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REVIEW ARTICLE

Exosome-based bioinks for 3D bioprinting applications in tissue engineering and regenerative medicine

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Abstract

Bioprinting is an emerging technology for tissue engineering and regenerative medicine. Despite its fast, accurate manufacture for tissues and organs *in vitro*, bioprinting has been seriously limited for biofabrication because of the restricted approaches to reproducing the extracellular matrix (ECM) with sufficient bioactivities for bioprinted cells. Exosomes are natural biological particles with proteins, lipids, or genetic materials. They have distinct properties and unique biological functions to manipulate cellular behaviors and cell fates, showing great potential to support cells for bioprinting. Here, we reviewed the recent progresses of exosome-advanced bioprinting for tissue engineering and regenerative medicine. Firstly, we offer an overview of the basics of exosomes and the current representative applications of exosomes in bone tissue engineering, immunological regulations, angiogenesis, and neural regenerations. Then, a brief introduction about the bioinks and the currently developed bioprinting methods is provided. We further give an in-depth review of the biomedical applications of bioprinting with exosomes, majorly in bone engineering, vascular engineering, therapy of neuron injury, and skin regeneration. We also conclude with an outlook on the challenges of the unmet needs of bioprinting cells with correspondent ECM environments through bioprinting with exosomes.

Keywords: Exosomes; Bioprinting; Bioinks; Regenerative medicine; Tissue engineering

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1. Introduction

Bioprinting is derived from three-dimensional (3D) printing technology. It can apply cells, proteins, DNA, and other biological materials into personalized 3D models or 3D biological functional structures through printing technology *in vitro*. Bioprinting operated with computer-assisted designs (CADs) has exhibited the potential to reproduce the complexity of native tissues in terms of the mechanical properties, the

specific structures, and the interactions between cells and their extracellular matrices (ECMs)[1-3].

Bioprinting has unique advantages for tissue engineering and regenerative medicine. Although tissue engineering and regenerative medicine are two different research topics, they share the common goal of generating tissues or organs, either *in vitro* (tissue engineering) or *in vivo* (regenerative medicine). Tissue engineering and regenerative medicine usually require the wise combination and organization of biomaterials, cells, and biological factors to fabricate structures to simulate (in tissue engineering) or to replace (in regenerative medicine) the targeted tissues or organs, thereby enabling drug testing, disease modeling, trauma repair, and reconstruction of tissue functions. The difficulty of these technologies lies in the spatial positioning of multiple types of cells and the deposition of different amounts of cells with ECMs or ECM mimics. In contrast, 3D bioprinting can precisely regulate the proportions, the positions, and even the densities of specific types of cells along with biomaterials for tissue reconstruction, fully demonstrating the advantages of bioprinting in terms of directed spatial manipulation and layer-by-layer material controls[4]. Therefore, bioprinting has been widely used, including muscle repair[5], vascular regeneration[6], bone injury treatment[7], and skin wound healing[8].

However, current bioprinting strategies still suffer from high costs, inconvenient *in vitro* cell culture and storage, as well as problems from multiple perspectives, such as nutritional acquisition, immune rejection, and maladaptation after *in vivo* implantation[2,9]. To address these problems, diverse strategies to fabricate engineered ECMs have been developed. One of the practical solutions comes from the usage of exosomes in bioink.

Exosomes are natural biological particles that transport proteins, lipids, or genetic materials to the recipient cells. They come from various sources and have certain biological functions of the parent cells, demonstrating potential immune privileges. Exosomes from different sources have distinct functions, which exhibit good potential for adaptation to various situations. At the same time, exosomes are easier to store than cells and can be easily applied to multiple systems[10,11]. Compared to employing living cells, bioprinting with exosomes can reduce *in vivo* rejection, achieve targeted exosome delivery, and overcome the regulatory and cost-effectiveness issues, thus addressing multiple challenges in tissue engineering. Therefore, bioprinting with exosomes is expected to advance the field of tissue engineering and regenerative medicine significantly.

In this review, we will discuss the current research progresses of the combination between bioprinting and exosomes in tissue engineering, highlighting the representative applications in bone engineering, vascular regeneration, nerve repair, and skin regeneration (Figure 1). At the same time, we will provide an outlook on the future research directions of bioprinting with exosomes.

2. Exosomes

2.1. The basics of exosomes

Exosomes were first discovered in sheep reticulocytes in 1983 but were once thought to be a cellular metabolic waste[12-14]. In 2007, Valadi *et al.* discovered that cells could exchange genetic materials via RNAs in exosomes[15], which ignited public interest in these new genetic information carriers. The 2013 Nobel Prize in Physiology or Medicine was awarded to the discoverers of the intracellular vesicular transport and control mechanism, highlighting the importance of the studies of exosomes. Since then, researchers have identified a variety of exosomes with different functions, and a growing number of scholars have begun to focus on the enormous potential and values of exosomes for tissue development, disease diagnosis, and therapeutics.

Exosomes are membrane vesicles released into the ECM by the fusion of intracellular multi-vesicular bodies with the cell membrane. They contain various proteins, lipids, and RNAs and are widely found in biological fluids. Exosomes have important roles in the transmission of materials and information between parent cells and offspring cells while retaining some of the biological functions of the parent cells[16]. In addition, exosomes also have reduced immunogenicity, enhanced permeability, and good retention effects, enabling them to modulate several complex biological activities (Figure 2)[17].

2.2. Current applications for exosomes

Exosomes have various biological functions (Figure 3)[18]. It can stimulate anti-tumor immune responses, aid angiogenesis in tumor metastasis[17], and play important roles in the propagation of misfolded proteins to influence the development of neuroinflammation in neurodegenerative diseases[19]. Exosomes are also rapidly evolving in the fields of immunomodulation, cancer therapy, and regenerative medicine.

2.2.1. Bone tissue engineering

In bone-related disease injuries, exosomes from mesenchymal stem cells (MSCs) can mediate cartilage repair by enhancing cell proliferation and infiltration, reducing apoptosis, and modulating immune responses[20]. A series of *in vivo* studies showed that the administration of exosomes from MSCs effectively reduced the production of pro-inflammatory cytokines in chondrocytes, increased the

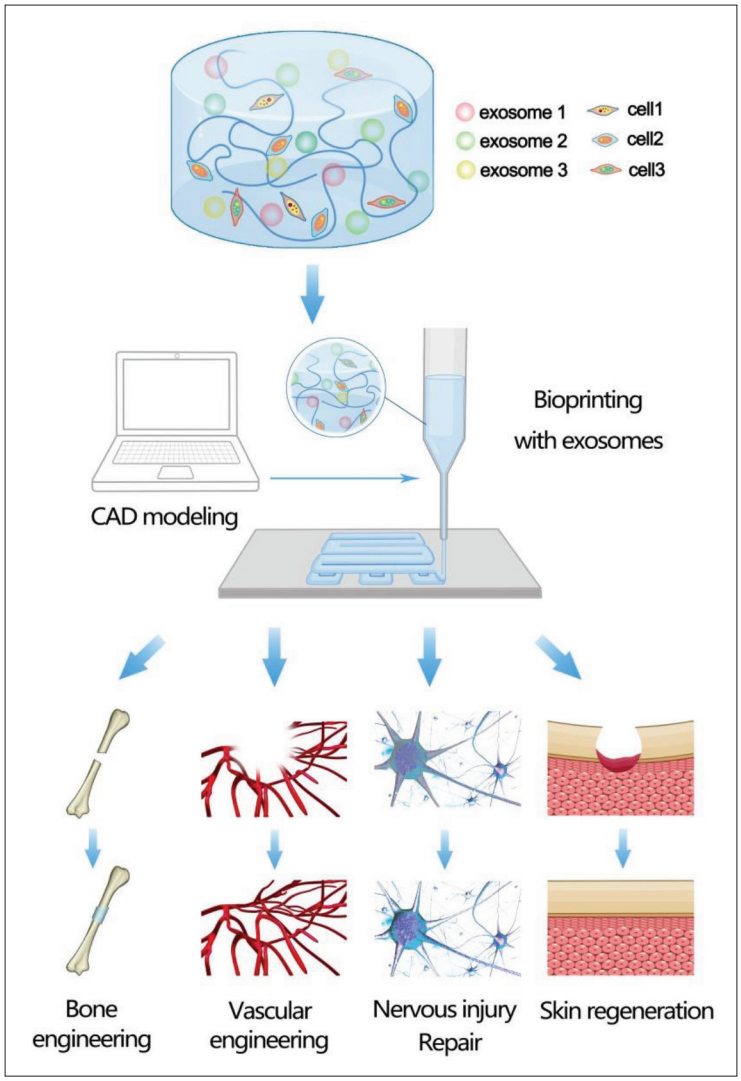

Figure 1. The general scheme of exosomes-advanced bioprinting and its applications. Through CAD modeling to recapitulate the structures of the damaged regions, exosomes can improve the bioprinted constructs for the applications of bone engineering, vascular engineering, nervous injury repair, and skin regeneration.

expression of ECM components in cartilage, and ultimately enhanced the regeneration of cartilage tissues[21-24].

