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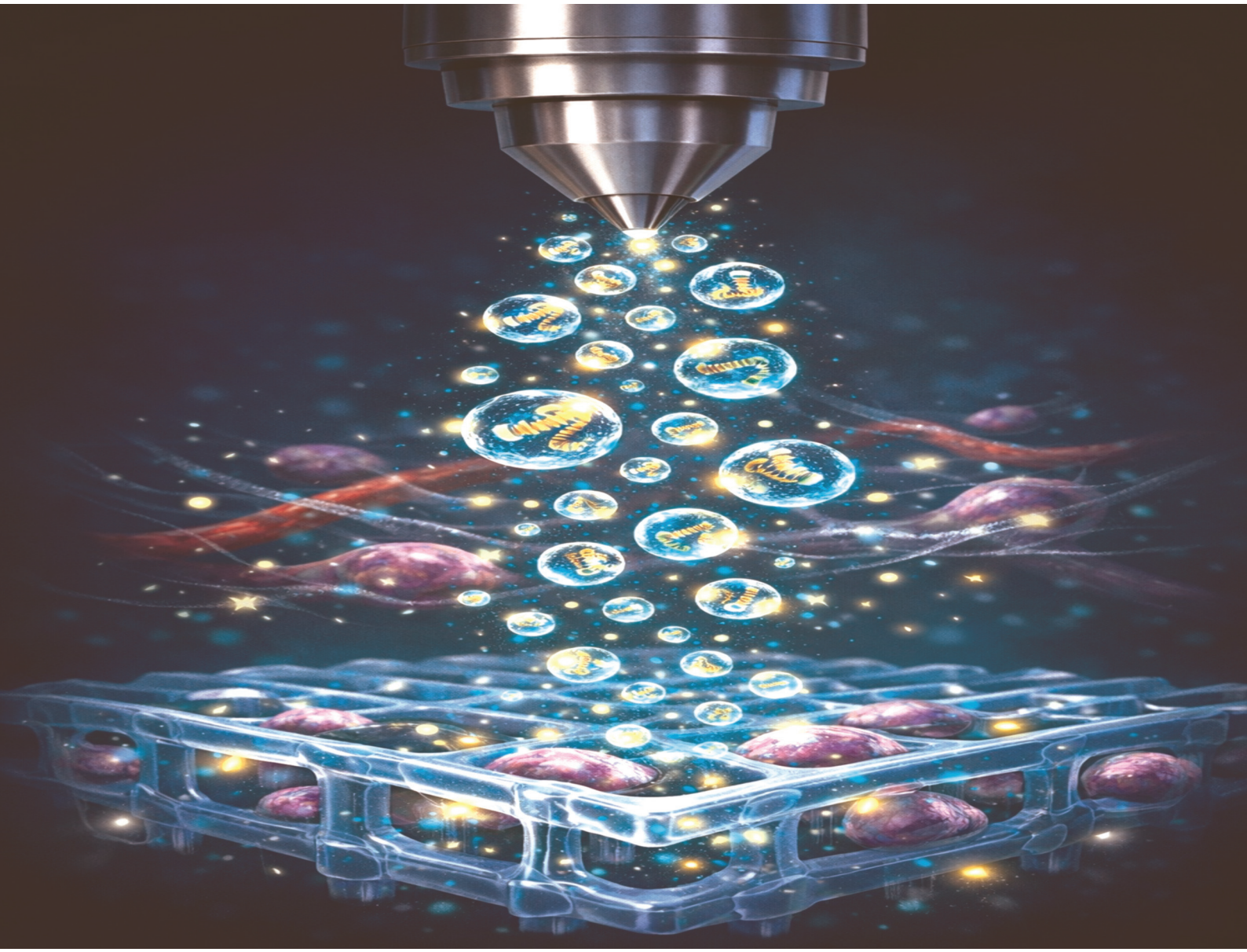

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REVIEW

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Bioprinting of Exosomes

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Exosomes (EXOs) help cells communicate by transporting proteins, lipids, and RNA. EXOs are being studied for diagnosis and treatment in oncology, regenerative medicine, and infectious diseases. EXOs' research is limited because isolating high-purity EXOs in sufficient quantities impairs their therapeutic value. Bioprinting, which uses 3D printing to produce complex living objects, opens new avenues for medical research. 3D bioprinting can overcome limitations in EXO research. Bioprinting-customized microenvironments can imitate the natural biological setting, increasing the production of EXOs and their research. Bioprinting has enabled the development of precise, reproducible 3D models to study EXOs' dynamics in a controlled setting. In three dimensions, bioprinted tissues can be used to study how EXOs affect cell-to-cell communication and disease progression. Additionally, bioprinted tissue models are essential for EXO-based therapeutic safety and efficacy testing. EXO bioprinting advances EXO isolation and application, clarifying their functions and therapeutic potential. Scientists enhance the scalability and precision of EXO production *via* bioprinting. By improving individualized EXO therapies, sophisticated printed models could revolutionize personalized medicine. Bioprinting technology is projected to revolutionize EXO modification and application, revolutionizing disease treatment and regenerative medicine. Overall, an emerging area of inquiry with great potential for cutting-edge biomedical research is presented, offering hope for more efficient therapeutic approaches leveraging Exos' biological processes.

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1. Introduction

Exosomes (EXOs), nanometric extracellular vesicles with several exciting potential applications, have attracted significant interest in regenerative medicine. Originating from the endosomal pathway, these membranes can contain a broad spectrum of bioactive compounds with proteins, nucleic acids, and lipids.[1] EXOs are vital since they affect different physiological mechanisms like immune regulation,[2,3] tissue repair,[4,5] cell cycle,[6,7] angiogenesis,[8,9] and cell migration.[10,11] EXOs have limitations in targeted delivery, stability, and scalability, despite their great therapeutic potential, thereby impairing their optimal use in tissue regeneration.[12,13] The challenges include limited cargo capacity, rapid blood clearance, and the need for accurate delivery to the target site. Such adverse effects highlight the need for creative ideas to improve the effectiveness of EXO-based treatments. One interesting approach for managing these problems is to combine EXOs with 3D bioprinting methods.[14,15] Integrating EXOs with 3D bioprinting techniques is a promising solution to addressing these challenges.[16] 3D bioprinting enables the precise spatial arrangement of cells and biomaterials, creating complex tissue structures that mimic the native tissue environment.[17–19] This technology offers fine spatiotemporal control and large submicron-scale resolution, which can be leveraged to implement directional gradient release of single or multiple biomimetic cues, including cell-derived EXOs. 3D bioprinting enables the precise spatial arrangement of cells and biomaterials, thereby forming complex tissue structures that mimic the native tissue environment.[20,21] 3D bioprinting empowers the development of scaffolds that can remodel the architectural, biochemical, and physical cues of target tissues. These scaffolds can be designed to incorporate EXOs directly within their structure or be functionalized with EXO-laden hydrogels that serve as localized delivery systems.[22,23] The ability to precisely control the spatial distribution of EXOs within a bioprinted construct enhances therapeutic effects by ensuring that these bioactive molecules are released in a manner that closely mimics natural physiological processes.[24] Moreover, recent advancements have demonstrated that 3D-printed microenvironment-specific bilayer scaffolds can efficiently accelerate the simul-

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taneous regeneration of cartilage and subchondral bone tissues in preclinical models. This innovative approach highlights the potential for EXO-laden structures to serve as effective platforms for complex zonal tissue regeneration, with attractive clinical translation potential.[25,26] In addition to their role in cartilage and bone regeneration, EXO-loaded 3D-printed structures have demonstrated potential applications across diverse tissue types, including complex, soft, and neural tissues. These constructs create a biomimetic environment that facilitates cell proliferation, differentiation, and tissue remodeling. In complex tissue regeneration, they promote structural integrity and expedite healing.[27,28] In soft tissue repair, they assist in controlling inflammation and facilitating vascularization.[29,30] Moreover, EXO-functionalized bioprinted scaffolds enhance neuroprotection and neuronal connectivity during neural regeneration, presenting novel opportunities for treating nerve injuries.[31] This adaptable method highlights the possibility of combining EXOs with 3D bioprinting to enhance regenerative medicine across several fields. In the following sections, we will thoroughly review the current literature on EXO-loaded bioprinted structures, emphasizing their applications and therapeutic potential. Initially, we will explore the importance of integrating EXOs into bioprinted constructs and the benefits of this integration in improving regenerative mechanisms.

2. Current 3D bioprinting methods in tissue engineering

This section will focus on the basic techniques involved in bioprinting, which are high-tech methods used to create three-dimensional structures of living cells and biomaterials. Bioprinting techniques, such as inkjet, extrusion-based, and laser-assisted printing, use different mechanisms to accumulate biological materials in layers. Such methods enable accurate placement of cells and their composition, thereby enabling the construction of complex tissue structures for regenerative medicine, drug testing, and targeted therapy.[31,32] Varying these techniques can offer additional possibilities, such as EXO bioprinting, which could change the landscape of tissue engineering and biomedical research. Fig. 1 shows a brief schematic of bioprinting methods.

