Received: 18 December 2023 | Revised: 5 February 2024 | Accepted: 11 February 2024

DOI: 10.1111/prd.12557

Periodontology 2000 WILEY

REVIEW ARTICLE

Understanding exosomes: Part 3—therapeutic + diagnostic potential in dentistry

Richard J. Miron[1,2] | Nathan E. Estrin[2,3] | Anton Sculean[1] | Yufeng Zhang[4]

[1]Department of Periodontology, University of Bern, Bern, Switzerland

[2]Advanced PRF Education, Venice, Florida, USA

[3]School of Dental Medicine, Lake Erie College of Osteopathic Medicine, Bradenton, Florida, USA

[4]Department of Oral Implantology, University of Wuhan, Wuhan, China

Correspondence
Richard J. Miron, Department of Periodontology, University of Bern, Bern, Switzerland.
Email: rick@themironlab.com

Abstract

Exosomes are the smallest subset of extracellular signaling vesicles secreted by most cells with the ability to communicate with other tissues and cell types over long distances. Their use in regenerative medicine has gained tremendous momentum recently due to their ability to be utilized as therapeutic options for a wide array of various diseases. Over 5000 publications are currently being published on this topic yearly, many of which in the dental space. This extensive review article is the first scoping review aimed at summarizing all therapeutic uses of exosomes in regenerative dentistry. A total of 944 articles were identified as using exosomes in the dental field for either their regenerative/therapeutic potential or for diagnostic purposes derived from the oral cavity. In total, 113 research articles were selected for their regenerative potential (102 in vitro, 60 in vivo, 50 studies included both). Therapeutic exosomes were most commonly derived from dental pulps, periodontal ligament cells, gingival fibroblasts, stem cells from exfoliated deciduous teeth, and the apical papilla which have all been shown to facilitate the regenerative potential of a number of tissues including bone, cementum, the periodontal ligament, nerves, aid in orthodontic tooth movement, and relieve temporomandibular joint disorders, among others. Results demonstrate that the use of exosomes led to positive outcomes in 100% of studies. In the bone field, exosomes were found to perform equally as well or better than rhBMP2 while significantly reducing inflammation. Periodontitis animal models were treated with simple gingival injections of exosomes and benefits were even observed when the exosomes were administered intravenously. Exosomes are much more stable than growth factors and were shown to be far more resistant against degradation by periodontal pathogens found routinely in a periodontitis environment. Comparative studies in the field of periodontal regeneration found better outcomes for exosomes even when compared to their native parent stem cells. In total 47 diagnostic studies revealed a role for salivary/crevicular fluid exosomes for the diagnosis of birth defects, cardiovascular disease, diabetes, gingival recession detection, gingivitis, irritable bowel syndrome, neurodegenerative disease, oral lichen planus, oral squamous cell carcinoma, oropharyngeal cancer detection, orthodontic root resorption,

Richard J. Miron and Nathan E. Estrin contributed equally to this work.

pancreatic cancer, periodontitis, peri-implantitis, Sjögren syndrome, and various systemic diseases. Hence, we characterize the exosomes as possessing "remarkable" potential, serving as a valuable tool for clinicians with significant advantages.

KEYWORDS
EVs, exosomes, extracellular vesicles, periosomes, regenerative medicine, ultracentrifugation

1 | INTRODUCTION

Exosomes are the smallest extracellular vesicles; they range in size from 30 to 150 nm and are found in the majority of bodily fluids.[1] They can transport functional miRNAs, coupled with the inherent biological functions of exosomes, which makes them a novel delivery platform for gene therapy.[2] Exosomes have many advantages as new drug delivery tools—specifically, they have the characteristics of low immunogenicity and high transport efficiency, they can regulate inflammation, and they can cross the blood–brain barrier (BBB).[3–5] They transport signaling molecules that drive a number of biological processes, including cell signaling, immunological responses, tumor metastasis, and other biological activities. Exosomes have now been shown in several investigations to have both diagnostic and therapeutic functions, in which their precise detection, separation, and quantification are crucial. Today, exosomes are one of the most highly researched topics in regenerative medicine, with over 5000 publications being published on the topic yearly. Part 1 of this 3-part review on exosomes focused on exosome biogenesis as well as their standard isolation techniques, including ultracentrifugation, microfluidics, immunoaffinity, precipitation, size-exclusion chromatography, and ultrafiltration technologies. The second part focused on the therapeutic potential of exosomes in medicine. This third part is divided into 2 major sections investigating all aspects of exosomes in dentistry, including (1) the therapeutic use of exosomes in dentistry and (2) the use of salivary exosomes as diagnostic biomarkers. The potential therapeutic uses of exosomes are as follows:

1. Angiogenesis
2. Bone regeneration
3. Cancer therapy
4. Cementoblast regeneration
5. Endo/pulp regeneration
6. Immune cell behavior
7. Mesenchymal stem cell behavior
8. Nerve regeneration
9. Oral pathogen counts
10. Orthodontic tooth movement/resorption
11. Periodontal disease/periodontitis
12. Temporomandibular joint (TMJ)
13. Other treatments

This scoping review is the first of its kind, with a broad objective to summarize the extensive regenerative potential of exosomes across a wide range of treatments within the dental field.

