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REVIEW ARTICLE

Exosome-integrated bioinks for 3D bioprinting in regenerative medicine: Design strategies and applications

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Abstract

Exosome-containing "hybrid bioinks" integrate the mechanical properties of 3D bioinks with the bioactive contents of extracellular vesicles, enabling the fabrication of bioactive tissue constructs amenable to 3D printing. Here, we summarize how 3D bioprinting hybrid bioinks can develop tissue constructs with regenerative capacity, immunomodulatory function, angiogenic ability, and cell differentiation guidance. Despite recent advances in integrating exosomes into bioinks, the rational design of these systems remains underdeveloped. This limitation arises not only from the intrinsic complexity of vesicle–matrix interactions, but also from the lack of standardized design principles and unified performance evaluation frameworks. This challenge is further compounded by source-dependent heterogeneity in exosomal composition and function. In addition, current systems offer limited control over loading efficiency, spatial distribution, and release kinetics, and there is still no consensus on how to assess post-fabrication vesicle stability, bioactivity retention, or therapeutic performance. Together, these limitations hinder reproducibility, cross-study comparability, and the establishment of predictive design rules for clinically translatable exosome-integrated bioinks. Therefore, this review critically discusses recent trends in the design of exosome-laden bioconstructs, including their major advantages and drawbacks. From a design perspective, key considerations include bioactive matrix engineering, exosome loading, triggered release, and construct integrity during biofabrication. Design parameters affecting the overall performance of the construct are discussed in light of the challenges and standardization issues of developing clinically relevant exosome-loaded bioengineered constructs. Moreover, recent progress in exosome isolation and characterization, and state-of-the-art enabling technologies are discussed. Therapeutic applications reviewed here include wound healing, musculoskeletal tissue engineering, neural repair, and cardiac regeneration, highlighting the diverse therapeutic potential of exosomes. In the last section, we outline programmable bioink integration, stimuli-responsive materials, and computational design approaches toward the future generation of biofabrication technologies for regenerative medicine and precision therapeutics.

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1. Introduction

Three-dimensional (3D) bioprinting has emerged as a transformative technology in tissue engineering and regenerative medicine, enabling the precise fabrication of cell-laden constructs with well-defined spatial architecture and compositional heterogeneity.[1-3] By integrating biomaterials, living cells, and bioactive molecules, 3D bioprinting provides an unprecedented platform to recapitulate key structural and functional features of native tissues, including gradients, vascular networks, and multicellular organization.[2-4] Over the past decade, significant progress has been achieved in printing modalities such as extrusion-based, inkjet-based, and light-assisted bioprinting, as well as in the development of advanced biomaterials tailored for biofabrication.[3,5] Despite these advances, the clinical translation of bioprinted constructs remains limited, largely due to the insufficient biological functionality of currently available bioinks.[4,6]

Bioinks play a pivotal role in determining the success of bioprinting processes, as they must simultaneously satisfy requirements for printability, mechanical stability, and biocompatibility.[7] Among various candidates, hydrogel-based bioinks—such as gelatin methacryloyl (GelMA), alginate, collagen, and their composites—have been extensively investigated due to their high water content and resemblance to the extracellular matrix.[6,8-10] These materials provide a supportive microenvironment for cell encapsulation and proliferation, and their physicochemical properties can be tuned through crosslinking mechanisms and compositional modifications. However, most conventional bioinks are primarily designed as passive structural matrices and lack intrinsic biological signaling capabilities.[4,6,11] As a result, they often rely on soluble growth factors or cytokines to enhance cellular responses.

Nevertheless, the use of growth factors in biofabrication is associated with several critical limitations. Growth factors typically exhibit short half-lives, rapid degradation, and limited stability under physiological conditions, which restrict their long-term efficacy.[11,12] In addition, their uncontrolled release can lead to off-target effects and potential safety concerns, while large-scale production remains costly and challenging.[12,13] These drawbacks highlight the need for alternative bioactive components that provide sustained, localized, and physiologically relevant signaling within bioprinted constructs. Compared with growth factors, exosomes offer a more stable and biologically complex signaling platform by delivering proteins, lipids, and nucleic acids in a protected vesicular form.[14,15] However, exosome-based systems still face important challenges, including source heterogeneity, rapid in vivo clearance, and the lack of standardized characterization and delivery frameworks. These limitations must be addressed before exosomes can be reliably integrated into bioink systems.[16-19]

In this context, exosomes and other extracellular vesicles have emerged as promising candidates for next-generation bioinks. Exosomes are nanoscale lipid bilayer vesicles (typically 30–150 nm in diameter) secreted by various cell types and serve as key mediators of intercellular communication by transporting proteins, lipids, and nucleic acids.[14,20,21] Accumulating evidence indicates that exosomes play critical roles in regulating multiple biological processes, including angiogenesis, immune modulation, and tissue regeneration.[20-22] Compared with traditional cell-based therapies, exosome-based approaches offer several advantages, such as reduced immunogenicity, lower risk of tumorigenicity, improved storage stability, and easier handling and standardization.[23-25] Importantly, exosomes can recapitulate many of the therapeutic effects of their parent cells while avoiding the complexities associated with live cell transplantation.

Despite their therapeutic potential, the direct administration of exosomes is often limited by rapid clearance, poor retention at target sites, and insufficient control over their spatial and temporal distribution in vivo.[17,26,27] These challenges have prompted increasing interest in the development of biomaterial-based delivery systems for exosomes. In particular, hydrogel-based scaffolds have been widely explored as carriers to encapsulate and protect exosomes, enabling sustained release and improved localization within target tissues.[8,17,28] Building upon these advances, the integration of exosomes into bioinks represents a logical and promising extension of current strategies, allowing for the fabrication of structurally defined, bioactive constructs with enhanced regenerative potential.

Recent studies have demonstrated that exosome-integrated bioinks can significantly improve the biological performance of bioprinted constructs. For example, exosome-loaded hydrogels have been shown to promote

angiogenesis and accelerate wound healing, enhance osteogenic differentiation in bone regeneration, and support neural repair in injury models.[6,17,29] These findings suggest that combining the structural precision of 3D bioprinting with the biological functionality of exosomes can lead to multifunctional scaffolds capable of actively modulating the cellular microenvironment. However, despite these encouraging results, the field remains in its early stages, and several critical challenges need to be addressed. Importantly, many current studies still evaluate exosome-integrated bioinks primarily through short-term regenerative outcomes, while insufficient attention is paid to mechanistic reproducibility, manufacturing scalability, and long-term translational feasibility. In many cases, improved tissue repair is reported without clearly distinguishing whether the observed therapeutic effects arise predominantly from exosome cargo activity, matrix-mediated microenvironmental modulation, altered immune responses, or synergistic interactions among these components. This ambiguity complicates cross-study comparison and limits the establishment of predictive design principles.

Moreover, the field continues to face a broader conceptual challenge: exosome-integrated bioinks are frequently presented as universally bioactive platforms, despite the likelihood that optimal vesicle source, matrix composition, release kinetics, and fabrication strategy may differ substantially across tissue types and pathological conditions. As a result, future progress will likely depend less on developing generalized "all-purpose" exosome bioinks and more on establishing tissue-specific and disease-specific design frameworks that integrate biological mechanisms, biomaterial engineering, and translational constraints into a unified fabrication strategy.

One of the major challenges lies in the lack of standardization across studies. Variations in exosome sources, isolation methods, and characterization techniques contribute to significant heterogeneity, making it difficult to compare results and establish reproducible protocols.[14,16] In addition, current integration strategies often suffer from limited control over exosome loading efficiency, distribution, and release kinetics within bioinks. Many systems exhibit an initial burst release, which may compromise long-term therapeutic efficacy.[6] Furthermore, the effects of bioprinting processes—such as shear stress during extrusion and crosslinking conditions—on exosome stability and functionality remain poorly understood.

Another critical limitation is the lack of standardized criteria for evaluating the performance of exosome-integrated bioinks. While various studies report improvements in cell proliferation, differentiation, or tissue regeneration, differences in experimental design, model systems, and outcome measurements hinder direct comparison and systematic analysis. This underscores the need for more standardized and quantitative approaches to evaluate both the physicochemical properties and biological performance of these systems.

Given these challenges, a comprehensive and critical understanding of current design strategies and applications is essential to guide future developments in this field. In this review, we provide a systematic overview of exosome-integrated bioinks for 3D bioprinting. We first summarize the fundamental properties and biological functions of exosomes in regenerative medicine. We then discuss commonly used bioink materials and their limitations, followed by a detailed analysis of current strategies for integrating exosomes into bioinks, including encapsulation, surface immobilization, and hybrid approaches. Furthermore, we highlight representative biomedical applications and examine key challenges related to standardization, scalability, and functional performance. Finally, we discuss future perspectives toward the development of advanced, multifunctional bioinks capable of achieving precise spatial and temporal control of biological signaling in next-generation bioprinting systems.

2. Exosomes in regenerative medicine

2.1. Biological characteristics of exosomes

Most cell types secrete nanoscale vesicles into the extracellular environment to mediate intercellular communication. Among extracellular vesicles, exosomes are typically 30–150 nm in diameter and originate from the multivesicular body of the late endosomal compartment (Figure 1A).[14,21,30,31] In the endosomal lumen, inward budding leads to the formation of intraluminal vesicles, which are released into the extracellular environment when the multivesicular body fuses with the plasma membrane to form an exosome.[14,21,31] Of particular interest is the vast array of proteins, lipids, and RNAs that are secreted and included in the exosome payload. Many of the bioactive species are stable and reflect the physiological state of the parent cell, including messenger RNAs (mRNAs), many non-coding RNAs, and a wide variety of microRNAs.[20,21,30,31]

Exosomes interact with recipient cells primarily through membrane fusion, receptor–ligand interactions, and endocytosis, thereby delivering functional cargo that alters cell signaling (Figure 1B).[20-22,32] The lipid bilayer that forms the exosome membrane encapsulates proteins and genetic material, protecting them from enzymatic degradation. Importantly, the exosome composition varies depending on the originating cell type and the environment in which

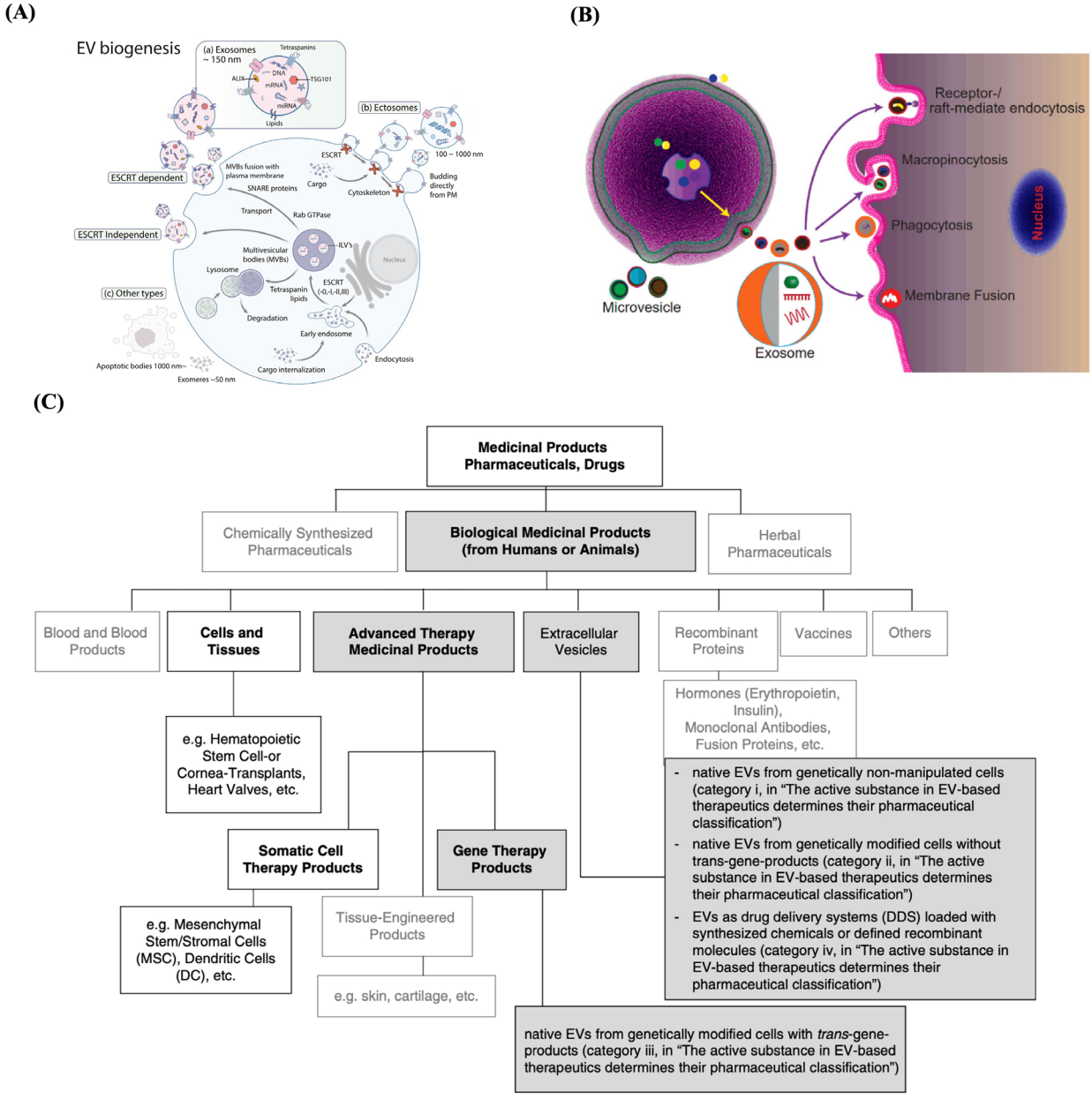

Figure 1. Overview of extracellular vesicle (EV) biogenesis, functional cargo transfer, and pharmaceutical classification. (A) Schematic illustration of EV biogenesis. Microvesicles are generated by outward budding of the plasma membrane, whereas exosomes originate from the endosomal pathway through the formation of early endosomes and multivesicular endosomes (MVEs), followed by the release of intraluminal vesicles into the extracellular space. Some MVEs are also directed to lysosomes for degradation. Modified from Yu *et al.*[31] (B) Representative strategies for evaluating EV-mediated functional cargo transfer to recipient cells, including fluorescence-tagged messenger RNA (mRNA), luciferase reporter systems, and Cre recombinase-based reporter assays. These approaches are used to monitor the delivery of EV cargo and its functional activity in recipient cells. Modified from Liu *et al.*[32] (C) Classification framework of EVs within medicinal products. EV-based therapeutics may be categorized according to their active substance, including native EVs from genetically non-manipulated cells, native EVs from genetically modified cells, and EVs used as drug delivery systems loaded with synthetic chemicals or recombinant molecules. Modified from Lener *et al.*[25]

they are generated, thereby ensuring a vast repertoire of functions. However, this variability hampers efforts to standardize and reproducibly harness their potential for therapeutic applications.[20,30,32]

2.2. Functional roles in tissue regeneration

Exosomes play multifaceted roles in tissue regeneration through dynamic interactions within the regenerative microenvironment.[22,23,33] They are widely recognized for their pro-angiogenic properties, contributing to the formation of new blood vessels—an essential process for effective tissue repair. In particular, exosomes derived from mesenchymal stem cells and other progenitor cells have been shown to enhance endothelial cell proliferation, migration, and tubulogenesis, thereby facilitating the development of functional vascular networks that support regeneration.[15,22,34]

Beyond their role in angiogenesis, exosomes also exert important immunomodulatory effects. They can influence immune cell behavior by promoting macrophage polarization toward the anti-inflammatory M2 phenotype, which helps suppress pro-inflammatory (M1) responses and creates a more favorable environment for tissue repair.[23,34] This ability to regulate immune responses is a key factor in successful regeneration.