2.2.2. Immunological regulation

The two main mechanisms of exosomes acting in immune regulation are their direct actions on the targeted cells to initiate downstream signals and the miRNA-mediated (indirect) regulations[18]. Cancer cell-derived exosomes can block the maturation and migration of dendritic cells through PD-L1 (programmed death ligand-1)[25]. Meanwhile, exosomes from tumors can inhibit RFXAP (regulatory factor X-associated protein), an essential transcription factor for MHC-II (major compatibility complex II) in dendritic cells, via miR-212-3p, thereby reducing MHC-II expression and inducing immune tolerance in dendritic cells[26].

2.2.3. Angiogenesis

Exosomes can also be involved in the differentiation of vascular cells, the promotion of blood flow restoration, and the formation of the capillary network during angiogenesis. For instance, MSC-exosomes can increase endothelial cell lumen formation and promote angiogenesis[27]. Subcutaneous injection of the exosomes from human umbilical MSCs in a nude mouse model significantly increased neovascularization around the infarct areas *in vivo*[28]. In addition, the exosomes from stem cells also have the ability to induce angiogenesis in the resting state[29].

2.2.4. Neural degeneration

Certain neuronal exosomes are involved in the accumulation of misfolded proteins in the brain and accelerate the progression of neurodegenerative disease,

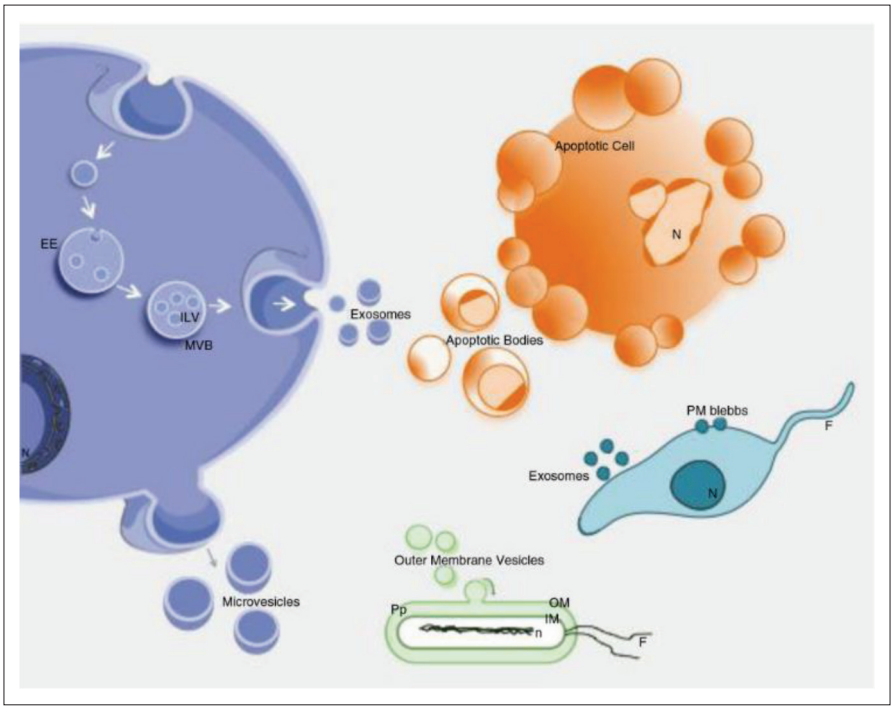

Figure 2. Biogenesis and release of extracellular vesicles. Extracellular vesicles can be broadly classified into three main classes: (a) Microvesicles are produced by outward budding and fission of the plasma membrane; (b) exosomes are formed within the endosomal network and released upon fusion of multi-vesicular bodies with the plasma membrane; (c) apoptotic bodies are released as blebs of cells undergoing apoptosis. EE: early endosome; F: flagella; ILV: intraluminal vesicles; IM: inner membrane; MVB: multi-vesicular body; N: Nucleus; n: nucleoid; OM: outer membrane; PM: plasma membrane ; Pp: periplasm. Reprinted with permission from ref.[17]. Copyright 2015 John Wiley and Sons, Inc.

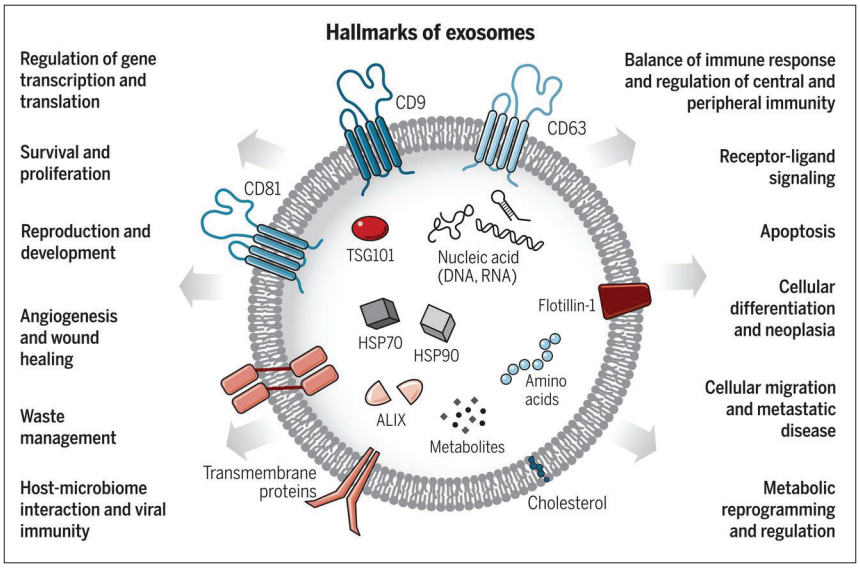

Figure 3. Exosomes: A cell-to-cell transit system in the human body with pleiotropic functions. Reprinted with permission from ref.[18]. Copyright 2020 American Association for the Advancement of Science.

while others can help remove these misfolded proteins and perform detoxification, showing the neuroprotective functions. Haney *et al.* developed an exosomal drug delivery system based on oxidase, which was tested both *in vivo* and *in vitro* with Parkinson's models and demonstrated the significant neuroprotective effects of this system, offering further possibilities for the treatment of neurodegenerative diseases[30].

2.3. Exosomes and decellularized extracellular matrix (dECM)

Recently, there has been another biologically derived material widely used in bioprinting called decellularized ECM (dECM). It is a combination of 3D scaffolds, proteins, and bioactive small molecules that remain after removing all or part of the cellular and nucleic acid components. It is typically derived from animal, human, or plant tissues[31]. While dECM can be used as a bioink in bioprinting, it differs significantly from exosomes due to the variations in their sources, collection methods, bioink preparation processes, and ink properties. Common methods for preparing dECM involve either vascular system perfusion or immersion/stirring[32]. During printing, collagen cross-linking serves as the primary mechanism of solidification. Therefore, it is mainly compatible with digital light processing or extrusion-based approaches[33].

Unlike dECM, exosomes can be collected using centrifugation techniques and subjected to surface processing[34]. By adding specific scaffolding proteins, cytokines, hydrogels, or other materials, exosomes can be turned into bioinks that primarily deliver signaling molecules, proteins, and nucleic acids. Exosomes contain signaling molecules that play a significant role in tissue repair, giving them potential for applications in bioprinting[35]. Compared to dECM, exosome therapy has fewer ethical limitations, lower immunogenicity, and reduced risks of ectopic transplantation[36]. Therefore, it has been applied to the bioprinting of different tissues or organs, such as blood vessels [37-39], bones[36,40], skin[41], nerves[42-44], corneas[45], etc. For instance, Zhang *et al.* developed a 3D PLA scaffold based on MSC exosomes[40]. It reduced pro-inflammatory markers and ROS (reactive oxygen species), showing immune regulation potential, and enhanced osteogenic differentiation, contributing to bone formation. Shafei *et al.* used an alginate hydrogel with Adipose-derived Stem Cells (ASC) exosomes as a bioactive scaffold[46]. It had beneficial effects on wound closure and promoted re-epithelialization. The applications of exosomes in different tissues will be discussed in detail later in the text. However, it should be noted that exosomes are highly sensitive to environmental factors such as temperature and pressure since they exist as extracellular vesicles, causing their storage and transport more challenging. Further explorations are also needed to understand the functional mechanisms of exosomes.