1.1 | Search strategy

An initial search using terms related to exosomes (exosomes, extracellular vesicles) and dentistry (dental, periodontal, pulpal, orthodontics, endodontics, periodontics, dentistry) was performed on September 1st, 2023. A total of 944 publications were initially retrieved. Of these, 290 were selected and further evaluated. Following the full-text screening, a total of 113 research articles that examined the regenerative potential of exosomes were included (102 in vitro studies, 60 in vivo studies, and 49 studies that included both in vitro and in vivo components). The included studies examined the therapeutic potential of exosomes for the treatment of dental tissues/diseases or when exosomes were isolated from dental sources. Forty-seven articles were selected and included as diagnostic biomarkers of exosomes for various diseases and illnesses.

2 | RESULTS

In total, 13 in vitro subsections and 8 in vivo sections were identified as key groups related to exosomes derived from preclinical studies. Seventeen subsectional groups were observed when exosomes from salivary and crevicular fluids were used for diagnostic purposes.

2.1 | Angiogenesis

In total, 12 of the included studies investigated the therapeutic potential of exosomes for angiogenesis in vitro,[6–17] while only 1 study investigated the regenerative potential of exosomes in vivo.[14] In total, 5 studies isolated exosomes from hPDSCs, 3 studies isolated exosomes from SHED, 2 studies isolated exosomes from SCAP, 1 study isolated exosomes from hPDLCs, and 1 study isolated exosomes from hBMSCs (Table 1). Each in vitro paper tested human-derived exosomes and utilized a target cell line of HUVECs. The results from this section revealed that several isolated exosomes from dental sources were able to promote HUVEC proliferation, VEGF expression, proangiogenic factors, tube formation and capillary formation capacity in vitro (Figure 1, Table 1). Figure 2 highlights the in vivo model utilized by Liu et al.,[14] demonstrating that even subcutaneously injected exosomes into the dorsal skin along the abdomen of female nude mice led to a significantly greater increase in new blood vessel formation.

TABLE 1 In vitro and in vivo therapeutic regenerative potential of various exosomes derived from multiple sources on angiogenesis.

Author–year	Source of exosomes	Target tissue of exosomes/Duration of the study	Main findings
Angiogenesis—in vitro			
Xian et al. (2018)[6]	Human dental pulp stem cells (DPSCs)-Exos	Endothelial cells from human umbilical veins (HUVECs) The culture medium was centrifuged at 300 ***g*** for 10 min followed by 2000 ***g*** for 10 min to remove the cells and debris. The supernatant was concentrated with a Ultra-15 Centrifugal Filter Unit. After centrifugation at 10000 ***g*** for 30 min to further remove the debris, the cell-free supernatant was centrifuged at 100 000 ***g*** for 1 h. The pellet was resuspended in PBS and centrifuged again at 100 000 ***g*** for 1 h. All procedures were conducted at 4°C	DPSC-Exos stimulated the growth of HUVEC cells, increased the expression of proangiogenic factors, and facilitated the creation of blood vessels in vitro. Furthermore, blocking p38 MAPK signaling improved DPSC-Exos' capacity for angiogenesis. The mRNA level for MMP9 increased 2-fold with DPSC-Exos when compared to control
Wu et al. (2019)[7]	Exfoliated deciduous teeth from humans (SHED)-Exos stem cells	HUVECs HUVECs were harvested, resuspended in EGM with or without exosomes, and seeded into 48-well plates coated with Matrigel	The results demonstrated that the levels of angiogenesis-related genes showed a 3–15 fold increase in mRNA levels of KDR, FGF2 and VEGF was reported
Zhang et al. (2020)[8]	Human periodontal ligament cells (hPDLCs)-Exos	HUVECs Exosomes cultured with HUVECs for 5 h	PDLC-Exos enhanced the formation of new blood vessels by promoting angiogenesis in HUVECs. Conversely, inhibiting the release of exosomes resulted in impaired angiogenesis of HUVECs. Exosomes carrying vascular endothelial growth factor-A (VEGFA) have been shown to have a vital role in facilitating communication between PDLSCs and HUVECs. Tube length increased 3 fold in the Exo-group after 14 days
Gardin et al. (2021)[9]	Human bone marrow stem cells (hBMSCs)-Exos	HUVECs Incubated for 4 h with HUVECs	Nanomodifications of titanium surfaces greatly enhanced releasing exosomes produced from mesenchymal stem cells sourced from human bone marrow (hBMSC-Exos). HUVECs effectively took up hBMSC-Exos, and thereafter enhanced their migration and differentiation. Certain angiogenic markers such as VEGFA was increased 4 fold over the baseline after 7 days
Huang et al. (2021)[10]	hDPSCs stimulated with/without Lipopolysaccharide (LPS)	HUVECs Exosomes were added and incubated for 4 h at 37°C	There was a significant difference between the LPS-Exo and non-LPS-Exo groups in the mRNA expression levels of VEFG and kinase-insert domain-containing receptors. In vitro experiments demonstrated that exosomes isolated from inflammatory hDPSCs had much higher proangiogenic capacities. The LPS-Exos increased migration by 250% and VEGF by 350%

