven continuous extrusion	20–1,000 $\mu\text{m}$ <sup>225</sup>	30 mPa·s–60 kPa·s <sup>225,227</sup>	40–80% <sup>227,309</sup>	Excellent compatibility with shear-thinning HA hydrogels; multi-material & composite capability; scalable for volumetric constructs <sup>225,227</sup>	Limited resolution for micro-features (<50 $\mu\text{m}$ ); homogeneous cell distribution within filaments; shear stress induces cell damage <sup>29,309</sup>	Primary technology for 3D wound dressings, cell-laden skin equivalents with multifunctional composites <sup>279,310</sup>
	Electrospinning	High-voltage electrostatic fiber drawing	50 nm–5 $\mu\text{m}$ <sup>233,235</sup>	100–2,000 mPa·s <sup>235</sup>	70–90% <sup>235,311</sup>	Produces extracellular matrix-mimetic nanofibrous mats from HA blends; high surface area for drug/cell interaction <sup>233,235</sup>	Limited 3D shape control; challenges in cell encapsulation; solvent/voltage biocompatibility <sup>235</sup>	Nanofibrous wound interface layers; often integrated with other technologies in hybrid constructs for enhanced biofunctionality <sup>233,238</sup>
Two-dimensional (Layer)	Digital light processing	Mask-projection photopolymerization of entire layers	<100 $\mu\text{m}$ <sup>28</sup>	1–200 mPa·s <sup>23,312</sup>	>75% <sup>242,313</sup>	High resolution and efficiency; ideal for photopolymerizable HA (e.g., HAMA); enables complex internal architectures and patient-specific geometries <sup>28</sup>	Restricted to photocrosslinkable resins; limited multi-material capability; photoinitiator cytotoxicity; poor heterogeneous capability <sup>28,93</sup>	High-fidelity acellular scaffolds with designed porosity/channels; patient-specific implants; micro-topographical guidance structures <sup>5</sup>

Abbreviations: 3D: Three-dimensional; HA: Hyaluronic acid; HAMA: Hyaluronic acid methacryloyl.



**Figure 6.** Electrospinning (A) and EBB of HA-based wound dressings (B, C). (A) Electrospinning of HA/PVA nanofibers crosslinked with maleic anhydride enhanced thermal stability and structural homogeneity, demonstrating their potential as a smart wound dressing with improved storage stability and controlled water absorption. Reprinted with permission from de Castro *et al.*<sup>252</sup> Copyright © 2022, American Chemical Society. (B) A methacrylated recombinant human collagen and HA composite hydrogel loaded with silver nanoclusters exhibits excellent printability, antibacterial activity, and biocompatibility, highlighting its potential as an *in situ* 3D bioprinted bioactive dressing for chronic diabetic wound repair. Scale bar: 6 mm. Reprinted with permission from Liang *et al.*<sup>262</sup> Copyright © 2023, American Chemical Society. (C) MSC-sEVs embedded in a 3D-printed MeHA hydrogel patch promote wound healing in a diabetic pressure ulcer model by enhancing re-epithelialization, angiogenesis, and innervation through sustained vesicle release. Reprinted from Ferroni *et al.*<sup>193</sup>

Abbreviations: AgNCs: Silver nanoclusters; EBB: Extrusion-based bioprinting; HA: Hyaluronic acid; HAMA: Hyaluronic acid methacryloyl; MA: Methacrylate anhydride; MeHA: Methacrylated hyaluronic acid; MSC-sEVs: Mesenchymal stem cell-derived small extracellular vesicles; PVA: Polyvinyl alcohol; RHC: Recombinant human collagen; RHCMA: Methacrylated recombinant human collagen.

components. This section sequentially reviews these two technological pathways. As illustrated in Figure 6, electrospinning and other bioprinting technologies are widely used in HA-based wound dressings.

### 5.1.1. Electrospinning of hyaluronic acid for wound dressing applications

As illustrated in Section 4.2.2, electrospinning is a cornerstone technique for producing wound dressings that replicate the nanofibrous architecture of native ECM. The efficient electrospinning of HA, however, requires mitigating its inherent physicochemical challenges, including high hydrophilicity, high surface tension, and strong inter-chain hydrogen bonding, which impede stable jet formation. As detailed in Section 3, successful processing is achieved through material engineering, primarily by blending HA with electrospinnable carrier polymers or by chemical derivatization. This subsection reviews the application of these engineered HA formulations in electrospun wound dressings, categorizing them by their primary functional enhancement.

A primary strategy involves blending HA with complementary natural polymers to create composite fibers with synergistic properties. For instance, combining anionic HA with cationic CS merges HA's pro-regenerative signaling with the inherent antimicrobial and hemostatic activity of CS. Foroozandeh *et al.*<sup>251</sup> demonstrated this by fabricating a nylon 6/HA/CS (N6/HA/CS) nanofibrous mat via one-step electrospinning. The introduction of HA and CS significantly improved the mat's hydrophilicity and water uptake. The optimal formulation (2 wt% CS) demonstrated effective antibacterial activity against *S. aureus* and *E. coli*, supported L929 fibroblast proliferation, and exhibited a balanced profile of tensile strength, porosity, and degradation, underscoring its potential as a cost-effective, multifunctional dressing.<sup>251</sup>

Blends with synthetic polymers, such as PVA, are equally prevalent, aimed at improving processability and mechanical integrity. Research here extends beyond simple blending to include stabilization strategies. As illustrated in Figure 6A, de Castro *et al.*<sup>252</sup> produced HA/PVA nanofiber membranes crosslinked with maleic anhydride and loaded with *Plantago major* extract. The crosslinking step was critical, enhancing thermal stability, preventing phase separation, and yielding uniform fibers, thereby highlighting the importance of post-processing modification for durable dressings.<sup>252</sup> In a complementary study, Ilomuanya *et al.*<sup>253</sup> systematically optimized far-field electrospun PVA/HA membranes, identifying an optimal formulation that maximized mucoadhesion and keratinocyte (human immortalized keratinocytes) viability,

illustrating how precise control over material parameters tailors performance for specific wound environments.

To combat infection, electrospun HA mats are functionalized as delivery vehicles for antimicrobial agents. For the management of diabetic wounds, Su *et al.*<sup>254</sup> engineered a core-shell fiber system via emulsion electrospinning, featuring a PCL shell encapsulating a core of HA, keratin, and metformin hydrochloride. This design facilitated sustained metformin hydrochloride release over 20 days. The hydrophilic HA and keratin components increased swelling and degradation rates, while the composite structure maintained excellent cytocompatibility and enhanced fibroblast proliferation, offering a sophisticated platform for localized therapy.<sup>254</sup> In an alternative approach, Yang *et al.*<sup>255</sup> incorporated the natural AMP  $\epsilon$ -polylysine (EPL) into OHA nanofiber mats. The OHA-EPL mats outperformed starch-based counterparts, exhibiting higher EPL loading (27.9% vs. 19.2%), superior fluid absorption (26.3 times vs. 15.1 times its weight), greater tensile strength (0.6 MPa vs. 0.3 MPa), and more rapid polymer erosion. These enhancements are attributable to HA's anionic character and high hydrophilicity, demonstrating its role in optimizing both the physicochemical and bioactive performance of antimicrobial dressings.<sup>255</sup>

The design of electrospun systems has evolved to include multilayer or composite architectures for controlled release. Gruppuso *et al.*<sup>256</sup> developed antibacterial multilayer matrices using two designs: a sequentially electrospun bilayer of PCL and HA/lactose-modified CS/polyethylene oxide, and a PCL mat coated with lactose-modified CS and HA layers. With rifampicin loaded in the PCL layer, both constructs exhibited favorable swelling and biocompatibility but offered distinct release profiles. The bilayer electrospun structure provided immediate polysaccharide release and faster antibiotic elution, whereas the coated system yielded a more sustained release, exemplifying the ability to engineer specific pharmacokinetics into the dressing architecture.<sup>256</sup>