In addition to their role as carriers of bioactive molecules, exosomes are involved in regulating cellular differentiation and extracellular matrix remodeling during tissue regeneration.[22,33] For example, in bone tissue, exosomes can promote osteogenic differentiation of stem cells, while in skin repair, they enhance fibroblast proliferation and collagen deposition. In the nervous system, exosomes have been reported to support neuronal survival and stimulate axonal growth, further highlighting their broad regenerative potential.[33,34]

2.3. Advantages over cell-based therapies

Compared with conventional cell-based therapies, exosome-based approaches offer several advantages for regenerative medicine. For example, they have lower immunogenicity as compared to native cells, are devoid of live cells, and express lower levels of major histocompatibility complex molecules, thus providing flexibility for usage in an allogeneic manner, reducing the risk of rejection.[24,25,35]

Exosomes offer a number of advantages in terms of safety. First, exosomes cannot proliferate, form tumors, or generate ectopic tissues, which are theoretical risks associated with allogeneic stem cell therapies. Secondly, exosomes are extremely small and therefore have the potential for rapid distribution and clearance from the body, in the worst-case scenario of adverse reaction. Thirdly, for therapeutic purposes, exosomes show advantages of stability and manipulability over "live" cells. Stored under appropriate conditions, they retain function long term and are much easier to manipulate, ship, and store than somatic cells. Many of the effects that would be provided by the parent cells can be delivered directly to the site of requirement by the bioactive cargo within the exosomes. In this way, exosomes offer a truly cell-free alternative. Overall, the various features described above make exosomes promising therapeutic tools for regenerative medicine, especially when combined with biomaterial scaffolds for efficient delivery (Figure 1C).[24,25,35]

2.4. Limitations and delivery challenges

Although naturally derived exosomes have attracted considerable attention as therapeutic agents, several inherent limitations still hinder their direct clinical translation. One of the primary challenges is their rapid clearance following systemic administration. Once introduced into the bloodstream, exosomes are quickly recognized and internalized by the mononuclear phagocyte system, leading to preferential accumulation in organs such as the liver and spleen. As a result, their in vivo half-life is markedly short, which restricts their retention at target sites and limits their overall therapeutic efficacy.[26,27,36] These pharmacokinetic constraints underscore the need for strategies that can enhance their localization and prolong their biological activity.

Beyond pharmacokinetics, exosome heterogeneity represents another major obstacle. Variations in donor cell type, culture conditions, and isolation protocols can produce exosome populations with markedly different compositions and functional properties. This variability poses significant challenges for reproducibility, especially in cross-laboratory comparisons and large-scale studies. To address this issue, the development of standardized protocols for exosome isolation, characterization, and quantification is essential. Emerging technologies, including ultrafiltration-based methods and microfluidic platforms, are being explored to improve consistency and analytical precision.[36,37]

Another critical limitation lies in the difficulty of achieving controlled delivery. Due to their nanoscale size, exosomes readily diffuse and become diluted after administration, particularly when delivered through conventional routes such as intravenous injection. This rapid dispersion makes it challenging to regulate their spatial distribution and temporal release in vivo, thereby reducing targeting efficiency and therapeutic impact.[26,27]

In response to these challenges, increasing attention has been directed toward integrating exosomes with biomaterial-based delivery systems. Platforms such as hydrogels and 3D-printed scaffolds offer a promising approach to improve exosome stability and enable localized, sustained release. By incorporating exosomes into bioinks for 3D bioprinting, it becomes possible to create structured, site-specific delivery systems that enhance retention and bioactivity.[1,2,37]

3. Bioinks for 3D bioprinting

Bioinks are central to 3D bioprinting because they serve as multifunctional materials that provide structural support while also regulating cellular behavior and tissue development. The design of bioinks requires the integration of rheological properties, crosslinking mechanisms, and biological functionality to achieve both high printing fidelity and effective tissue regeneration. Figure 2 summarizes representative applications of 3D bioprinting in tissue engineering. Tissue-specific bioinks have been used to fabricate constructs for osteochondral, fibrous, and skin tissue regeneration, while cell-laden bioprinted scaffolds can also be primed in vitro to enhance their regenerative potential for cartilage and bone repair in vivo.[4,5,38] In recent years, the field has shifted from conventional material-based classifications toward a function-driven design paradigm in which bioinks are engineered to interact actively with the cellular microenvironment and respond to biological stimuli.[3,4,38]

Traditionally, 3D bioprinting has focused on cell-laden bioinks, in which living cells are encapsulated within hydrogels to contribute directly to tissue formation. These systems aim to recapitulate native tissue architecture by combining biomaterials with stem cells or primary cells, enabling cell proliferation, differentiation, and extracellular matrix deposition. However, several limitations remain, including reduced cell viability due to shear stress during printing, heterogeneous cell distribution, limited long-term control over cell behavior, and challenges related to immune compatibility and regulatory approval.[39] Furthermore, issues of scalability and storage significantly hinder clinical translation.[7,39]

To address these limitations, increasing attention has been directed toward cell-free biofabrication strategies, in which bioactive components such as growth factors or extracellular vesicles are incorporated into biomaterials to modulate cellular responses without requiring live-cell transplantation. This shift highlights the importance of developing advanced bioink systems that not only support printing but also provide biologically instructive cues.[17,38]

3.1. Rheological and printability-driven bioinks

Rheological properties are fundamental determinants of bioink performance and directly influence printability, resolution, and cell viability. Among these, shear-thinning behavior is particularly critical in extrusion-based bioprinting. Shear-thinning bioinks exhibit reduced viscosity under applied shear stress during extrusion and rapidly recover their viscosity after deposition, thereby maintaining structural fidelity while minimizing mechanical damage to encapsulated cells.[4,6] In addition to shear-thinning, yield stress and viscoelasticity are essential for stabilizing printed structures. Bioinks with appropriate yield stress can maintain their geometry without collapse, while viscoelastic behavior affects filament formation and interlayer adhesion.[6,40] However, optimizing these properties often involves trade-offs. This trade-off is particularly important in exosome-integrated systems because rheological optimization for print fidelity does not necessarily align with optimal preservation of vesicle functionality. For example, increasing polymer concentration or viscosity may improve structural resolution and retention, yet simultaneously reduce matrix permeability, hinder exosome diffusion, and limit cell–vesicle interactions after printing. Conversely, highly permissive hydrogels may facilitate biological exchange but compromise construct stability and long-term spatial control. Therefore, maximizing a single material parameter is unlikely to produce biologically optimal constructs. Instead, future exosome–bioink design may require multi-objective optimization strategies that balance printability, vesicle preservation, controlled release, and cellular accessibility simultaneously.

Recent strategies have incorporated nanocomposite systems, such as nanoclay or cellulose nanofibers, to improve rheological behavior and mechanical stability.[6,41] While these approaches enhance printability, they do not inherently provide biological functionality, emphasizing the need for integrating bioactive components into bioink systems.

3.2. Crosslinking mechanism-based bioinks

Crosslinking mechanisms are critical for stabilizing printed constructs and determining mechanical properties, degradation rates, and cellular responses. Among these, photo-crosslinkable bioinks such as GelMA and polyethylene glycol diacrylate (PEGDA) are widely used due to their rapid gelation and high spatial resolution.[8] These systems enable precise tuning of mechanical properties through light exposure; however, potential phototoxicity and UV-induced cell damage remain concerns.

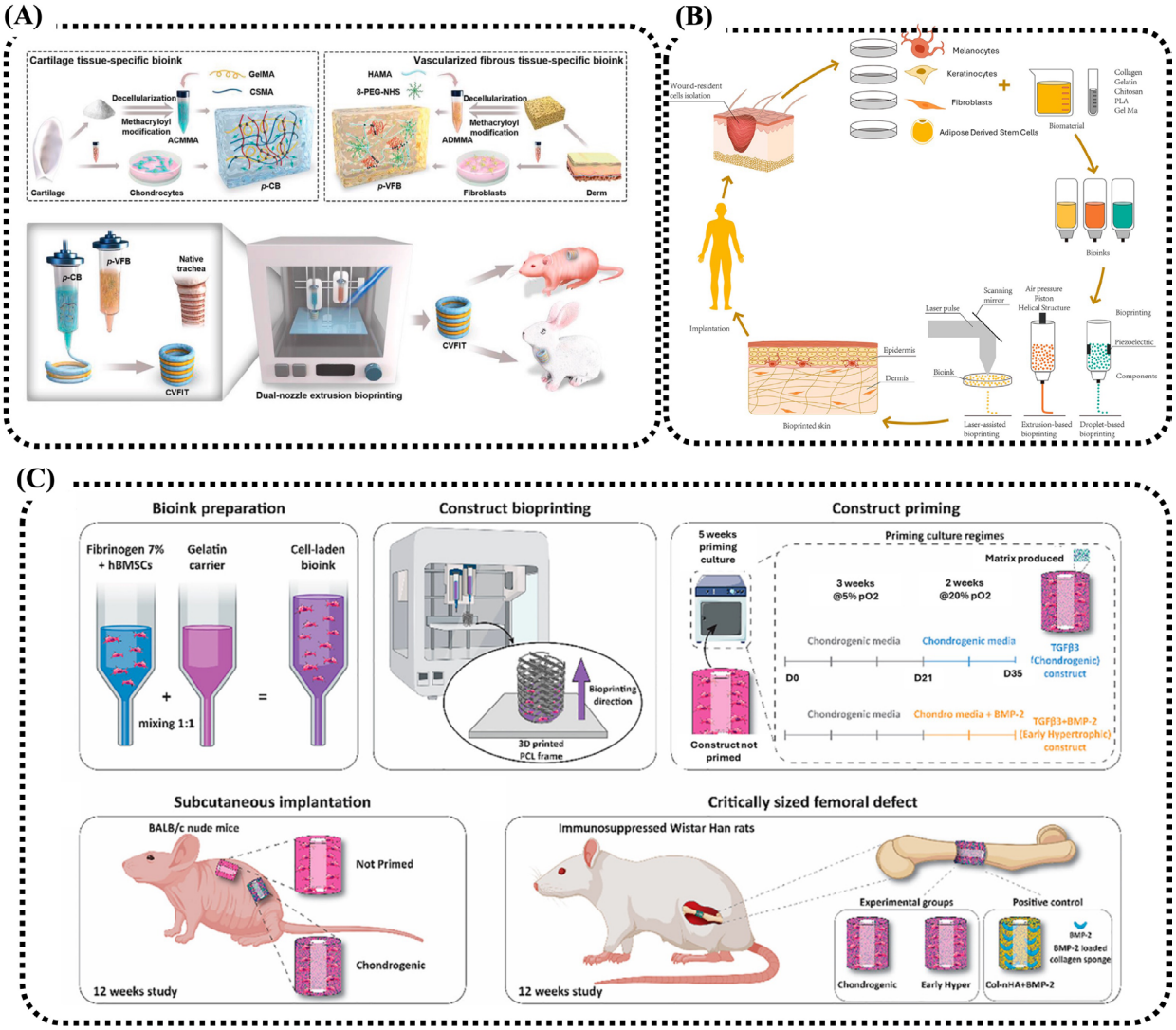

Figure 2. Representative strategies for 3D bioprinting in tissue engineering and regenerative medicine. (A) Schematic illustration of dual-nozzle extrusion bioprinting using tissue-specific bioinks for osteochondral and vascularized fibrous tissue regeneration. Decellularized extracellular matrix (dECM)-based bioinks are chemically modified and combined with appropriate cell types to fabricate composite constructs, which are subsequently evaluated in vivo. (B) Workflow for bioprinted skin fabrication. Skin-resident cells, including melanocytes, keratinocytes, fibroblasts, and adipose-derived stem cells, are combined with biomaterials to generate bioinks, which are then processed through different bioprinting modalities, such as laser-assisted, extrusion-based, and droplet-based bioprinting, for skin reconstruction and implantation. (C) Strategy for fabrication and priming of cell-laden bioprinted constructs for cartilage and bone repair. Bioinks containing fibrinogen, gelatin carrier, and human bone marrow mesenchymal stem cells are printed into a 3D frame, followed by in vitro priming under defined culture conditions to induce chondrogenic or hypertrophic differentiation. The resulting constructs are then assessed in subcutaneous implantation and in a critically sized femoral defect model. Reprinted from Mirsky *et al.*[38]

Ionic crosslinking systems, exemplified by alginate-based bioinks, offer rapid gelation through interactions with divalent cations such as calcium ions.[42] These systems provide excellent printability and structural integrity but lack intrinsic cell adhesion sites, limiting biological interactions unless modified.

Thermally responsive bioinks, including gelatin and poly(N-isopropylacrylamide (PNIPAM)-based systems, undergo temperature-dependent sol–gel transitions, enabling mild cell encapsulation. However, their mechanical stability at physiological temperatures is often insufficient, requiring secondary crosslinking strategies.[43] Overall, while crosslinking strategies have significantly improved structural stability, achieving a balance between

cytocompatibility, mechanical strength, and biological functionality remains a major challenge.

3.3. Stimuli-responsive bioinks

To better mimic the dynamic behavior of native tissues, stimuli-responsive bioinks have been developed to respond to environmental cues such as pH, temperature, and enzymatic activity. These systems represent a shift toward adaptive biomaterials that can modulate their properties in response to biological signals.[44-46] For example, enzyme-responsive bioinks, particularly those sensitive to matrix metalloproteinases (MMPs), allow cell-mediated degradation and remodeling, facilitating tissue regeneration. Similarly, pH-responsive systems enable controlled release of therapeutic agents in acidic environments, such as tumors or inflamed tissues. Temperature-responsive materials provide reversible phase transitions, enabling dynamic control of scaffold stiffness and permeability. Despite their advantages, these systems often require complex material design and precise control of responsiveness to ensure predictable behavior in vivo. Nonetheless, stimuli-responsive bioinks provide an important platform for integrating advanced bioactive components.

3.4. Dynamic and 4D bioinks

The concept of 4D bioprinting introduces time-dependent functionality into 3D bioprinting, allowing constructs to undergo controlled transformations after fabrication.[46,47] Dynamic bioinks are designed to respond to environmental stimuli, leading to changes in shape, mechanical properties, or biological activity over time. Examples include shape-memory polymers and swelling-based hydrogels that enable self-folding or morphing structures. These systems are particularly promising for applications such as vascular and organ modeling, where dynamic structural adaptation is required. However, 4D bioinks remain in early stages of development. Challenges include controlling transformation kinetics, ensuring compatibility with living cells, and integrating biological signaling with dynamic material behavior. Further research is needed to translate these systems into practical biomedical applications.