(Continues)

TABLE 1 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes/Duration of the study	Main findings
Wu et al. (2021)[11]	SHED	HUVECs The supernatant was centrifuged at 2000 *g* × 10 min. Next, the supernatant was centrifuged at 10 000 *g* × 30 min. The supernatant was then ultracentrifuged at 100 000 *g* × 70 min. Finally, the pellet was washed using PBS by ultracentrifugation at 100 000 *g* × 70 min	The in vitro tube formation experiment showed SHED-Exos increased HUVEC angiogenicity. MiR-26a regulates the TGF-β/SMAD2/3 signaling pathway to improve blood vessel development in HUVECs. In the SHED-Exo group, the expression of VEGF, PDGF, and Angiogenin nearly doubled when compared to the SHED group alone
Li et al. (2022)[12]	hDPSCs in hypoxic (Hypo-Exos) and normoxic (Nor-Exos) environments	HUVECs Exosomes were isolated from the culture media of DPSCs cultured under normoxia or hypoxia. Exosome were isolated by centrifugation at 300 *g* × 10 min followed by 2000 *g* × 10 min to remove the cells. After centrifugation at 10 000 *g* × 30 min to eliminate the debris, the supernatant was concentrated with an Ultra-15 Centrifugal Filter Unit. Subsequently, the concentrated supernatant was centrifuged at 100 000 *g* × 1 h. The pellets were resuspended in PBS and centrifuged at 100 000 *g* × 1 h. All procedures were conducted at 4°C	NorExos improved the in vitro migration, proliferation, and tube formation of HUVECs. The proteomics analysis found that Hypo-Exos significantly changed the expression of 79 proteins. It was shown that hypoxia-preconditioned DPSCs, Hypo-Exos, and inflammatory dental pulp all had elevated levels of LOXL2
Lin et al. (2022)[13]	Exosomes produced from apical papilla (SCAP) human stem cells	HUVECs The labeled exosomes were cocultured with HUVECs for 12 h	Exosomes produced from SCAPs stimulated HUVEC angiogenesis in hypoxic environments by expressing miR-126, which in turn inhibited SPRED1 and triggered the ERK signaling cascade. Vascular tube totals increased by over 50% in the Exo group and over 2 fold when miR-126 were compared in a migration assay
Liu et al. (2022)[14]	Human SHED preconditioned under hypoxia (Hypo-Exos) and from SHED cells cultivated normally (Norm-Exos)	HUVECs Exosomes were added to HUVECs and incubated at 37°C for 24 h	Hypo-Exos boosted endothelial cell proliferation, migration, and tube formation in a lab environment more than Norm-Exos. Hypo-Exos increased in vitro wound closure by an extra 50%
Zhou et al. (2022)[15]	hDPSCs-Exos	HUVECs The cell culture supernatant was harvested and subjected to two centrifugation steps (500 *g* for 10 min at 25°C; 2000 *g* for 30 min at 25°C) to remove dead cells and cell debris. Exosome pellets were purified from the cleared supernatant by ultracentrifugation at 100 000 *g* for 1 h at 4°C	The DPSC-Exos accelerated wound healing by promoting neovascularization. The DPSC-Exos improved HUVEC migration, proliferation, and capillary formation
Liu et al. (2023)[16]	SCAP-Exos under hypoxia	HUVECs HUVECs were cultured with exosomes for 12 h	The angiogenesis capacities of HUVECs were significantly boosted in vitro by exosomes derived from SCAPs that were trained under hypoxic conditions. This augmentation was shown using a tube formation test and RT-qPCR of angiogenesis-related molecular markers. The migration of Hypo-Exos was improved by 200% over the baseline and VEGF increased by over 350%

TABLE 1 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes/Duration of the study	Main findings
Shi et al. (2023)[17]	hDPSC-Exos	HUVECs After 24 h, the culture medium was collected and sequentially centrifuged at 300 ***g*** for 10 min, 3000 ***g*** for 10 min and 10 000 ***g*** for 30 min. Then, cellular debris was removed and the supernatants were collected. To precipitate exosomes, the supernatants were ultracentrifuged for 2 h at 100 000 ***g***	The hDPSC-Exos stimulated the growth, movement, and creation of capillaries in HUVECs in a manner that depended on the dosage, achieved via activating the PI3K/AKT signaling pathway. The increase was routinely 3-fold higher in the Exo group at the highest doses