2.1. Micro-extrusion-based bioprinting

Micro-extrusion-based bioprinting is widely accepted in the area. In producing three-dimensional constructs, bio-ink is continuously extruded through a nozzle. The bioink used in most applications is composed of hydrogels, as well as other materials with the required mechanical properties and biocompatibility. This approach produces three-dimensional con-

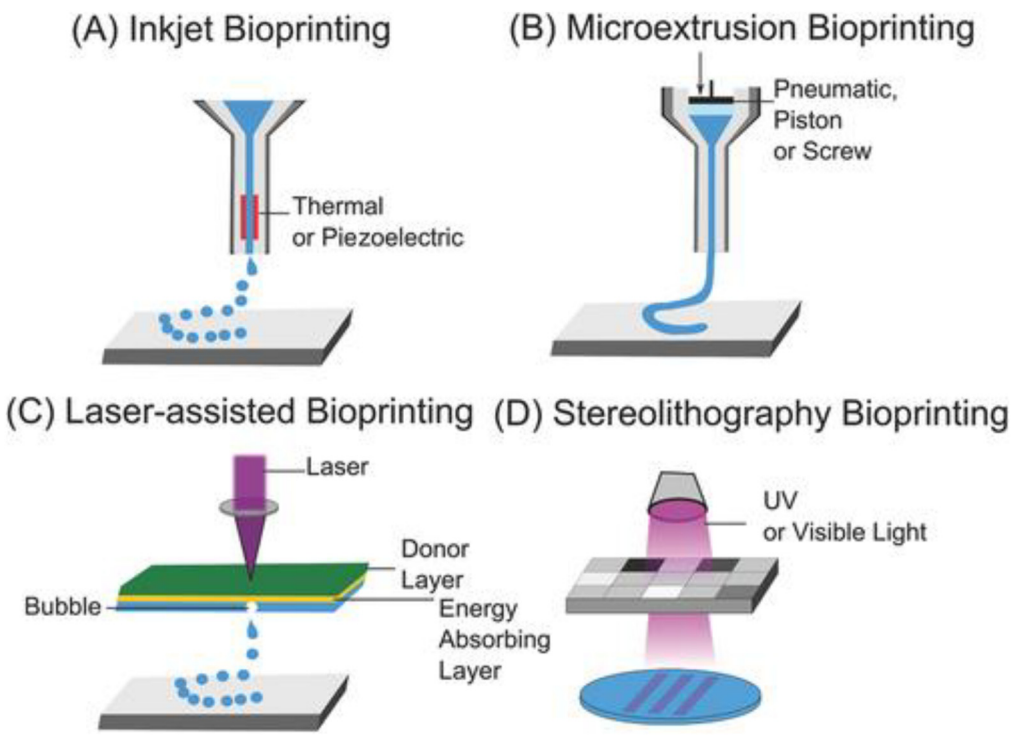

Fig. 1 Examples of different bioprinting methods: (A) inkjet bioprinters deposit minute droplets of the hydrogel and cells to construct tissue layer-by-layer. (B) Microextrusion bioprinters dispense a cell-embedded liquid solution under pneumatic or manual pressure. (C) Laser-assisted bioprinting employs a laser to rapidly heat a donor layer (green), creating a bubble that propels the bio-ink onto the substrate. (D) Stereolithography bioprinters utilize UV or visible light to cross-link bioinks layer-by-layer to create a 3D structure selectively. Adapted from Foyt *et al.*[33] "Exploiting Advanced Hydrogel Technologies to Address Key Challenges in Regenerative Medicine", *Advanced Healthcare Materials,* https://doi.org/10.1002/adhm.201700939, under the cc 4.0 License. Copyright 2026.

structs using a computer-based system through filamentous bioinks in an extruded form. However, such a system typically has lower resolution than inkjet or stereolithography systems, and cellular viability is also affected by shear stress, especially during printing.[34,35]

2.2. Inkjet bioprinting

Another technique of inkjet bioprinting uses a drop-on-demand method to apply bioink droplets to a surface rather than pre-coating the surface with ink. This is achieved *via* thermal or piezoelectric actuation, and such a method provides high accuracy and resolution in constructing and printing several cell types at once. However, it is constrained to low-viscosity bio-inks, which are limited in number and may encounter cell viability issues due to shear stress during droplet filling.[36]

2.3. Stereolithography bioprinting

Stereolithography (SLA) enables the fabrication of intricate structures by curing layers of photopolymerizable bioinks with light, making it suitable for many applications. One more layer of sophistication is added to SLA by using UV lasers or digital light projectors to polymerize and solidify the bioink into the required shape. This method offers high resolution and prevents intricate design, but SLA cover provision for photopolymer materials, which are often not biocompatible, must also be done. It is even more time-consuming and costly due to equipment use.[37,38]

2.4. Laser-assisted bioprinting

Laser bioprinting utilizes a concentrated laser beam to project bio-ink from the donor slide onto a substrate without direct contact. The metal layer beneath the donor slide is heated by a single light beam, causing the bio-ink droplets to disperse onto it. This technology offers higher spatial resolution and reduces mechanical stress on cells during printing, while enabling greater material versatility. Nevertheless, it requires a more complex configuration than alternative methods and incurs higher costs associated with laser technology. Direct bioprinting involves the deposition of bioinks without scaffolding materials, enabling the formation of tissue-like structures that closely resemble natural environments. This method directly prints cells or tissue aggregates into specified configurations, facilitating self-organization and extracellular matrix deposition after printing. This approach reduces the necessity for scaffold materials, enhancing integration with host tissues following transplantation. Still, precise regulation of bio-ink characteristics and environmental parameters is essential to address these difficulties. However, careful control over bio-ink properties and environmental conditions is required while facing challenges in achieving structural stability without scaffolding.[39,40]

In conclusion, the various techniques of 3D bioprinting offer unique advantages and limitations, making them suitable for different applications in tissue engineering and regenerative medicine. While microextrusion-based bioprinting remains the most widely used method due to its versatility and cost-effectiveness, advancements in inkjet, stereolithography, and laser-assisted techniques pave the way for more precise and complex tissue constructs. Continued research into these methods will be crucial for overcoming current challenges and enhancing their clinical applicability in creating functional tissues and organs.

3. Integrating bioprinting with EXO research for advanced tissue regeneration

By incorporating EXOs into bio-ink formulations used in bioprinting, a controlled and sustained release of EXOs within the printed constructs can be achieved.[41] Moreover, incorporating EXOs into the bio-ink used for 3D bioprinting goes beyond a sustained release strategy; it becomes a means to orchestrate complex cellular interactions within the printed structures. EXOs, with their cargo of bioactive molecules, act as molecular messengers, influencing neighboring cells and signaling cascades crucial for tissue regeneration.[42] The controlled deposition of EXO-laden bio-ink establishes microenvironments that promote cellular differentiation, migration, and proliferation, fostering a more robust, orchestrated regenerative response.[42] Furthermore, the combination of EXOs and 3D bioprinting offers the potential for biofabrication of customized constructs tailored to specific tissue types and therapeutic goals.[16] Researchers can modulate the concentration and distribution of EXOs within the bioink to fine-tune the regenerative potential of the printed structures. This customized technique helps create tissue constructs with improved biological functionality that meet the complex requirements of various regenerative applications. Finally, the combination of EXOs with 3D bioprinting goes beyond the typical concept of sustained release to become a dynamic interaction between bioactive molecules and the microenvironment. This revolutionary method addresses the challenges of tissue regeneration and leverages the synergistic power of both technologies to open new avenues for tailored, effective regenerative medicine. Application of bioprinting of EXO-embodied for the following sections.

3.1. Applications of bioprinting for EXO-driven joint and bone regeneration

Human mesenchymal stem cells (MSCs) produce exosomes through a complex process, which are essential for repairing and replacing damaged bone and cartilage. Optimal tissue repair is fostered by promoting many cellular processes, such as cell migration, growth, the assembly of structural components, the reduction of inflammation, and the stimulation of the release of healing proteins.[43,44] These MSC EXOs are packed with a rich assortment of proteins and RNA, which empower them to orchestrate these complex healing processes effectively. Furthermore, a growing number of studies have

examined the therapeutic effects of MSC-derived EXOs for bone tissue regeneration and their usage in experimental and clinical trials of therapeutic angiogenesis.[45]

EXOs have been shown to stimulate endothelial cell proliferation and vessel formation *via* exosomal miR-129,[46] miR-136,[47] and miR-423-5p,[46] respectively. Additionally, there is a clear possibility that additional molecules, including proteins, localized inside/on the surface of EXOs, are transported into the target cells' interiors or directly stimulate endothelial cells through EXO-to-cell communication. A progressive cartilage regeneration study by EXOs showed that, in as little as 2 weeks, they begin regenerating new tissue and repairing damaged cartilage, maximizing recruitment, reducing cell mortality, and increasing cell proliferation, all while coordinating with increased matrix production. This process is facilitated by the EXO's influence on specific genes: PCNA and FGF-2, which are associated with cell growth, and Survivin and Bcl-2, which help prevent cell death. Activation of the AKT and ERK pathways *via* adenosine production enhances cell proliferation and migration. In addition, treatment leads to a more regenerative immune environment, increasing CD163+ M2 macrophages over CD86+ M1 macrophages and decreasing pro-inflammatory IL-1β. This points to a targeted approach for regenerative therapies by manipulating these pathways and immune profiles.[48] These kinds of studies demonstrate that bioactive EXO-based biomaterials are worth developing and have a wide range of potential applications in medicine, as mentioned in the introduction. Several studies have also examined the development of 3D-printed scaffolds coated or encapsulated with EXOs.[49,50] The cell-free bio-printed hydrogels laden with EXOs can provide EXO retention at specific bone defect sites and enhance repair of the defect areas. In this way, small molecules, mRNA, and drugs can act as a load in EXOs, resulting in efficient delivery to defective sites.[51–53] The 3D bioprinted constructs, loaded with EXOs, can provide targeted drug delivery to the defective sites[53,54] The 3D bioprinted constructs, loaded with EXOs, can provide targeted drug delivery to the defective sites.[53,54] Han *et al.*'s study investigated the application of 3D bioprinted scaffolds made from gelatin methacryloyl (GelMA) and small extracellular vesicles (sEVs) sourced from human periodontal ligament cells (hPDLCs) and human gingival fibroblasts (hGFs) to promote the differentiation of human buccal fat pad-derived mesenchymal stromal cells (hBFP-MSCs).[55] The findings indicated that hBFP-MSCs cultured on GelMA scaffolds infused with hPDLC-sEVs showed improved cell adhesion, mechanotransduction, and differentiation into ligamentous, osteogenic, and cementogenic lineages compared to hGF-sEVs or GelMA alone. The findings indicate that 3D bioprinted GelMA/hPDLC-sEVs scaffolds are a promising cell-free approach for periodontal regeneration. Other studies have suggested that imine-mediated *ortho*-nitrobenzyl alcohol-based (HA-NB) photocrosslinked hyaluronic acid hydrogels can serve as effective EXO-holding systems.[56–58] Another photoinduced imine crosslinking (PIC) hydrogel glue has been used to encapsulate EXOs, creating an EXO-complex hydrogel tissue patch. The researchers showed that this tissue patch can successfully maintain EXOs at the defect site, as it readily binds to natural cartilage tissue. It has been suggested that this tissue patch, a unique cell-free companion, can be used to fully regenerate tissues and organs. Light-induced imine-crosslinked hydrogels, which have demonstrated remarkable biocompatibility, can integrate with cartilage and are convenient to use. These hydrogels might be utilized as EXO scaffolds to create tissue patches devoid of cells for cartilage regeneration. Tissue patches devoid of cells can bind EXOs produced by stem cells and favorably regulate the growth of chondrocytes and human bone marrow stem cells *in vitro*. Furthermore, a cell-free tissue patch can attach to its cartilage matrix, and promote the attachment of new cells at the defect site, and eventually aid in the repair and regeneration of cartilage defects.[59] Building on these advances, a specific strategy involves encapsulating GDF-5-preconditioned synovial MSC-derived EXOs (G-Exos) within glycyrrhizic acid/methacrylate-acylated hyaluronic acid (GA/HA) hydrogels fabricated using Digital Light Processing (DLP) bioprinting.[60] By stimulating the Kdm2a/SOX2 axis, GDF-5 preconditioning EXOs with miR-383-3p enhances chondrogenic differentiation. DLP-printed GA/HA scaffolds are sponge-like, with holes, and function well with living organisms. It uniformly releases and distributes G-EXOs over 20 days while active. *In vivo* implantation in a rat cartilage damage model promoted cartilage regeneration, GAG and COL-2 deposition, subchondral bone repair (as indicated by BV/TV and BMD), and improved the collagen architecture. This flexible platform provides structural support and biochemical signals, making it ideal for cartilage repair alone.