Similar to exosomes, dECM may also contain signaling molecules that can regulate cell behaviors and promote cell adhesion, migration, and differentiation[47,48]. In addition, research has demonstrated that dECM materials have potential applications in tissue remodeling and organ regeneration by enhancing cellular functions and promoting a variety of tissue remodeling processes[49]. The dECMs from different sources of the tissues have shown higher tissue-specific heterogeneities for their potential applications compared to the applications of exosomes. Currently, many dECM inks are derived from porcine tissues[50,51], raising questions about their biocompatibilities for later clinical applications. Additionally, their overall biocompatibilities also require further investigation since dECM materials are extracted from real biological tissues that are influenced by age, health status, and environmental factors. Batch variations and instabilities are also issues that need addressing.

In summary, both dECM and exosomes have the potential for applications in tissue engineering. However, further research is needed to explore their applications and overcome their respective limitations.

3. Bioprinting

3D printing technology, as an emerging field in regenerative medicine, is showing great potential compared to traditional technologies. It has offered a huge possibility for digital designs with low manufacturing costs. Derived from 3D printing technology, bioprinting, especially organ printing, has developed rapidly in recent years. With technological advances, plenty of great demonstrations have been fulfilled in various fields, such as bone engineering, artificial vascular, nerve injury treatment, skin regeneration, and so on. In order to meet the distinct functions required in these different applications, the appropriate bioinks and printing methods need to be designed and engineered.

3.1. Bioink

Bioink is an important factor that directly affects cell survival and biomaterial constituent in tissue engineering and regenerative medicine. Hydrogel is a commonly used bioink which can mimic the physical characteristics of ECMs in the body. Generally, two types of hydrogels are used for bioink: natural-derived hydrogels and synthetic hydrogels. The materials of natural-derived hydrogels are mainly generated from the body of the organisms, such as collagen, alginate, agarose, hyaluronic acid, etc. On the other hand, a synthetic hydrogel is usually synthesized by chemical methods. Its physical and chemical properties are usually controllable to meet specific requirements for bioprinting, such as good biocompatibility for a high cell survival rate and optimal viscosity for high printing resolution. Commonly used synthetic bioink includes polyethylene glycol (PEG) and Pluronics F127. For the specific introductions of bioinks, please refer to the review by Barrs *et al.*[52] In addition, according to the preparation methods of hydrogels, they can be divided into physically

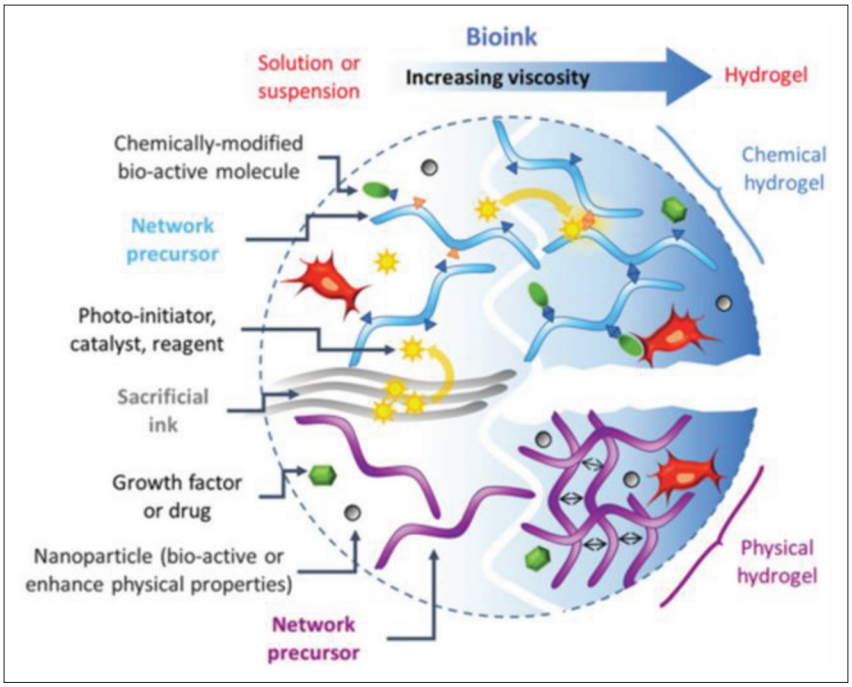

Figure 4. Bioink systems for printing. Reprinted with permission from ref.[53]. Copyright 2019 Royal Society of Chemistry.

cross-linked hydrogels and chemically cross-linked hydrogels (Figure 4). The specific principles and examples for hydrogel preparations can refer to the review by Valot *et al.*[53].

3.2. Printing methods

There are several distinct available printing methods. Here, we briefly introduce three printing methods categorized according to ASTM standards, which are extrusion-based, jetting-based, and vat photopolymerization (VP)-based bioprinting.

3.2.1. Extrusion-based bioprinting

One of the most common bioprinting methods is extrusion-based bioprinting (Figure 5A). It combines a fluid distribution system and an automated robotic system. The fluid-dispensing system can be driven by a pneumatic-, mechanical-(piston or screw-driven), or solenoid-based system[54]. The working procedure of extrusion-based bioprinting includes three steps: (i) the hot-melt material (as the bioink) is liquidized through the heater; (ii) the bioink is pumped into filaments and sent to the hot-melt nozzle; (iii) the nozzle head squeezes out of the bioink. Through printing, CAD layered data can control the path during the squeezing out of bioink and place it in the specified positions to solidify. The printed materials can bond with the surrounding materials, stacking layer by layer[55]. Therefore, a stable bioink is needed for this approach. However, this technology has shortcomings, such as a limited cell survival rate and relatively low resolutions[56].

3.2.2. Jetting-based bioprinting

Inkjet bioprinting is derived from commercial two-dimensional (2D) inkjet printing technologies (Figure 5B). The main difference between this technology and extrusion-based bioprinting is that the bioink is produced at a point where the nozzle head hits. Therefore, jetting-based bioprinting has the advantage of high resolution. For jetting-based bioprinting, its bioink must be liquid-like in case of blocking the nozzle. Besides, the viscosity of biological ink is also difficult to control[57]. Meanwhile, the side effect brought by high resolution is slow printing speed. In addition, the discontinuous droplets can also lead to a weak mechanical strength of the bioprinted structure[58]. Therefore, it is recommended to adjust the mechanical properties of bioink to guarantee the printing quality. For instance, Suntornnond *et al.* modified GelMA through saponification and heat treatment, effectively improving its printability and biocompatibility in thermal injection printing[59].

3.2.3. Vat photopolymerization (VP)-based bioprinting

The working principle of VP-based bioprinting (laser-assisted bioprinting [LAB]) is that the laser focuses on the glass plate absorption layer to produce a high-pressure liquid foam and to push the cells to the acceptable

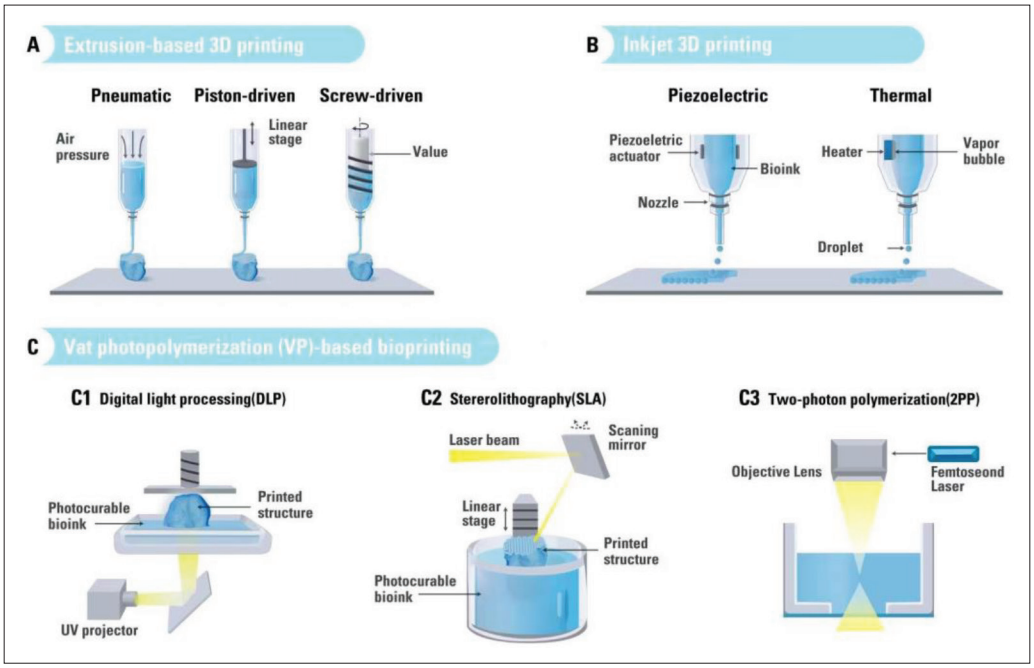

Figure 5. Representative demonstrations of 3D bioprinting methods. (A) Extrusion-based 3D printing; (B) Inkjet 3D printing; (C) Vat photopolymerization (VP)-based bioprinting: C1, digital light processing (DLP); C2, stereolithography (SLA); C3, two-photon polymerization (2PP)[61].

substrate[60]. The VP-based bioprinting is divided into stereolithography (SLA), digital light processing (DLP), and two-photon polymerization (2PP)[61].