Aqueous stability is a critical requirement for HA-based dressings, often addressed through crosslinking. Xue *et al.*<sup>257</sup> applied a periodate oxidation-adipic acid dihydrazide crosslinking strategy to HA nanofibers. This biocompatible reaction achieved a crosslinking density of 75.7%, resulting in exceptional water resistance that maintained fibrous morphology for 14 days in simulated body fluid and possessed a wet tensile strength of 0.8 MPa. Notably, the mats were non-cytotoxic despite residual aldehyde groups, validating the approach for creating durable, biocompatible HA dressings.<sup>257</sup>



Finally, electrospinning was integrated into the fabrication of bioinspired, structurally-graded scaffolds that bridge the categories of dressing and substitute. Chen *et al.*<sup>258</sup> created a fetal dermis-inspired bilayer scaffold by combining a porous PCL/collagen/HA substrate (fabricated by porogen leaching) with an aligned PCL nanofiber membrane (fabricated by electrospinning). This biomimetic design, which mimics the oriented ECM of fetal skin, supported fibroblast proliferation and exhibited favorable biocompatibility, indicating its potential as a regenerative template for healing with reduced scarring.<sup>258</sup>

In summary, electrospinning offers a versatile, well-established platform for manufacturing HA-based wound dressings with enhanced functionality. Through material blending, bioactive agent incorporation, chemical stabilization, and architectural innovation, electrospun HA mats have been engineered to actively address key challenges in wound healing, including infection, inflammation, moisture imbalance, and mechanical support. This positions them as a significant advancement over passive wound covers.

### 5.1.2. Extrusion-based bioprinting of hyaluronic acid as wound dressings

While electrospinning excels in producing nanofibrous, ECM-mimetic mats that serve as advanced wound interfaces, its limitations in precise 3D architectural control, spatial patterning of multiple materials, and direct cell encapsulation underscore the necessity for complementary fabrication strategies.<sup>259</sup> EBB with hybrid derivatives has therefore emerged as a viable bioprinting technology for wound dressing. It enables the precise fabrication of HA-based wound dressings with customized geometries, controlled porosity, compartmentalized functionality, and integrated therapeutic agents.<sup>260</sup> By leveraging the engineered rheological and crosslinking properties of HA bioinks (Section 3.2), EBB enables the fabrication of dressings that extend beyond passive barriers to become active, programmable therapeutic platforms.

Due to its intrinsic advantages, EBB is the most versatile and widely adopted method for fabricating volumetric HA-based dressings, owing to its broad compatibility with viscoelastic hydrogels and composites.<sup>227</sup> A key application is the fabrication of mechanically stable, porous hydrogel patches designed for exudate management and controlled drug release. For example, Si *et al.*<sup>261</sup> used EBB to produce a double-crosslinked wound dressing from HA-SH and HAMA. By modulating the HA-SH/HAMA ratio, hydrogels with tunable rheological properties, swelling behavior, and degradation rates were obtained. The optimized formulation exhibited controlled degradation

(approximately 90% over 11 days) and functioned as an effective carrier for the sustained release of the antibiotic nafcillin, demonstrating the capacity of EBB to integrate long-acting antimicrobial functionality into dressings.<sup>261</sup> This approach addresses the need for prolonged antibacterial activity in chronic wound management, overcoming the burst-release limitations associated with many conventional films or sponges.

Moreover, EBB allows precise spatial organization and controlled release of bioactive agents within an HA matrix. For example, Puertas-Bartolomé *et al.*<sup>259</sup> illustrated this by developing a bioactive bioink containing catechol-functionalized polymeric nanoparticles within a carboxymethyl CS (CMCS) and HA matrix. Using reactive mixing during EBB, they bioprinted scaffolds with homogeneous nanoparticle distribution and sustained release kinetics. The catechol nanoparticles provided antioxidant and anti-inflammatory activities, while the HA-based hydrogel offered a supportive, degradable matrix. This work highlights the potential of EBB to fabricate HA dressings capable of delivering not only drugs but also advanced nanocarriers that modulate key wound healing processes, such as inflammation and angiogenesis.<sup>259</sup>

While EBB is well-suited for processing composite and particle-laden bioinks, photocrosslinkable bioinks play a significant role in creating HA-based dressings with high structural fidelity and intricate microarchitectures that guide cellular responses and manage the wound microenvironment. As illustrated in Figure 6B, Liang *et al.*<sup>262</sup> utilized photocrosslinkable bioinks to develop an *in situ*-printable hydrogel system for chronic diabetic wound repair. Their bioink consisted of methacrylated recombinant human collagen and HAMA, loaded with ultra-small silver nanoclusters. The photocrosslinkable formulation enabled rapid UV crosslinking, yielding hydrogels with defined porosity, favorable mechanical properties, and high shape fidelity. The embedded silver nanoclusters conferred deep tissue penetration and broad-spectrum antibacterial activity.<sup>262</sup>

The functional scope of bioprinted HA dressings can be further expanded by incorporating conductive or photoactive components to enable advanced therapeutic modalities. Zhao *et al.*<sup>263</sup> developed an EBB-compatible ink composed of gelatin, alginate, HA, and a cationic conjugated polymer. Strong electrostatic interactions between the cationic conjugated polymer and the anionic biopolymer matrix ensured uniformity and printability of the ink. The resulting artificial skin patches exhibited effective photodynamic therapy-based antibacterial activity against *S. aureus* under visible light irradiation. Additionally, covalent modification with a laminin-derived



peptide (ASG81) enhanced cell adhesion and migration.<sup>263</sup> This work demonstrates how material design combined with bioprinting can produce HA-based dressings that integrate targeted anti-infection mechanisms with pro-regenerative biochemical cues.

A significant advancement enabled by these bioprinting techniques is the fabrication of cell-instructive and cell-laden HA constructs, representing a shift toward dressings that actively support regeneration by delivering therapeutic cells or their bioactive secretions. Ferroni and colleagues<sup>264</sup> have systematically explored this approach using EBB to produce MeHA patches as delivery vehicles for MSC-derived vesicles. Initial work showed that MeHA patches loaded with exosomes enhanced the proliferation, migration, and angiogenic potential of human dermal fibroblasts and endothelial cells *in vitro*.<sup>264</sup> As illustrated in Figure 6C, a subsequent study employed a similar strategy to deliver MSC-sEVs from bioreactor-expanded MSCs. These sEV-laden MeHA patches not only reproduced pro-regenerative effects *in vitro* but also accelerated wound closure, epithelialization, and angiogenesis in a diabetic mouse pressure ulcer model *in vivo*.<sup>193</sup> This progression from *in vitro* validation to *in vivo* efficacy in a complex disease model supports the translational potential of bioprinted, vesicle-delivering HA dressings. Similarly, advancing beyond acellular cargoes, Guan *et al.*<sup>265</sup> used EBB to fabricate pro-angiogenic patches from gelatin methacryloyl (GelMA) and HAMA, covalently conjugated with the QHREDGS peptide. The 3D bioprinted structure enabled prolonged peptide release and conferred potent angiogenic properties, improving wound healing *in vivo*.<sup>265</sup> These examples demonstrate how bioprinting enables the creation of bioactive, instructive HA-based matrices that can orchestrate complex cellular responses.