In addition, EXOs have been shown in many investigations to include functioning mitochondria and other components of the mitochondria;[60–62] In addition, EXOs have been demonstrated in many investigations to include functioning mitochondria and other components of the mitochondria;[60–62] Chen *et al.* examined the impact of EXOs produced from bone-marrow stem cells (BMSCs) on mitochondrial homeostasis.[61] They created a 3D scaffold printed using EXOs, GelMA, and ECM. They discovered that this design encourages the prolonged release of exons and chondrocyte migration to the defective location. The findings imply that BMSCs can repair damage caused by oxidative stress and mitochondrial dysfunction in damaged cartilage. Using the decellularized tissue-specific extracellular matrix in 3D-printed EXO-laden cell-free constructs is a recent, novel approach that has attracted significant attention. In a study, Li *et al.* present a novel approach to simultaneous cartilage and bone restoration using 3D printing of a microenvironment biomimetic scaffold based on tissue-specific dECM in association with MSC-derived EXOs. The study demonstrates that sustained release of MSC-derived EXOs greatly promotes healing of osteochondral injuries, with evidence of the scaffold's enhanced chondrogenic and osteogenic properties *in vitro*. The findings suggest that combining 3D dECM-based microenvironment-specific biomimetics encapsulated with bioactive EXOs can serve as a novel cell-free recipe for stem cell therapy, with implications for clinical

translation and the targeted regeneration of complex tissues.[26] MSC-EXOs combined with scaffold materials and bioactive factors can also play a more significant role in promoting bone differentiation; Sun *et al.* investigated the application of three-dimensional (3D) printing to fabricate porous scaffolds using bioceramic-induced macrophage EXOs (BC-Exos) for bone repair. The research indicates that 3D-printed BC-Exo scaffolds exhibit immunomodulatory properties and promote osteogenesis and angiogenesis *via* sustained EXO release. The BC-EXOs in the printed scaffolds influenced macrophage polarization and the expression of chemokines that recruit bone marrow mesenchymal stem cells (BMSCs) and endothelial cells. Scaffolds containing BC-EXOs derived from macrophages with a mixed phenotype markedly boosted the osteogenic differentiation and immunosuppressive capabilities of BMSCs. They enhanced the angiogenic activity of human umbilical vein endothelial cells *in vitro*.[62]

As it has been discussed before, EXOs have received significant attention, especially in bone tissue engineering. They utilize the proteins, lipids, and RNA in their vesicles to modulate osteoblast proliferation and function, facilitating angiogenesis, osteogenic differentiation, and matrix mineralization to support bone regeneration. Integrating EXOs with bone tissue engineering materials not only fosters an optimal environment for EXO activity but also mitigates the drawbacks of these materials, including protracted angiogenesis and communication barriers among cells. Nevertheless, EXOs have a short half-life and undergo rapid metabolism upon injection; hence, the scaffold must "save" and "retain" the EXOs. Although 3D printing and composite materials have recently emerged, offering many possibilities for EXO attachment, specific challenges may arise. It is still difficult to control EXO release using scaffolds. Although delayed release is possible, consistent release has not been achieved, and the optimal release rate for bone development remains unknown. Further difficulties arise from matching scaffold degradation rates to bone-healing cycles. Rapidly dissolving scaffolds may not entirely correct bone abnormalities; however, slowly degrading scaffolds may prevent bone regeneration (Table 1 and Fig. 2).

3.2. Applications of bioprinting for EXO-driven soft tissue regeneration

Although some human organs, like the liver, can regenerate relatively competently. For the most part, however, the regenerative capacity of human biology remains somewhat limited, especially when compared to other species. Factors contributing to these regenerative limitations are diverse, but to name a few: some tissues may not be able to regenerate rapidly because the cells forming them are highly differentiated and have limited proliferative capacity.[69,70] Other regenerative capacities may be limited due to the low vascular supply to the tissue itself.[71] Additionally, the regenerative mechanisms of human biology do not always favor functional regeneration. In many cases, the healing procedure presents fibrotic reactions, inflammation, and tissue adhesions, all of which result in permanent loss of functional tissue.[72,73] Finally, since the regenerative process is long and energy-consuming, any problem that could disrupt the energy metabolism or perfusion of the tissue can cause severe disruptions to tissue healing, as is the case with diabetes mellitus (DM).[74] Therefore, developing new ways to promote functional tissue recovery without permanent function loss is a vital goal for regenerative medicine and Bioprinting can help isolate EXOs in the target area. It can also extend the EXO's life spans, making EXOs better accompany the naturally slow-occurring tissue regeneration process. For example, the Exos@GelMA + PCL biohybrid scaffold facilitates adipose regeneration by combining polycaprolactone (PCL) structural support with adipose-derived mesenchymal stem cell (ADSC) EXOs (ADSC-Exos) to promote M2 macrophage polarization. This change in the immune system reduces the growth of fibrous tissue that is often seen with polymeric scaffolds. After 12 weeks, the adipose tissue area percentage is significantly higher (46.26%) than in controls.[75] As mentioned earlier, sufficient perfusion of the targeted tissue is critically important for functional tissue recovery, and EXOs might be useful. EXOs possess strong angiogenesis-inducing capabilities that can be further enhanced by 3-D bioprinting techniques.[8,76] For example, a study utilized a GelMA/ALG-based 3D scaffold platform laden with HUVEC-isolated EVs from different oxygen conditions (normoxia/hypoxia serum and normoxia/hypoxia with a serum-free medium). By bioprinting 10-fold tall layers of a consecutively aligned 200 μM hydrogel, researchers managed to get a structure similar to the parallel stretching of arteries and veins and home the EVs to host vascular structures.[77] Vasculature-like scaffolds laden with EVs were then transplanted subcutaneously at different positions in the dorsal muscle of mice. In immunodeficient mice, after 60 min, an extensive network of new vasculature was observed only in the EV-laden materials. More importantly, however, the newly formed vasculature infiltrated the bio-printed constructs and partially followed the parallel stretches of the gel fibers. This effect was absent with the shapeless bulk implant.[77] As expected, the serum-free hypoxia group showed the greatest pro-vasculature effect, creating a robust network of veins with some branching and connecting to larger vasculatures. However, in immunocompetent CL57/BL6 mice, the host immune reaction caused a rapid hydrogel resorption, significantly disrupting the formation of a suitable vascular network. A potential solution to immune-mediated damage to scaffolds could be using ECM-based scaffolds, which possess innate biocompatibility. For long-term conditions like diabetic foot ulcers (DFU), LncRNA-MALAT1-engineered EXOs are incorporated into hyaluronic acid/platelet-rich plasma (HA/PRP) hydrogels using 3D bioprinting. This speeds up anti-inflammatory and angiogenic actions, which boost wound healing rates (up to 82.43%).[78] Indeed, Wen *et al.* designed an inverse opal porous ECM (ioECM) scaffold that could potentially excrete EXOs. The ioECM was acquired by guiding animals' biology to create a porous ECM scaffold by surgically inserting PCL microspheres as a sacrificial template.[79] The ioECM's porosity allowed BMCSs to cover much