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Angeogenesis—in vivo			
Liu et al. (2022)[14]	Human hypoxic-preconditioned SHED cells (Hypo-Exos) and normally grown SHED cells (Norm-Exos) are the two cell types that are mentioned in the text	The angiogenesis ability was assessed using Murine Matrigel plug tests in vivo. Four-week-old female BALB/C nude mice were given one single subcutaneous injection of exosomes into the dorsal skin along the midline of their abdomens. The final assay was assessed 10 days later	The in vivo testing revealed an increased production of VEGF and a greater quantity of luminal structures that exhibited positive staining for CD31 in the Hypo-Exos group. Vessels around the implant increased by over 50% in the Hypo-Exo group

Abbreviations: BALB/C, albino, laboratory-bred strain of mouse; DPSCs, Dental Pulp Stem Cells; ERK, Extracellular Signal-regulated Kinases; Exo, Exosome; FGF2, Fibroblast Growth Factor-2; hDPSCs, human DPSCS; hPDLCs, human PDLCs; HUVECs, Human Umbilical Vein Endothelial Cells; Hypo-Exos; KDR, Kinase Insert Domain Receptor; LOXL2, Lysol oxidase-like-2; LPS, Lipopolysaccharide; LPS-exo, exosome with LPS; MAPK, mitogen-activated protein kinase; miR, microRNA; Non-LPS-exo, exosome without LPS; Nor-exo, exosomes under normoxia; P13K, Phosphoinositide 3-kinase, AKT1, set of three serine/threonine-specific protein kinases; PDLCs, Periodontal Ligament Stem Cells; RT-qPCR, Reverse transcription-quantitative polymerase chain reaction; SCAPs, Stem Cells from the Apical Papilla; SDF-1, Stromal Cell Derived Factor-1; SHED, Stem cells from Human Exfoliated Deciduous teeth; SMAD, Suppresor of Mothers against Decapentalegic; SPRED1, Sprouty Related EVH1 Domain Containing 1; TGF-β, Transforming Growth Factor-β; Ti, Titanium; VEGFA, Vascular endothelial growth factor A.