In the SLA system (Figure 5C1), two different methods can be used for optical solidification: (i) top-down printing approach, that is, the scanning laser solidification above the vat cures a layer of resin on the build platform, and it is lowered into the vat to repeat the curing process; (ii) bottom-up printing approach, that is, the scanning laser is located at the bottom of the vat, and the build platform is raised above the bioresin vat via a "peeling" step between each printed layer. DLP (Figure 5C2) is another method for optically curing biological resin. Using digital micromirror devices (DMD) in DLP helps to obtain a layer of optical solid resin instead of single-point solidification in SLA. 2PP (Figure 5C3) process is caused by three-order non-linear absorption within the focal region; the beam of the flying laser is closely focused on the photoresist (liquid biological resin) on the glass coverslip with an oil-immersion objective lens to fabricate high-resolution 3D structures beyond the optical diffraction limit by moving the focused beam within the photoresist.

The advantage of VP-based bioprinting is that the nozzle is open; thus, there is no nozzle blockage issue. At the same time, the cell damage is limited, leading to a 95% cell survival rate[62]. However, the effect of laser on cells is not well-defined yet, which may have considerable cytotoxicity[63].

4. Biomedical applications of bioprinting with exosomes

4.1. Bone engineering

Traditional bone transplantation is still one of the common ways to treat bone damage or loss. However, due to the body's rejection, allografts are likely to cause a series of complications[64], so their application is limited. Applying tissue engineering approaches (e.g., bioprinting or organ printing) to repair bone damage and loss is a relatively novel way and also a hot research topic at present.

However, the bioactivities of several traditional bioink for bone repair are limited[65]. Meanwhile, exosomes with the size of 50–120 nm have relatively high biocompatibility and a strong ability to promote bone formation, providing a new idea for the strategies of bone regeneration. Great bone specificity and strong bone regeneration properties make exosomes significantly valuable for therapeutics, which can enhance bone growth to treat clinical bone diseases[66]. Therefore, loading exosomes into bioink for bone tissue bioprinting has become one of the practical options to build highly bioactive structures for bone repair.

Sun *et al.* applied 3D printing technology to construct porous scaffolds with β-tricalcium phosphate (β-TCP) bioceramic-induced macrophage exosomes[36]. The system exhibited a predefined structure and a persistent release of exosomes, displaying improved effects in immunomodulatory and osteogenesis/angiogenesis

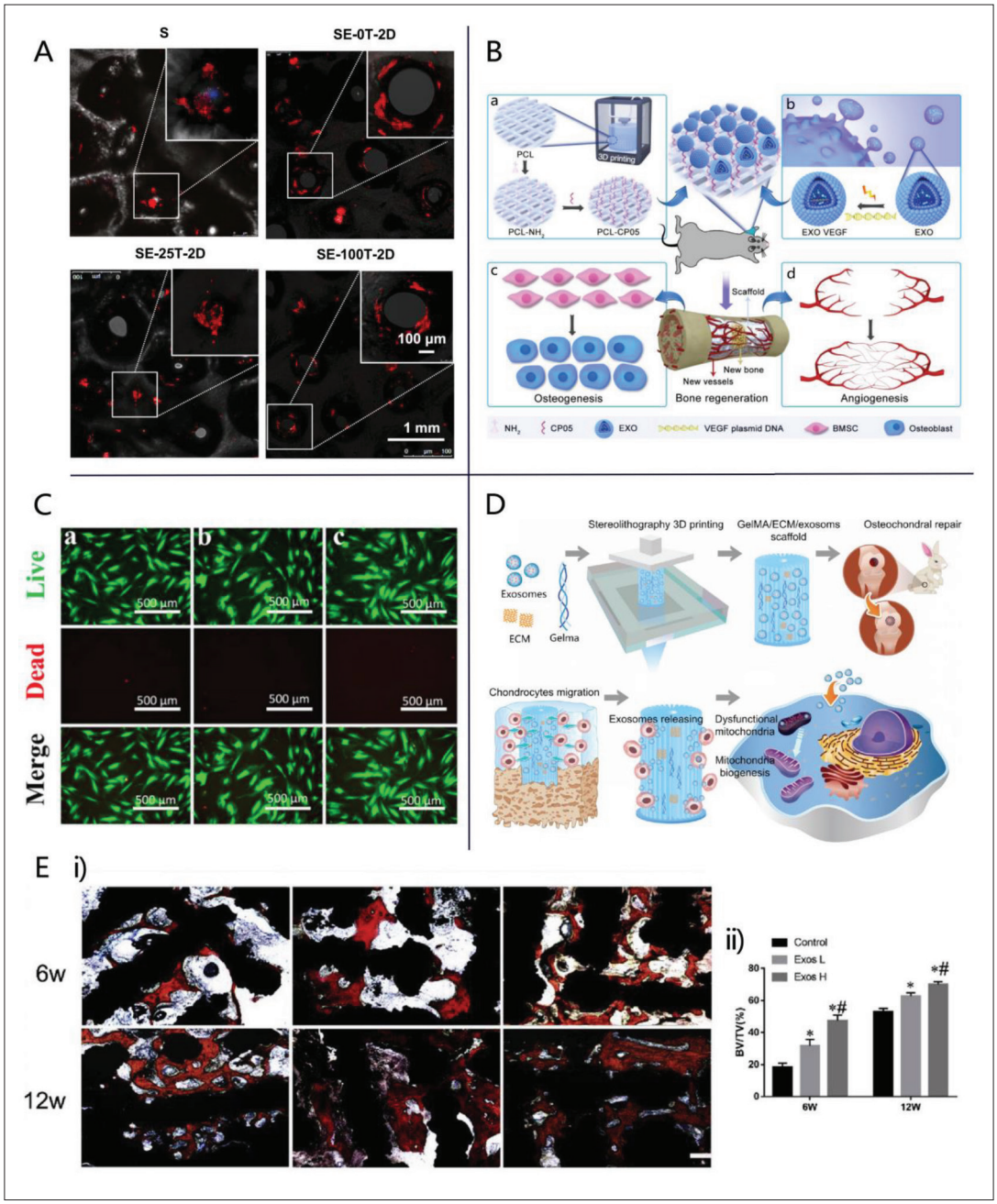

Figure 6. Bioprinting with exosomes for biomedical applications in bone engineering. (A) Representative fluorescence images of Human umbilical Vein Endothelial Cells (HUVECs) on scaffolds on day 1. Cells were stained with TRITC–phalloidin (red). Adapted with permission from ref.[36]. Copyright 2021 Springer Nature Limited. (B) General scheme of engineered exosome-enhanced therapies on osteogenesis and angiogenesis. (a) 3D-printed porous PCL scaffolds were modified with 1,6-hexanediamine to generate the amino group on PCL scaffolds that were subsequently modified with the exosomal anchor peptide CP05. (b) Engineered exosomes were fabricated by encapsulating the VEGF plasmid DNA into ATDC5-derived exosomes. The well-designed bone scaffolds were constructed by combining the engineered exosomes with the CP05-modified 3D-printed scaffolds, and eventually implanted into a rat radial defect model to promote osteogenesis (c) and angiogenesis (d). Adapted with permission from ref.[67]. Copyright 2021 Ivyspring International Publisher. (C) Live/dead assay of human bone marrow stem cells (hBMSCs) co-cultured with different scaffolds for 3 days. The cells were stained with fluorescein diacetate (FDA, green) and propidium iodide (PI, red) and were examined by confocal microscopy. Green indicated living cells, and dead cells were labeled as red fluorescence. Group a = control, group b = PLA scaffold, group c = PLA-Exo scaffold. Adapted with permission from ref.[40]. Copyright 2022 BioMed Central Ltd., part of Springer Nature. (D) Schematic illustration of the one-step operation system for facilitating osteochondral defect regeneration. Adapted with permission from ref.[68]. Copyright 2019 Ivyspring International Publisher. (E) Effect of different scaffolds on bone regeneration. (i) Van Gieson staining results in the different groups at 6 and 12 weeks. (ii) Quantitative analysis of BV/TV in the different groups[36-40]. Adapted with permission from ref[69]. Copyright 2020 Elsevier LTD.