Table 1 Summarys of findings reported for EXO-driven joint and bone regeneration

Study type	Study model	EXO source	Bioprinting technique	Observation	Remarks	Ref.
In vivo & *in vitro*	OC defective New Zealand rabbits & hBMSCs	hIPC-derived MSCs	*In situ* formed acellular hydrogel glue tissue patch EHG through combining SC-EXO with PIC hydrogel glue	EHG tissue patch can seamlessly integrate with native cartilage and effectively retain EXOs at defect sites. This promotes cartilage repair and regeneration.	The SC-Exos encapsulating hydrogel glue tissue patch provides a novel cell-free material with great practical value for extensive tissue and organ repair.	59
Human study & *in vivo*	Human OA samples & rabbit OA model	BMSCs	Radially oriented extracellular matrix by desktop stereolithography	The design promoted prolonged release of EXOs and chondrocyte migration to the defective cartilage region.	The findings imply that BMSCs can repair damage caused by oxidative stress and mitochondrial dysfunction in damaged cartilage.	61
In vivo	OC defective SD rats	MSCs	MSC-Exo laden cell-free dECM double-network hydrogel scaffold.	Sustained release of MSC-derived Exos significantly promotes osteochondral injury healing, with evidence of enhanced chondrogenic and osteogenic properties of the scaffold *in vitro*	A combination of 3D dECM-based microenvironment-specific biomimetics encapsulated with bioactive Exos can serve as a novel cell-free recipe for stem cell therapy	26
In vitro	BMSCs & HUVECs	BC-Exos	Extruded 3D hyaluronic acid/alginate scaffold	The BC-Exos in the printed scaffolds modulated macrophages and promoted the recruitment of BMSCs and endothelial cells.	Cell-free 3D-printed scaffolds with macrophage Exos promote tissue regeneration and offer a novel approach for bone repair.	62
In vivo & *in vitro*	SD rats & ADMSCs, BMSCs	NGF-stimulated MSCs	3D-printed hierarchical porous scaffold	N-Exos enhanced the osteogenic potential of osteo-reparative cells. The N-Exos-functionalized hierarchical porous scaffold significantly induced neurovascular structure formation and innervated bone regeneration in a rat model with a distal femoral defect.	The N-Exos-functionalized hierarchical porous scaffold developed in this research holds promise as a neurovascular-promoting bone repair scaffold with potential clinical use.	63
In vivo & *in vitro*	SD rats & BMSCs	BMSCs	Oxygen-supplying composite scaffold, with the encapsulation of calcium peroxide in a polylactic acid three-dimensional (3D) printing construct (CPS)	Exos promoted the proliferation of BMSCs, alleviated inflammation, and exhibited excellent osteogenic properties.	This osteogenic functional composite scaffold provides a highly efficient solution for bone repair.	64
In vivo & *in vitro*	WJMSCs & New Zealand male white rabbits	WJMSCs	3D-printed calcium silicate (CS) scaffolds with lithium (Li)	Li-modified CS scaffolds promoted osteochondral regeneration, and paracrine Exos influenced the osteochondrogenic capabilities of the scaffolds.	The scaffold can be easily modified for tissue engineering, and further evaluation is needed to understand the mechanisms behind these surface modifications.	65
In vivo & *in vitro*	hBMSCs & RAW264.7 & CBD rabbits	Bioactive serum Exos (sEXOs)	3D-printed strontium–titanium scaffold	The SrTi Sc + BF EXO composite significantly accelerated bone repair through osteoconduction, osteoinduction, and revascularization in the radial CBD of rabbits.	This research expands the sources and biomedical applications of specifically functionalized Exos and offers a thorough and clinically viable approach for treating significant bone defects.	66
In vivo & *in vitro*	L929 & bone defective rabbit	Bioactive serum EXOs (BF EXO)	3D-printed porous zinc scaffold	Incorporating Exos improved the zinc scaffold's ability to stimulate osteogenic cell activity and reduce osteoclast activity.	This study highlights that combining degradable porous zinc scaffolds with BF Exo represents a practical, biocompatible approach for treating bone defects.	67
In vivo & *in vitro*	BMSCs & the UMRCT rabbit model	Tendon stem cell-derived EXOs (TSC-Exos)	3D-printed polycaprolactone (PCL)–based scaffold	TSC-Exos-S improved the proliferation, migration, and tenogenic differentiation of rabbit BMSCs *in vitro*.	TSC-Exos-S may be a potential strategy for repairing UMRCTs with severely retracted musculotendinous tissues and significant tendinous tissue defects.	68

MSCs, mesenchymal stem cells; BMSCs, bone marrow stem cells; GelMA, gelatin methacryloyl; HUVEC, human umbilical vein endothelial cells; SC-Exos, stem cell EXOs; dECM, decellularized extracellular matrix; OC, osteochondral; SD, Sprague-Dawley; OA, osteoarthritis; VJMSC, Wharton's jelly mesenchymal stromal cells; N-Exos, the NGF-stimulated MSC-derived EXO.

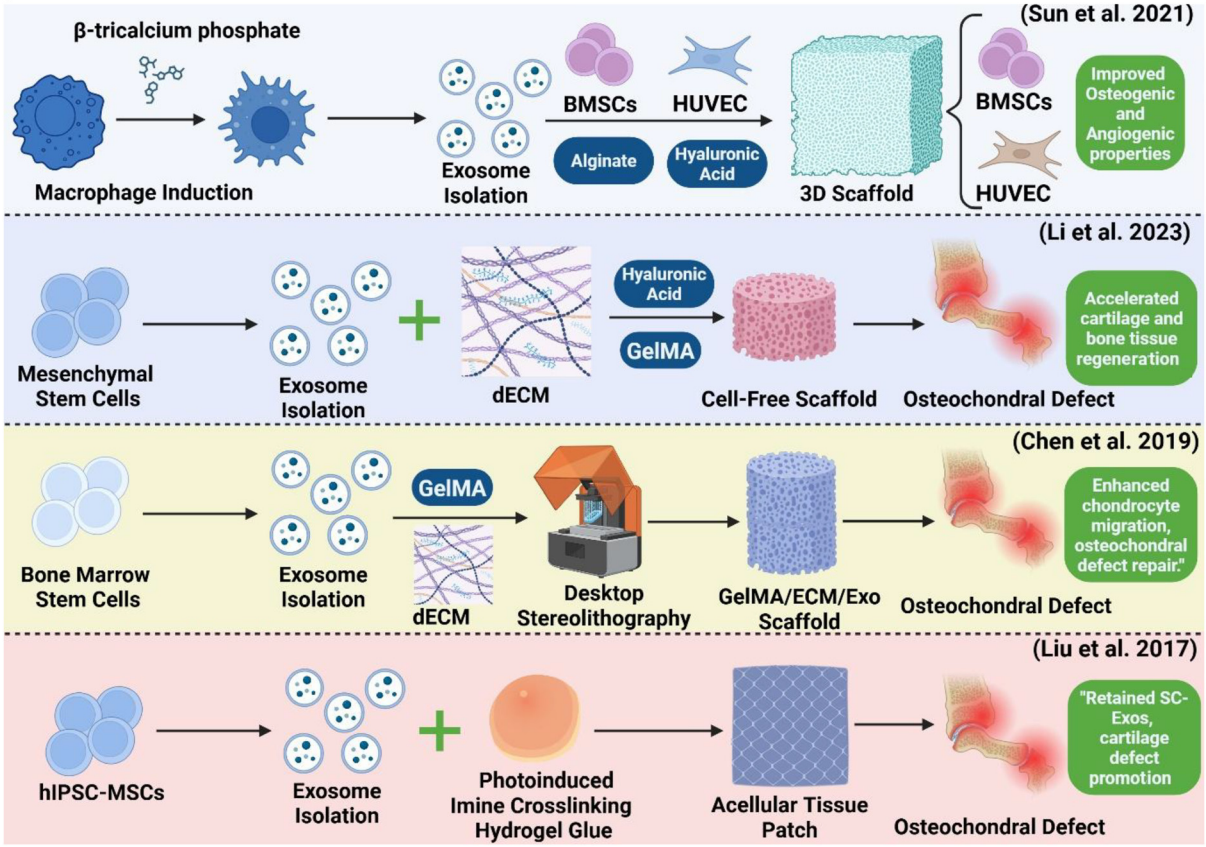

Fig. 2 Use of Bio-printing in EXO-driven joint/bone regeneration: EXO-based scaffolding strategies for promoting osteochondral defect repair. Conceptual diagram summarizing recent joint/bone tissue engineering strategies. EXOs purified from macrophages, mesenchymal stem cells, and bone marrow stem cells are integrated into different scaffolds, including 3D scaffolds made from alginate and hyaluronic acid, cell-free gelatin methacryloyl/decellularized extracellular matrix scaffolds, and 3D-printed radially oriented gelatin methacryloyl scaffolds. These scaffolds promote osteogenic and angiogenic properties, accelerate tissue regeneration, enhance chondrocyte migration, and effectively repair osteochondral defects. Additionally, an acellular tissue patch combined with stem cell-derived EXOs and a photoinduced imine crosslinking hydrogel glue promotes cartilage repair, highlighting the versatility of EXO-based therapies (MSCs, mesenchymal stem cells; BMSCs, bone marrow stem cells; GelMA, gelatin methacryloyl; dECM, decellularized extracellular matrix; HUVEC, human umbilical vein endothelial cells; SC-Exo, stem cell EXOs). The schemes are illustrated based on the studies by Sun *et al.*,[62] Li *et al.*,[26] Chen *et al.*[61] and Liu *et al.*[59] Created with Biorender. Copyright 2026.