3.5. Bioactive and cell-instructive bioinks

Despite advances in their mechanical and responsive properties, conventional bioinks remain limited by their lack of intrinsic biological signaling. Traditional strategies have often relied on the incorporation of soluble growth factors to regulate cell behavior; however, these molecules are constrained by rapid degradation, short half-lives, and limited control over release kinetics.[11] To overcome these limitations, a new generation of bioactive and cell-instructive bioinks has been developed to integrate biologically functional cues directly into material systems.[48] These approaches include peptide-functionalized hydrogels, extracellular matrix-derived bioinks, synthetic nucleic acid-based systems, and extracellular vesicle-based bioinks.[15,17]

Among these, peptide-functionalized hydrogels offer a highly defined and modular strategy for directing cell behavior. By presenting specific adhesive or signaling motifs, these systems can regulate receptor-mediated interactions with relatively high molecular precision. This design flexibility is advantageous when a narrowly defined biological response is desired, such as enhanced cell adhesion, lineage bias, or receptor-specific signaling. However, the bioactivity of peptide-functionalized systems is often limited to a relatively small number of pathways, which may restrict their ability to reproduce the broader signaling complexity of native regenerative microenvironments.

A second emerging approach involves synthetic mRNA-loaded hydrogels or related nucleic acid-based biomaterials that can induce transient intracellular expression of therapeutic proteins after delivery. These systems are attractive because they allow local and programmable production of growth factors and other functional molecules directly within the engineered construct. In principle, this offers greater temporal flexibility than direct protein delivery and may reduce the instability associated with exogenous growth factor supplementation. Nevertheless, mRNA-based systems still depend on efficient intracellular delivery, translation efficiency, and protection against degradation, and their biological output is typically focused on selected encoded factors rather than broader endogenous signaling networks.

In contrast, exosome-integrated bioinks provide a distinct instructive strategy by delivering a multicomponent and naturally protected signaling package that includes proteins, lipids, mRNAs, and regulatory non-coding RNAs.[14,16,20,21,36,49] Rather than activating only one or a few predefined pathways, exosomes can modulate multiple regenerative processes simultaneously, including angiogenesis, immune regulation, extracellular matrix remodeling, and lineage-specific differentiation. This broader signaling repertoire is a major conceptual advantage in tissue engineering, where regeneration often depends on coordinated and dynamic interactions among multiple cell types and biological pathways. However, this conceptual advantage should also be interpreted cautiously. Although exosomes are frequently described as inherently biomimetic and multifunctional therapeutic systems, increasing signaling complexity does not

automatically guarantee superior regenerative outcomes. In some contexts, highly heterogeneous vesicle cargo may introduce unpredictable biological variability, off-target signaling, or inconsistent therapeutic responses across batches and disease models. Therefore, greater biological complexity may improve physiological relevance, but it may also reduce controllability and standardization. This tension between biomimicry and engineering precision represents one of the central unresolved challenges in exosome-integrated biofabrication.

Compared with peptide- or mRNA-based systems, exosome-integrated bioinks may therefore provide a more physiologically relevant and multifunctional form of biological instruction. Their vesicular structure also offers intrinsic protection of cargo, which may improve local retention of bioactivity relative to freely soluble molecules. At the same time, exosome-based systems remain more difficult to standardize because their composition depends strongly on donor cell source, culture conditions, and isolation methods.[16-19] In this sense, exosome-integrated bioinks occupy a unique position within the broader instructive biomaterials landscape: they offer broader and more biomimetic signaling than many alternative systems, but at the cost of increased complexity in characterization, reproducibility, and translational control.

Overall, the evolution of bioink design is moving beyond passive structural support toward active and programmable instructive biomaterials. During this transition, exosome-integrated bioinks stand out not only for their bioactivity but also for combining structural biofabrication with multiscale biological communication. A comparative summary of these advanced bioink systems, including peptide-functionalized, nucleic acid-based, and exosome-integrated platforms, is provided in Table 1.

Table 1. Comparative overview of advanced bioink systems based on functional design principles

Bioink category	Representative materials	Representative strategy	Main bioactive mechanism	Key advantages	Main limitations	Relevance to exosome-integrated bioinks	Refs.
Rheology- and printability-driven bioinks	Alginate, gelatin, nanoclay, cellulose nanofibers	Shear-thinning hydrogels, nanocomposite inks	Primarily structural support and print fidelity	Good printability, shape fidelity, tunable mechanics	Limited intrinsic biological signaling	Provide the physical platform, but usually require added instructional components	6,7
Crosslinking mechanism-based bioinks	GelMA, PEGDA, alginate, gelatin, PNIPAM	Photo-crosslinkable, ionic, or thermal systems	Structural stabilization through network formation	Rapid gelation, tunable stiffness, adaptable fabrication	Crosslinking conditions may affect cytocompatibility and cargo stability	Important for preserving exosome integrity during printing and post-print maturation	8,42,43
Stimuli-responsive bioinks	pH-responsive polymers, enzyme-sensitive hydrogels, thermoresponsive polymers	pH-, enzyme-, or temperature-responsive systems	Triggered material response and controlled release	Dynamic behavior, on-demand release, adaptive function	Complex design and variable in vivo predictability	Highly relevant for spatiotemporal control of exosome release	44,45
Dynamic/4D bioinks	Shape-memory polymers, swelling hydrogels, responsive composite matrices	Shape-morphing or time-responsive constructs	Time-dependent structural or functional transformation	Mimic tissue dynamics, enable adaptive constructs	Limited maturity, difficult transformation control	Could support staged or region-specific exosome presentation	46,47

(*Cont'd...*)

Table 1. (***Continued***)

Bioink category	Representative materials	Representative strategy	Main bioactive mechanism	Key advantages	Main limitations	Relevance to exosome-integrated bioinks	Refs.
Peptide-functionalized bioinks	RGD-modified hydrogels, IKVAV-functionalized matrices, peptide-grafted PEG hydrogels	Motif-functionalized hydrogels	Receptor-specific signaling and cell adhesion control	High molecular precision, modularity, tunable bioactivity	Often limited to selected pathways; narrower biological output	Useful benchmark systems, but generally lack the multicomponent signaling complexity of exosomes	[50,51]
Synthetic nucleic acid-based bioinks	mRNA-loaded hydrogels, RNA–polymer complexes, nucleic acid-functionalized biomaterials	RNA- or mRNA-loaded hydrogels and related biomaterials	Local intracellular expression of encoded therapeutic factors	Programmable, transient expression, temporal and spatial control	Delivery efficiency, degradation risk, pathway-specific output	Offer potent instructive signaling but are typically less biomimetic than vesicle-mediated cargo delivery	[52,53]
ECM-derived bioinks	dECM, tissue-specific ECM hydrogels	Tissue-specific decellularized ECM systems	Native-like matrix cues and tissue-mimetic interactions	Good biological relevance, support for cell–matrix communication	Batch variability, weaker reproducibility, variable mechanics	Complementary to exosome systems and often useful as supportive matrices	[4,14,16,49]
Exosome-integrated bioinks	GelMA, alginate, dECM, PEG-based hybrid hydrogels, exosome-loaded composite matrices	Encapsulation, surface immobilization, or hybrid exosome incorporation	Multicomponent vesicular signaling via proteins, lipids, mRNAs, and regulatory RNAs	Broad and physiologically relevant bioactivity, protected cargo, simultaneous modulation of multiple regenerative pathways	Source heterogeneity, batch variability, difficult standardization, complex quality control	Represent a uniquely multifunctional instructive platform for regenerative biofabrication	[14,16,18,19]

Abbreviations: dECM: Decellularized extracellular matrix; ECM: Extracellular matrix; mRNA: Messenger RNA; PEG: Polyethylene glycol; PEGDA: Poly(ethylene glycol) diacrylate; PNIPAM: Poly(N-isopropylacrylamide).

4. Design strategies for exosome-integrated bioinks in 3D bioprinting

From a systems-engineering perspective, the development of exosome-integrated bioinks should not be interpreted as a simple combination of hydrogels and extracellular vesicles, but rather as a dynamically coupled therapeutic system in which matrix architecture, vesicle presentation, transport behavior, degradation kinetics, and cellular responses continuously influence one another across multiple spatial and temporal scales. Recent reviews consistently indicate that the performance of these systems depends on how effectively researchers coordinate several interrelated variables, including matrix composition, vesicle incorporation mode, release behavior, preservation of vesicle activity, and translational manufacturability.[3-5,38] More importantly, exosome-integrated bioinks should not be defined merely by the incorporation of extracellular vesicles into printable hydrogels, but by whether the overall biofabrication system can actively regulate vesicle retention, presentation, accessibility, and post-print biological function.

This strategic perspective is especially important because exosomes differ fundamentally from conventional soluble additives such as single growth factors. Their therapeutic value lies in their ability to deliver a multicomponent signaling package, including proteins, lipids, mRNAs, and regulatory RNAs, which can simultaneously influence multiple biological pathways.[14,16,20] However, this same complexity also makes their integration more challenging. Importantly, these variables cannot be optimized independently because changes in one parameter frequently alter the behavior of the overall fabrication system. For example, improving matrix stiffness may enhance structural fidelity and vesicle

retention while simultaneously reducing permeability, cellular infiltration, and exosome accessibility. Likewise, stronger vesicle immobilization may prolong local retention while limiting uptake efficiency and downstream biological interaction. A matrix formulation optimized for print fidelity may not adequately preserve vesicle integrity; systems with high loading efficiency may still exhibit excessive burst release; and a delivery system that improves retention may inadvertently reduce vesicle accessibility to recipient cells. Therefore, successful exosome–bioink engineering requires systems-level design logic capable of balancing structural, physicochemical, transport-related, and biological requirements across the entire fabrication workflow (Figure 3A).[3-5,14,16,20,38]

Accordingly, exosome-integrated bioinks should be designed as coordinated therapeutic systems in which matrix engineering, vesicle incorporation, release behavior, stability preservation, and translational standardization are treated as interdependent design dimensions rather than isolated formulation variables. From this perspective, the design of exosome-integrated bioinks can be organized into five major strategic dimensions: matrix design, exosome incorporation strategy, release-control strategy, stability-preservation strategy, and translational standardization strategy.

4.1. Matrix design strategies for exosome-compatible bioinks

The first design strategy concerns the engineering of an exosome-compatible matrix. In conventional bioprinting, the matrix is often optimized primarily for rheology, print fidelity, and post-print mechanical stability. In exosome-integrated systems, however, the matrix plays a broader role: it functions simultaneously as a printable medium, a protective carrier, and a regulator of vesicle diffusion and cell–vesicle interaction.[4,5,7] This means that matrix design should not be treated as a neutral background variable, but as a central determinant of therapeutic performance.

Recent literature identifies hydrogel-based matrices as the dominant platform because they offer high water content, tunable crosslinking, and relatively mild processing conditions.[3-5,7,8,38] Among these, GelMA, alginate, collagen, decellularized extracellular matrix, and polyethylene glycol (PEG)-based hybrid systems are the most commonly discussed exosome-compatible materials.[7,8,42] However, no currently available matrix system fully satisfies the competing requirements of printability, vesicle preservation, biological accessibility, and long-term structural stability. GelMA provides strong geometric control and adjustable mechanics, making it attractive for high-resolution bioprinting, but its photo-crosslinking conditions may threaten vesicle integrity if not carefully optimized. Alginate supports gentle ionic gelation and good printability, but its low intrinsic bioactivity and limited cell adhesion often require blending with more interactive biomaterials. Decellularized extracellular matrix-based systems offer tissue-specific bio cues and may better support native-like vesicle presentation, but they frequently suffer from compositional variability and weaker reproducibility.[8,42,54]

A key trend in recent reviews is the shift from viewing the matrix as a passive storage phase to an interactive exosome-regulating environment.[17,20,36] This is an important conceptual advance. The matrix can influence how long exosomes remain retained, whether they aggregate, how rapidly they are released, and how readily surrounding cells can access them. Consequently, matrix selection should be guided not only by printability but also by vesicle compatibility, permeability, degradation profile, and tissue-mimetic signaling. From this standpoint, matrix design becomes the foundational strategy that governs all subsequent aspects of exosome integration. From a systems-engineering perspective, the matrix should therefore not be viewed merely as a carrier phase, but rather as a regulatory interface that dynamically mediates interactions among exosomes, infiltrating cells, extracellular fluid transport, and mechanical cues. This conceptual shift is important because it reframes matrix design from passive material selection toward active control of biological communication within the printed construct.

4.2. Exosome incorporation strategies

The second design dimension concerns how exosomes are introduced into the bioink system. Current approaches can be broadly classified into encapsulation-based, surface-associated, and hybrid integration strategies.[3-6,14,17,36,38] Although this classification appears simple, the choice of incorporation mode has major consequences for loading homogeneity, bioactivity retention, release kinetics, and spatial localization.

Encapsulation remains the most widely used strategy because it is experimentally straightforward and compatible with a wide range of hydrogel systems.[4,5] In this approach, exosomes are mixed into the prepolymer solution or hydrogel precursor before printing, resulting in relatively homogeneous distribution throughout the printed construct. This strategy is easy to implement and often preserves vesicle integrity under mild mixing conditions. However, recent reviews repeatedly emphasize that encapsulation is often accompanied by weak vesicle–matrix interactions, which in turn promote rapid early

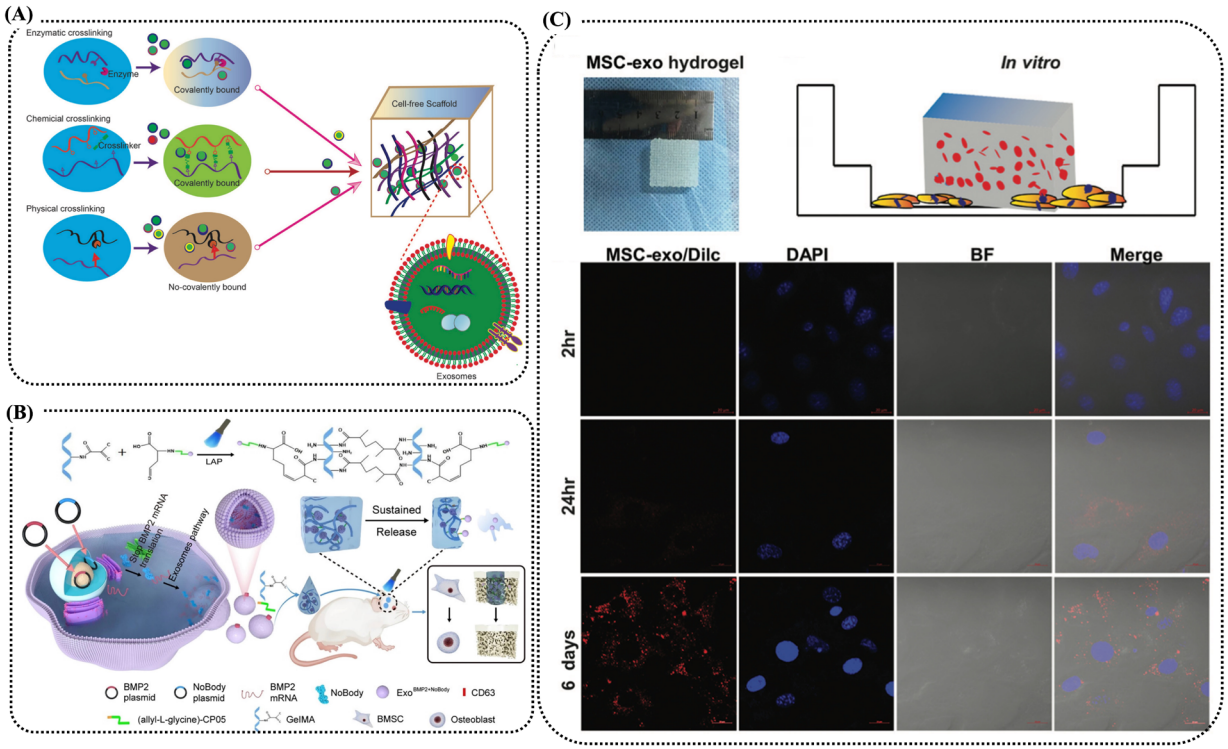

Figure 3. Representative strategies for incorporating exosomes into hydrogel-based delivery systems for regenerative applications. (A) Schematic illustration of exosome immobilization within cell-free scaffolds through enzymatic, chemical, or physical crosslinking. Depending on the loading strategy, exosomes can be incorporated through covalent or non-covalent interactions, enabling their retention within the scaffold matrix. Modified from Huang *et al.*[54] (B) Engineered exosome-loaded hydrogel platform for sustained delivery of therapeutic cargo. Exosomes derived from genetically modified donor cells carrying osteogenic signals are incorporated into a gelatin methacryloyl (GelMA)-based hydrogel, allowing prolonged release and enhanced bone regeneration in vivo. Modified from Hu *et al.*[55] (C) Characterization of a mesenchymal stem cell (MSC)-exosome hydrogel and its in vitro release and uptake behavior. The upper panels show the gross appearance of the MSC-exosome hydrogel and a schematic of exosome release in vitro. The lower panels present fluorescence images of Dil-labeled MSC-derived exosomes released from the hydrogel and taken up by recipient cells over time, at 2 h, 24 h, and 6 days. Modified from Hu *et al.*[55]

diffusion and a pronounced burst-release profile.[3,4,17,38] Thus, although encapsulation simplifies fabrication, it may compromise temporal control.