In summary, EBB technology enables the fabrication of HA-based wound dressings with precise architectural control, material complexity, and integrated biofunctionality. It supports the development of dressings that are: (i) geometrically tailored to patient-specific wounds; (ii) functionally programmed with spatially organized cues; (iii) capable of sustained or stimuli-responsive release; and (iv) cell-instructive through the delivery of therapeutic cells or paracrine signals. This shift from static barriers to dynamic, multifunctional interfaces represents a substantial advance. Nevertheless, while such advanced dressings actively modulate the wound microenvironment, their design often prioritizes specific functional delivery over explicit replication of the full structural and cellular hierarchy of native skin. This function-oriented approach provides a foundation for the next frontier: the use of 3D bioprinting to fabricate

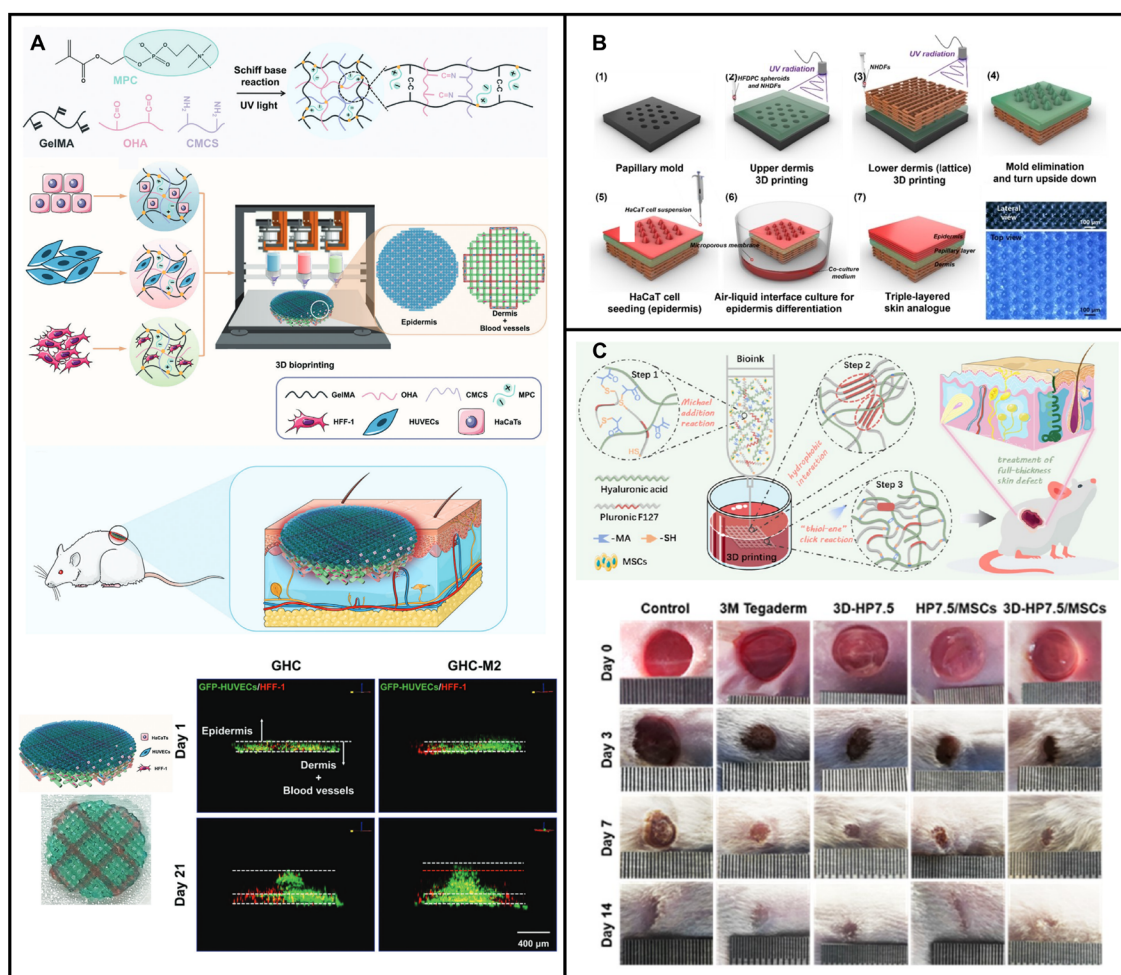
HA-based skin substitutes that biomimetically reconstruct epidermal and dermal layers with high histological and functional fidelity.

### 5.2. Three-dimensional bioprinted hyaluronic acid-based skin substitutes: Toward structural and functional regeneration

While advanced wound dressings actively modulate the wound microenvironment to facilitate the body's intrinsic healing, they are insufficient for reconstructing the anatomical and functional complexity of full-thickness skin defects. Chronic, non-healing wounds (e.g., diabetic ulcers, severe burns) and extensive traumatic injuries present a fundamental biological challenge: the native regenerative capacity is often severely compromised due to factors such as sustained inflammation, ischemia, neuropathy, or microbial colonization.<sup>5,224</sup> In these scenarios, the wound healing cascade is disrupted or stalled, rendering the body unable to regenerate functional tissue autonomously. Consequently, while HA-based dressings can provide critical support in maintaining moisture, delivering antimicrobials, and reducing inflammation, their therapeutic scope is inherently limited to modulating the existing microenvironment. They lack the intrinsic architectural blueprint and cellular complexity necessary to directly orchestrate the *de novo* formation of a multilayered, vascularized, and innervated tissue.<sup>266</sup>

This limitation underscores the imperative to evolve from function-oriented interfaces to structure-instructive templates. The goal thus shifts decisively toward engineering skin substitutes that serve as regenerative blueprints. Unlike dressings, which act from the wound surface inward, a true skin substitute is designed to integrate into the defect as a living, instructive scaffold that actively guides cellular processes: providing topographical and biochemical cues for keratinocyte stratification to form a competent epidermal barrier, offering a 3D dermal matrix for fibroblast infiltration, alignment, and organized collagen deposition, and, most critically, presenting predefined vascular channels or endothelial cell niches to drive rapid anastomosis and perfusion.<sup>267</sup> Only through such architectural and cellular guidance can the substitute overcome the diffusion-limited necrosis that plagues avascular grafts and achieve functional integration with the host. Therefore, the fabrication of HA-based skin substitutes via 3D bioprinting represents a paradigm shift from wound management to tissue restoration, targeting the reconstruction of both form and function in the most challenging clinical contexts.<sup>125</sup>

Three-dimensional bioprinting represents a pivotal technology for this purpose, as it enables the precise spatial



**Figure 7.** Bioprinting of HA-based skin substitutes. (A) EBB of a double-layer ionic-conductive skin scaffold using GHCM bioink enhances wound healing by improving vascularization and re-epithelialization. Scale bar: 400  $\mu$ m. Reprinted with permission from Wang *et al.*<sup>268</sup> Copyright © 2025, Wiley. (B) EBB of skin equivalents with hair follicle structures and epidermal-papillary-dermal layers using GelMA/HAMA hydrogels enhances structural mimicry and hair-inductive potency. Scale bar: 100  $\mu$ m. Reprinted with permission from Kang *et al.*<sup>[272]</sup> Copyright © 2022, Wiley. (C) A stepwise multi-crosslinking bioink combining HAMA and PF127-SH enables embedded 3D bioprinting of cell-laden scaffolds that accelerate full-thickness wound healing by enhancing cell viability and promoting tissue regeneration. Reprinted with permission from Hao *et al.*<sup>[275]</sup> Copyright © 2023, American Chemical Society. Abbreviations: CMCS: Carboxymethyl chitosan; EBB: Extrusion-based bioprinting; GelMA: Gelatin methacryloyl; HaCaTs: Human immortalized keratinocytes; HAMA: Hyaluronic acid methacryloyl; HFF-1: Human foreskin fibroblasts; HUVECs: Human umbilical vein endothelial cells; MPC: 2-methacryloyloxyethyl phosphorylcholine; OHA: Oxidized hyaluronic acid.

orchestration of multiple biomaterial matrices and living cell types to recapitulate the multilayered architecture, cellular heterogeneity, and functional gradients of native skin.<sup>1</sup> Within this framework, HA-based bioinks are indispensable, providing a biocompatible and bioactive matrix that closely mimics the native dermal GAG milieu. This subsection critically examines recent advances in 3D bioprinting of HA-based skin substitutes, focusing on stratified tissue design, the regeneration of functional skin appendages, and strategies to enhance vascularization. Furthermore, it discusses the evolution from *ex vivo* fabrication to *in situ* bioprinting as a pathway for clinical

translation. As illustrated in Figure 7, 3D bioprinting technologies have been widely used to fabricate HA-based skin substitutes.