more area and live longer than non-porous control scaffolds. Additionally, the ioEMC scaffold-assisted delivery of BMCS was applied to mice with critical hindlimb ischemia. The BMSC-laden ioEMC scaffold significantly slowed necrosis rates and allowed most mice to retain their hindlimbs.[79] Finally, using ioEMC allowed BMCS to promote the strongest blood vessel formation and muscle tissue regeneration effect compared to saline, cell therapy-only, and control scaffolds. However, muscle tissues are relatively well-vascularized, making the patches applied to muscles relatively easier to perfuse. However, ease of perfusability is an exception rather than a rule. So, it is safe to say that perfusing the patch and ensuring the survival of EXOs and cells remains a huge challenge for most applications. Additionally, materials engineered from biological inks such as gelatin and alginate are susceptible to excess shear stress, which can severely affect the viability of cells inside the constructs. One potential solution could be the augmentation of EXOs with cell survival factors, which was the case with the study by Barr *et al*. In the study, macrophage-derived EVs were engineered to steadily secrete miR-199a-3p, an important miRNA for cell survival, with the EVs incorporated into an extrusion-printed Alginate 3D cardiac patch. *In vitro* studies revealed that engineered EVs allowed neonatal cardiomyocytes encapsulated inside the patch to survive, proliferate and present an angiogenic potential significantly more prominent than no-EV controls.[80] Sustaining cell viability becomes even more challenging when targeted tissue presents with large ischemic areas and ischemic heart diseases, which is a prototypical example. Other than the perfusion issue, cardiac patches should be able to adapt to the heart's unique contractile and electrical functions. Yang *et al.* developed a biomaterial-free, "net mold patch" cardiac tissue from IPSC-derived cardiomyocytes, human cardiac fibroblasts, and HUVECs. The patch showed real heart tissue-like electrical integration and could contract synchronously.[81] Additionally, compared to a single cardiomyocyte layer, the net mold patch tissue was significantly more vascularized, an observation partly attributed to higher EV expression in the mold patch.[81]

Finally, the net mold patch successfully sustained cardiac function with higher ejection fraction and lower-end systolic and diastolic diameter in LAD ligation MI model.[81] The use of biomaterial-free patches allowed a significant level of cell viability. However, the patch size was limited, and further research is required to develop material-free patches in clinical size. Another team of researchers successfully engineered a clinically relevant cardiac patch that is large enough to apply to larger mammals.[82] This was partly achieved by making patches more resistant to hypoxia with better perfusion through co-culturing hiPSC-derived CMs with endothelial cells and smooth muscle cells. Then, the cell mixture was seeded into a 3D scaffolding and placed into a dynamic rocking platform for mechanical stimulation. Finally, the end-product patches were transplanted into the LAD occlusion-induced infarcted heart of female pigs after 60 minutes of occlusion. 4 weeks after the surgery, hCMPs were able to successfully improve left ventricle end-systolic volume, ejection fraction of the left ventricle and systolic pressure, and prevent cardiac remodeling when compared to "blank" patches and the MI only group.[82] Finally, the hCMPs were reported to be able to secrete EXOs that boost ECs' angiogenic activity, protect CMs from hypoxic damage, and encourage CM proliferation and cell-cycle progression.[82] The results gathered hold great promise for many patients suffering from post-infarct consequences of MI. However, further delaying the patch intervention may reflect a real-life MI scenario as inevitably considerable time is lost between onset of symptoms to definitive intervention in most of the MI cases. Just like the heart construct, the tracheal construct for trachea repair has issues with permutability, an issue that becomes worse because the natural trachea has limited vascular supply.[71,83] Shen *et al.* proposed a solution by fabricating a cell-free, endothelial precursor cell-EXO loaded GelMA/PCL tissue-engineered trachea (TET) to enable perfusion through the graft.[83] In a chorioallantoic membrane model of segmental tracheal deficits, as early as two days after implantation, the PCL/GelMA-Exos group showed profuse microvascular development around and on the surface of the implants, in contrast to the PCL scaffold group with only some vascularization around the perimeter of the scaffold. The vascularization effect seen on the 2nd day was much more prominent in the 2-month post-op, with a vivid red luminal tracheal wall that allows unobstructed airflow and exhibits more cellular adherence and proliferation than the dead, colorless mucous structure observed in the PCL-only group.[83] Other than the perfusion issue, just like its biological counterpart, TETs require a functioning epithelial layer, as without epithelia, TET recipient animals face the risk of severe complications.[84] Zhang *et al.* addressed this epithelization issue by fabricating a 3D double-layered trachea by loading multipotent tracheal basal cells (TBCs) into the innermost layer and loading 3T3-J2 cells into the outer layer.[85] By bringing two cell lines together, which was normally impossible since they require different culturing protocols, inner TBCs could benefit from secreted EXOs of 3T3-J2 and proliferate and cover the graft area in about 4 weeks. Although it was much faster than the 3T3-J2 control group, it was still slower than expected by the researcher. Still, the TET double-layered trachea stayed unobstructed for 6 months post-op, and the recipients showed significantly fewer symptoms.[85] Another scenario where re-epithelization could be impacted is the microvasculopathic conditions seen in DM's hyperglycemic conditions. Elevated blood sugar levels cause microvasculopathy, damaging small blood vessels and impairing wound blood flow.[86] This compromised circulation, coupled with inflammation triggered by the body's response to high glucose, hinders the delivery of essential nutrients, delaying the healing process. EXOs, with their angiogenic properties, have the potential to be utilized in diabetic wound healing. For example, in a study, the researchers could fabricate a wound-healing patch using cryogenic 3D printing technology.[87] Cryogenic printing produces micropores that enhance surface roughness and scaffold porosity and enable rapid 3D development by freezing the water in the extruded and lyophilized slurry. A type of decellularized ECM recognized for its multifunctional qualities was utilized for the bio-ink MBG (mesoporous bioactive glass) and SIS (small intestine submucosa). Final hydrogel scaffolds, SIS/MBG@EXOs, showed a potent capacity to release EXOs obtained from BMSCs in a biocompatible manner for a prolonged duration.[87] The cell studies demonstrated that these scaffolds significantly improved HUVECs' capacity for migration, proliferation, and angiogenic potential. Further animal investigations demonstrated that using SIS/MBG@Exos hydrogel scaffolds increased blood perfusion, decreased wound length, and promoted collagen deposition.[87] Another critical area in which EXOs can enhance bioprinting-aided cellular therapy is intra-uterine adhesions. For example, a study on rats utilized a GelMA-based scaffold in which the porosity was augmented with polyethylene oxide.[88] The scaffolds were loaded with ADSCs and showed a strong ability to increase HUVEC line migration, vessel-forming capacity, and cell proliferation. More importantly, comparison with only EXO-loaded scaffolds revealed that the scaffolds themselves can potentially increase the effective lifetime of encapsulated ADSCs or EXOs and provide protection while regulating cell activities. Finally, in the *in vivo* studies, it was observed that by decreasing the inflammatory response, encouraging cell proliferation, and enhancing neovascularization, ADSC-loaded scaffolds introduced into the wounded uterus significantly enhanced functional endometrial regeneration.[88] Furthermore, studies focusing on Tissue-Engineered Dermis (TED) demonstrate that mechanical stress applied during fabrication enhances the secretion of fibroblast-derived EXOs (HDF-Exos), which in turn promotes angiogenesis, HUVEC proliferation, migration, and tubular structure formation, a process crucial for graft survival. These applications underscore the necessity of 3D bioprinting in providing biomechanical support and a sustained delivery platform for exosomal bioactive components to reprogram the hostile regenerative microenvironment. Overall, the research on EXO-loaded scaffolds and cell-based therapies presents promising avenues for advancing regenerative medicine in treating cardiovascular

Table 2 Summary of findings reported for EXO-driven soft tissue regeneration

Study type	Study model	EXO source	Bioprinting technique	Observation	Remarks	Ref.
In vivo	NSG mice	HUVEC	Extruded GelMA with ten-layer-thick constructs with consecutively aligned, organized fibers for each layer.	Parallel-stretched fibers enabled precise control over the spatial localization of EVs within a 3D matrix, ultimately enhancing EVs' intrinsic angiogenic potential.	The printing technique could enhance biofabricated tissues by promoting rapid engraftment in scarred areas and facilitating quick revascularization of ischemic tissues.	77
In vitro	Neonatal rat cardiac muscle cells	THP1-derived MΦ cells	RGD-peptide modified alginate extruded with the FRESH approach.	This includes engineered EVs in bioink-yielded cardiac patches with threefold greater cell viability. After 5 days, the cardiac path exhibited elevated metabolic activities and fewer apoptotic cells.	The results could enhance 3D bioprinting outcomes, creating more viable cellular constructs that integrate effectively with the host after *in vivo* implantation.	80
In vivo	LAD-ligated Lewis nude rats	hIPCSs	Biomaterial free, net-mold patches produced by cell spheroids.	Net-mold patches enhanced function, maturation, and vascularization after implantation in infarcted rats.	The results show a significant advancement in the production of therapeutically acceptable tissue grafts by using just hiPSC-generated cells.	81
In vivo	Infarcted swine heart	hIPCSs	Fibrin scaffolded, cell suspended cardiac patch	Significant decrease in infarct size, myocardial wall stress, myocardial hypertrophy, and apoptosis in the periscar border zone of the myocardium were linked to hCMP transplantation.	Human cardiac muscle patches offer a promising future for myocardial infarction therapy, with scalable patches that reduce infarct size and improve heart function in preclinical models.	82
In vivo	Female rabbits	EPCs	Extruded GelMA/PCL scaffold	Vascular endothelial progenitor EXO/ polycaprolactone to create a new tracheal stent that successfully stimulates angiogenesis for tracheal repair has been engineered	The results imply that PCL/GelMA-EXO scaffolds hold great promise as a method for tissue-engineered trachea fast vascularization and for clinical transplantation in the future.	83
In vivo	New Zealand white rabbits	3T3-J2	Bi-lineage TET with the implantation of autologous chondrocytes on the scaffold's outer layer and TBCs on its inner layer.	A new culture strategy using EXOs from feeder cells significantly improves TBC proliferation, accelerates TET epithelialization/ciliation, and enhances early prognosis transplantation outcomes.	Future studies should focus on figuring out how 3T3-J2 generated EXOs cause TBCs to expand quickly and how to enhance the ciliated differentiation of TBCs that have been seeded.	85
In vivo	STZ-induced diabetic SD rats	BMSCs	Extrusion-based cryogenic 3D printing/SIS & MBG as bioink	SIS/MBG@Exos hydrogel scaffolds speed up diabetic wound healing by improving blood flow and stimulating the angiogenesis process.	SIS/MBG@Exos hydrogel scaffolds, presenting a promising new approach for treating diabetic wounds.	87
In vivo	Rat model of intrauterine adhesion	ADSCs	A microfluidic-based approach presents an injectable porous hydrogel scaffold with customizable shapes.	By decreasing the inflammatory response, encouraging cell proliferation, and enhancing neovascularization, ADSC-loaded scaffolds into the wounded uterus significantly boosted functional endometrial regeneration.	The findings suggest that these 3D-printed porous scaffolds show promise as stem cell delivery systems, and the proposed strategy could also be effective in other tissue repair applications.	88
In vivo & *in vitro*	32 Balb/c mice & HaCaTs & HUVECs	ADMSC Exos	3D-printed microfiber culture	3D-Exos significantly increased the proliferation and migration of HACAT and HUVEC cells. Additionally, HA-loaded 3D-Exos provided better support for burn wound healing, accelerating healing rates and improving collagen remodeling.	The results support the expanded use of high-quality, high-yield 3D EXOs going forward and introduce a novel approach for treating severe burns.	88
In vitro	HDF & HUVEC	hMSC	Methacrylated hyaluronic acid (MeHA) based 3D bioprinting	MeHA patches infused with hMSC-EXOs enhanced the proliferation, migration, angiogenic capacity, and expression of specific markers associated with wound healing in human fibroblasts and endothelial cells.	MeHA hMSC-EXO patches have the potential to be a groundbreaking and effective smart wound dressing with bioactive properties for future use, thanks to their favorable mechanical and biological traits.	88
In vivo & *in vitro*	Male BALB/c mice & HaCat	ADMSC Exos	Collagen (COL), platelet-rich plasma (PRP) based 3D scaffold	The cellular scaffold lowered inflammation levels and enhanced wound healing by facilitating cell proliferation and angiogenesis. Proteomic analysis indicated that the EXOs displayed remarkable anti-inflammatory and pro-angiogenic effects within collagen/platelet-rich plasma scaffolds.	The suggested approach offers a novel therapeutic strategy and a theoretical foundation for tissue regeneration and wound healing.	88