Surface-associated strategies attempt to solve this problem by attaching exosomes to scaffold surfaces through electrostatic adsorption, affinity interactions, or chemical conjugation.[6,14,36] The key conceptual advantage of this approach is that vesicles are no longer merely trapped within the bulk hydrogel volume; instead, they can be presented at specific locations and potentially patterned across the construct. This is particularly attractive for layered tissues or interfaces where signaling gradients matter. However, stronger binding is not always beneficial. If the interaction masks membrane proteins or alters vesicle accessibility, uptake by recipient cells may be reduced. Therefore, incorporation design must balance retention against biological availability.

Hybrid strategies combine these two approaches, typically using a fraction of vesicles for immediate bulk presentation and another fraction for more persistent surface-anchored delivery.[3,4,6,14,38] A representative 2025 example is the study by Lou *et al.*[56], who developed a 3D-printed hydrogel scaffold incorporating exosomes derived from human skeletal stem cells identified by single-cell RNA sequencing for osteochondral regeneration. In this work, exosome source optimization, scaffold architecture, and matrix-based local delivery were integrated into a single biofabrication platform. The study showed that the exosome-loaded printed scaffold promoted both cartilage and subchondral bone regeneration more effectively than the comparison groups. These findings indicate that the exosome incorporation strategy should be considered together with vesicle source and scaffold function, rather than as an isolated formulation variable.[56] Importantly,

this example moves beyond simply mixing exosomes into a printable material and instead shows how hybrid design can coordinate biological specificity, structural support, and localized vesicle presentation within one engineered construct.

From a design perspective, this shift toward hybrid incorporation is highly significant. It suggests that future exosome-integrated bioinks should not be developed merely to maximize loading efficiency, but to deliberately regulate where exosomes are positioned, how long they are retained, and when they become biologically available. In this sense, hybrid incorporation strategies may represent an early transition from passive exosome encapsulation toward programmable control of vesicle presentation within tissue-specific regenerative microenvironments.

4.3. Release-control and spatiotemporal design strategies

A third major strategy is the control of exosome release behavior in space and time. This design dimension is central because extracellular vesicles are limited clinically by their rapid clearance and poor retention following direct administration.[20,21] Embedding them into bioinks is therefore not only a way to localize vesicles but also to reprogram their pharmacokinetics.

In early exosome-loaded hydrogels, release was mainly diffusion-governed, meaning vesicles escaped quickly through hydrated polymer networks (Figure 3B,C).[55] While this can provide an early burst of bioactivity, it often leads to inefficient utilization and poor long-term signaling.[4,5] Nevertheless, burst release should not always be interpreted as undesirable. In certain regenerative settings, including acute inflammation, ischemic injury, or early wound repair, an initial high-concentration exosome pulse may be beneficial for rapidly modulating immune responses and initiating tissue remodeling cascades. Consequently, the optimal release profile is likely to be application-dependent rather than universally sustained. This highlights the need for context-specific release engineering rather than assuming that prolonged release alone represents the ideal therapeutic strategy. More recent design strategies have shifted toward degradation-mediated and stimuli-responsive release systems.[17,21,45] In these systems, vesicle liberation depends on matrix breakdown, cell-secreted enzymes, pH changes, or other local signals.

One particularly promising future direction is the incorporation of MMP-sensitive linkers or degradable peptide motifs into exosome-containing hydrogels. This strategy is especially attractive because MMP activity is tightly associated with extracellular matrix remodeling, inflammation, angiogenesis, and wound repair. In principle, such systems could enable biologically coupled release, in which exosome liberation is triggered by cell-mediated remodeling activity rather than by passive diffusion alone. Under baseline conditions, exosomes would remain relatively retained within the hydrogel matrix; however, when infiltrating or activated cells secrete MMPs during tissue repair, cleavage of MMP-sensitive motifs would locally release vesicles into the surrounding microenvironment. This would create a more demand-driven release profile and potentially improve temporal coordination between vesicle availability and regenerative signaling requirements.[45,50,53]

The strategic significance of this shift is considerable. A diffusion-dominated system treats the matrix as a temporary container, whereas a stimuli-responsive system treats it as an active regulator of vesicle presentation. Recent reviews emphasize that advanced exosome bioprinting should move toward spatiotemporally controlled delivery, including multilayer constructs, gradient printing, and compartmentalized hydrogels.[17,36,45] Thus, a release-control strategy should be understood not simply as a formulation parameter but as a core design principle that determines whether exosomes act as a short-lived additive or as a sustained instructive signal within the printed construct.

4.4. Stability-preservation strategies during fabrication and storage

The fourth design strategy concerns the preservation of exosome stability and bioactivity during fabrication, crosslinking, and storage. This issue is particularly important because exosomes are structurally delicate lipid vesicles whose biological function depends on membrane integrity, accessible surface proteins, and preservation of internal cargo.[14-16]

Recent reviews underscore that exosome stability can be compromised at multiple stages: during pre-print mixing, during extrusion or droplet generation, during photo- or chemical crosslinking, and during post-print storage.[3-5,38] An additional, but closely related, consideration is the influence of the bioprinting modality on exosome preservation. Different 3D bioprinting techniques may expose vesicles to distinct physical stresses, thereby affecting membrane integrity, cargo stability, and downstream biological function. In extrusion-based bioprinting, exosomes may be subjected to prolonged shear stress, pressure gradients, and repeated deformation during passage through the nozzle, potentially increasing the risk of membrane disruption or aggregation. Inkjet-based bioprinting may reduce continuous shear exposure, but droplet generation can still impose transient mechanical stress and, in some systems, localized

thermal effects that may alter vesicle stability. Laser-assisted bioprinting avoids nozzle-associated clogging and offers high spatial precision, yet concerns remain regarding local energy exposure, process complexity, and the limited availability of direct evidence on exosome preservation under these conditions. From a translational manufacturing perspective, printing modality should not be regarded merely as a fabrication preference, but as a critical process variable capable of directly influencing vesicle integrity, cargo preservation, and downstream therapeutic function.[57-59] Accordingly, future studies should compare different printing modalities using standardized post-print characterization metrics, including particle size distribution, membrane-marker retention, cargo integrity, and functional bioassays, to determine which fabrication strategies are most compatible with preserving exosome activity. Importantly, preservation of particle size or canonical membrane markers alone should not be considered sufficient evidence of retained therapeutic functionality. Exosomes may maintain apparent structural integrity while experiencing substantial alterations in cargo composition, membrane accessibility, or downstream signaling capacity after fabrication. Therefore, future evaluation frameworks should incorporate functional bioassays that directly assess biological activity rather than relying exclusively on physicochemical characterization. Mechanical shear, localized thermal or energy exposure, and reactive species generated during crosslinking may all contribute to vesicle damage, cargo degradation, or loss of bioactivity, while storage conditions can further affect vesicle potency and reproducibility.[15,24]

This makes stability-preservation a true design axis rather than a secondary technical concern. Strategies proposed in recent literature include mild crosslinking conditions, reduced photoinitiator exposure, gentle extrusion settings, and protective hydrogel environments that limit direct vesicle exposure to harmful stresses.[4,5] More conceptually, some authors now argue that vesicle preservation should be considered upstream, beginning with donor-cell conditioning and isolation quality, rather than only at the printing stage.[20,36,60] This broader view is important because exosome potency can be affected throughout the entire production-to-print pipeline. Accordingly, a high-quality exosome–bioink design should include stability-oriented evaluation metrics such as particle size distribution, membrane-marker retention, post-print cargo integrity, and functional bioassays. Without such metrics, it remains difficult to distinguish whether poor performance results from biological inefficacy or from fabrication-induced damage.

4.5. Translational, manufacturing, and standardization strategies

The fifth design strategy is translational in nature: the system must be manufacturable, reproducible, and standardizable. This point is increasingly emphasized in recent reviews, which repeatedly identify the same bottlenecks: low exosome yield, heterogeneity across donor cells, variation in isolation methods, incomplete quality control, and poor comparability between studies.[3-5,38,60-62]

Recent literature therefore supports a quality-by-design framework in which exosome source selection, isolation method, characterization criteria, matrix formulation, and release testing are integrated into a reproducible workflow.[36,49,60,62] This is a major conceptual advance. Rather than treating exosomes as an experimental additive appended to printable gels, the field is moving toward treating the entire platform as a controlled therapeutic product.

Standardization should include not only Minimal Information for Studies of Extracellular Vesicles (MISEV)-compliant vesicle characterization, but also print-specific quality controls such as post-fabrication vesicle retention, functional recovery assays, and release benchmarking under biologically relevant conditions.[16,49,60] In addition, future work will likely require closer integration of predictive modeling, scalable manufacturing, and automated formulation optimization.[49,63] In this sense, translational design is not separate from scientific design; it is the framework that will determine whether exosome–bioink systems remain promising academic constructs or become clinically meaningful biofabrication platforms. Taken together, these five strategic dimensions indicate that exosome-integrated bioinks are evolving from passive biomaterial formulations toward programmable therapeutic systems capable of coordinating structural support, vesicle-mediated signaling, and adaptive regenerative function within complex tissue environments. A comparative summary of these strategic dimensions is provided in Table 2.

5. Biomedical applications of exosome-integrated bioinks

The biomedical value of exosome-integrated bioinks lies in their ability to combine architectural control from 3D bioprinting with multimodal biological signaling from extracellular vesicles. Recent application studies suggest that this platform is particularly well-suited for tissues in which regeneration depends on synchronized control of inflammation, angiogenesis, matrix remodeling, and

Table 2. Design strategies for exosome-integrated bioinks

Design dimension	Core strategy	Representative approach	Main benefit	Key limitation	Refs.
Matrix design	Tune rheology, crosslinking, and exosome–matrix interaction	GelMA, alginate, dECM, PEG-based hybrids	Print fidelity with vesicle-compatible microenvironment	Trade-off between retention and vesicle accessibility	3-5,38
Incorporation mode	Encapsulation, surface association, or hybrid loading	Physical embedding, tethering, compartmentalized presentation	Better control of localization and biological presentation	Burst release or reduced accessibility	3-5,14,38
Release control	Engineer diffusion-, degradation-, or stimulus-governed release	MMP-responsive, pH-responsive, multilayer bioinks	Sustained and spatially resolved delivery	Complex optimization and limited benchmarking	3,14,20,34,38
Stability preservation	Minimize fabrication-induced vesicle damage	Mild crosslinking, protective matrices, optimized printing parameters	Better post-print bioactivity and cargo retention	Lack of standardized assays	3,5,16,34,38
Translational standardization	Build reproducible, scalable exosome–bioink workflows	Quality-by-design, standardized characterization, release benchmarking	Improved comparability and clinical readiness	Field-wide heterogeneity remains high	3,5,16,34,38

Abbreviations: dECM: Decellularized extracellular matrix; GelMA: Gelatin methacryloyl; MMP: Matrix metalloproteinase; PEG: Polyethylene glycol.

lineage-specific differentiation. At present, the strongest direct evidence is found in skin/wound repair, bone and osteochondral regeneration, and neural repair, whereas cardiovascular and vascular applications remain promising but less mature from a strictly bioprinted exosome–bioink perspective. This distinction is important, as it reflects not only the maturity of the field but also the differing biological and engineering requirements across tissue systems.[56,64,65] At the same time, not all regenerative applications may benefit equally from exosome-integrated bioprinting. Tissues requiring highly organized structural, electrical, or mechanical integration—such as myocardium or load-bearing osteochondral interfaces—may impose substantially greater design constraints than soft tissue repair applications. Consequently, the translational maturity of exosome–bioink systems should be evaluated not only by biological response but also by the structural, mechanical, and electrophysiological complexity of the target tissue environment.

5.1. Wound healing and skin regeneration

Wound healing is currently the most mature application area for exosome-integrated bioinks because effective repair requires coordinated modulation of angiogenesis, inflammation, re-epithelialization, and extracellular matrix remodeling. A work by Dutta *et al.*[64] moved beyond simple exosome loading and instead used a 3D bioprinting-enabled exosome-production strategy, where immunomodulatory alginate/gelatin/polydopamine-based inks promoted M2 macrophage polarization and subsequent M2-exosome generation. The resulting collagen/decellularized extracellular matrix/M2-exosome (COL@dECM/M2-Exo) construct supported vascularized wound healing, epidermal remodeling, and even hair follicle induction in vivo. Mechanistically, this system was associated with activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and peroxisome proliferator-activated receptor (PPAR) signaling pathways during macrophage polarization, along with suppression of mitogen-activated protein kinase (MAPK)-related inflammatory remodeling in downstream tissue regeneration, demonstrating how bioink design can influence both exosome origin and therapeutic output (Figure 4A–C).[64]

A second notable advance is the 2025 *MALAT1*-engineered exosome bioprinted hydrogel for diabetic wound healing.[65] In that study, *MALAT1*-overexpressing bone marrow mesenchymal stem cell-derived exosomes were encapsulated within a hyaluronic acid/platelet-rich plasma bioink and 3D-printed into a hydrogel exhibiting an extracellular matrix-like architecture, high biocompatibility, and enhanced exosome stability.