The fabrication of biomimetic skin substitutes relies on constructing a stratified architecture that supports distinct epidermal and dermal compartments. EBB has been widely employed due to its versatility in depositing viscoelastic, cell-laden HA-based bioinks. A common strategy is to design dual-layer or multi-material constructs. As illustrated in Figure 7A, Wang *et al.*<sup>268</sup> developed a double-layer ionic-conductive skin scaffold using a bioink composed of GelMA, OHA, CMCS, and the

zwitterionic (2-methacryloyloxyethyl) phosphorylcholine. The rigid GelMA network provided shape fidelity, while the dynamic OHA-CMCS network ensured printability. The scaffold, integrating human fibroblasts, endothelial cells, and keratinocytes to form vascularized dermal and epidermal layers, exhibited ionic conductivity that mimicked the electrical signaling of natural skin, accelerating re-epithelialization, collagen deposition, and angiogenesis *in vivo*.<sup>268</sup> Similarly, Bettendorf *et al.*<sup>269</sup> systematically evaluated HA-containing bioinks (alginate/GelMA) for co-printing human immortalized keratinocytes and adipose-derived stem cells. The resulting biphasic constructs demonstrated high cell viability and facilitated keratinocyte migration out of the GelMA matrix, highlighting their potential as therapeutic grafts for large chronic wounds.<sup>269</sup> These examples illustrate how EBB combined with composite HA bioinks can fabricate stratified, cell-laden structures that actively participate in tissue regeneration.

Achieving high-fidelity anatomical replication requires mimicking the critical dermal-epidermal junction, particularly its undulating rete ridge morphology, which is essential for mechanical stability, nutrient exchange, and scar prevention. Admane *et al.*<sup>270</sup> addressed this using a silk-gelatin bioink in an EBB process to bioprint a full-thickness human skin model that recapitulated the undulated dermal-epidermal junction. Extensive transcriptomic and proteomic analyses revealed that the bioprinted construct exhibited a high degree of similarity to native human skin, activating pathways related to skin development, ECM organization, and keratinization.<sup>270</sup> This study underscores the importance of microscale architectural fidelity, enabled by precise bioprinting, for eliciting physiologically relevant cellular signaling and differentiation.

The regeneration of functional skin appendages, such as hair follicles and sebaceous glands, represents a significant frontier for restoring complete skin function and aesthetics. Ma *et al.*<sup>271</sup> and Kang *et al.*<sup>272</sup> both employed HAMA in combination with GelMA to engineer skin equivalents with hair follicle potential. As illustrated in Figure 7B, Kang *et al.*<sup>272</sup> optimized a GelMA/HAMA bioink to recapitulate the native collagen-to-GAG ratio. Constructs incorporating epidermal/papillary dermal layers and hair follicle dermal papilla cell spheroids spontaneously developed hair pore structures and maintained hair-inductive potency.<sup>272</sup> Ma *et al.*<sup>271</sup> advanced this concept by co-printing epidermal stem cells and skin-derived precursors within a GelMA/HAMA hydrogel. Upon transplantation, the prefabricated artificial skin promoted rapid wound closure and the regeneration of complete skin appendages, including hair follicles, blood vessels, and sebaceous glands.<sup>271</sup> These

studies demonstrate that the biochemical composition and 3D microenvironment provided by HA-based composite hydrogels can support the complex epithelial-mesenchymal interactions necessary for appendage morphogenesis.

Vascularization remains a paramount challenge for the survival and integration of thick, engineered skin grafts. Bioprinting strategies are being devised to pre-form perfusable vascular networks within HA-based dermal substitutes. Zhang *et al.*<sup>273</sup> engineered a dermal substitute using a GelMA-HAMA-fibrin composite bioink. By applying confined mechanical forces during *in vitro* culture through 3D bioprinted frameworks, they induced the alignment and maturation of encapsulated endothelial cell networks. This prevascularized construct facilitated rapid anastomosis with host vasculature upon transplantation, enhancing graft perfusion, epithelialization, and collagen deposition.<sup>273</sup> In a different approach, Zhou *et al.*<sup>274</sup> utilized a sacrificial templating strategy within a catechol-functionalized HA/alginate ink. They printed a scaffold with embedded gelatin fibers, which were subsequently eluted at 37 °C to create defined microchannels. These channels were then infused with a thrombin-free fibrinogen gel containing human dermal fibroblasts, with human immortalized keratinocytes seeded on top to form a full-thickness skin model. The microchannels significantly enhanced nutrient diffusion and cell proliferation.<sup>274</sup> These works highlight the convergence of advanced bioink design and innovative bioprinting strategies to address the vascularization barrier.

The functional performance of bioprinted skin substitutes is ultimately assessed in clinically relevant wound models. Studies are progressively employing more stringent *in vivo* evaluations. As illustrated in Figure 7C, Hao *et al.*<sup>275</sup> developed a stepwise, multi-crosslinking bioink based on HAMA and thermosensitive Pluronic F127-SH for embedded 3D bioprinting. This strategy allowed the bioprinting of complex structures in a support bath at 37 °C, followed by photochemical crosslinking. The cell-laden bioprinted hydrogels significantly promoted full-thickness wound healing in mice by modulating inflammation and accelerating angiogenesis.<sup>275</sup> Zhou *et al.*<sup>250</sup> leveraged the high resolution of DLP to print a functional living skin construct from a GelMA/HAnorbornene/LAP bioink. Featuring interconnected microchannels for mass transport, the construct promoted dermal regeneration with skin appendages in a porcine model.<sup>250</sup> DLP enables the rapid fabrication of constructs with intricate, predefined architectures that are difficult to achieve with extrusion alone.

A transformative direction aimed at bridging laboratory

research and clinical application is *in situ* bioprinting, which is the direct deposition of bioinks onto a wound bed in a surgical setting. Hakimi *et al.*<sup>276</sup> pioneered this approach with a handheld skin bioprinter. This device deposited bioinks (including alginate, fibrin, collagen, and HA) and cells conformally onto wound surfaces through a microfluidic cartridge, with rapid crosslinking provided by a co-deposited crosslinker.<sup>276</sup> Proof-of-concept demonstrations in murine and porcine excisional wound models confirmed the feasibility of depositing homogeneous or architected sheets onto irregular, compliant wound beds subject to motion. While this initial study focused on acellular hemostatic sheets, the platform is inherently compatible with cell-laden HA-based bioinks. This technology circumvents the need for prefabrication, scaffold handling, and the complex logistics of traditional skin grafts, thereby presenting a paradigm shift toward personalized, point-of-care regenerative therapy.