(Figure 6A). It provided a novel inspiration for bone regeneration and the design of therapeutic biomaterials with improved immune regulations.

Of the essential elements of tissue engineering, cell seeding is important for inducing effective tissue regeneration. However, cell-based tissue engineering approaches also have many drawbacks, such as limited sources and restricted expansion capacities of donor cells, immune rejection, and many others. Thus, cell-free tissue engineering has been extensively explored in regenerative medicine as a safe, effective, and off-the-shelf strategy. Zha *et al.* developed a cell-free tissue engineering system using functional exosomes instead of seeding cells[67]. Gene-activated engineered exosomes were constructed by using ATDC5-derived exosomes to encapsulate the VEGF gene. The specific exosomal-anchored peptide CP05 acted as a flexible linker, effectively linking the engineered exosome nanoparticles with 3D-printed porous bone scaffolds (Figure 6B). The scaffolds were tested to effectively induce the bulk of vascularized bone regeneration, illuminating the potential of functional exosomes in acellular tissue engineering.

MSCs have strong proliferative ability and multi-directional differentiation potential. It can secrete cytokines through paracrine effects to repair tissues. Zhang *et al.* developed a bioactive 3D PLA scaffold using an exosome-based strategy[40]. PLA-Exo scaffold can reduce the expression of the pro-inflammatory markers and limit the production of the ROS, indicating its immunoregulatory potential. Meanwhile, the authors confirmed that it could enhance osteogenic differentiation in the osteogenic tests, showing potential applications in bone tissue regeneration (Figure 6C).

Similarly, Chen *et al.* also designed a bioscaffold for delivering MSC exosomes. They fabricated a 3D-printed cartilage ECM/gelatin methacrylate/exosome scaffold with radially oriented channels using desktop-stereolithography technology (Figure 6D), which significantly facilitated cartilage regeneration in the animal model[68]. At the same time, the 3D-printed radial exosome scaffolds also can be a promising strategy for early osteoarthritis treatment.

In addition to the direct combination of bone bioprinting and osteogenic exosomes for bone tissue repair, the following system also offered new opportunities for the applications of exosomes in bone bioprinting. Wu *et al.* added the Schwann cell-derived exosomes to bone marrow stromal cell culture environments and found they could effectively promote the migration, proliferation, and differentiation of bone marrow stromal cells[69] (Figure 6E). In addition, the combination of exosomes and porous Ti6Al4V implants provided both mechanical support and open pores, exhibiting good biological activity. It was also a therapeutic strategy with a high potential for treating bone defects.

Exosomes are considered a powerful supplement for cell therapy in regenerative medicine for their excellent biocompatibility, efficient cell internalization, and strong load capacities. However, exosomes still have some shortcomings, such as low yield, unstable efficiency, and lacking drug delivery routes, which can affect the further applications of biomolecular carriers. Zha *et al.* developed a novel exosome-mimetics, which had a similar structure and biomarkers in comparison with the routine exosomes[70]. It can be generated with a high yield and has been applied to construct an engineered gene-activated matrix for local therapy, opening up a new idea for using exosomes.

Furthermore, some researchers have also provided new ideas to improve the therapeutic efficacy of exosomes. Li *et al.* developed a stem cell-mediated gene therapy in which mediator MSCs were genetically engineered by the bone morphogenetic protein-2 gene to produce exosomes with enhanced bone regeneration potency[71]. The accelerating effect in bone healing and good biocompatibility suggested the potential clinical application of this strategy if applied with bioprinting.

In summary, though exosomes have been widely studied, the research of 3D bioprinting with exosomes has been initiated in bone tissue engineering and holds great potential in tissue regeneration.

4.2. Vascular engineering

Exosome bioprinting technology has also been applied to vascular engineering. The application of exosomes in 3D printing can promote the sustained release of exosomes and improve their biological activity. Sun *et al.* used 3D printing technology to construct porous scaffolds for macrophage exosomes (BC-Exos) induced by β-tricalcium phosphate (phosphate bioceramics), and realized 3D-printed BC-Exo scaffolds. The system had a predefined structure and enabled the continuous release of exosomes (Figure 7A)[36]. It also showed significant immunomodulatory effects and improved osteogenesis/angiogenesis properties. This design of a cell-free 3D-printed scaffold using biomaterials to activate macrophage exosomes has increased the application of immune cell-derived exosomes in tissue regeneration and provided a new direction for the design of bioprinted systems. This study suggested that the 3D printing of bioceramics-induced macrophage exosomes can be a useful strategy for tissue engineering and regenerative medicine.

In terms of the clinical application of exosomes derived from bone marrow MSCs, Zhang *et al.* developed a system

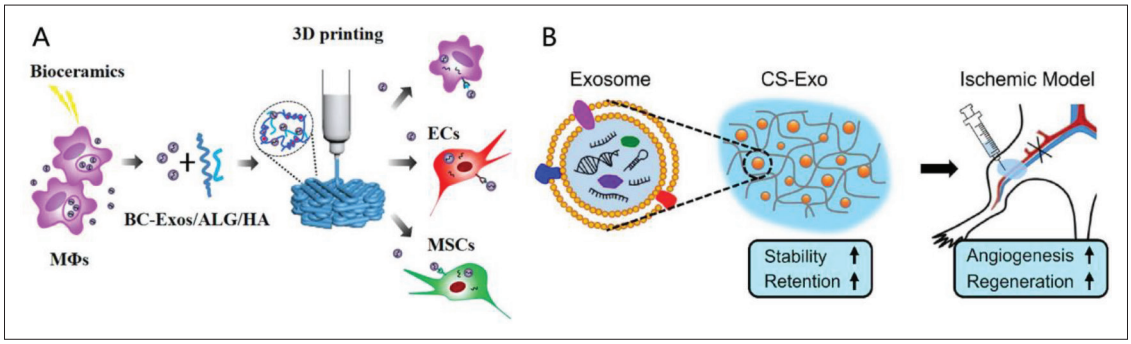

Figure 7. Exosomes promote bioprinting for vascular engineering. (A) Schematic illustration showing the preparation of bioceramic-induced macrophage exosomes (BC-Exos) and 3D printing of BC-Exos for immunomodulation, osteogenesis, and angiogenesis of macrophages, mesenchymal stem cells (MSCs), and endothelial cells. Adapted with permission from ref.[36]. Copyright 2021 Springer Nature Limited. (B) Schematic illustration of hP-MSCs-derived exosomes incorporated with CS hydrogel for muscle regeneration. Adapted with permission from ref.[72]. Copyright 2018 American Chemical Society.

combining exosomes with chitosan hydrogels to enhance the therapeutic effect of human placenta-derived MSCs (hP-MSCs) derived exosomes[72] (Figure 7B). The chitosan hydrogels had the characteristics that can enhance the retention and stability of exosomes. In addition, the hydrogels also further improved the therapeutic effect of angiogenesis in hindlimb ischemia, as shown by firefly luciferase imaging. This strategy may be of great practical value for cell-free therapy.

4.3. Nerve injury repair

Nervous injury includes central nervous injury and peripheral nerve injury. It has the characteristics of high incidence and inaccurate pathogenesis; furthermore, it still lacks a clear treatment strategy yet[73]. In recent years, many researchers have focused on bioprinting scaffolds with exosomes. These systems have the following advantages: (A) They can effectively maintain the exosomes of the damaged part and retain its performance and structural characteristics. (B) The exosomes are released into ECM to adjust the phenotype of neighboring cells. (C) It can be combined with the injured tissues to support the migration of neighboring cells. Once the neighboring cells migrate to the biological scaffold, the exosomes can be absorbed to promote tissue regeneration[74].