In diabetic wound models, this construct significantly improved healing outcomes, achieving approximately 82% wound closure. These effects were accompanied by increased vascular endothelial growth factor (VEGF) expression, reduced tumor necrosis factor-α (TNF-α) levels, elevated interleukin-10 (IL-10), and enhanced extracellular matrix reconstruction. Importantly, this study highlights a key trend in the field: exosome-integrated bioinks are evolving from passive delivery platforms into engineered signaling systems, in which vesicle content is intentionally modified to target disease-specific microenvironments (Figure 4D,E).[65]

These studies collectively refine the mechanistic understanding of why exosome-integrated bioinks outperform conventional wound dressings. One reason wound healing has emerged as one of the most successful application areas is that soft tissue repair depends strongly on dynamic paracrine communication rather than on highly ordered mechanical reconstruction. As a result, the biological advantages of exosome-mediated signaling can be translated more directly into therapeutic benefit without requiring the level of structural precision necessary in load-bearing or electrically active tissues. Rather than acting through a single pathway, they simultaneously provide pro-angiogenic, anti-inflammatory, and matrix-remodeling signals. However, translational challenges remain, including variability in exosome sources, differences in engineering strategies, and a lack of standardized release profiles. It also remains unclear whether increasingly complex exosome-engineering strategies consistently provide clinically meaningful advantages over simpler hydrogel-based wound healing systems. Future work should therefore emphasize quantitative evaluation of

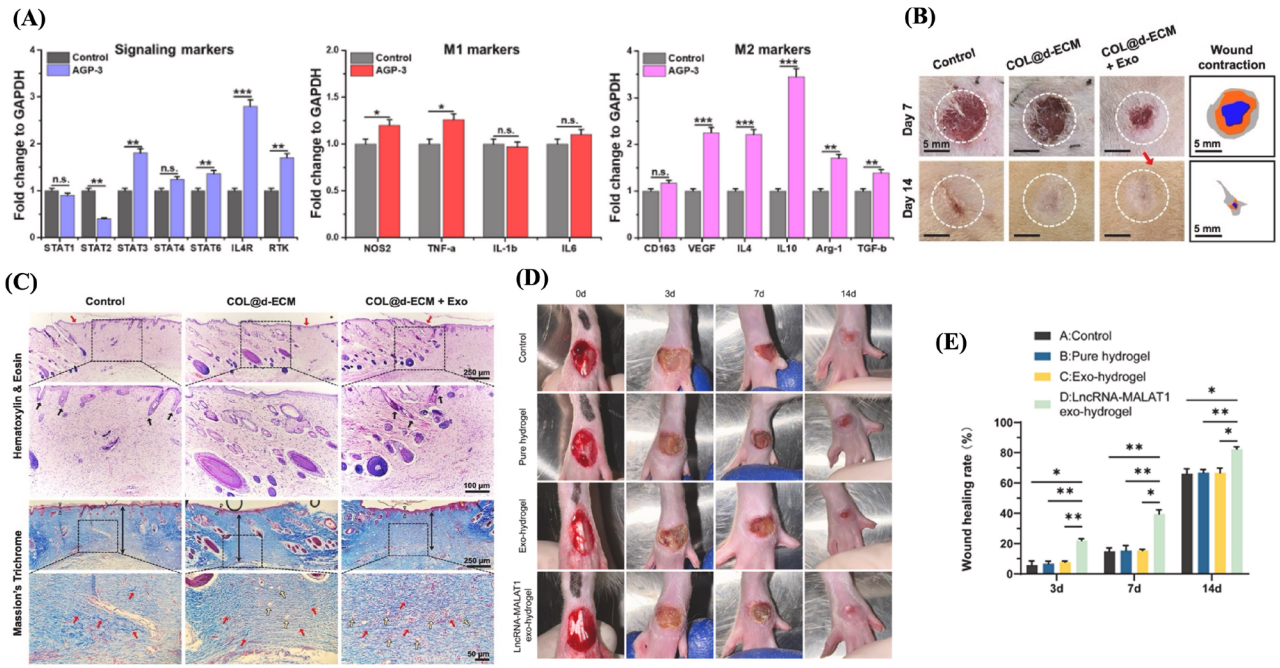

Figure 4. Exosome-based biomaterial platforms promote wound healing through immunomodulation and tissue regeneration. (A) Gene expression analysis of macrophage polarization-related markers following AGP-3 treatment. Signaling markers, M1-associated markers, and M2-associated markers were evaluated, showing increased expression of anti-inflammatory and pro-regenerative M2 markers. Modified from Dutta *et al.*[64] (B) Representative gross images of wounds treated with the control, COL@dECM, or COL@dECM + Exo at days 7 and 14, demonstrating accelerated wound closure in the exosome-loaded group. Modified from Dutta *et al.*[64] (C) Histological evaluation of wound tissues by hematoxylin and eosin staining and Masson's trichrome staining. Compared with control and COL@dECM groups, the COL@dECM + exo group showed improved re-epithelialization, tissue regeneration, and collagen deposition. Modified from Dutta *et al.*[64] (D) Representative photographs of wound-healing progression in animals treated with control, pure hydrogel, exo-hydrogel, or lncRNA-MALAT1 exo-hydrogel over 0, 3, 7, and 14 days. Modified and reprinted with permission from Yingying Shi *et al.*[65] Copyright © 2025 Elsevier. (E) Quantitative analysis of wound healing rates showed that exosome-hydrogel treatment, particularly the lncRNA-MALAT1 exo-hydrogel group, significantly promoted wound closure compared with the control group. Modified from Shi *et al.*[65]

exosome retention, release kinetics, and reproducibility across batches.[65]

5.2. Bone and osteochondral regeneration

Bone and osteochondral regeneration represent the second major application domain, with recent studies demonstrating a shift toward microenvironment-specific exosome biofabrication strategies. In 2025, Lou *et al.*[56] reported a 3D-printed hydrogel scaffold incorporating exosomes derived from human skeletal stem cells, identified through single-cell RNA sequencing. As shown in Figure 5A–C, in osteochondral defect models, this scaffold promoted simultaneous cartilage and subchondral bone regeneration, with enhanced chondrogenesis attributed to the miR-214-3p/Jagged canonical notch ligand 2 (JAG20 signaling axis. This work highlights that exosome source selection is not a trivial variable, but rather a critical determinant of regenerative outcomes that should be optimized based on target tissue requirements.

Similarly, Zhu *et al.*[66] developed a 3D-printed β-tricalcium phosphate (β-TCP) scaffold loaded with exosomes derived from baicalin-preconditioned bone marrow mesenchymal stem cells, demonstrating enhanced endothelial cell migration, angiogenesis, and reduced inflammation through paired-related homeobox 2 (PRRX2)-mediated signaling (Figure 5D,F). Their study emphasizes that bone regeneration should be viewed not merely as an osteogenic process but rather as a coordinated interaction between osteogenesis, angiogenesis, and immune modulation.

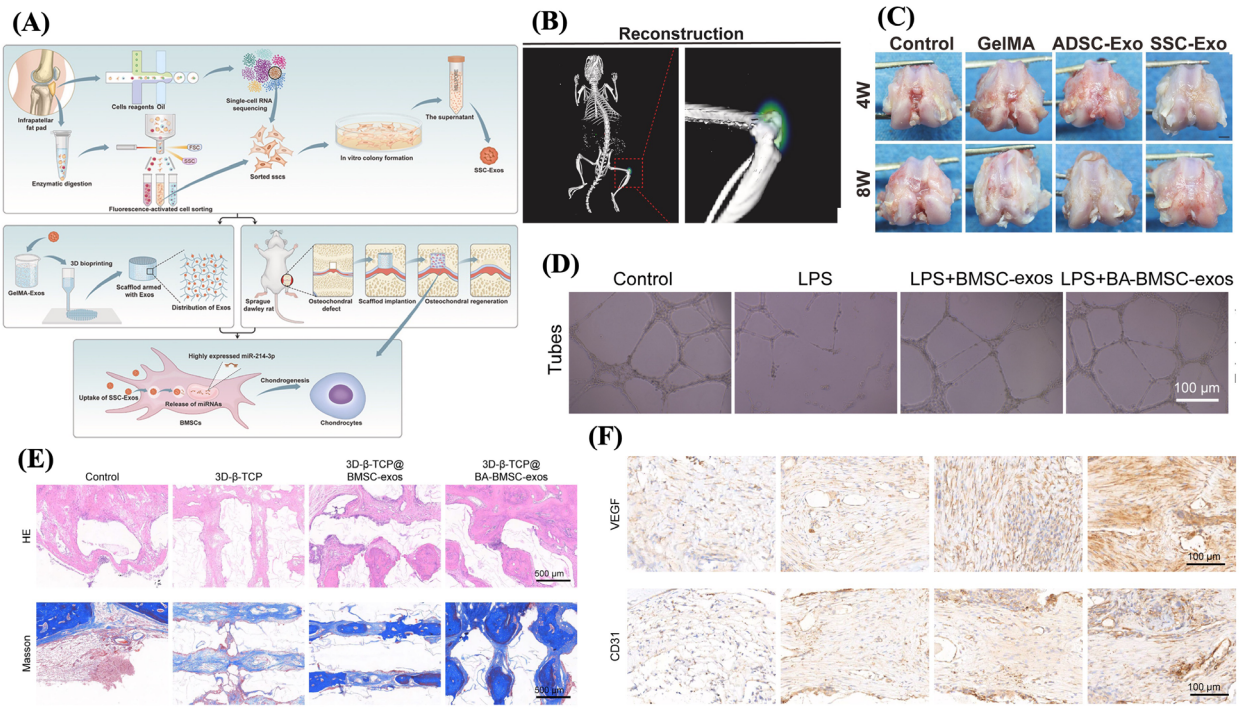

Figure 5. Stem cell-derived exosome-loaded biomaterial scaffolds promote osteochondral and bone tissue regeneration. (A) Schematic illustration of the preparation and application of skeletal stem cell-derived exosomes (SSC-Exos) incorporated into a gelatin methacryloyl (GelMA)-based bioink for 3D bioprinting. Exosomes were isolated from sorted SSCs, mixed with GelMA, and applied to osteochondral defects, where exosomal cargo was proposed to enhance chondrogenic differentiation and tissue regeneration. Modified from Lou *et al.*[56] (B) Three-dimensional reconstruction image showing the osteochondral defect model used for in vivo evaluation. Modified from Lou *et al.*[56] (C) Representative gross images of osteochondral repair in the control, GelMA, adipose-derived stem cells exosome (ADSC-Exo), and SSC-Exo groups at 4 and 8 weeks, showing improved defect repair in exosome-treated groups. Modified from Lou *et al.*[56] (D) In vitro tube formation assay demonstrating the pro-angiogenic effects of bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) and baicalin-pretreated bone marrow mesenchymal stem cell-derived exosomes (BA-BMSC-Exos) under inflammatory conditions. Modified from Zhu *et al.*[66] (E) Histological evaluation of bone defect repair by hematoxylin and eosin and Masson's trichrome staining in the control, 3D-β-tricalcium phosphate (3D-β-TCP), 3D-β-TCP@BMSC-Exos, and 3D-β-TCP@BA-BMSC-Exos groups. Exosome-loaded scaffolds showed enhanced tissue regeneration and matrix deposition. Modified from Zhu *et al.*[66] (F) Immunohistochemical staining of vascular endothelial growth factor (VEGF) and CD31, indicating enhanced angiogenesis in defects treated with exosome-functionalized scaffolds. Modified from Zhu *et al.*[66]

Another emerging strategy involves combining exosomes with biomimetic structural scaffolds, such as titanium trabecular architectures, together with controlled release systems.[67] In this context, hypoxia-preconditioned exosomes were incorporated into PEGDA/GelMA microgels to achieve sustained release, mimicking both the structural and biochemical features of native bone tissue. This dual-biomimetic approach represents a key innovation direction in the field. Nevertheless, bone and osteochondral applications remain significantly more demanding than soft tissue repair because successful translation requires simultaneous coordination of mechanical strength, vascular integration, and long-term structural remodeling. This creates a fundamental challenge for exosome-integrated bioinks, as material systems optimized for biological signaling are not always compatible with the mechanical requirements of load-bearing tissue environments. Overall, current evidence suggests that the most effective strategy for bone regeneration is no longer simply "exosome + scaffold," but rather source-optimized exosomes combined with tissue-specific architecture and controlled release systems. Remaining challenges include achieving sufficient mechanical strength for load-bearing applications and standardizing exosome characterization across studies.[66,67]

5.3. Cartilage and musculoskeletal regeneration

Cartilage regeneration presents distinct challenges due to its avascular nature, limited self-healing capacity, and requirement for zonal organization. Exosome-integrated bioinks offer a promising solution by providing sustained delivery of bioactive signals that promote chondrogenesis and matrix production.[56,68]

The osteochondral study by Lou *et al.* is particularly relevant here (Figure 6). They demonstrated enhanced cartilage regeneration alongside subchondral bone repair, outperforming exosomes derived from alternative sources. These comparative results underscore the importance of exosome origin in determining therapeutic efficacy. Additionally, recent work has shown that exosome-

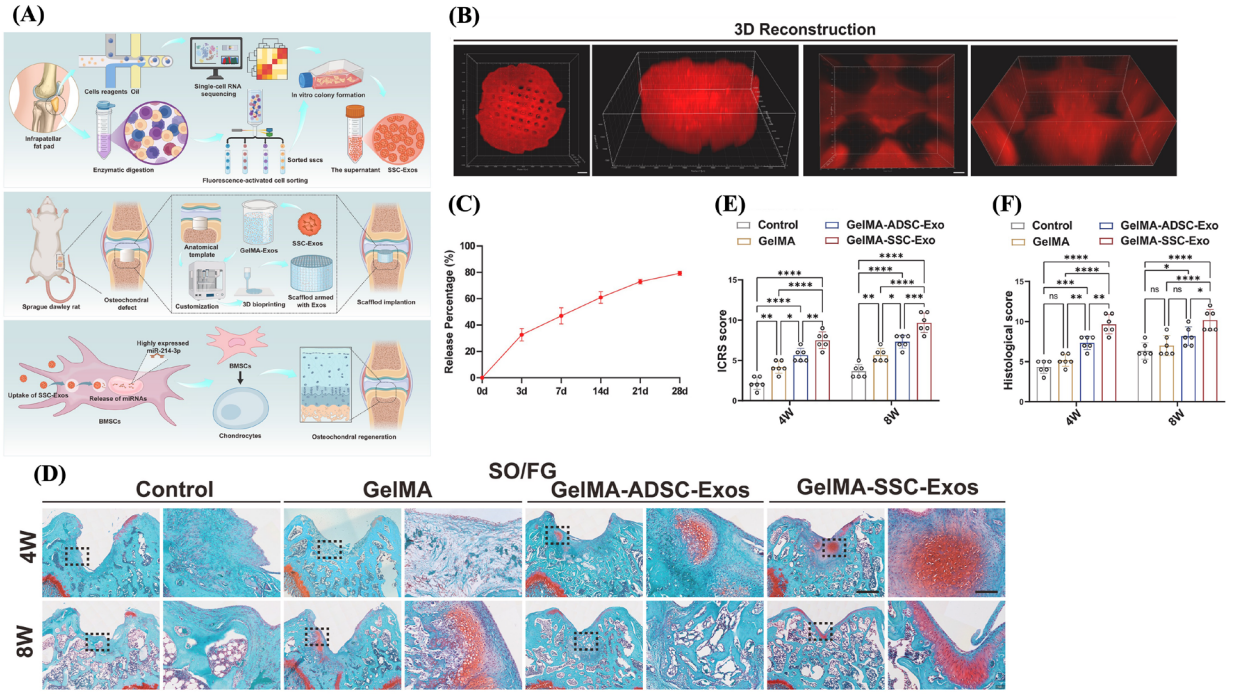

Figure 6. Skeletal stem cell (SSC)-derived exosome-loaded gelatin methacryloyl (GelMA) scaffolds promote osteochondral regeneration. (A) Schematic illustration of the construction of a biomimetic 3D-printed scaffold loaded with SSC exosomes (SSC-Exos) for osteochondral regeneration. (B) Confocal and reconstruction images showing the uniform distribution of exosomes (red) within the scaffold. Scale bar: 1 mm (whole view); 0.1 mm (enlarged view). (C) Quantification of fluorescence intensity. (D) Representative images of safranin O/fast green staining. (E) International Cartilage Repair Society (ICRS) scores for repaired tissues in each group after 4 and 8 weeks post-operation. (F) Histological scores for repaired tissues in each group after 4 and 8 weeks post-operation. $n = 6$ per group. Data are shown as the mean ± SD ($n \geq 3$). Modified from Lou *et al.*[56]

functionalized hydrogels can enhance cartilage repair by improving exosome delivery efficiency and creating a more chondroinductive microenvironment.[68] Although not always implemented within fully bioprinted systems, these studies provide critical insight into how bioink design can be optimized for cartilage-specific applications.