Overall, 3D bioprinting of HA-based constructs has advanced the field of skin substitutes from simple cellularized sheets toward anatomically precise, functionally enhanced, and clinically translatable grafts. The integration of HA into composite bioinks provides a foundational matrix that supports cell viability, differentiation, and biomimetic signaling. Current research is multidimensional, focusing on: (i) enhancing structural biomimicry through multilayer bioprinting and replication of the dermal-epidermal junction; (ii) engineering higher-order functionality such as appendage regeneration and pre-vascularization; (iii) validating efficacy in advanced disease and large animal models; and (iv) innovating delivery modalities like *in situ* bioprinting for clinical deployment. The synergy between increasingly sophisticated HA biomaterial science and evolving bioprinting technologies continues to narrow the gap between engineered skin substitutes and native skin.

## 6. Discussion and future perspectives

The preceding sections have systematically detailed the rationale for employing HA as a central biomaterial platform in wound therapy, based on its inherent bioactivity, versatile chemical modifiability, and foundational role within the native ECM.<sup>14</sup> Through strategic molecular engineering, including chemical derivatization, biofunctionalization, and composite formulation, the intrinsic limitations of native HA have been addressed, yielding a diverse array of advanced biomaterials with tailored mechanical properties, degradation profiles, and multifunctional capabilities. These material innovations have transitioned to clinical practice as standardized commercial dressings, which primarily function as protective, hydrating, and antimicrobial barriers. The advent of 3D bioprinting has further enabled the spatial and architectural programming

of these engineered HA materials, facilitating the fabrication of constructs ranging from advanced dressings to stratified, cell-laden skin equivalents.

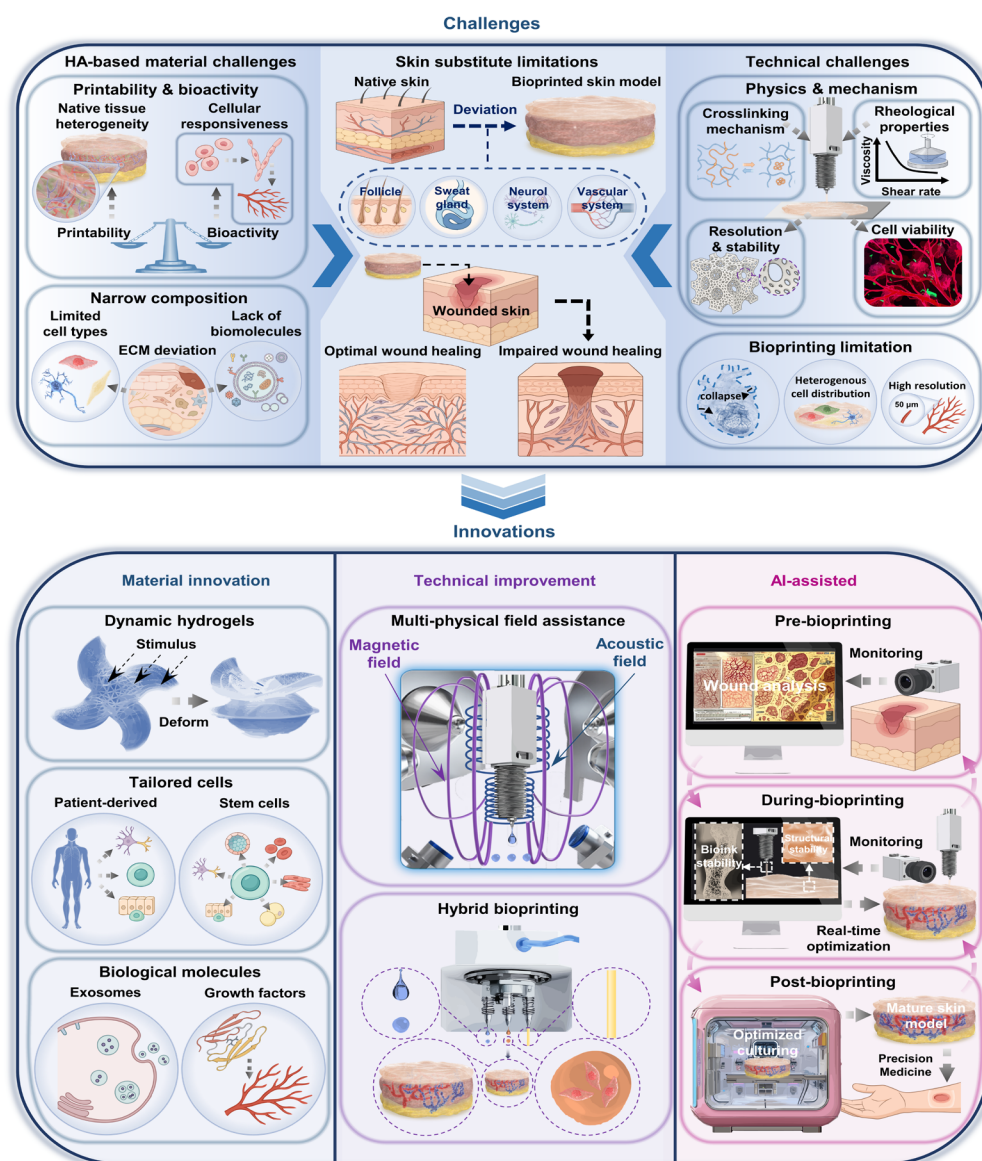
Nevertheless, a critical appraisal of the current state of the art reveals a persistent translational gap. Existing HA-based constructs, even those fabricated via advanced bioprinting, largely fail to recapitulate the structural hierarchy, dynamic reciprocity, and functional complexity of native, healthy skin. The predominant outputs remain sophisticated wound dressings rather than fully regenerative tissue substitutes. This discrepancy stems from interconnected challenges spanning material science, biofabrication technology, and biological design principles. Materially, engineering HA for printability often compromises its native structure–function relationship. Chemical modifications (e.g., methacrylation) and crosslinking strategies essential for mechanical integrity can alter the MW profile and bioactivity of HA, while potentially introducing cytotoxic residues from initiators or crosslinkers.<sup>21</sup> This creates a fundamental conflict: the processed material may lose the context-dependent signaling (e.g., pro- versus anti-inflammatory, angiogenic versus quiescent) inherent to the native HA's MW spectrum, as detailed in Section 2. Architecturally, current HA-based constructs do not replicate skin's functional complexity. As evidenced in vascularized skin models<sup>5,277</sup>, even advanced bioprinted substitutes lack hierarchical, anastomosis-capable vasculature, functional skin appendages (e.g., hair follicles, sweat glands). It must be emphasized that appendage regeneration remains an early-stage research frontier; promising results from highly regenerative models, such as mice, are species-dependent and not yet translatable to humans. Furthermore, these substitutes lack integrated neuro-immune networks. These omissions limit their function to sophisticated dressings rather than true organ replacements, failing to restore sensation, thermoregulation, and immune competence. Technologically, bioprinting modalities present inherent trade-offs. EBB offers structural robustness but struggles to achieve resolutions (<50 µm) in capillary networks.<sup>221,278</sup> DLP achieves high resolution but lacks control over multi-material and cellular heterogeneity.<sup>28</sup> Droplet-based methods enable precise patterning but yield mechanically weak constructs unsuitable for volumetric grafting.<sup>210</sup> Consequently, a single technology cannot concurrently deliver the resolution, heterogeneity, and structural integrity required to biomimic full skin architecture. Additionally, practical manufacturing challenges such as achieving reliable sterilization and long-term storage stability of HA biomaterials and constructs, alongside ensuring batch-to-batch consistency in material properties and printing reproducibility, remain critical barriers to



scalable clinical translation.