Liu *et al.* prepared 3D-printed collagen/silk fibroin/hypoxia-pretreated human umbilical cord MSCs (HUCMSCs)-derived exosomes scaffolds (3D-CS-HMExos) and implanted it into the injured brain of the small hunting dog to treat traumatic brain injury (TBI)[75]. The experimental results showed that the method could effectively promote nerve regeneration after TBI and, at the same time, inhibit neuritis and the apoptosis of neurotic cells, providing a new strategy for treating TBI (Figure 8A). Additionally, Hsu *et al.* developed alginate and HUCMSCs exosomes[78]. The scaffold had anti-inoculation, anti-inflammatory, and neurotrophic effects. It also has been shown that the pain caused by L5/6 spinal nerve ligation (SNL) can be treated by this scaffold.

Rao *et al.* combined biodegradable chitin with exosomes derived from the gums to treat the sciatic defects of rats[79]. The study results showed that exosomes were effective for treating nerve injuries, and it was a method of nerve regeneration with excellent performance. Han *et al.* proposed a controllable 3D external hydrogel mixed microneedle array patch to achieve the method of repairing spinal cord injury (Figure 8B)[76]. They cultivated the exogenous body of 3D mesenchymal stem cells (MSC-EXO). Different from other studies of the single-layer (2D) exterior body of hydraulic gel, 3D-EXO can maintain the stem nature and improve the effects of MSCs. In addition, the study also compared the effects of 2D and 3D exosomes on the treatment of nerve repair, and the results showed that the 3D exosomes have higher therapeutic efficiency. This result also revealed that biological 3D printing could effectively improve the efficiency of the repair.

At present, the demonstrations of biological 3D printing technology with exosomes are still limited, but many studies have been put into treatment with exosomes and hydrogels. Liu *et al.* tested the rigidity of hydrogels in loaded exosomes in nerve repair[80]. The study showed that soft hydrogel can better repair peripheral nerve damage. This result also revealed the selection criteria of hydrogels in nerve repair. Li *et al.* used exosomes derived from the human MSC in an EXOO-PGEL (EXOO-PGEL) for peptides[77]. The tissue nerve-derived injury has developed an innovative strategy for exogenous physical delivery for spinal cord injury treatment (Figure 8C). Wang *et al.* used retinal ganglion cells of rats (RGC) exosomes as nano-sized vesicles, loading PACAP38 through exosomes anchoring peptide CP05 (Exopacap38) to treat external injuries

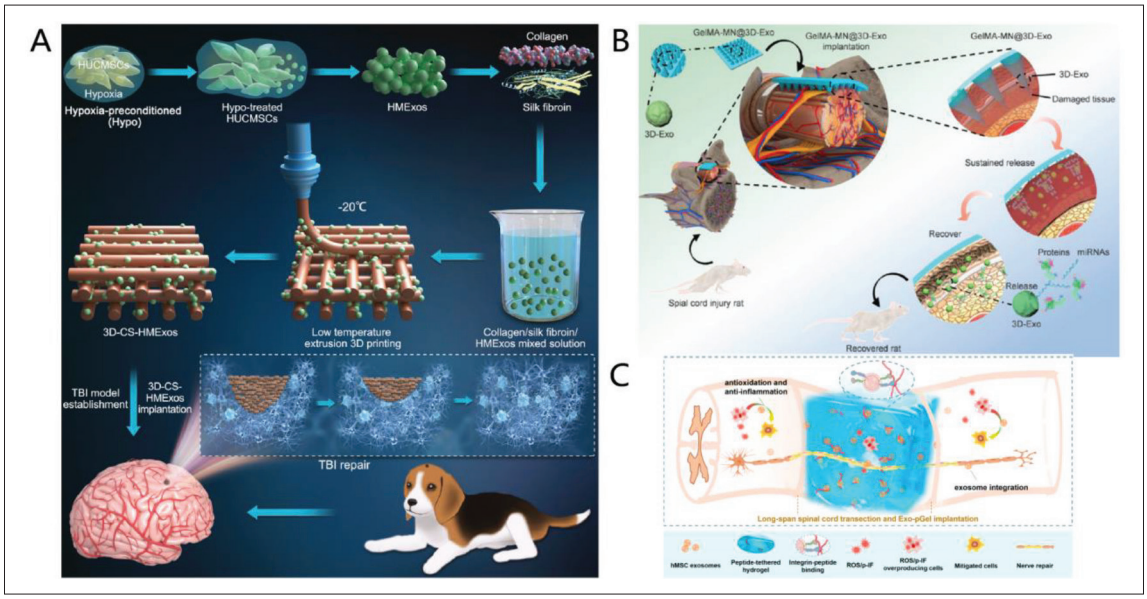

Figure 8. The applications of biomedical engineering for nerve injury repair. (A) 3D-printed collagen/silk fibroin/hypoxia-pretreated human umbilical cord mesenchymal stem cells (HUCMSCs)-derived exosomes scaffolds (3D-CS-HMExos) and implanted in the injured brain of the small hunting dog, which used to treat traumatic brain injury (TBI). Adapted with permission from ref.[75]. Copyright 2022 Frontiers Media S. A. (B) The controllable three-dimensional exterior hydrogel mixed microneedle array patch to achieve the repair of spinal cord injury. Adapted with permission from ref.[76]. Copyright 2022 American Chemical Society. (C) The exogenous body derived from human MSC was fixed in an exo-PGEL (Exo-PGEL) that promoted spinal cord regeneration and recovery of hind limbs. Adapted with permission from ref.[77]. Copyright 2020 American Chemical Society.

(traumatic optic neuropathy[TON])[81]. It overcame the shortcomings of low tissue penetration and a short half-life period and improved the transfer efficiency of neurogenic peptides.

Bioprinting technology with exosomes is an innovative strategy for treating nerve damage. Compared with other bioink, exosomes can effectively improve bioactivity. The potential of exosomes in treating neural diseases, including those related to neurotransmitters, is evident from their nature discussed in the first chapter and this section.

4.4. Skin regeneration

Conventional means of skin repair include topical application of relevant drugs, exposure to lasers, and skin grafting[82,83]. In recent years, researchers have discovered the linkage between exosomes and skin diseases[84,85]. They found that exosomes can participate in the physiological and pathological processes of the skin, such as regulating the secretion of pro-inflammatory cytokines in the microenvironment of skin, promoting vascularization and collagen deposition in some skin defect diseases, as well as regulating the proliferation and differentiation of skin fibroblasts[86-88]. Most importantly, people have discovered that exosomes positively affect skin regeneration and repair.

Shafei *et al.* used an alginate hydrogel loaded with adipose stem cell-derived exosomes as a bioactive scaffold and found that it not only had good effects on wound closure, but also promoted a high degree of re-epithelialization [46]. They characterized the physical and biochemical properties of the hydrogel and found that the prepared exosome-hydrogel had excellent biodegradability and biocompatibility. The exosome-hydrogel significantly improved wound closure, collagen synthesis, and angiogenesis in the wound area. The results of this study also provided a cell-free therapeutic strategy for wound healing treatments using composite structures of exosomes-encapsulated alginate hydrogels, showing its great potential for application in bioprinting.

One of the common complications of diabetes is impaired wound healing, characterized by inadequate angiogenesis and susceptibility to infections, which can lead to non-healing chronic diabetic ulcers[89]. Wang *et al.* prepared a polysaccharide-based dressing (FEP) exosome-contained scaffold dressing with heat-sensitive, injectable, adhesive, self-healing, antibacterial, hemostatic, and UV shielding properties to stimulate early angiogenesis in diabetic wounds[90]. It has been demonstrated that the system thus promoted skin healing and reconstruction. The scaffold can chronically release exosomes from adipose stem cells, enhancing the proliferation, migration, and tubular formation of stimulated HUVECs, and promoting diabetic wound healing. The synergistic effects of these stimulatory responses promoted granulation tissue

formation and collagen deposition, driving wound re-epithelization and significantly increasing the regeneration of skin appendages. In addition, it also reduces the formation of scar tissue. Exosome-loaded FEP hydrogels have shown great potential in promoting diabetic wound healing, providing strong evidence for their clinical potential in skin regeneration.

Exosomes combined with bioprinting for skin tissue engineering may mainly refer to the exosome-contained, bioprinted scaffolds to aid wound closure and promote the stable release of therapeutic exosomes in predesigned areas. The combination improves therapeutic efficacy and further takes advantage of the high bioactivity of exosomes[91]. Although it is still at an early stage of development and more focus on the research is needed, based on the current demonstrated examples about the combination of exosomes and hydrogels, it may be possible to modify the designs of exosome-loaded hydrogels and apply them to the preparation of bioinks, thus building high-activity exosome-bioprinting systems, which will be one of the promising research directions in the field of regenerative medicine in the near future.