Recent literature trends further suggest that osteochondral regeneration is becoming a major testing ground for spatially controlled exosome delivery, including gradient scaffolds and multilayer biofabrication strategies. However, significant challenges remain, including the need for improved mechanical properties, better integration with host tissue, and more rigorous comparisons across different exosome sources and material systems.[56,68]

5.4. Neural regeneration

Neural regeneration is emerging as one of the most mechanistically advanced applications of exosome-integrated bioprinting. In 2025, Zhang *et al.*[68] developed a 3D-bioprinted alginate dialdehyde–gelatin hydrogel scaffold loaded with neural stem cell-derived extracellular vesicles for traumatic brain injury treatment. This scaffold enabled sustained extracellular vesicle release for up to 28 days, reduced lesion volume, and improved behavioral recovery. Mechanistically, these effects were associated with activation of AMP-activated protein kinase (AMPK)–unc-51-like autophagy activating kinase 1 (ULK1)-mediated autophagy and suppression of the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) inflammatory pathway, providing strong molecular evidence for exosome-mediated neural repair.

A complementary study by Zhang *et al.*[69], shown in Figure 7, utilized a 3D-printed exosome-functionalized brain extracellular matrix hydrogel for intracerebral hemorrhage. This system demonstrated sustained exosome release, reduced inflammation, improved extracellular matrix remodeling, and decreased neuronal apoptosis. Importantly, this work highlights that exosome-based systems can regulate not only neuronal survival but also the early inflammatory environment following neural injury.

More recently, Sun *et al.*[70] reported that 3D-bioprinted mesenchymal stem cell-derived exosomes significantly enhanced peripheral nerve regeneration through the miR-26b-5p/phosphatase and tensin homolog (PTEN)/phosphatidylinositol 3-kinase–protein kinase B (PI3K–AKT) signaling pathway, promoting macrophage polarization and Schwann cell function. Their study introduced a critical concept: 3D culture and bioprinting can influence exosome potency itself, not just delivery efficiency. Compared with mechanically demanding tissues, neural repair may represent a particularly favorable application environment for exosome-integrated bioinks, as functional recovery depends heavily on immune modulation, neuroprotection, extracellular matrix preservation, and sustained biochemical signaling rather than on bulk mechanical reinforcement. Together, these findings suggest that exosome-integrated bioinks are particularly suited for neural repair scenarios requiring immune modulation, extracellular matrix preservation, and sustained signaling. However, challenges remain in achieving precise neural network organization and long-term functional integration.[68-70]

5.5. Cardiovascular and vascular applications

Cardiovascular and vascular applications represent a promising but still developing area for exosome-integrated bioinks. Current studies are more commonly focused on exosome-releasing scaffolds rather than fully integrated bioprinted bioinks, reflecting the early stage of this field.[71-73] As shown in Figure 8A,B, Hashemi *et al.*[71] developed a 3D-printed conductive chitosan–polyaniline scaffold with exosome release for enhanced angiogenesis and cardiomyocyte protection. In this system, the printed conductive scaffold provided structural and electroactive support, while the released exosomes promoted endothelial cell function and protected cardiomyocytes under hypoxic conditions. Their study is important because it illustrates how exosome delivery can be combined with electrically active biomaterials to address both vascular and myocardial requirements within a single biofabricated construct. It suggests that combining exosomes with conductive biomaterials may address both biological and electrophysiological requirements in cardiac repair. However, cardiovascular regeneration remains one of the most difficult translational targets for exosome-integrated bioinks, as therapeutic success depends on synchronized electrical conductivity, vascular integration, mechanical coupling, and long-term tissue organization. These requirements substantially exceed the biological signaling demands observed in simpler regenerative applications such as wound healing. Consequently, although exosome-mediated signaling may improve local repair responses, it remains uncertain whether current exosome–bioink systems alone can achieve the level of structural-functional integration necessary for clinically meaningful cardiac regeneration.

Another approach involves oxygen-generating exosome-loaded hydrogels, which can alleviate hypoxia and improve tissue repair after myocardial infarction (Figure 8C,D).[72] This system integrated several design elements that are highly relevant to cardiac regeneration, including local oxygen generation, conductive biomaterial

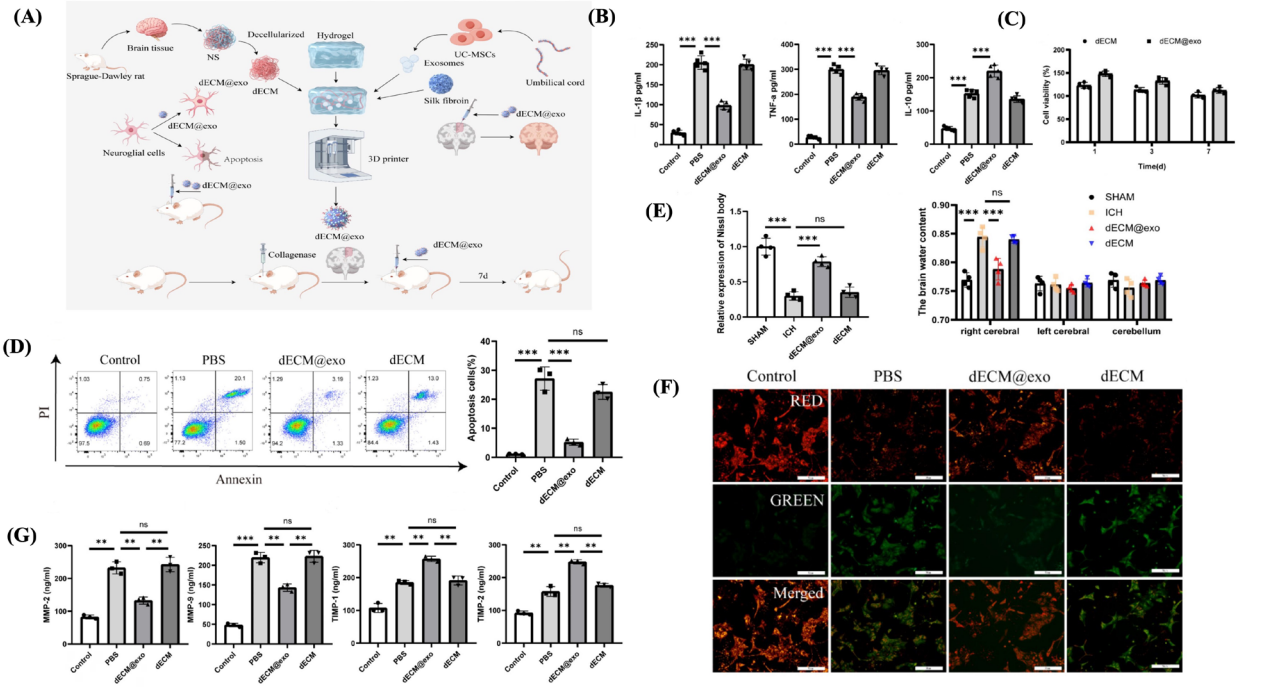

Figure 7. Exosome-functionalized brain decellularized extracellular matrix (dECM) bioink promotes neuroprotection and brain tissue repair after cerebral injury. (A) Schematic illustration of the preparation of dECM@Exo bioink. dECM was obtained from rat brain tissue and combined with umbilical cord mesenchymal stem cell-derived exosomes (UC-MSC-Exos) and silk fibroin to generate a printable hydrogel for neural tissue repair. (B) Expression levels of inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10), in different treatment groups, showing that dECM@Exo modulated the inflammatory response after injury. (C) Evaluation of neurological function and brain edema. Behavioral scores and brain water content analysis indicated improved functional recovery and reduced cerebral edema in the dECM@Exo-treated group. (D) Flow cytometric analysis of apoptosis in different groups, together with quantitative assessment of apoptotic cells, demonstrating that dECM@Exo reduced cell apoptosis compared with control treatments. (E) Quantification of neuronal and tissue repair-related parameters, showing enhanced regenerative responses following dECM@Exo treatment. (F) Representative fluorescence images of neural tissue staining in the control, phosphate-buffered saline (PBS), dECM@Exo, and dECM groups, illustrating improved cell survival and tissue repair in the dECM@Exo group. (G) Quantitative analysis of immunofluorescence results, including microtubule-associated protein 2 (MAP2), myelin basic protein (MBP), neuronal nuclei (NeuN), and (terminal deoxynucleotidyl transferase dUTP nick end labeling) TUNEL-related markers, indicating enhanced neuronal preservation, remyelination, and reduced apoptosis after dECM@exo treatment. Modified from Zhang *et al.*[69]

support, and exosome-mediated biological signaling. Rather than serving merely as a passive depot, the hydrogel was designed to regulate the local microenvironment while simultaneously improving exosome retention and therapeutic efficacy in ischemic tissue. This study therefore represents an important step toward multifunctional exosome-integrated cardiovascular biomaterials.[72] In addition, Enayati *et al.*[73] reported a 3D-printed cardiac patch coated with human extracellular matrix hydrogel, demonstrating improved cell adhesion and immune compatibility. Although exosomes were not directly incorporated into this particular system, the study is still relevant because it highlights the ongoing movement toward more biomimetic and clinically oriented cardiac biofabrication strategies. In the context of exosome-integrated bioinks, such extracellular matrix-informed patch designs provide a logical foundation for future incorporation of extracellular vesicle-mediated signaling into bioprinted cardiac constructs.

Taken together, these studies suggest that cardiovascular biofabrication is transitioning from simple exosome-releasing materials toward more integrated systems that combine structural support, conductive or oxygen-regulating functions, and exosome-mediated bioactivity. However, compared with other regenerative applications, the number of studies involving truly programmable and fully integrated 3D-bioprinted exosome–bioinks for cardiac repair remains limited. Future progress in this area will likely depend on the development of cardiovascular-specific bioinks capable of coordinating exosome retention, spatial presentation, electrical compatibility, and long-term therapeutic function in a single printed platform.[71-73]

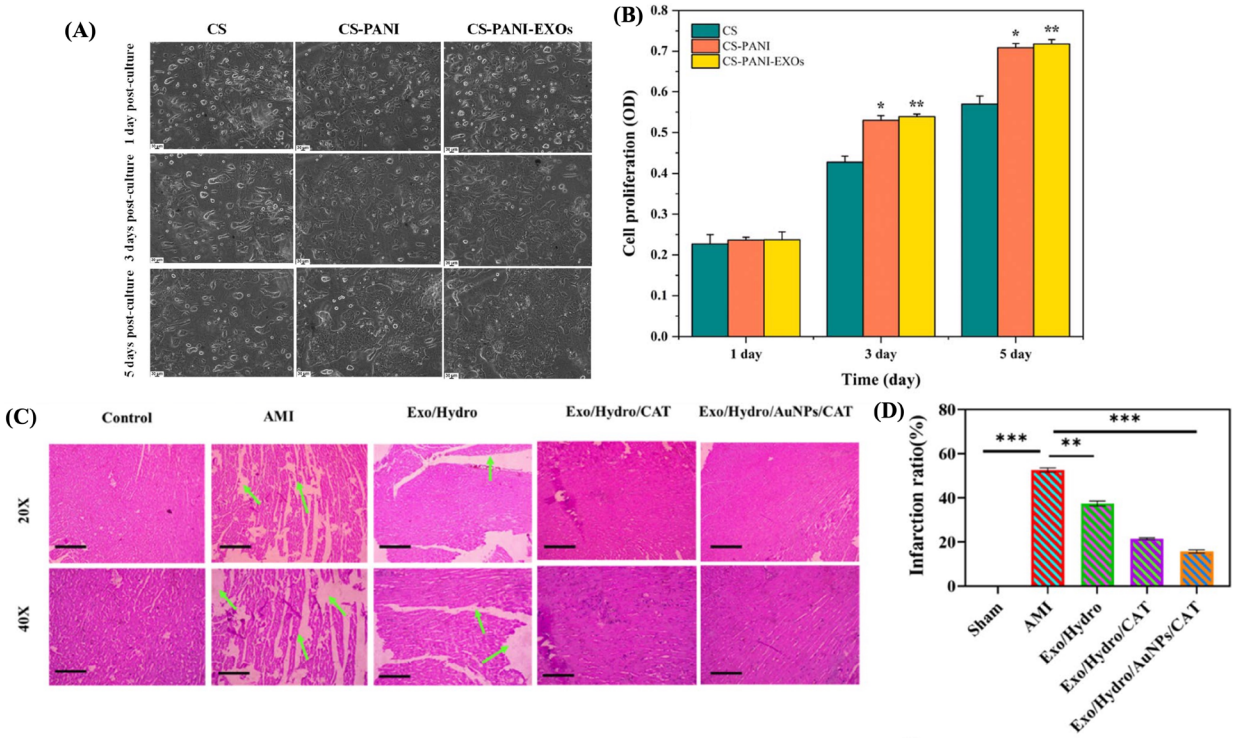

Figure 8. Exosome-functionalized biomaterials enhance cell proliferation and myocardial repair. (A) Representative microscopic images of cells cultured on chitosan (CS), chitosan-polyaniline (CS-PANI), and chitosan-polyaniline-exosomes (CS-PANI-EXOs) scaffolds for 1, 3, and 5 days, showing improved cell attachment and growth on the exosome-loaded scaffold. Modified from Hashemi *et al.*[71] (B) Quantitative analysis of cell proliferation at different time points. Compared with CS alone, CS-PANI and CS-PANI-EXOs significantly promoted cell growth, with the highest proliferation observed in the CS-PANI-EXOs group. Modified from Hashemi *et al.*[71] (C) Histological evaluation of myocardial tissue in the control, acute myocardial infarction (AMI), exosome-loaded hydrogel (Exo/Hydro), exosome and catalase-loaded hydrogel (Exo/Hydro/CAT), and exosome with gold nanoparticles and catalase-loaded hydrogel (Exo/Hydro/AuNPs/CAT) groups at low and high magnification. Exosome-containing hydrogel treatments reduced tissue damage and improved myocardial preservation compared with the AMI group. Modified from Xu *et al.*[72] (D) Quantitative analysis of infarction ratio, showing that exosome-loaded hydrogel formulations, particularly Exo/Hydro/AuNPs/CAT, significantly reduced infarct size. Modified from Xu *et al.*[72]

Taken together, current evidence suggests that the translational potential of exosome-integrated bioinks may depend strongly on tissue-specific regenerative complexity. Applications dominated by paracrine signaling and immune modulation currently appear more immediately translatable, whereas tissues requiring highly organized structural, mechanical, or electrophysiological integration continue to present major engineering and biological barriers. This distinction highlights the need for application-specific design frameworks rather than assuming that a single exosome–bioink strategy can be generalized across all regenerative contexts.