Therefore, the trajectory toward clinically impactful, regenerative HA-based skin constructs necessitates a paradigm shift from incremental improvements to synergistic, systems-level innovation. Future progress depends on the convergent evolution of three core domains: (i) the development of next-generation HA biomaterials that function as dynamic, instructive microenvironments rather than passive scaffolds; (ii) the advancement of bioprinting technologies through

hybridization and novel fabrication paradigms to achieve unprecedented biomimicry; and (iii) the integration of artificial intelligence (AI) and data-driven science to personalize, optimize, and predict stages of the therapeutic workflow, from wound assessment to long-term outcome. The following sections delineate a forward-looking roadmap, articulating specific research avenues within these domains to bridge existing gaps and accelerate the translation of 3D bioprinted HA constructs from bench to bedside. Furthermore, the clinical translation is also



**Figure 8.** From challenges to integrated solutions in three-dimensional bioprinted hyaluronic acid (HA)-based skin regeneration. Bridging material limitations, structural simplicity, and bioprinting constraints with next-generation bioinks, hybrid bioprinting, and intelligent systems. Abbreviations: AI: Artificial intelligence; ECM: Extracellular matrix.

discussed. As shown in Figure 8, to address the current limitations in skin substitutes, including material and technical challenges, that contribute to suboptimal wound healing outcomes, innovations in dynamic biomaterials, patient-specific cells, biological molecules, multi-physical field assistance, hybrid bioprinting techniques, and AI-assisted manufacturing jointly pave the way for more physiologically relevant skin substitutes and precise medicine.

### 6.1. Biomaterial innovations: Advanced hyaluronic acid-based biomaterials

While the innovations outlined below represent exciting research directions, their translation to clinical wound care faces significant hurdles. The manufacturing consistency, sterility assurance, and long-term stability of dynamic HA networks under storage conditions remain largely unvalidated. The regulatory pathway for 'smart' biomaterials with stimulus-responsive behavior remains undefined. Moreover, the cost-effectiveness of these advanced systems relative to existing HA-based products must be demonstrated to support widespread adoption in healthcare systems. To transition HA from a versatile biopolymer into a cornerstone for regenerative skin substitutes, material innovations must extend beyond conventional chemical modifications. Future strategies should focus on creating multifunctional biomaterial systems that provide structural support while dynamically orchestrating the wound healing cascade. This evolution encompasses three synergistic fronts: developing biomimetic and adaptive crosslinking networks, recapitulating HA's native signaling dynamics, and strategically integrating advanced regenerative agents.

Firstly, advancing crosslinking strategies toward dynamic and bio-adaptive networks is essential. Current approaches often rely on static, irreversible bonds, yielding hydrogels with fixed properties that limit remodeling in response to cellular activity and the evolving wound microenvironment.<sup>13</sup> Integrating dynamic covalent crosslinking via reversible bonds, such as imines, boronic esters, or disulfides, offers a promising approach. These networks provide shear-thinning behavior for printability and rapid self-healing post-deposition. Their reversible nature facilitates stress relaxation and enables gradual, cell-mediated remodeling, thereby mimicking reciprocal cell-ECM interactions and potentially mitigating fibrotic encapsulation.<sup>12</sup> Concurrently, refining crosslinking mechanisms for enhanced cytocompatibility is imperative. This involves optimizing enzymatic crosslinking (e.g., using HRP with HA-Tyr) for spatiotemporal control

under physiological conditions, advancing catalyst-free bioorthogonal "click" reactions (e.g., strain-promoted azide-alkyne cycloaddition), and developing novel photoinitiators activatable by tissue-penetrating, low-energy light to reduce phototoxicity.<sup>21</sup>

Secondly, biomaterial design must actively recapitulate the spatiotemporal MW spectrum of HA, which is central to its physiological function. As detailed in Section 2, HMW-HA promotes tissue homeostasis and anti-inflammatory signaling, while LMW-HA can drive inflammation, proliferation, and angiogenesis.<sup>49</sup> Current biomaterials typically employ a single, modified HA derivative, failing to encode this critical temporal signaling logic. Future systems should incorporate strategies for programmed MW transitions. This could involve co-printing biomaterials containing HA of distinct, predefined MWs (e.g., HMW-HA > 1 MDa and LMW-HA < 250 kDa) to establish spatial biochemical gradients. A more sophisticated approach entails engineering HA backbones with stimuli-responsive, labile linkers that degrade in response to wound-specific cues, such as elevated levels of MMPs or ROS.<sup>11</sup> Such a design would enable a construct to initially present a stabilizing HMW-HA matrix, which subsequently undergoes controlled fragmentation to locally release pro-regenerative LMW-HA signals, thereby propelling healing from the inflammatory to the proliferative phase.<sup>10</sup>

Thirdly, to amplify regenerative potential, HA matrices should evolve into sophisticated carriers for advanced therapeutic agents. While encapsulating patient-specific stem cells (e.g., induced pluripotent stem cells, MSCs) remains a pathway<sup>10,121</sup>, logistical and safety challenges associated with cell therapy necessitate alternative strategies. Cell-derived nanotherapeutics, particularly MSC-sEVs, present a promising opportunity.<sup>193</sup> These natural nanocarriers can be pre-loaded into HA hydrogels to deliver a complex cocktail of therapeutic microRNAs, cytokines, and growth factors, orchestrating key processes such as angiogenesis, macrophage polarization toward a pro-healing (M2) phenotype, and fibroblast activation without the risks of cell transplantation. Beyond serving as passive reservoirs, the HA matrix can be engineered as an active signaling platform. For instance, covalent conjugation of heparin-mimicking peptides or strategic sulfation can create high-affinity binding sites for critical heparin-binding growth factors (e.g., VEGF, fibroblast growth factor 2), protecting them from degradation and presenting them in a stable, matrix-bound manner to enhance localized bioavailability and signaling precision.<sup>122,136</sup>



## 6.2. Technical convergence: Advancing biofabrication toward personalization

Overcoming the architectural simplicity of current constructs requires a fundamental leap in biofabrication capabilities. To translate advanced biofabrication concepts into viable HA-based therapies, several material-specific challenges must be tackled concurrently. As no single bioprinting technology possesses the resolution, material diversity, and structural control necessary to replicate skin's hierarchical organization, the path forward lies in the strategic convergence of complementary technologies and exploration of next-generation fabrication paradigms, integrated with multi-physical field assistance.

A primary strategy involves developing integrated, hybrid multi-process bioprinting platforms. These systems synergistically combine the strengths of distinct modalities to overcome individual limitations. For example, a highly effective configuration involves sequential integration of EBB with high-resolution droplet-based bioprinting (inkjet or LIFT).<sup>279,280</sup> In this workflow, EBB first fabricates the bulk, mechanically robust dermal scaffold from a composite HA-based biomaterial. Subsequently, inkjet or LIFT precisely patterns low-viscosity biomaterials containing epidermal keratinocytes, melanocytes, or vascular endothelial cells onto predefined scaffold regions with micrometer-scale accuracy. This approach decouples macro-structural fabrication from micro-cellular patterning challenges. For HA-based systems, this necessitates the development of multi-material HA bioinks with orthogonal crosslinking mechanisms to prevent interfacial delamination during sequential printing. Similarly, combining electrospinning with EBB allows creation of constructs featuring an electrospun, nanofibrous HA-polymer blend membrane mimicking native basement membrane topography and surface area seamlessly integrated with a 3D bioprinted, cell-laden hydrogel layer providing volumetric architecture and cellular niches.<sup>260</sup> Furthermore, adopting coaxial and multi-material extrusion printheads is critical for single-step fabrication of heterogeneous, functionally graded structures, such as hollow, perfusable channel networks or scaffolds with controlled stiffness and composition gradients.<sup>221,231</sup>