4.5. Other applications

4.5.1. Corneal repair

Currently, bioprinting with exosomes has not yet been systematically studied in the field of corneal repair. However, there has been relatively in-depth research on using exosomes for the treatment of corneal injury. For instance, Samaeekia *et al.* isolated corneal mesenchymal stem cell (cMSCs) exosomes from humans and found a significant healing effect of the exosomes on corneal epithelial cell damage[92]. Shojaati *et al.* demonstrated that exosomes produced by corneal stromal stem cells (CSSC) inhibited the formation of fibrotic scarring after corneal injury and stimulated the regeneration of transparent stromal tissue[93].

Several different 3D bioprinting strategies have been developed for the fabrication of corneal donor graft materials. Sorkio *et al.* used stem cells and laser-assisted bioprinting to produce a 3D corneal tissue that mimicked the structure of natural corneal tissue. The cells in the structure maintained good viability[94]. Isaacson *et al.* used a low-viscosity bioink made from sodium alginate and methacrylated type I collagen to produce an artificial corneal substitute by 3D bioprinting, which also had good biocompatibilities and bioactivities[95].

4.5.2. Oral repair

The oral cavity contains various tissues, such as teeth, jaw, gums, oral mucosa, gland, and cartilage. Stem cells in these tissues can secrete different functional exosomes, and these exosomes have different biological effects. For example, studies have found that exosomes secreted from dental pulp stem cells (DPSCs-E) inhibited the differentiation of $CD4^+$T cells into T helper 17 cells (Th17) and reduced the secretions of pro-inflammatory factors IL-17 and TNF-α, while promoted the polarization of $CD4^+$T cells into Treg and increased the release of anti-inflammatory factors IL-10 and TGF-β[96]. Wei *et al.* used stem cells from human exfoliated deciduous teeth (SHED)-derived exosomes (SHED-Exo) in the bone loss area caused by periodontitis in a mouse model[97]. SHED-Exo specifically promoted BMSCs osteogenesis and inhibited adipogenesis. In addition, SHED-Exo can further promote osteogenic differentiation and bone formation in BMSCs.

Meanwhile, in dental medicine, biological 3D printing technology has been widely used to cure diseases such as tooth osteo-deficiency. Tian *et al.* mixed sodium alginate (SA), gelatin (Gel), and nano-hydroxyapatite (na-HA) to prepare a hydrogel composite[98]. Human periodontal ligament stem cells (HPDLSCs) were mixed with SA/Gel/na-HA printing slurry to create a "bioink" to prepare SA/Gel/na-HA/hPDLSCs cell bioscaffolds. The results showed that the SA/GEL/N-HA composite hydrogel had good streaming performance and was suitable for printing. Cell biological stent had good biocompatibility and was conducive to the osteoma of HPDLSCs.

In all, there have been great potential for the use of exogenous biological printing for corneal repair and oral repair, while the current research applications still have great challenges from the perspective of real clinical applications.

5. Summary

3D bioprinting technology is a new field that has emerged in recent years. However, their translational application in the clinic is still lagging due to their limited ability to produce bioprinted constructs with the necessary biological activities to integrate with host tissues. Exosomes have high biological activity as an important medium of information transmission in organisms. They have become one of the potential materials for application in bioprinting systems. Combining exosomes as bioink with bioprinting technology can compensate for the lack of biological activity of traditional 3D bioprinting. Traditional 3D bioprinting bioinks for scaffold manufacturing include alginate, gelatin, and collagen. Gelatin is a soluble protein compound obtained by partial hydrolysis of collagen, which is the main fibrin component in bone, cartilage, and skin[99], while natural alginate is a bioinert material (i.e., it lacks cell adhesion part) with limited biodegradation[100-102]. Compared with the above biological materials, exosomes have shown higher biological activity and targeting in cell signaling and drug release[103]. Therefore, exosomes can

effectively affect the activity of cells and the development of tissues through multiple signaling pathways without causing immune responses. In addition, some stem cell-derived exosomes also have exhibited immunomodulatory and tissue regeneration abilities[104]. From the perspective of enhancing the biological activity of biological ink by exosomes, this paper briefly introduced exosomes and 3D bioprinting technology and summarized the application and progress of exosomes and 3D bioprinting technology in bone, blood vessel, nerve, and skin in recent years. At present, there have been many studies on the use of exosome hydrogels for the treatment of diseases. This paper briefly combed them and outlined their advantages and disadvantages, as detailed in Table 1.

However, despite the exciting progress to date, several challenges remain. Firstly, good-quality exosomes should be obtained before printing. However, the current challenge lies not only in the isolation of exosomes before preparing exosome-rich bioinks. At present, the methods for extracting exosomes mainly include differential centrifugation[105], volume exclusion chromatography[106], and immunoaffinity capture[107]. Each method has advantages and disadvantages, but the extraction method to be used is determined by the sample source and the intended use of exosomes. A few studies have been conducted on the rapid and accurate preparation of bioactive exosomes with a low loss rate[108]. Secondly, during the printing process, it is necessary to ensure that the exosomes are put into the bioprinting system while maintaining sufficient biological activity. For instance, integrating exosomes with bone tissue engineering materials gives rise to a favorable environment for exosomes to exert their functions[24]. However, it has also been found that exosomes cannot exert sustained function due to their short half-life. Although the combination of exosomes and liposomes can complement each other, sustained release of exosomes has not been demonstrated to accumulate in the bone marrow[109]. In the area of angiogenesis, there have been cases in which chitosan hydrogels have been used to improve the stability of proteins and microRNAs in exosomes significantly. However, the release of exosomes depended on the permeation and biodegradation of chitosan hydrogel, which made this process difficult to control[72]. A class of peptide-modified adhesive hydrogels (Exo-pGel) therapies provided a promising strategy for the effective treatment of central nervous system diseases based on exosome implantation. In another study, the combination of size exclusion chromatography and ultracentrifugation was used, and before ultracentrifugation, the qEV column was used to exclude microvesicles from the total extracellular vesicles (EVs), and the highly bioactive exosomes were successfully isolated and purified[77]. The exosomes, after being added to biomaterials, can enhance the osteogenic ability of the body and treat neurological diseases. However, maintaining the biological activity of exosomes is still a great challenge. Thirdly, 3D printing can print a variety of tissues and organs, but the current applications of exosomes are limited, and the studies on their functions and clinical applications are inadequate. This paper mainly focuses on the fields of bone engineering, vascular engineering, nerve injury treatment, and skin regeneration. In conclusion, the applications of exosomes to promote bioprinting in tissue engineering and regenerative medicine in other fields such as liver tissue regeneration, kidney tissue regeneration, and lung tissue regeneration have great potential and need to be developed.

Table 1. Summaries of recent studies in the four fields of bone, skin, nerve, and vascular using exosomal hydrogels to treat diseases

Field	Source of exosomes	Components of hydrogel	Diseases treated	Results-pros	Results-cons	Reference
Bone	BMSC-exosomes	SIS hydrogels doped with 3-(3,4-dihydroxyphenyl) propionic acid	Fracture	Enhanced bioactivity and promoted the active role of exosomes in osteogenic differentiation of BMSCs.	Need to enhance the mechanical properties.	[110]
	BMSC-exosomes	Alginate-dopamine, chondroitin sulfate, and regenerating filament protein	Cartilage defects	Induced differentiation of BMSCs into chondrocytes to repair the chondral defects in the patellar groove of rats.	Need to evaluate further safety and efficacy.	[111]
	M2D-Exos	HA@SDF-1α/M2D-Exos hydrogel	Fracture	Had local antibacterial activity; promoted the proliferation of hMSCs and HUVECs; stimulates ECM mineralization; promoted osteogenesis and angiogenesis; ultimately promoted fracture healing.	Need to evaluate the influence of different types of injuries or the severity of fractures on the repair results.	[112]
	PDLSCs-Exos	Alginate-gelatin cross-linked hydrogel	Alveolar bone defect	Repaired alveolar bone defects in rats.	The detection of exosomes in hydrogels was not ideal.	[113]
	hMSC-exosomes	Coral hydroxyapatite/ filamentous protein/glycolic chitosan/bi-functionalized polyethylene glycol hydrogel	Fracture	Promoted bone healing, bone morphogenetic protein 2 (BMP2) deposition, bone collagen deposition and maturation, and angiogenesis in SD rat model.	Need a negative control group to prove the effects of the hydrogel.	[114]
Skin	hMSC- exosomes	Poloxam 407, carboxymethyl chitosan, natural non-toxic cross-linker Genipin	Full thickness skin wounds	Had good wound protection effect and moisture retention function without stimulating or pro-inflammatory effects on wound; biodegradable and dual-sensitive (thermo and pH-sensitive); improved wound healing and tissue regeneration of skin appendages.	Need to test the sensitivity of the hydrogel to UV light and to evaluate the penetration of the UV light through skin.	[41]
Nerve	hMSC-exosomes	Peptide-modified adhesive hydrogel	Spinal cord regeneration	Induced efficient, comprehensive cell mitigation to the injured microenvironment	The loss of stem cells restricted the treatment effect	[77]
	BMSC-exosomes	GMPE (GelMA-polypyrrole) hydrogels	Spinal cord injury	Inhibited inflammation; enhanced the recruitment of NSCs; promoted the regeneration of neurons and myelin-associated axons	Did not verify the recovery of the hydrogel in the later stage of the mouse model	[42]
	ADSC-Exo	Thermosensitive hydroxyethyl chitosan/sodium β-glycerophosphate hydrogel	Post-operative erectile dysfunction induced by cavernous nerve injury (CNI-ED)	Enhanced retention of ADSC-Exo at the site of injury; significant improved the erectile function in CNI-ED rats.	No test of the degree of degradation of the hydrogel.	[43]
	MSC-Exos	Photocrosslinked hyaluronic acid methacrylate hydrogel	Peripheral nerve injury (PNI)	Soft hydrogel-regulated exosome rapid release; better repaired PNI and motor dysfunction *in vivo* compared to rigid hydrogel.	Need to analyze the mechanism of the exosome-loaded hydrogels inhibiting inflammation.	[80]