Table 2 *(Contd.)*

Study type	Study model	EXO source	Bioprinting technique	Observation	Remarks	Ref.
In vivo & *in vitro*	HUVECs & HDMECs	MSCs	3D-printed scaffold-perfusion bioreactor system	Perfusion bioreactor culture markedly enhanced the production of MSC EVs compared to traditional cell culture methods. Furthermore, MSC EVs produced with the perfusion bioreactor system considerably accelerated wound healing in a diabetic mouse model compared to those treated with MSC EVs derived from flask cell culture.	This research provides a promising approach for addressing a significant bottleneck in the translation of extracellular vesicles, offering the ability to be tailored for specific applications and improved alongside advancements in 3D printing technologies.	88

ADSCs, adipocyte-derived stem cells; SIS, small intestinal submucosa; MBG, mesoporous bioactive glass; EXO, EXO; BMSCs, bone marrow stem cells; SD, Sprague-Dawley; STZ, streptozotocin; TBC, tracheal basal cells; TET, tissue-engineered trachea; PCL, polycaprolactone; GelMA, gelatin-methacrylyol; EPC, endothelial progenitor cells; hCMPs, human cardiac muscle patches; hIPSCs, human induced pluripotent stem cells; MΦ, macrophage; EVs, extracellular vesicles; HUVECs, human umbilical vein endothelial cells; ADMSCs, adipose mesenchymal stem cells.

diseases, tracheal deficits, diabetic complications, and intrauterine adhesions. Further exploration and clinical translation of these strategies may lead to significant advancements in regenerative therapies for various medical conditions (Table 2 and Fig. 3).

3.3 Applications of 3D bioprinting for EXO-driven neural regeneration

The limited self-regenerative capacity of neural tissue after injury presents a significant challenge, and innovative strategies are needed to promote effective repair and regeneration. Bioprinting, combined with EXOs' therapeutic potential, offers a promising approach to address this challenge. The role of EXO in intercellular communication and its therapeutic potential for neural regeneration are increasingly recognized. EXOs contain a variety of bioactive molecules, including proteins, mRNAs, and miRNAs, which can promote nerve regeneration, neuroprotection, and neuroplasticity.[89–91] The challenge of limited self-regeneration in neural tissues following injury necessitates innovative therapeutic strategies. The convergence of bioprinting and EXO-based therapies offers a promising avenue for addressing both TBI (Traumatic Brain Injury) and SCI (Spinal Cord Injury). In the context of TBI, bioprinted scaffolds loaded with engineered EXOs can serve multiple functions to enhance recovery. These scaffolds provide structural support to the damaged brain tissue and act as carriers for the EXOs, enabling sustained release of therapeutic cargo and promoting tissue ingrowth. Specifically, neural stem cell (NSC)-derived EXOs, preconditioned with factors like interferon-gamma (IFN-γ) or insulin-like growth factor 1 (IGF-1), have shown particular promise. IFN-γ preconditioning can help modulate the inflammatory response, while IGF-1 enhances neurogenesis. EXOs, when combined with materials such as collagen and chitosan, facilitate the formation of a biocompatible and biomimetic scaffold.[92,93] These scaffolds can improve neurological function, reduce inflammation, promote nerve fiber growth, and enhance tissue remodeling at the lesion site. Studies have demonstrated that the use of 3D-printed collagen/chitosan scaffolds with IFN-γ preconditioned EXOs can significantly reduce the cavity area at the injury site, improve nerve fiber and myelin sheath regeneration, promote endogenous neuroregeneration, and reduce glial scarring. In addition, hypoxia-pretreated mesenchymal stem cell (MSC)-derived EXOs within collagen/silk fibroin scaffolds also exhibit enhanced angiogenesis, decreased apoptosis, and improved functional recovery in canine TBI models.[93,94] The use of low-temperature 3D printing is essential to preserve the bioactivity of EXOs, ensuring that their therapeutic potential is not compromised during the fabrication process. Furthermore, BDNF-stimulated human umbilical cord MSC-derived EXOs, combined with collagen/chitosan scaffolds, can promote neural network repair in TBI (Fig. 4).[95] These approaches highlight the potential of combined EXO and bioprinting therapies to mitigate the complex pathological processes associated with TBI. In another TBI study on murine models, AG@NSC-EV (neural stem cell-derived extracellular vesicles composited in

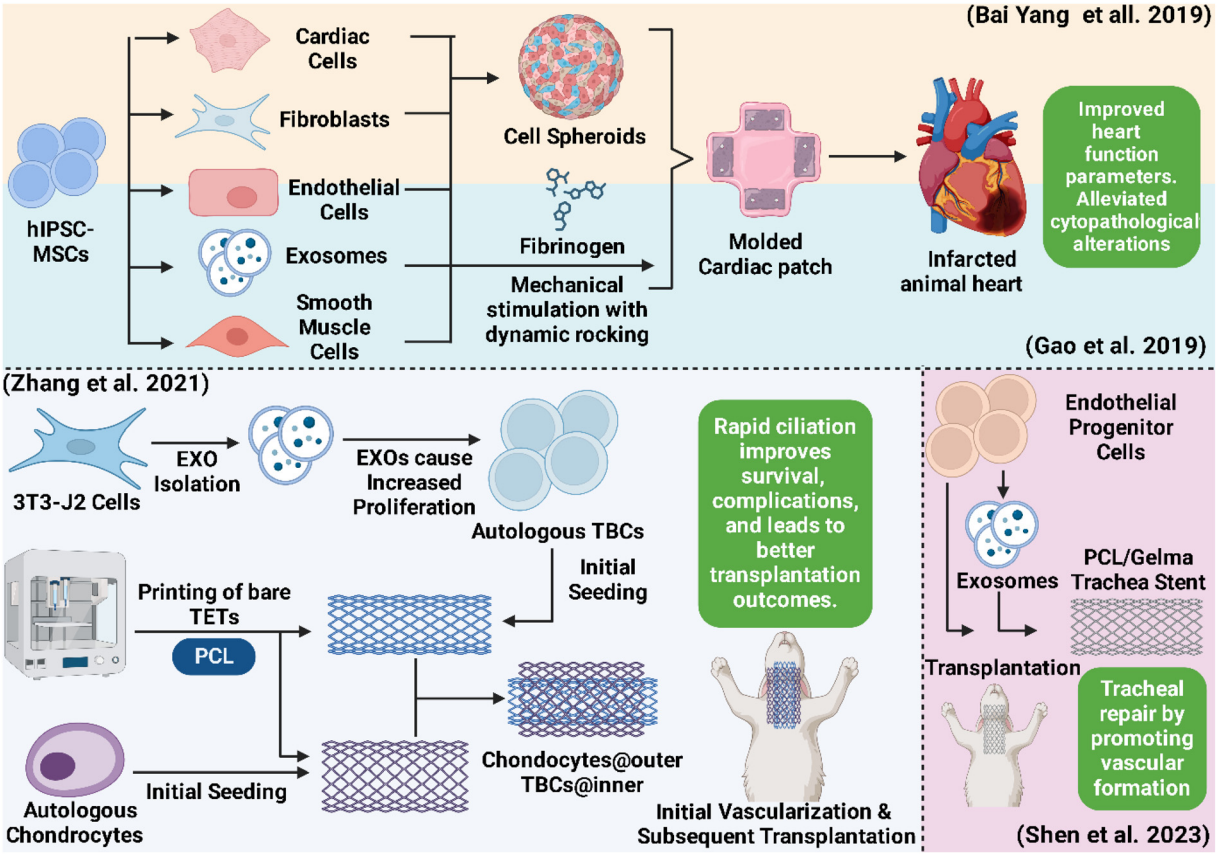

Fig. 3 Applications of bioprinting in EXO-driven soft tissue regeneration. Conceptual diagram summarizing recent soft tissue engineering strategies. The EXOs derived from cardiac, endothelial progenitor, and other cell types are combined with various scaffolds, such as PCL/Gelma tracheal stents and cardiac patches, to promote vascularization, proliferation, and effective tissue repair. These approaches leverage cell differentiation, mechanical stimulation, and EXO-mediated signaling to accelerate regeneration and improve functional outcomes in preclinical transplantation models. (EXO, exosomes; TBCs, tracheal basal cells; PCL, polycaprolactone; GelMA, gelatin methacrylate; hIPSC, human induced pluripotent stem cells; MSCs, mesenchymal stem cells). The schemes are illustrated based on the studies by Bay Yang *et al.*,[81] Gao *et al.*,[82] Shen *et al.*[83] and Zhang *et al.*[85] Created with Biorender. Copyright 2026.