6. Remaining challenges and translational bottlenecks

Despite rapid progress in exosome-integrated bioinks for 3D bioprinting, several critical challenges remain to be addressed before these systems can achieve widespread translational and clinical impact. A broader concern is that the field may currently be advancing faster in technological diversification than in mechanistic convergence. Although increasingly sophisticated exosome–bioink systems are being reported, there is still limited consensus regarding which design variables most strongly determine therapeutic performance across different regenerative contexts. As a result, many studies continue to rely on empirical optimization rather than predictive engineering principles, making it difficult to establish transferable design rules or standardized translational benchmarks. These challenges arise from the convergence of extracellular vesicle biology, biomaterials engineering, and manufacturing science, underscoring the need for more coordinated design strategies that can integrate biological functionality, material performance, and manufacturing scalability.[18,19,58]

One of the most significant unresolved issues is the heterogeneity of exosome sources. Exosomes derived from different cell types, culture conditions, and isolation protocols exhibit substantial variability in size distribution, molecular cargo, and biological activity.[18,19] Even subtle differences in donor-cell conditions, such as oxygen tension or culture configuration, can significantly alter vesicle function. This variability complicates reproducibility and limits cross-study comparability. From a practical biofabrication perspective, exosome isolation methods should therefore be selected not only for yield, but also for their ability to preserve vesicle integrity and downstream biological function. For exosome-integrated bioinks, where vesicles must remain functional throughout formulation, printing, and post-print release, size-exclusion chromatography (SEC) is generally more compatible with maintaining vesicle purity and functional integrity than conventional ultracentrifugation, which may increase vesicle aggregation, co-isolation of non-vesicular proteins, or mechanical stress-related damage. Accordingly, SEC-based workflows may be preferable when the goal is to preserve vesicle bioactivity during bioprinting-related processing. In line with the MISEV guidelines, isolation should also be complemented by standardized characterization of particle size and concentration, vesicle morphology, canonical protein markers, and functional bioactivity. Therefore, future research must emphasize standardized characterization frameworks, including multi-omics profiling and function-based benchmarking, to establish reproducible design rules.[16,18,19,60]

A second major challenge is the limited control over loading efficiency and release kinetics. Although hydrogel-based bioinks improve exosome retention compared to direct injection, many systems still exhibit burst release behavior and unpredictable long-term delivery profiles.[58,74] Furthermore, the interactions between exosomes and polymer networks remain insufficiently understood, particularly in relation to matrix degradation and vesicle diffusion. Addressing this issue will require the integration of quantitative transport modeling, advanced material design, and real-time release monitoring, enabling predictive control over delivery rather than empirical optimization.

Another key bottleneck lies in the preservation of exosome integrity during fabrication and storage. Exosomes are highly sensitive to mechanical stress, reactive species, and environmental conditions, all of which may compromise membrane integrity and cargo functionality.[16,19] However, systematic studies evaluating how bioprinting parameters influence exosome stability remain limited. The field currently lacks standardized evaluation methods to assess vesicle functionality after printing. Future work should establish robust post-fabrication characterization pipelines, including structural, molecular, and functional assays.

From a translational perspective, scalability and reproducibility represent major obstacles. Clinical applications require large-scale production of exosomes with consistent quality, as well as standardized bioink formulations that can be reproduced across batches.[18,58] This is further complicated by the complexity of integrating biological materials with advanced fabrication technologies. Adoption of quality-by-design frameworks, automated manufacturing systems, and standardized production protocols will be critical for advancing toward clinical translation. Finally, regulatory challenges must also be considered. Exosome-integrated bioinks fall within a hybrid category that overlaps biologics, biomaterials, and advanced therapy products, making regulatory pathways unclear.[33,58] Clear guidelines for characterization, safety, and quality control will be essential for enabling clinical adoption.

Regulatory translation of exosome–bioink composite products remains challenging because no major agency currently offers a dedicated exosome–bioink pathway, and classification is generally determined on a case-by-case basis according to the product's principal mechanism of action and constituent parts. In the United States, the Food and Drug Administration (FDA) states that exosome products intended to treat disease generally require approval and that no exosome products are currently approved. When exosomes are incorporated into a printed scaffold or hydrogel, the product may fall under biologic/device combination product regulation, in which case sponsors may seek jurisdictional clarification through the Office of Combination Products using a pre-request for designation (Pre-RFD) or formal RFD. Investigational submissions are typically made as an investigational new drug (IND) when the product has a drug or biologic primary mode of action, or as an investigational device exemption (IDE) when it has a device primary mode of action; corresponding marketing submissions generally follow the primary mode of action (PMOA)-based pathway (e.g., biologics license application/new drug application [BLA/NDA] for biologic- or drug-led products, or premarket approval [PMA]/De Novo/510[k] for device-led products).[75-78]

In the European Union (EU), exosome–bioink composites likewise require case-by-case classification. If the product falls within the advanced therapy medicinal product framework, sponsors may request a scientific recommendation on advanced therapy medicinal product (ATMP) classification from the Committee for Advanced

Therapies. Where a printed exosome-containing construct is classified as an ATMP or a combined ATMP, clinical development proceeds through the EU Clinical Trials Regulation system, with trial applications submitted through the clinical trials information system, and marketing authorization is pursued through the centralized procedure. However, because exosome-containing biomaterial constructs do not fit neatly into existing cell- or tissue-based ATMP definitions, early regulatory classification is especially important in Europe.[79-82]

In China, no exosome-specific regulatory pathway has yet been formally established for exosome–bioink composite products. At present, such products are generally expected to enter the National Medical Products Administration (NMPA)/Center for Drug Evaluation (CDE) system through the existing biologics and/or drug–device combination review framework, with development typically aligned to IND and NDA-type submission routes when regulated as therapeutic biologics. Recent official CDE/NMPA reporting confirms that drug–device combination applications are already being handled within the current technical-review system, underscoring that exosome–bioink composites are likely to be regulated through existing combination-product mechanisms until more specific guidance becomes available.[83] More fundamentally, the regulatory uncertainty surrounding exosome-integrated bioinks reflects a deeper conceptual issue: these systems do not fit neatly into traditional categories of biologics, devices, or biomaterials because their therapeutic activity emerges from the interaction among structural scaffolds, vesicle-mediated signaling, and dynamic host responses. This hybrid biological-engineering nature may ultimately require new evaluation paradigms that extend beyond conventional single-component regulatory frameworks.

7. Emerging trends and future perspectives

Beyond regenerative therapy, exosome-integrated bioinks also hold considerable promise in disease modeling and drug screening, where their value lies in the ability to combine the structural controllability of 3D bioprinting with the biological complexity of extracellular vesicle-mediated signaling. Conventional two-dimensional culture systems often fail to reproduce the tissue-specific architecture, extracellular matrix context, and dynamic cell–cell communication found in vivo, which limits their predictive relevance for both mechanistic studies and therapeutic screening.[38,84] By contrast, 3D bioprinted constructs can provide spatially organized microenvironments with controllable geometry, matrix composition, and multicellular distribution, thereby creating more physiologically relevant in vitro models.[38,49,84]

In this context, the integration of exosomes into bioinks introduces an additional layer of biological realism. Because exosomes carry proteins, lipids, mRNAs, and regulatory non-coding RNAs that reflect the state of their parent cells, they can serve not only as therapeutic agents but also as functional mediators of pathological signaling.[14,15,20-22] This feature is particularly relevant for disease modeling, where exosome-integrated bioinks could be used to reconstruct pathological microenvironments associated with chronic wounds, osteochondral degeneration, neuroinflammation, myocardial infarction, fibrosis, or tumor progression. For example, disease-associated exosomes derived from inflammatory, fibrotic, ischemic, or tumor-like cell populations could be embedded within printed matrices to reproduce local paracrine signaling conditions that are difficult to mimic using soluble factors alone.[14,15,20-22,33] In this way, bioprinted exosome-integrated systems could help investigators study how extracellular vesicle signaling contributes to disease initiation, progression, and tissue remodeling in a spatially controlled setting.

Such platforms may be particularly useful for disease-specific microenvironment models. In osteochondral disease modeling, for example, exosome-integrated bioinks could be used to reproduce catabolic and inflammatory communication between cartilage, subchondral bone, and immune cells. In neural disorders, printed brain-mimetic matrices containing inflammatory or injury-associated exosomes may provide a more realistic system for studying neuroinflammation, neuronal stress responses, and repair mechanisms. In cardiovascular disease, exosome-bearing conductive or oxygen-regulating bioinks may enable the construction of ischemic cardiac models that better capture the interplay among hypoxia, inflammation, extracellular matrix remodeling, and cell survival. These applications would allow exosome-mediated signaling to be investigated as an active biological variable rather than as a passive background factor.

Exosome-integrated bioinks also have strong potential in drug screening, because they may improve the predictive value of in vitro platforms compared with conventional monolayer culture and simplified hydrogel systems. A major limitation of traditional screening models is that they often fail to recapitulate local signaling gradients, extracellular matrix barriers, and multicellular interactions, all of which can strongly influence therapeutic response.[38,84] By incorporating exosomes into printed tissue models, researchers may be able to create screening systems that more faithfully reflect tissue-specific or disease-specific signaling states. This approach is especially relevant for evaluating drugs that target inflammation, angiogenesis, fibrosis, extracellular matrix remodeling, or intercellular

communication pathways. In such settings, exosome-integrated constructs could be used not only to test therapeutic efficacy but also to assess how candidate drugs alter extracellular vesicle release, uptake, cargo activity, or downstream paracrine effects.

Another promising direction is the development of patient-specific screening systems. Because exosomes retain features of their donor-cell origin, combining patient-derived cells with patient-derived or disease-matched exosomes in bioprinted matrices may enable more personalized in vitro disease models. Such systems could be used to stratify therapeutic response, identify responder versus non-responder phenotypes, or evaluate individualized regenerative and anti-inflammatory treatments. This possibility is especially attractive in disorders characterized by strong biological heterogeneity, such as osteoarthritis, chronic diabetic wounds, neurodegeneration, and cardiovascular injury. In the long term, exosome-integrated bioinks may therefore support a transition from generalized biofabrication platforms toward precision disease models that integrate tissue architecture, extracellular matrix features, and biologically relevant vesicle signaling within a single experimental system.[49,54,57,58]

Despite this promise, several challenges remain before disease modeling and drug screening can become mature application areas for exosome-integrated bioinks. These include the need for standardized exosome sources, reproducible loading methods, quantitative control of vesicle distribution and release, and robust metrics for evaluating post-print vesicle bioactivity.[57,58] In addition, future research should move beyond proof-of-concept demonstrations and establish comparative benchmarks showing whether exosome-integrated models truly outperform conventional 3D cultures in terms of physiological relevance, response prediction, and translational utility. Nevertheless, the convergence of 3D bioprinting, advanced bioink design, and extracellular vesicle biology suggests that disease modeling and drug screening may become two of the most important non-therapeutic application directions of exosome-integrated biofabrication in the coming years.

8. Conclusion

Exosome-integrated bioinks represent a promising direction in 3D bioprinting for regenerative medicine, combining the structural controllability of biofabrication with the multifunctional signaling capacity of extracellular vesicles. Compared with conventional bioinks, these systems offer a more instructive platform for regulating angiogenesis, immune modulation, extracellular matrix remodeling, and tissue-specific regeneration. This review highlights that their performance depends on the coordinated design of the entire biofabrication system, particularly with respect to matrix selection, exosome incorporation, release behavior, stability preservation, and translational standardization. Although encouraging progress has been reported in wound healing, musculoskeletal regeneration, neural repair, and cardiovascular applications, the field remains limited by exosome heterogeneity, incomplete standardization, insufficient control over release and post-print function, and unresolved manufacturing and regulatory challenges. Future advances will require more rigorous design frameworks, MISEV-aligned characterization, and improved integration of responsive biomaterials, scalable processing, and post-print functional validation. In a broader sense, the future of exosome-integrated bioinks may depend less on simply increasing biological complexity and more on achieving controllable biological orchestration. The next generation of biofabrication systems will likely require tighter integration of vesicle biology, materials science, computational modeling, and translational manufacturing to create constructs capable of dynamically interacting with evolving tissue microenvironments. Accordingly, the field is gradually shifting from the development of "bioactive materials" toward the engineering of programmable regenerative systems. Overall, exosome-integrated bioinks should be regarded not simply as vesicle-loaded hydrogels, but as engineered therapeutic systems with strong potential to advance next-generation regenerative biofabrication and precision tissue engineering.

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Conflict of interest

The authors declare no conflicts of interest.

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References

1. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol*. 2014;32(8):773-785.

   doi: 10.1038/nbt.2958

2. Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. *Biotechnol Adv*. 2016;34(4):422-434.

   doi: 10.1016/j.biotechadv.2015.12.011

3. Groll J, Boland T, Blunk T, *et al*. Biofabrication: reappraising the definition of an evolving field. *Biofabrication*. 2016;8(1):013001.

   doi: 10.1088/1758-5090/8/1/013001

4. Daly AC, Prendergast ME, Hughes AJ, Burdick JA. Bioprinting for the biologist. *Cell*. 2021;184(1):18-32.

   doi: 10.1016/j.cell.2020.12.002

5. Heinrich MA, Liu W, Jimenez A, *et al*. 3D bioprinting: from benches to translational applications. *Small*. 2019;15(23):1805510.

   doi: 10.1002/smll.201805510D

6. Chimene D, Lennox KK, Kaunas RR, Gaharwar AK. Advanced bioinks for 3D printing: a materials science perspective. *Ann Biomed Eng*. 2016;44(6):2090-2102.

   doi: 10.1007/s10439-016-1638-y

7. Hospodiuk M, Dey M, Sosnoski D, Ozbolat IT. The bioink: A comprehensive review on bioprintable materials. *Biotechnol Adv*. 2017;35(2):217-239.

   doi: 10.1016/j.biotechadv.2016.12.006

8. Yue K, Trujillo-de Santiago G, Alvarez MM, *et al*. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials*. 2015;73:254-271.

   doi: 10.1016/j.biomaterials.2015.08.045

9. Zhu J, Marchant RE. Design properties of hydrogel tissue-engineering scaffolds. *Expert Rev Med Devices* 2011;8(5):607-626.

   doi: 10.1586/erd.11.27

10. Liu W, Zhang YS, Heinrich MA, *et al*. Rapid continuous multimaterial extrusion bioprinting. *Adv Mater*. 2017;29(3):1604630.

    doi: 10.1002/adma.201604630

11. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface*. 2011;8(55):153-170.

    doi: 10.1098/rsif.2010.0223

12. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. *Pharm Res*. 2003;20(8):1103-1112.

    doi: 10.1023/A:1025034925152

13. Uebersax L, Merkle HP, Meinel L. Insulin-like growth factor I releasing silk fibroin scaffolds induce chondrogenic differentiation of human mesenchymal stem cells. *J Control Release*. 2008;127(1):12-21.

    doi: 10.1016/j.jconrel.2007.11.006

14. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):eaau6977.

    doi: 10.1126/science.aau6977

15. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci*. 2019;9:19.

    doi: 10.1186/s13578-019-0282-2

16. Théry C, Witwer KW, Aikawa E, *et al*. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7(1):1535750.

    doi: 10.1080/20013078.2018.1535750

17. Liao W, Du Y, Zhang C, *et al*. Exosomes: The next generation of endogenous nanomaterials for advanced drug delivery and therapy. *Acta Biomater*. 2019;86:1-14.