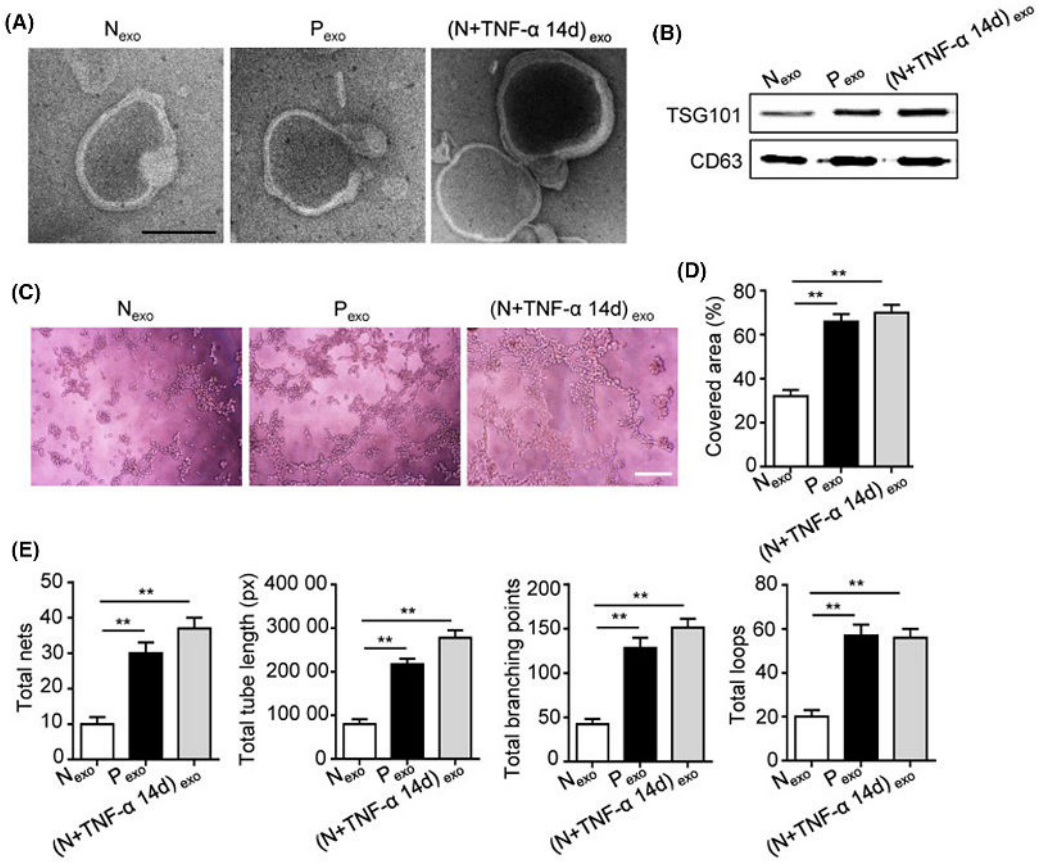

FIGURE 1 Impact of PDLSCs on angiogenesis in inflammatory circumstances via the exosome-mediated pathway. (A) SEM was used to identify the secretion of exosomes. Scale bar: 100 nm. (B) TSG101 and CD63, two exosome-specific markers, were investigated using western blot. Exosomes produced from PDLSCs were used to treat HUVECs, which resulted in tube formation (C). After that, Image J was used to examine the total length of the tubes, total branching points, total loops, covered area, and total nets (D, E). Scale bar: 200 μm. $^{**}p < 0.01$. One-way analysis of variance (ANOVA). Reprinted with permission from Zhang et al.[8]

2.2 | Bone regeneration

In total, 28 studies investigated bone regeneration preclinically, of which 28 studies investigated the therapeutic potential of exosomes in vitro, while 11 studies investigated the therapeutic potential of exosomes in vivo (11 of the studies used both in vitro and in vivo methods). Based on the in vitro data, a total of 9 different derived exosomes were isolated as follows: 6 studies isolated PDLC-Exos, 6 studies isolated BMSC-Exos, 5 studies isolated macrophage-Exos, 3 studies isolated DPSC-Exos, 3 studies isolated SHED-Exos, 2 studies isolated ADSC-Exos, and one study each isolated gingival MSC-Exos, SC derived-Exos, and ginseng-derived-Exos (Table 2). In the majority of studies (61% or 17/28 studies), exosomes were isolated from humans; additionally, exosomes were isolated from mice in 6 studies (primarily RAW264.7 cells), rats in 4 studies (primarily rat BMSCs) and plant-based exosomes in one study (ginseng). All target cells examined in vitro demonstrated the stimulatory effects of exosomes on osteogenic differentiation, with the exception of research conducted by Seo et al.,[41] who investigated the impact of bone marrow-derived macrophages on osteoclast inhibition by exosomes. The target tissue was BMSCs in 27/28 studies, of which 12 were performed on human BMSCs, whereas 7 studies were performed on rats and 9 were performed on mice. All studies demonstrated favorable outcomes utilizing exosomes. One of the reported findings also depicted that, based on the fact that exosomes can avoid immune recognition, several cross-species results demonstrated excellent and favorable outcomes.

Based on the in vivo data, a total of 6 different sources of exosomes were isolated as follows: 5 studies isolated BMSC-Exos, 2 studies isolated DPSC-Exos, 1 study isolated macrophage-Exos, 1 study isolated adipose-derived-Exos, 1 study isolated SC derived-Exos, and 1 study isolated ginseng-derived-Exos (Table 2). In just over half of the studies (6/11 studies), exosomes were isolated from humans, whereas exosomes were isolated from rats in 3 studies (primarily rat BMSCs), mice in 1 study and plants in 1 study (ginseng). Of the 11 in vivo models, 8 tested calvarial defect models (4 rat studies and 4 mouse studies), 1 tested white rabbit lateral femoral defects, 1 tested rat alveolar bone defects and 1 tested rat ramus defects. All of the included studies concluded