Beyond hybrid systems, adopting emerging volumetric and four-dimensional bioprinting paradigms is essential to transcend the constraints of layer-by-layer fabrication. For soft, cell-laden HA hydrogels lacking intrinsic mechanical support, embedded 3D bioprinting, which deposits bioinks within a yield stress support bath, is indispensable.<sup>243</sup> This technique enables freeform fabrication of complex, overhanging structures (e.g., budding hair follicle germs)

that would otherwise collapse. Volumetric bioprinting represents a revolutionary advance by solidifying entire 3D structures from a rotating vial of photosensitive resin within seconds, eliminating layer-related artifacts and drastically reducing shear stress on encapsulated cells.<sup>281</sup> Applied to HA-based resins (e.g., HAMA), this could enable rapid production of patient-specific, high-fidelity scaffolds. Concurrently, four-dimensional bioprinting, where printed constructs undergo predictable, time-dependent shape or functional changes in response to stimuli, aligns with smart material design. By printing with HA-based shape-memory polymers or contractile hydrogels, a flat, easily handled scaffold could be designed to self-fold or contract upon implantation, conforming to irregular wound cavities and applying beneficial mechanical cues to healing tissue.<sup>243</sup>

To further enhance microarchitectural control and functionality, integrating multi-physical field assistance during or post-bioprinting is promising.<sup>282</sup> This involves employing external fields to guide component assembly and behavior within the HA matrix.<sup>283</sup> Acoustic focusing could pattern cells or functional nanoparticles into specific spatial arrangements within a printed filament before gelation.<sup>284</sup> Magnetic field guidance, utilizing cells or scaffolds doped with superparamagnetic nanoparticles, offers a tool to align endothelial cells along predefined paths to form nascent vascular networks or orient neural crest cells for peripheral nerve regeneration.<sup>285</sup> Electrical stimulation, when applied to constructs incorporating conductive HA composites (e.g., with graphene or polypyrrole), can enhance migration, proliferation, and maturation of electrically excitable cells (e.g., neurons, myofibroblasts) and has been shown to promote angiogenic responses.<sup>178</sup> These field-assisted techniques can introduce microstructural order and biomimicry difficult to achieve through bioprinting mechanics alone.

## 6.3. Artificial intelligence-enhanced workflow: Toward predictive and autonomous systems

The complexity of wound pathophysiology, combined with the high-dimensional parameter space governing HA ink formulation and bioprinting processes, presents a challenge that transcends conventional empirical optimization. The complexity of HA ink formulation and bioprinting poses a high-dimensional optimization problem well-suited to AI/machine learning. However, its application must be grounded in HA-specific material databases and wound healing biology. This complexity establishes an ideal domain for integrating AI and machine learning. Strategic incorporation of data-driven intelligence holds transformative potential to revolutionize the therapeutic pipeline, from patient-specific diagnosis and scaffold

design to optimized fabrication and post-operative monitoring, enabling a closed-loop, precision medicine paradigm for advanced wound repair.<sup>286</sup>

The initial impact of AI lies in accelerating development and optimization cycles for HA inks and bioprinting processes.<sup>287</sup> The traditional trial-and-error approach to formulating inks with ideal rheological, mechanical, and biological properties is slow and resource-intensive. Generative machine learning models, trained on curated datasets correlating HA chemical structures, composite compositions, processing parameters, and final construct outcomes (e.g., print fidelity, cell viability, degradation kinetics), can serve as predictive engines.<sup>288</sup> These models can rapidly identify promising biomaterial ink formulations from a vast combinatorial space or engage in inverse design, proposing novel material compositions meeting predefined target performance criteria. During fabrication, AI enables real-time, adaptive process control. Integrating in-line monitoring systems (e.g., high-speed vision, rheometric sensors) with algorithms such as convolutional neural networks allows the bioprinting system to continuously assess extruded filament quality, droplet formation, or photopolymerization uniformity.<sup>287</sup> This facilitates instantaneous anomaly detection (e.g., inconsistent strand diameter, potential clogging) and autonomous, dynamic adjustment of printing parameters (e.g., pressure, nozzle speed, light intensity) to correct deviations and ensure robust, repeatable manufacturing of high-fidelity constructs.<sup>289</sup> For HA-based EBB, predictive models could correlate HA's MW, degree of modification, and composite composition with printability metrics (e.g., shape fidelity, strand uniformity) to accelerate ink development.

Moving upstream in the clinical workflow, AI bridges nuanced wound diagnostics and the generation of personalized therapeutic blueprints. Effective treatment begins with accurate assessment. Advanced computer vision and machine learning algorithms can analyze multi-modal wound images, incorporating data from standard photography, thermal imaging, hyperspectral imaging, or optical coherence tomography.<sup>290</sup> These systems perform automated, quantitative analysis, surpassing subjective human evaluation in consistency, segmenting wound boundaries, classifying tissue types (viable vs. necrotic), quantifying exudate and biofilm presence, and assessing perfusion status. This objective diagnostic dataset serves as the foundation for generative design.<sup>291</sup> An AI-driven design engine can utilize this input to intelligently synthesize an optimal internal microarchitecture, including spatially varying porosity for guided cell infiltration, patterning gradients of biochemical cues (e.g.,

growth factors, specific MW HA), and computationally optimizing prevascular channel network layouts to maximize perfusion potential upon integration, all tailored to specific wound pathophysiology and patient healing profile. As a representative example, machine learning could be implemented as follows:

- (i) Inputs: Multi-modal wound images and patient metadata (e.g., diabetes status, wound duration) are collected.
- (ii) Model output: A convolutional neural network segments the wound area, classifies tissue type (granulation, slough, necrosis), and predicts a "healing propensity score."
- (iii) Validation endpoint: The predicted healing score is correlated with the observed and measured percentage of wound closure in four weeks, and the model's precision-recall performance is evaluated against clinician annotations.

The most forward-looking application involves developing intelligent constructs and closed-loop therapeutic ecosystems. Future iterations of HA-based dressings or substitutes could be engineered as responsive systems incorporating embedded biosensors to continuously monitor local wound microenvironmental parameters (e.g., pH, temperature, moisture, specific biomarkers of infection or inflammation).<sup>292</sup> Streaming telemetric data to cloud-based machine learning analytics platforms enables remote, real-time assessment of healing. More significantly, it can enable a closed-loop therapeutic response: algorithmic detection of rising pro-inflammatory cytokine signatures or bacterial bioburden could trigger controlled, on-demand release of anti-inflammatory drugs or antimicrobial agents from the construct.<sup>286</sup> Similarly, in bioreactors used for *ex vivo* maturation of bioprinted skin grafts, AI controllers can manage complex, multi-parameter conditioning protocols.<sup>293</sup> Integrating real-time sensor feedback on metabolites, oxygen tension, and tissue contractility allows dynamic tailoring of perfusion dynamics, mechanical stimulation regimens, and biochemical cues to steer tissue development toward a desired, functional phenotype.<sup>294,295</sup>

Notwithstanding its transformative potential, integrating AI into wound theragnostics is constrained by significant technical and practical limitations. Key challenges include (i) the current inability of AI models to replicate nuanced, context-aware clinical reasoning and therapeutic decision-making of experienced physicians in complex, multi-factorial chronic wound scenarios; and (ii) technical gaps in reliable wound assessment, as many contemporary vision-based algorithms rely on limited data modalities (e.g., RGB images alone), compromising

accuracy in evaluating parameters like wound depth, tissue composition, and infection severity compared to integrated clinical judgment.<sup>286,296</sup> Consequently, at present, AI should be positioned as a compensatory and facilitative tool, aiding data quantification, pattern recognition, and workflow efficiency, to augment, not replace, clinician expertise.