alginate dialdehyde-gelatin hydrogel scaffolds) exhibited mechanical properties compatible with brain tissue (approximately 600–800 Pa) and achieved sustained NSC-EV release kinetics, with approximately 80% cumulative release by day 28. These systems improve neurofunctional recovery and decrease lesion volume by activating AMPK-ULK1-mediated autophagy, which then inhibits the cGAS-STING signaling pathway (Fig. 4).[96]

In treating SCI, bioprinted scaffolds incorporating EXOs provide mechanical support and deliver therapeutic molecules to the injury site. Individualized biomimetic scaffolds, created using multimodal imaging and 3D printing, allow for a customized approach that considers the unique injury characteristics. These scaffolds are designed for sustained release, biodegradability, and biocompatibility and are often loaded with mesenchymal stem cell (MSC)-derived EXOs. These EXOs can help alleviate inflammation and reduce scar formation by targeting the deposition of extracellular matrix (ECM) molecules. Furthermore, siRNAs targeting PTEN can be introduced into EXOs to enhance neuronal repair and regeneration. These engineered EXOs can induce endogenous neuronal regeneration and enhance axonal growth through the PTEN/PI3K/AKT/mTOR signaling pathway. Additionally, the implantation of these scaffolds can bridge the severed ends, providing spatial signals that modulate the organization of neural cells and facilitate the reconstruction of neural networks.[97] The integration of plant-derived EXOs, such as those from *Lycium barbarum* L. loaded with isoliquiritigenin (ISL), into 3D-printed hydrogels has also shown promising results in promoting neural differentiation and reducing inflammation and oxidative stress (Fig. 5).[98] These findings demonstrate the potential of EXOs and bioprinting to promote nerve regeneration and restore nerve conduction in SCI. Applying EXOs in bioprinted scaffolds is a significant step forward in treating TBI and SCI by improving nerve regeneration, reducing inflammation and scar tissue, and improving functional recovery. Out of TBI and SCI, Zhang *et al.* showed that bioprinting of EXOs could overcome limitations associated with conventional treatments for Intracerebral Hemorrhage (ICH).[99] They studied the encapsulation of mesenchymal stem cell-derived EXOs

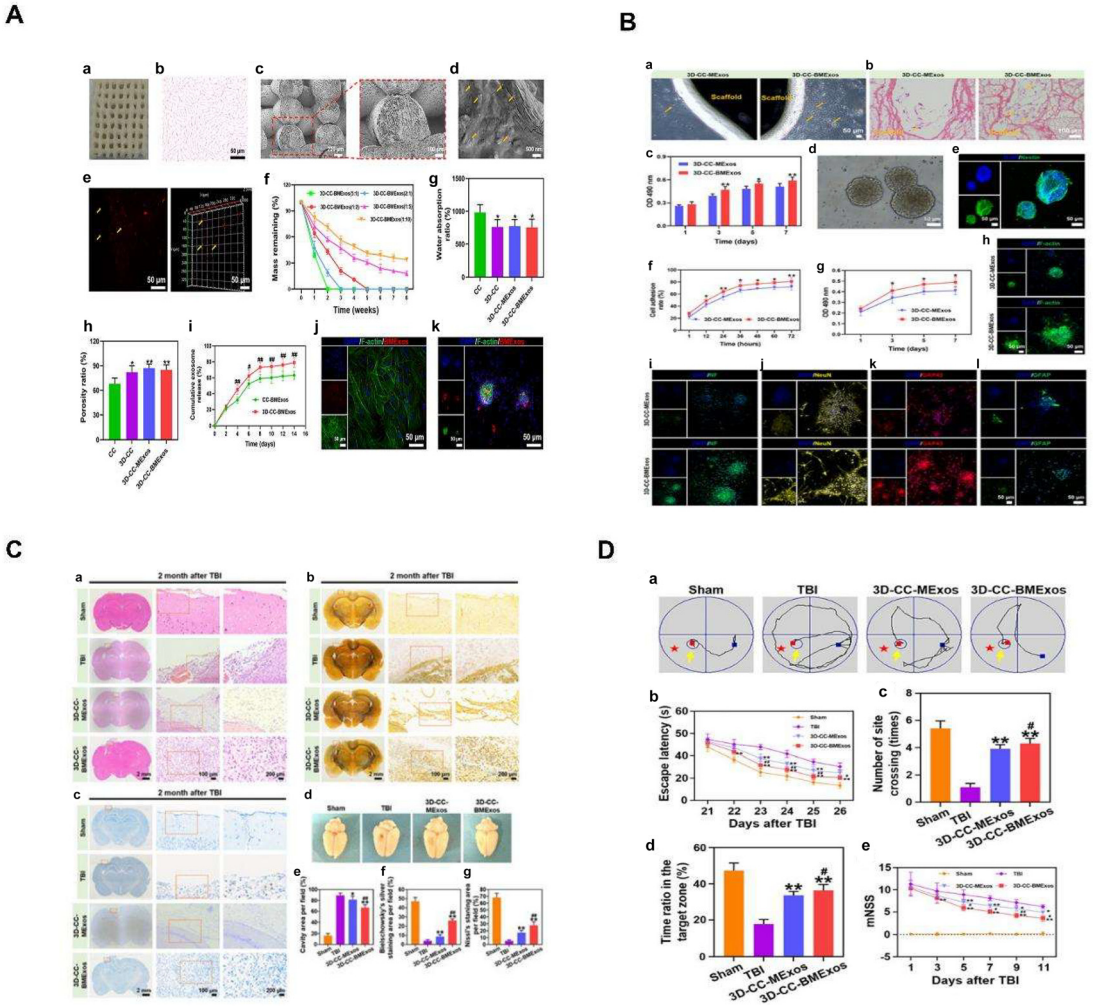

Fig. 4 Repair of the neural networks *in vivo via* BDNF stimulated MSC-derived EXOs embedded into 3D printed collagen/chitosan scaffolds. Characteristics of composite scaffolds. (A) Representative images of 3D-CC-BMExos under general observation (a), H&E staining (b), and SEM imaging (c and d). The pores of 3D-CC-BMExos were well interconnected, and BMExos were evenly distributed within the scaffold (d). (e) 3D immunofluorescence images showing the distribution of BMExos in 3D-CC-BMExos. (f) Degradation rate of 3D-CC-BMExos with different collagen/chitosan mass ratios measured at 2, 4, 6, and 8 weeks after transplantation. (g and h) Compared with CC scaffolds, 3D-printed composite scaffolds showed a lower water absorption ratio and a higher porosity ratio. (i) Cumulative release profile of BMExos from CC-BMExos and 3D-CC-BMExos over 14 days. (j and k) Representative immunofluorescence images of F-actin/PKH26-labeled BMExos in HUCMSCs (j) and NSCs (k). Biocompatibility test of scaffolds. (B) Representative images of HUCMSCs cocultured with 3D-CC-MExos or 3D-CC-BMExos under phase-contrast microscopy (a) and H&E staining (b). (c) MTT assay results of HUCMSCs cocultured with 3D-CC-MExos and 3D-CC-BMExos. (d) Optical microscopy image showing spherical morphology of NSCs. (e) Representative immunofluorescence images of NSCs stained with a nestin antibody (green). (f) Cell adhesion rates of NSCs at different time points after coculture with scaffolds. (g) MTT assay results of NSCs cocultured with 3D-CC-MExos and 3D-CC-BMExos. (h–l) Representative immunofluorescence images of NSCs stained for F-actin (h), NF (i), NeuN (j), GAP42 (k), and GFAP (l) at 7 days after coculture. The recovery of cognitive function and sensorimotor function among the four groups. (C)(a) Representative H&E-stained images of brain slices among the four groups showing histomorphological differences at 2 months post-TBI. (b) Representative Bielschowsky's silver staining images of brain slices among the four groups showing differences in nerve fiber alterations at 2 months post-TBI. (c) Representative Nissl staining images of brain slices among the four groups showing differences in neuronal cell body numbers at 2 months post-TBI. (d) General observation of the injury cavity across the four groups. (e) Quantitative measurement of the cavity area per field at the defect site. (f) Quantitative measurement of Bielschowsky's silver staining area per field at the defect site. (g) Quantitative measurement of Nissl staining area per field at the defect site. (D) (a) Representative images of the search route in the spatial learning stage, with the yellow arrow indicating the platform and the red star marking the target zone. (b) Analysis of escape latency during the spatial learning stage. (c and d) Analysis of the number of site crossings (c) and the time ratio in the target zone (d) in the spatial memory stage. (e) mNSS assessment at different time points after TBI. 3D-CC-BMExos therapy promoted histomorphological recovery at the injury site after TBI. (3D-CC-BMExos: three-dimensional collagen–chitosan composite scaffold loaded with bone marrow-derived EXOs. CC: collagen–chitosan. BMExos: bone marrow-derived EXOs. SEM: scanning electron microscopy. H&E: hematoxylin and eosin. HUCMSCs: human umbilical cord mesenchymal stem cells. NSCs: neural stem cells. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (cell viability assay). F-actin: filamentous actin. PKH26: fluorescent dye used for membrane labeling. NF: neurofilament. NeuN: neuronal nuclear protein. GAP42: growth-associated protein 42. GFAP: glial fibrillary acidic protein. TBI: traumatic brain injury. mNSS: modified neurological severity score.) Adapted from Liu *et al.*,[95] integrated printed BDNF-stimulated HUCMSC-derived EXOs/collagen/chitosan biological scaffolds with 3D printing technology promoted the remodelling of neural networks after traumatic brain injury, *Regenerative Biomaterials*, https://doi.org/10.1093/rb/rbac085, under the CC 4.0 Common license, Copyright 2026.