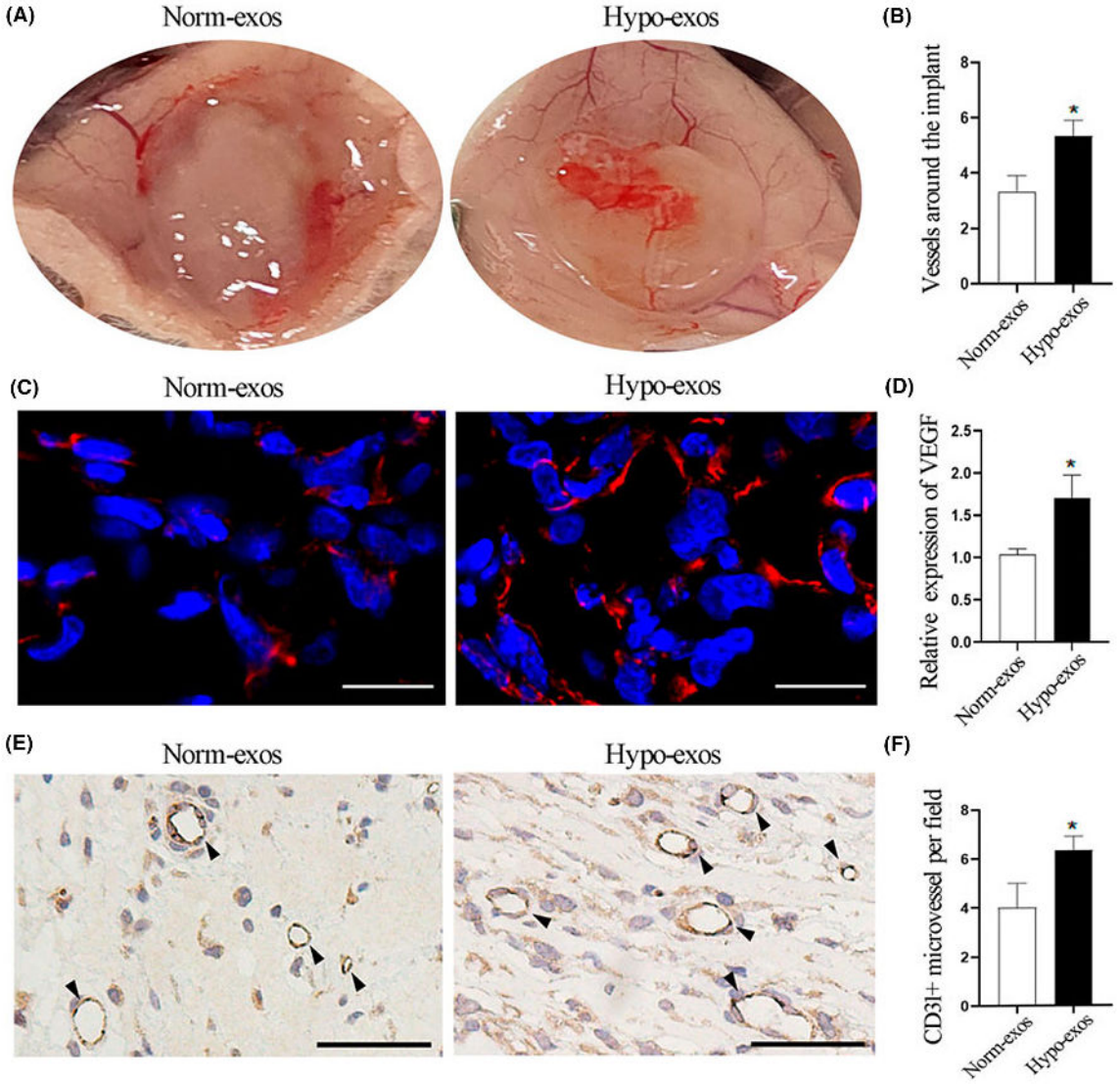

FIGURE 2 Exosomes encourage the in vivo development of microvessels. (A) Typical pictures of Matrigel plugs placed under the skin in mice for 10 days. (B) measurement of the quantity of microvessels around the implanted plugs. (C) VEGF immunofluorescence labelling on implanted Matrigel plugs with Norm- or Hypo-expos. Scale bar: 500 μm. (D) VEGF expression in Matrigel plugs quantified. (E) In matrigel plugs with Hypo-expos, CD31 immunofluorescence labeling reveals increased neovasculature (Scale bar = 500 μm). (F) Quantification of CD31-stained tubular structures. *$p < 0.05$. Reprinted with permission from Liu et al.[14]

that exosomes derived from any of the above-mentioned sources promoted osteoblast differentiation and improved new bone formation in vivo.

Two noteworthy preclinical in vitro studies demonstrated interesting outcomes while investigating exosomes on in vitro osteoblast activity. A study by Lan et al.[32] found that utilizing PDLC-Exos not only promoted proliferation and migration of the human osteoblast cell line hFOB1.19 but also led to significantly faster wound healing scratch assay closure, as depicted in Figure 3. To investigate the regenerative potential of exosomes, Lee et al.[40] compared DPSC-Exos to rhBMP2 and found that the exosomes were just as prolific as rhBMP2 on osteogenic differentiation in vitro (Figure 4). Furthermore, each of the animal models demonstrated favorable bone regeneration outcomes. A study by He et al.[38] demonstrated complete regeneration of a critical-size ramus defect using exosomes (Figure 5).

2.3 | Cancer therapy

Two studies investigated the use of exosomes in cancer therapies in vitro (Table 3). While these studies are still in their infancy, both utilized either an immortalized cell line or exosomes packed with a chemotherapeutic agent (gemcitabine) and showed that both were able to attenuate carcinoma cell lines (human carcinoma cell line TR146[45] and human pancreatic cell line[46]).