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	SHED-Exo	Bioactive antioxidant poly(citric acid-gallic acid)-based hybrid hydrogel	Traumatic brain injury (TBI)	Thermosensitive, injectable, self-healing, antioxidant, low cytotoxicity, and ultralong sustained release of SHED-Exo; enhanced the therapeutic potential of SHED-Exo.	Did not achieve the effective influence on the prognosis of traumatic brain injury (TBI).	[44]
	Hypoxia-stimulated MSCs-derived exosomes (sub-exo)	Binding peptide PPFLMLLKGSTR-modified hyaluronic acid hydrogel	Traumatic Spinal Cord Injury (SCI)	Improved angiogenesis, nerve regeneration, and functional motor restoration; Enhanced the therapeutic regenerative outcomes of MSC-derived exosomes, promoting the regeneration of the injured spinal cord.	No statistical differences were displayed between hypo-Exo and Exo.	[115]
Blood vessel	Gingival mesenchymal stem cells(GMSCs)-Exos	Chitosan/Silk Hydrogel Sponge	Diabetes	Promoted the healing of Streptozotocin (STZ)-induced diabetic rat skin wounds, promoted the re-epithelialization, deposition, and remodeling of ECM, and promoted angiogenesis and neurite growth.	Clinical trial data are lacking.	[116]
	Umbilical cord mesenchymal stem cell-derived exosomes (UMSC-Exo)	PGN hydrogel (PA-GHRPS peptide+peptide NapFF)	Myocardial infarction	Improved myocardial function by reducing inflammation, fibrosis, and apoptosis and promoting angiogenesis.	The mechanical properties and stability need to be further improved.	[117]
	ADSC-exo	Four-arm SH-PEG cross-linked with Ag^+ hydrogel	Thin endometrium	Promoted angiogenesis and endometrial regeneration, facilitating the recovery of fertility.	The purity and quality of exosomes need to be further studied and evaluated to ensure their safety and efficacy.	[38]
	AMSCs-exo	Anti-microbial peptide-based FHE hydrogel	Diabetic wound	Promoted neovascularization and cell proliferation; accelerated granulation, re-epithelialization, and collagen remodeling at the trauma site; accelerated the healing process of diabetic wounds.	Need to address the cost and safety of the method.	[118]
	Macrophage-exo	Alginate+hyaluronic acid	*In vitro* model	Improved the angiogenic activity of HUVECs.	Need to study the relationship between the biological activity and therapeutic effect with the morphology and structure of bioceramic-induced macrophage exosomes (BC-Exos).	[36]

Table 2. Summaries of the studies discussed in the four fields of bone, skin, nerve, and vascular using exosomal bioprinting to treat diseases

Field	Source of exosomes	Components of hydrogel	Diseases treated	Results-Pros	Results-Cons	Reference
Bone	Macrophage-exo	Sodium alginate and hyaluronic acid.	Regular immuno-modulation, osteogenesis, and angiogenesis	Distinct immunomodulatory effects and osteogenesis/angiogenesis	Systemic immunogenicity or off-target effects is not clear.	[36]
	ATDC5 (a chondrogenic progenitor cell line)-exo	PCL (polycaprolactone)	Large segmental bone defects	Excellent biocompatibility; enhanced therapy	Need to improve the production and purification techniques of exosomes	[67]
	HBMSCs-exo	PLA (Polylactic acid)	Osteogenic and immunoregulatory	Reduced the pro-inflammatory markers and ROS production in inflammatory macrophages and enhanced osteogenic differentiation	Need to improve its biofunctionality	[40]
	BMSCs-exo	Decellularized cartilage ECM, and gelatin methacrylate (GelMA) hydrogel	Osteoarthritis	Efficient for cells and tissues ingrowth, nutrients and waste exchange, ECM deposition and cell interaction	Need to determine the mechanisms of the immune response towards allografts	[68]
	Schwann cells-exo	Titanium (Ti) alloys	Bone defects	Provided both mechanical support and open pores; exhibited good biological activity	Need to check the mechanism underlying the effects of SC-derived exosomes	[69]
	ATDC5(a chondrogenic progenitor cell line)-exo	Soft chitosan (CS) and hard poly lactic acid(PLA)	vascularized osteogenesis	Equivalent functions but higher yields in comparison with the routine exosomes	Need to verify its reliability and effectiveness	[70]
Blood vessel	Macrophage-exo	Alginate + hyaluronic acid	In vitro model	Improved angiogenic activity	Need to optimize and study the morphology and structure of BC-exosomes	[36]
	MSC-derived exo	Chitosan hydrogel +hP-MSCs	Hindlimb ischemia	Enhanced the retention and stability of exosomes; enhanced the therapeutic effect	Difficult to control the release of exosomes	[72]
Nerve	HUCMSCs-derived exo	Collagen/silk fibroin scaffolds	Traumatic brain injury (TBI)	Promote the nerve regeneration after TBI; Inhibited neuritis and the apoptosis of neurotic cells	Insufficient vessel growth associated with injury	[75]
	HUCMSCs-derived exo	Sponge-like alginate scaffold	Nerve injury-induced pain	Slowly releases HUCMSCs exosomes	The loss of stem cells restricts the treatment effect	[78]
	Gingival mesenchymal stem cells-exo	Chitin conduits	Peripheral nerve injury	Increased the number and diameter of nerve fibers and promoted myelin formation	Cannot act in the body for a long time	[79]
	MSCs-exo	GelMA hydrogel	Spinal cord injury (SCI)	Reduced SCI-induced inflammation and glial scarring	Cannot be effectively released in the body for a long time	[76]
	MSC-exos	Photocrosslinked hyaluronic acid methacrylate hydrogel	Peripheral nerve injury (PNI)	Regulated exosome rapid release to repair PNI and motor dysfunction in vivo	Need to analyze the mechanism of exosome-loaded hydrogels inhibiting inflammation	[80]
	hMSC- exo	Peptide-modified adhesive hydrogel	Spinal cord regeneration	Provided an exosome-encapsulated ECM to the injured nerve tissue; induced efficient cell mitigation to the injured sites	The loss of stem cells restricts the treatment effect	[77]
Skin	ASCs-exo	Alginate	Full-thickness skin wounds	Excellent biodegradability and biocompatibility; no side effect or infection; improved vascularization; enhanced wound closure, re-epithelialization, collagen deposition and angiogenesis	Rapid clearance rate and relatively short half-life *in vivo*; Function is relatively too single	[46]
	ADSCs-exo	FEP hydrogel (F127 + polyethylenimine + aldehyde pullulan)	Diabetic wounds	Promoted the granulation tissue formation and collagen formation; generated the skin appendages, and accelerated the healing process in diabetic wounds	The effectiveness of therapy in both types of diabetes is not clear	[90]
Oral	Human periodontal ligament stem cells-exo	Sodium alginate (SA), gelatin (Gel), and nano-hydroxyapatite (na-HA)	Bone defects	Had good rheological properties to be suitable for printing; Had good biocompatibility for osteogenic differentiation of hPDLSCs.	No test of the degree of degradation of the hydrogel and the continuously release of exosomes	[98]

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Conflict of interest

The authors declare no conflict of interest.

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