To transition from a facilitative tool to a robust, clinically reliable partner, future development must prioritize creating high-fidelity, multi-modal datasets and advancing algorithm sophistication. This necessitates sustained, interdisciplinary collaboration to curate large-scale, well-annotated datasets encompassing diverse patient populations and wound etiologies.<sup>297</sup> These datasets must integrate multi-modal data streams beyond imagery, including histopathology, microbiology, patient omics, and longitudinal healing outcomes. Beyond data collection, research should leverage advanced techniques such as data augmentation, transfer learning, and federated learning to improve model robustness, generalizability, and privacy preservation. Most pivotally, there is a need to transcend purely correlative models by developing physics- and biology-informed machine learning frameworks. Integrating fundamental principles of wound healing biomechanics, tissue growth dynamics, and biomaterial-tissue interactions into the model architecture would shift AI from pattern recognition toward a more predictive, mechanistically grounded simulation, enhancing the reliability and interpretability of predictions for scaffold design and healing trajectory forecasting.<sup>297</sup>

Parallel to technical advancement, ethical, regulatory, and human-centered dimensions of AI integration demand proactive engagement. Clear ethical guidelines and evolving regulatory frameworks must govern patient data privacy, security, informed consent for data use in model training, and accountability for AI-assisted clinical decisions.<sup>298</sup> Algorithmic transparency and explainability must be embedded into the development lifecycle to foster clinical trust and meet regulatory standards. Patient autonomy must remain paramount; limitations and potential risks associated with AI-assisted care must be communicated transparently, ensuring patients are fully informed and retain consent rights regarding AI's role in their treatment.<sup>299</sup>

In summary, as AI technology matures and gains responsible access to richer, multidimensional personal health data, its synergistic convergence with 3D bioprinting and intelligent HA biomaterials will unlock significant opportunities. This triad promises to catalyze a paradigm shift in wound management, from reactive, standardized practice to proactive, predictive, and deeply personalized

therapeutic continuum. By navigating intertwined technical, clinical, and ethical landscapes, the field can progress toward AI-enhanced systems that collaboratively deliver intelligent, adaptive, and patient-specific regenerative therapies, ultimately aiming to restore quality of life for patients with severe and complex skin defects. Finally, it is crucial to recognize that most bioprinted HA constructs remain at an early, proof-of-concept stage. Their clinical translation depends on overcoming pivotal challenges beyond technical performance, including robust, scalable manufacturing to ensure batch-to-batch consistency, sterilization compatibility that preserves HA bioactivity, and regulatory clarity for these complex, often cell-laden combination products.

#### **6.4. Navigating the path to clinical translation: Regulatory and manufacturing considerations**

The promising *in vitro* and preclinical performance of 3D bioprinted HA constructs must ultimately be evaluated within the rigorous framework of clinical translation. Moving from clinical application potential to approved therapies requires successfully navigating a series of interconnected regulatory, manufacturing, and validation challenges.

A primary hurdle is defining the regulatory status of these advanced constructs. Depending on their final composition (acellular vs. cell-laden), primary mode of action (scaffold vs. drug delivery), and claims (wound management vs. regeneration), bioprinted HA products may be classified by agencies like the United States' Food and Drug Administration as medical devices (class II or III), combination products (device + biologic), or even as advanced therapy medicinal products if they contain viable cells. Each classification has distinct, often lengthy pathways for approval (e.g., pre-market notification [510(k)], pre-market approval, or biologics license application). For "smart" dressings with biosensing or stimuli-responsive drug release functionalities, they may fall under emerging regulations for software as a medical device or adaptive devices, adding further complexity.<sup>300</sup>

Prior to human trials, comprehensive validation in accordance with recognized standards (e.g., ISO 10993 for the biological evaluation of medical devices, ASTM F2902 for the assessment of absorbable biomaterials) is mandatory.<sup>300</sup> This includes exhaustive testing for sterility, pyrogenicity, cytotoxicity, sensitization, and systemic toxicity. For HA-based materials, special attention must be paid to the immunogenicity and long-term biocompatibility of degradation products from engineered derivatives, as highlighted in Section 3.1. Animal studies must move beyond proof-of-concept healing in healthy rodents to

include robust, clinically relevant disease models (e.g., diabetic, ischemic, or infected wounds in large animals) that better predict human responses. Demonstrating not only safety but also statistically significant superiority or non-inferiority over standard-of-care treatments is a key requirement for regulatory approval and market adoption.<sup>301</sup>

Scaling laboratory biofabrication to good manufacturing practice standards presents a formidable challenge. It demands strict control over every input, from the source and purity of HA (which can vary by origin and extraction method) to the consistency of chemical modification reactions.<sup>302</sup> The bioprinting process itself must be transformed from a bespoke, research-grade operation into a reproducible, validated, and automated manufacturing process with in-process quality controls. Key parameters requiring standardization include bioink rheology, printing fidelity, crosslinking efficiency, terminal sterilization methods (which must not degrade HA or its bioactivity), and final product sterility and shelf-life. For personalized “smart” dressings, the regulatory and logistical framework for point-of-care manufacturing remains largely undefined, posing significant questions about real-time quality assurance and liability.<sup>300</sup>

Beyond regulatory approval, successful translation depends on a viable business model. A clear intellectual property strategy protecting the unique material formulations, bioink compositions, and printing processes is essential. Furthermore, demonstrating not only efficacy but also cost-effectiveness compared to existing therapies is critical for securing reimbursement from healthcare payers, which is the ultimate determinant of widespread clinical adoption.<sup>303</sup>

In summary, while the scientific vision for intelligent, bioprinted HA wound therapies is compelling, a parallel, equally critical focus must be placed on addressing these translational “valley of death” challenges. Future research must adopt a design-for-translation mindset from the outset, engaging early with regulatory scientists, process engineers, and health economists to ensure that groundbreaking laboratory innovations can successfully make the journey to the patient’s bedside.

## 7. Conclusion

The convergence of advanced HA engineering and 3D bioprinting offers a transformative pathway for wound healing, enabling the fabrication of bioactive, structurally defined constructs that actively guide tissue regeneration. Through chemical modification, biofunctionalization, and hybrid compositing, HA has evolved from a simple

polysaccharide into a versatile, multifunctional biomaterial platform. 0D, 1D, and 2D bioprinting technologies have further translated these materials into personalized wound dressings and stratified skin substitutes with controlled architecture and biochemical signaling. Despite promising progress, challenges remain in fully replicating the structural and functional complexity of native skin, including hierarchical vascularization and appendage regeneration for clinical translation. Future advances should focus on integrating smarter, stimuli-responsive HA systems, multi-modal and volumetric bioprinting strategies, and AI-enhanced design and monitoring, collectively driving the field toward truly regenerative, patient-specific wound solutions.

## Abbreviations

Abbreviation	Full name
0D	Zero-dimensional
1D	One-dimensional
2D	Two-dimensional
Abbreviation	Full name
3D	Three-dimensional
AI	Artificial intelligence
CD44	Cluster of differentiation 44
CS	Chitosan
CMCS	Carboxymethyl chitosan
DLP	Digital light processing
EBB	Extrusion-based bioprinting
ECM	Extracellular matrix
EGF	Epidermal growth factor
GelMA	Methacrylated gelatin
HA	Hyaluronic acid
HAMA	Hyaluronic acid methacryloyl
HRP	Horseradish peroxidase
IL-1 $\beta$	Interleukin-1 $\beta$
LIFT	Laser-induced forward transfer
MSCs	Mesenchymal stem/stromal cells
PCL	Polycaprolactone
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
ROS	Reactive oxygen species
sEVs	Small extracellular vesicles
TGF- $\beta$	Transforming growth factor- $\beta$
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
VEGF	Vascular endothelial growth factor

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## Conflict of interest

The authors declare they have no competing interests.

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