TABLE 2 In vitro and in vivo therapeutic regenerative potential of various exosomes derived from multiple sources on bone regeneration.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Bone regeneration—in vitro			
Li et al. (2018)[18]	Human adipose-derived stem cell (hADSC-Exos)	hBMSCs Exosome pellets were incubated with hBMSCs for 2, 24, 48, and 72 h, respectively	Human bone marrow-derived mesenchymal stem cells (hBMSCs) were shown to be activated in an in vitro experiment by the steady and progressive release of exosomes from the PLGA/pDA scaffold. ALP activity was increased roughly 2 fold in the Exo-group at 7 and 14 days post-culture. Exos also significantly increased BMSC recruitment roughly 3 fold
Du et al. (2019)[19]	Mouse Exos derived from 3T3L1 preadipocytes (3T3L1-Exos)	Mouse 3T3L1 cell line Cells were stimulated with 2 μg/mL 3T3L1-exo for 24 h prior to analysis	This work validated that the addition of 3T3L1 exosomes accelerated the process of osteogenic differentiation in 3T3L1 preadipocytes. This was shown by the increased expression of genes related to osteogenic differentiation including over a 2-fold increase in ALP, Runx, Osterix, OCN and BSP, as well as a higher intensity of Alizarin Red staining
Takeuchi et al. (2019)[44]	hBMSCs-Exos	hBMSCs Cells were cultured with BMSC-Exo (5 μg/mL) + DMEM(−) (Exo-CM) or with MSC-CM for 1–14 days depending on the test	hBMSC-Exos increased the cell migration and differentiation including upregulation of many genes related to osteogenesis and angiogenesis. As a whole, a 50% increase in many genes related to osteogenesis were reported including ALP, Col1, OCN, OPN, and VEGF
Wu et al. (2019)[7]	SHED-Exos	Rat BMSCs Two different concentrations of exosomes (5 μg/mL and 10 μg/mL) were used to evaluate in vitro behavior	Although compound C mitigated the favorable effects on BMSCs, the findings demonstrated that exosome treatment enhanced the expression of osteogenesis-associated genes (COL1, RUNX2, and OPN) and phosphorylated (p)-AMPK. Migrated cell numbers was increased by over 50%, whereas ALP activity was increased by 2 fold. Both Runx2 and OPN showed over a 2-fold increase in mRNA expression levels
Huang et al. (2020)[22]	hBMSC-Exos	hBMSCs 6×106 EVs were added into culture for every 30000 HMSCs and extracted 48 h later	Isolating hBMSC-Exos separately enhanced the osteogenic differentiation of hBMSCs. VEGF, OSX, Runx2, BMP6 and BMP9 increased between 2 and 3 fold, whereas BMP2 increased over 6 fold
Ivica et al. (2020)[20]	hDPSC-Exos	hBMSCs Cells were culture in DMEM +10% FBS + exosomes (10 μL)	The study demonstrates that exosomes exert chemotactic effects on BMSCs and enhance their proliferation. A suggestion was made that using exosomes produced from pulp, together with a fibrin gel, might be a potent combination for advancing cell-free regenerative treatments and providing a novel approach to filling dental hard tissues. Migrated cells increased by a 2-fold versus controls
Jiang and Xu (2020)[23]	Human gingival MSCs (hGMSC-Exos)	Mouse preosteoblast MC3T3-E1 Cultured in a medium supplemented with or without GMSCs-Exos	GMSC-Exos promoted osteogenic differentiation in MC3T3-E1 cells by easing the migration of preosteoblasts and allowing them to differentiate into cells that create bone. Alizarin red staining, alkaline phosphatase (ALP) activity, and gene expression levels increased. Over a 2 fold increase in the in vitro scratch wound healing assay was observed. mRNA levels of ALP, BMP2, OCN, and Runx2 were all increased between 5 and 7 fold
Kang et al. (2020)[24]	Mouse macrophage Line J774A.1 as (M0), M1 and M2 polarized macrophage-derived-Exos	Mouse MSCs EVs were diluted and standardized to cell number and particle number that every 100 μL of EV suspension contained approximately 8 Å ~ 108 EVs	The functional analysis of miR-155, which is concentrated in M1 macrophage exosomes, revealed a decrease in the osteogenic differentiation of MSCs. Reductions in the expression of BMP2, BMP9, and RUNX2 relative to the control group served as an indicator of this reduction. Alternatively, osteoinductive gene expression in MSCs was upregulated after treatment with the M2-macrophage EV-enriched miR-378a mimic
Li et al. (2020)[21]	hBMSC-Exos from the jaw bone	hBMSCs from iliac crest bone A co-culture model was presented with both cell types Exos and hBMSCs	Osteogenic gene expression, ALP activity, and ARS staining were all enhanced in vitro by exosomes produced by BMSCs-J. Transmission electron microscopy and nanoparticle tracking analysis were used to establish the existence of exosomes in the culture supernatants of BMSCs. ALP went up 2 fold whereas Runx2 and OSX were increased between 50 and 100%