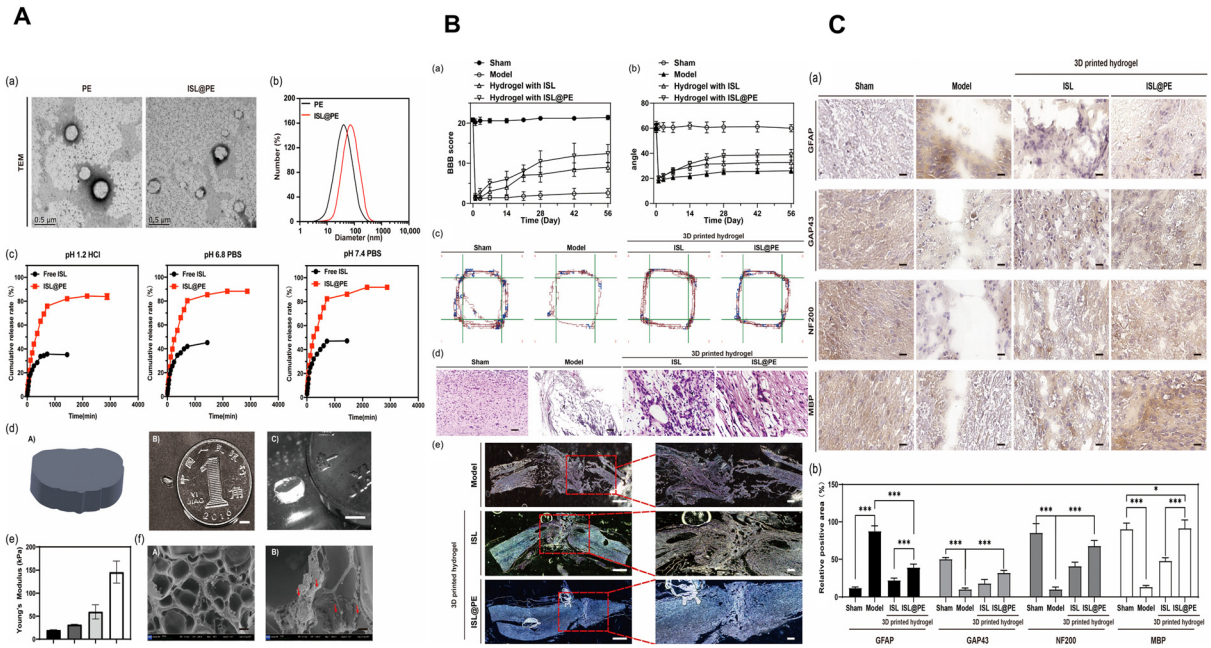

Fig. 5 Enhanced neural regeneration and restoration of the neural network after the SCI *via* plant-derived EXO embedded inside the 3D printed hydrogels: characterization of plant-derived EXOs (PE) and isoliquiritigenin (ISL)-loaded plant-derived EXOs (ISL@PE) within the hydrogel. (A) Model design of a spinal cord-mimicking segment: (a) transmission electron micrographs of PE and ISL@PE; (b) size distribution analysis of PE and ISL@PE; (c) *in vitro* cumulative release rate of ISL@PE and free ISL at pH 1.2, 6.8, and 7.4, (d) 3D-printed spinal cord hydrogel scaffold; (B) 3D-printed hydrogel scaffold (scale bar = 3 mm): (a) Basso–Beattie–Bresnahan (BBB) locomotor rating scale assessment; (b) inclined plane test; (c) open field test; (d) Nissl staining (scale bar = 50 μm); and (e) H&E staining (scale bar = 50 μm). Immune staining of GFAP, GAP43, NF200, and MBP proteins in the injured spinal cord across different treatment groups. (C) 3D-printed hydrogel scaffold (scale bar = 3 mm): (a) immunohistochemical staining of relevant protein expressions in different groups and (b) quantitative analysis of immunohistochemical staining, showing higher expression levels of GAP43, NF200, and MBP in the spinal cord treated with the 3D-printed hydrogel containing ISL@PE compared to the hydrogel containing ISL alone (scale bar = 50 μm) (PE: plant-derived EXOs. ISL: isoliquiritigenin. ISL@PE: isoliquiritigenin-loaded plant-derived EXOs. GelMA: gelatin methacryloyl. BBB: Basso–Beattie–Bresnahan (locomotor rating scale). GFAP: glial fibrillary acidic protein. GAP43: growth-associated protein 43. NF200: Neurofilament 200. MBP: myelin basic protein)). Adapted from Wang *et al.*,[98] plant-derived EXOs extracted from *Lycium barbarum* L. loaded with isoliquiritigenin promote spinal cord injury repair based on 3D printed bionic scaffolds, *Bioengineering and Translational Medicine*, https://doi.org/10.1002/btm2.10646, under the CC 4.0 Common license. Copyright 2026.

(hUCMSC-exos) within the decellularized brain matrix (dECM) composites (dECM@exo), to enable localized and sustained delivery of EXOs. The dECM@exo scaffold combined the bioactivity of dECM with the adjustable mechanics of GelMA/silk fibroin. It reduced inflammation by blocking the TLR4/NF-κB signaling pathway, lowering neuronal apoptosis, and reducing damage. These above-mentioned constructs are meant to help neurons regenerate by mimicking the natural neural microenvironment. However, regulatory and scalability issues still hinder their use in clinical settings for CNS disorders. The need for custom bioinks, which are typically composed of natural polymers and extracellular matrix components, highlights material-related issues that hinder the accurate replication of *in vivo* conditions.[100]

4. Novel approaches in EXO-driven bioprinting and the future perspectives

Combining 3D bioprinting with EXO-based therapies has considerably broadened the scope of regenerative medicine, tackling intricate issues surpassing conventional applications such as bone regeneration and vascularization. EXO-based methodologies are now being investigated for several therapeutic applications, including targeted drug delivery, cancer treatment, and control of cellular behavior. A significant work by Yerneni *et al.*[101] enhanced EXOs by functionalizing oligonucleotide tethers, facilitating the spatially regulated distribution of therapeutic agents, including small compounds and large proteins, while preserving EXO functionality. This scalable and flexible approach presents promising new prospects for personalized treatment.

Integrating bioprinting technologies into EXO-driven research offers promising opportunities to revolutionize medicine. However, several roadblocks must be overcome to realize its full potential. One of the primary challenges is the technical complexity and cost of bioprinting equipment, which can limit widespread adoption. Additionally, significant hurdles are related to the standardization and optimization of bioprinting parameters necessary to produce viable, functional tissues for EXO studies consistently. Moreover, accurate bioengineering models that mimic natural cellular environments,

essential for analyzing EXO behavior, remain a critical challenge. The fidelity of these models to human biology is vital for understanding the functional dynamics of EXOs, particularly in disease contexts.

Furthermore, EXO-driven systems have demonstrated potential in inducing targeted cell death and modulating the immune response, reinforcing their use in immunotherapy. Yerneni *et al.*[101,102] Employed an inkjet-based fabrication technique to establish durable EXO microenvironments, illustrating the capacity to control C2C12 myogenesis in a dose-dependent fashion. This spatially defined methodology enables meticulous regulation of cellular behavior, highlighting the adaptability of 3D-printed EXO-based therapeutics in modulating tissue regeneration, differentiation, and repair. These advancements underscore the significant promise of EXO-mediated bioprinting in enhancing targeted therapeutics for various medical disorders, such as cancer, tissue regeneration, and immune system modulation.

The fusion of bioprinting and EXO-driven research is expected to create a new paradigm in regenerative medicine, personalized therapies, and precision diagnostics. As these technological advances converge, 3D bioprinting is poised to become a cornerstone in the study of EXOs, enhancing the development of EXO-based therapeutics and diagnostics, by bioprinting tissues that closely replicate human tissue.

In disease states, researchers will have unprecedented opportunities to test the efficacy and safety of EXO-driven therapies in preclinical settings, thus revolutionizing the treatment of complex diseases.

5. Conclusions

In conclusion, integrating bioprinting with EXO therapies represents a significant advancement in regenerative medicine. By leveraging the capabilities of 3D bioprinting, researchers can create precise and functional tissue models that incorporate EXO-laden bioinks. These constructs enable controlled and sustained EXO release, which enhances tissue regeneration, angiogenesis, and targeted therapeutic delivery. The use of EXO-based bioprinted scaffolds shows great promise in treating a variety of hard-to-heal and poorly perfused tissues, offering improved outcomes in osteochondral, cardiac, tracheal and neuronal repairs. Despite challenges in standardization, scalability, and tissue complexity, the potential of bioprinting to revolutionize exosomal research and therapeutic applications is immense. As technological advances continue, bioprinting could become a cornerstone for personalized medicine, providing novel, effective treatment options for complex medical conditions and facilitating the translation of EXO-based therapies from the laboratory to the clinic.

Conflicts of interest

There are no conflicts to declare.

Data availability

No new datasets were generated or analysed in this study. This manuscript is a review article and relies exclusively on previously published research. All data supporting the findings are available within the cited literature listed in the References section.

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