    doi: 10.1016/j.actbio.2018.12.045

18. Wang Q, Liu Y, Zhang S, *et al*. Exosome-based bioinks for 3D bioprinting applications in tissue engineering and regenerative medicine. *Int J Bioprinting* 2023;9(6):0114.

    doi: 10.36922/ijb.0114

19. Camacho-Cardenosa M, Pulido-Escribano V, Estrella-Guisado G, *et al*. Bioprinted Hydrogels as Vehicles for the Application of Extracellular Vesicles in Regenerative Medicine. *Gels*. 2025;11(3).

    doi: 10.3390/gels11030191

20. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. 2019;88(1):487-514.

    doi: 10.1146/annurev-biochem-013118-111902

21. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular

vesicles for cell-to-cell communication. *Nat Cell Biol*. 2019;21(1):9-17.

doi: 10.1038/s41556-018-0250-9

22. Tkach M, Théry C. Communication by extracellular vesicles: where we are and where we need to go. *Cell*. 2016;164(6):1226-1232.

doi: 10.1016/j.cell.2016.01.043

23. Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells*. 2017;35(4):851-858.

doi: 10.1002/stem.2575

24. Witwer KW, Van Balkom BW, Bruno S, *et al*. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. *J Extracell Vesicles*. 2019;8(1):1609206.

doi: 10.1080/20013078.2019.1609206

25. Lener T, Gimona M, Aigner L, *et al*. Applying extracellular vesicles based therapeutics in clinical trials–an ISEV position paper. *J Extracell Vesicles*. 2015;4(1):30087.

doi: 10.3402/jev.v4.30087

26. Wiklander OP, Nordin JZ, O'Loughlin A, *et al*. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J Extracell Vesicles*. 2015;4(1):26316.

doi: 10.3402/jev.v4.26316

27. Smyth T, Kullberg M, Malik N, *et al*. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. *J Control Release*. 2015;199:145-55.

doi: 10.1016/j.jconrel.2014.12.013

28. Liu XQ, Sheng R. The Role of Exosomes as Endogenous Nanocarriers for Targeted Drug Delivery: Isolation, Engineering, and Clinical Progress in Neurological and Other Diseases. *J Integr Neurosci*. 2025;24(12):47443.

doi: 10.31083/JIN47443

29. Zhang K, Zhao X, Chen X, *et al*. Enhanced therapeutic effects of mesenchymal stem cell-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl Mater Interfaces*. 2018;10(36):30081-30091.

doi: 10.1021/acsami.8b08449

30. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol*. 2013;200(4):373-83.

doi: 10.1083/jcb.201211138

31. Yu J, Sane S, Kim JE, *et al*. Biogenesis and delivery of extracellular vesicles: harnessing the power of EVs for diagnostics and therapeutics. *Front Mol Biosci*. 2023;10:1330400.

doi: 10.3389/fmolb.2023.1330400

32. Liu Q, Huang J, Xia J, Liang Y, Li G. Tracking tools of extracellular vesicles for biomedical research. *Front Bioeng Biotechnol*. 2022;10:943712.

doi: 10.3389/fbioe.2022.943712

33. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev*. 2016;106:148-156.

doi: 10.1016/j.addr.2016.02.006

34. Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells*. 2019;8(7).

doi: 10.3390/cells8070727

35. Armstrong JP, Holme MN, Stevens MM. Re-engineering extracellular vesicles as smart nanoscale therapeutics. *ACS Nano*. 2017;11(1):69-83.

doi: 10.1021/acsnano.6b07607

36. Liang Y, Lehrich BM, Zheng S, Lu M. Emerging methods in biomarker identification for extracellular vesicle-based liquid biopsy. *J Extracell Vesicles*. 2021;10(7):e12090.

doi: 10.1002/jev2.12090

37. Nguyen TH, Duong CM, Nguyen X-H, Than UTT. Mesenchymal stem cell-derived extracellular vesicles for osteoarthritis treatment: extracellular matrix protection, chondrocyte and osteocyte physiology, pain and inflammation management. *Cells*. 2021;10(11):2887.

doi: 10.3390/cells10112887

38. Mirsky NA, Ehlen QT, Greenfield JA, *et al*. Three-Dimensional Bioprinting: A Comprehensive Review for Applications in Tissue Engineering and Regenerative Medicine. *Bioengineering*. 2024;11(8).

doi: 10.3390/bioengineering11080777

39. Ozbolat IT, Hospodiuk M. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials*. 2016;76:321-43.

doi: 10.1016/j.biomaterials.2015.10.076

40. Highley CB, Rodell CB, Burdick JA. Direct 3D Printing of Shear-Thinning Hydrogels into Self-Healing Hydrogels. *Adv Mater*. 2015;27(34):5075-5079.

41. Gaharwar AK, Peppas NA, Khademhosseini A. Nanocomposite hydrogels for biomedical applications. *Biotechnol Bioeng*. 2014;111(3):441-53.

doi: 10.1002/bit.25160

42. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. *Prog Polym Sci*. 2012;37(1):106-126.

doi: 10.1016/j.progpolymsci.2011.06.003

43. Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. *Eur J Pharm Biopharm*.

2008;68(1):34-45.

doi: 10.1016/j.ejpb.2007.02.025

44. Stuart MA, Huck WT, Genzer J, *et al*. Emerging applications of stimuli-responsive polymer materials. *Nat Mater*. 2010;9(2):101-113.

doi: 10.1038/nmat2614

45. Wu Z, Shi G, Li L, *et al*. Recent advances in smart responsive hydrogel microspheres for tissue regeneration: preparation, characteristics and applications. *Mater Horiz*. 2025;12(21):8943-8988.

doi: 10.1039/d5mh01020a

46. Miao S, Castro N, Nowicki M, *et al*. 4D printing of polymeric materials for tissue and organ regeneration. *Mater Today*. 2017;20(10):577-591.

doi: 10.1016/j.mattod.2017.06.005

47. Gladman AS, Matsumoto EA, Nuzzo RG, Mahadevan L, Lewis JA. Biomimetic 4D printing. *Nat Mater*. 2016;15(4):413-418.

doi: 10.1038/nmat4544

48. Suvarna P, Likhith K, Nison M, *et al*. Responsive to revolutionary: thermoresponsive hydrogels pioneering drug delivery and tissue engineering. *Mater Des*. 2026;266:115973.

doi: 10.1016/j.matdes.2026.115973

49. Huang J, Jia S, Liang R, *et al*. Construction of organoids using bioprinting technology: a frontier exploration of cartilage repair. *J Orthop Translat*. 2025;54:37-50.

doi: 10.1016/j.jot.2025.06.020

50. Zhu J. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials*. 2010;31(17):4639-56.

doi: 10.1016/j.biomaterials.2010.02.044

51. Li G, Luo Y, Hu Z, *et al*. Recent Advances in Peptide-Functionalized Hydrogels for Bone Tissue Engineering. *ACS Biomater Sci Eng*. 2025;11(4):1970-1989.

doi: 10.1021/acsbiomaterials.4c02198

52. Zhong R, Talebian S, Mendes BB, *et al*. Hydrogels for RNA delivery. *Nat Mater*. 2023;22(7):818-831.

doi: 10.1038/s41563-023-01472-w

53. Andree L, Yang F, Brock R, Leeuwenburgh SCG. Designing biomaterials for the delivery of RNA therapeutics to stimulate bone healing. *Mater Today Bio*. 2021;10:100105.

doi: 10.1016/j.mtbio.2021.100105

54. Huang J, Xiong J, Yang L, *et al*. Cell-free exosome-laden scaffolds for tissue repair. *Nanoscale*. 2021;13(19):8740-8750.

doi: 10.1039/d1nr01314a

55. Hu W, Wang W, Chen Z, Chen Y, Wang Z. Engineered exosomes and composite biomaterials for tissue regeneration. *Theranostics*. 2024;14(5):2099-2126.

doi: 10.7150/thno.93088

56. Lou W, Qiu X, Qin Y, *et al*. 3D-printed advanced scaffold armed with exosomes derived from human skeletal stem cell identified by single-cell RNA sequencing enhances osteochondral regeneration. *Bioact Mater*. 2025;51:231-256.

doi: 10.1016/j.bioactmat.2025.04.028

57. Hosseini Karimi SN, Sarisozen B, Oguz F, Sokullu E. Bioprinting of Exosomes. *Biomater Sci*. 2026;14(9):2154-2171.

doi: 10.1039/d5bm01754h

58. Quinteira R, Gimondi S, Melica ME, *et al*. 3D bioprinting meets nanotherapeutics: a vehicle for sustained extracellular vesicle delivery. *Biomaterials*. 2026;328:123851.

doi: 10.1016/j.biomaterials.2025.123851

59. Selvam S, Midhun BT, Bhowmick T, Chandru A. Bioprinting of exosomes: Prospects and challenges for clinical applications. *Int J Bioprint*. 2023;9(2):690.

doi: 10.18063/ijb.690

60. Witwer KW, Théry C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J Extracellular Vesicle*. 2019;8(1).

doi: 10.1080/20013078.2019.1648167

61. Zhang J, Tian X, Li Y, *et al*. Stem cell-derived exosomes: a comprehensive review of biomedical applications, challenges, and future directions. *Int. J. Nanomed*. 2025:10857-10905.

doi: 10.2147/IJN.S527137

62. Zhang Y, Zhao L, Li Y, *et al*. Advanced extracellular vesicle bioinformatic nanomaterials: from enrichment, decoding to clinical diagnostics. *J Nanobiotechnology*. 2023;21(1):366.

doi: 10.1186/s12951-023-02127-3

63. Moradi A, Khosravi N, Khanyile BS, Sharifianjazi F, Sorkhabi M. 3D Bioprinting Bioinks: an up-to-date overview. *J Compos Compd*. 2024;6(20).

doi: 10.61186/jcc.6.3.3

64. Dutta SD, An JM, Hexiu J, *et al*. 3D bioprinting of engineered exosomes secreted from M2-polarized macrophages through immunomodulatory biomaterial promotes in vivo wound healing and angiogenesis. *Bioact Mater*. 2025;45:345-362.

doi: 10.1016/j.bioactmat.2024.11.026

65. Shi Y, Chen L, Wang Y, *et al*. 3D-bioprinted hydrogel with MALAT1 exosomes for diabetic wound healing. *J Radiat Res Appl Sci*. 2025;18(4):101949.

doi: 10.1016/j.jrras.2025.101949

66. Zhu H, Cheng K, Tian M, *et al*. 3D-printed scaffold loaded with baicalin exosomes promotes bone defect repair via mediating PRRX2 to alleviate inflammation. *iScience*. 2026;29(1).

doi: 10.1016/j.isci.2025.113565 External Link

67. Luo L, Zheng W, Li J, *et al*. 3D-Printed Titanium Trabecular Scaffolds with Sustained Release of Hypoxia-Induced Exosomes for Dual-Mimetic Bone Regeneration. *Adv Sci*. 2025;12(23):e2500599.

doi: 10.1002/advs.202500599

68. Zhang L, Chen G, Pang J, *et al*. 3D bioprinted NSC-EVs hydrogel scaffold promotes sustained functional repair via autophagy activation after TBI in mice. *Chem Eng J*. 2025:167836.

doi: 10.1016/j.cej.2025.167836

69. Zhang A, Sun B, Nan C, *et al*. Effects of 3D-printed exosome-functionalized brain acellular matrix hydrogel on neuroinflammation in rats following cerebral hemorrhage. *Stem Cell Res Ther*. 2025;16(1):196.

doi: 10.1186/s13287-025-04332-3

70. Sun T, Huang L, Sun Q, *et al*. 3D-bioprinted mesenchymal stem cell-derived exosomes reprogram macrophages via PI3K/AKT to accelerate nerve regeneration. *J Transl Med*. 2026;24(1).

doi: 10.1186/s12967-026-07845-4

71. Hashemi A, Ezati M, Zumberg I, *et al*. In vitro evaluation of 3D-printed conductive chitosan-polyaniline scaffolds with exosome release for enhanced angiogenesis and cardiomyocyte protection. *RSC Adv*. 2025;15(21):16826-16844.

doi: 10.1039/d5ra02940f

72. Xu Z, Hong W, Mo Y, *et al*. Stem cells derived exosome laden oxygen generating hydrogel composites with good electrical conductivity for the tissue-repairing process of post-myocardial infarction. *J Nanobiotechnol*. 2025;23(1):213.

doi: 10.1186/s12951-025-03289-y

73. Enayati M, Riedmüller C, Rohringer S, *et al*. 3D-printed cardiac patch coated with human extracellular matrix hydrogel: Significant improvement of cell adhesion and immune reaction. *Int J Bioprint*. 2025;11(1):532.

doi: 10.36922/ijb.6383

74. Zheng Y, Pan C, Xu P, Liu K. Hydrogel-mediated extracellular vesicles for enhanced wound healing: the latest progress, and their prospects for 3D bioprinting. *J Nanobiotechnol*. 2024;22(1):57.

doi: 10.1186/s12951-024-02315-9

75. Consumer Alert on Regenerative Medicine Products Including Stem Cells and Exosomes. Food and Drug Administration. Accessed May 25, 2026. https://www.fda.gov/vaccines-blood-biologics/consumers-biologics/consumer-alert-regenerative-medicine-products-including-stem-cells-and-exosomes

76. Public Safety Notification on Exosome Products. Food and Drug Administration. Accessed May 25, 2026. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products

77. Frequently Asked Questions About Combination Products. Food and Drug Administration. Accessed May 25, 2026. https://www.fda.gov/combination-products/about-combination-products/frequently-asked-questions-about-combination-products

78. RFD Process. Food and Drug Administration. Accessed May 25, 2026. https://www.fda.gov/combination-products/rfd-process

79. Clinical Trials Information System. European Medicines Agency. Accessed May 25, 2026. https://www.ema.europa.eu/en/human-regulatory-overview/research-development/clinical-trials-human-medicines/clinical-trials-information-system?utm_source=chatgpt.com

80. Advanced therapy medicinal products: Overview. European Medicines Agency. Accessed May 25, 2026. https://www.ema.europa.eu/en/human-regulatory-overview/advanced-therapy-medicinal-products-overview

81. Advanced therapies: marketing authorisation. European Medicines Agency. Accessed May 25, 2026. https://www.ema.europa.eu/en/human-regulatory-overview/marketing-authorisation/advanced-therapies-marketing-authorisation?utm_source=chatgpt.com

82. Scientific recommendations on classification of advanced therapy medicinal products. European Medicines Agency. Accessed May 25, 2026. https://www.ema.europa.eu/en/human-regulatory-overview/marketing-authorisation/advanced-therapies-marketing-authorisation/scientific-recommendations-classification-advanced-therapy-medicinal-products

83. 2023 Drug Evaluation Report. National Medical Products Administration. Accessed May 25, 2026. https://www.nmpa.gov.cn/directory/web/nmpa/images/1707039824076019627.pdf [In Chinese]

84. Mancha Sánchez E, Gómez-Blanco JC, López Nieto E, *et al*. Hydrogels for bioprinting: A systematic review of hydrogels synthesis, bioprinting parameters, and bioprinted structures behavior. *Front Bioeng Biotechnol*. 2020;8:776.

doi: 10.3389/fbioe.2020.00776