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Liu et al. (2020)[25]	hPDLCs-Exos	Rat BMSCs Exosomes were added to the BMSC culture medium with 6 h of incubation to measure the uptake. Differentation assays were performed using BMSCs exposed to 50 μg/mL exosomes derived from undifferentiated PDLSCs (Exos_NC) and osteogenically differentiated PDLSCs of different time points (Exos_D3, Exos_D7, Exos_D14)	Exosomal miRNAs derived from osteogenic differentiated PDLSCs promoted the osteogenic differentiation of bone marrow stem cells (BMSCs). This finding suggests that exosomal miRNAs of PDLSCs may play a regulatory function in osteogenesis. OM + Exos_D14 had the most prolific osteogenic differentiation potential when compared to all other groups
Swanson et al. (2020)[26]	hDPSCs-Exos	hBMSCs Labeled EXOs was added to 800 μL of culture media and incubated for 30 min	Mineralization results from the controlled release of exosomes obtained from osteogenic hDPSCs, which is similar to the exogenous delivery of identical exosomes in human and mouse BMSCs. A roughly 10 fold increase in OCN and BSP mRNA levels at 14 days and alizarin red staining was observed at 21 days
Wei et al. (2020)[27]	SHED-expos	Mouse BMSCs The supernatant was collected and centrifuged at 300 ***g*** for 10 min, and 2000 ***g*** for 10 min. Upon removing non-adherent cells and debris, conditioned medium (CM) was collected and named SHED-CM. SHED-CM and BMSCs culture medium were mixed as 1:1 and treated BMSCs in the following experiments. EVs were further purified by ultracentrifugation at 10 000 ***g*** for 60 min. The supernatant was filtered through a 0.22-μm filter (Millipore) to remove the microvesicles and ultracentrifuged at 100 000 ***g*** for 70 min. The pellet was washed with PBS to eliminate contamination of proteins and centrifuged again at 100 000 ***g*** for 70 min	SHED-Exo exhibited moderate inhibition of apoptosis while particularly promoting the process of osteogenesis in BMSCs and inhibiting adipogenesis. The co-cultivation of SHED-Exos with BMSCs resulted in a considerable increase in the expression of osteogenic marker genes, ALP activity, and Alizarin Red S staining
Wu et al. (2020)[28]	Rat Schwann cells-Exos (SC-Exos)	Rat BMSCs SC-Exos were co-cultured with rat BMSCs. 105 cells/mL and incubated with the following factors: (1) pure a-MEM (control); (2) exosome-containing medium (107 particles/mL, Exos L); or (3) exosome-containing medium (108 particles/mL, Exos H)	Exosomes produced from stem cells enhanced the migration, proliferation, and differentiation of bone marrow-derived mesenchymal stem cells. ALP activity was increased over 3 fold in the Exo-high group. mRNA levels of ALP, Runx2, Col1, and OPN was increased 2 fold

(Continues)

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Luo et al. (2021)[29]	SHED-Exos	hBMSCs EVs collected after 24 h, 48 h, and 72 h	NTA data showed that sonication caused SHEDs-Exos collected at 24 h, 48 h, and 72 h (EV24, EV48, and EV72) to reorganize into smaller sizes, suggesting that they were secreted as exosomes. Moreover, according to the results of the ELISA tests, several EV groups were discovered to have high levels of various growth factors, such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), and fibroblast growth factor-2 (FGF-2)
Zhang et al. (2021)[30]	Mouse RAW264.7 macrophages-Exos	Mouse MC3T3-E1 cells Cells were co-cultured together and the secreted exosomes were studied for their ability to induce osteogenic differentiation	Exosomes produced from RAW264.7 cells stimulated the process of osteogenic development and mineralization in MC3T3-E1 cells. The research gave a more detailed explanation of the process by which implants integrate with bone and offered new knowledge about the impact of exosomes on this integration. These findings may be used as a reference to enhance the clinical outcomes of implant integration and increase the success rate of implants
Chen et al. (2022)[31]	Mouse M2-like macrophages (M2-Exos)	1. Mouse BMSCs 2. Mouse BM-macrophages Exos were added to the culture medium of BMSCs and BMDM for 6 h with confirmed uptake. Then the cells were assessed under various in vitro scenarios	Exosomes enhanced the process of bone marrow stromal cells (BMSCs) differentiating into bone-forming cells while simultaneously inhibiting the production of bone marrow-derived macrophages (BMDM) that break down bone tissue. It was found that M2-Exos improved ALP and Collagen1 by 3 fold as well as OCN and Runx2 over 25 fold. The number of osteoclasts was reduced over 6 fold
Lan et al. (2022)[32]	hPDLSCs-Exos	Human osteoblast cell line hFOB1.19 Exo were incubated with hFOB 1.19 cells at 37°C for 3 h for internalization analysis. The PDLSC-Exos were added to culture medium for cell experiments	PDLSC-Exos have the ability to enhance the growth, movement, and bone-forming differentiation of hFOB1.19 cells. They also prevent cell death caused by H_2O_2 and activate the PI3K/AKT and MEK/ERK signaling pathways. The relative migration rate increased 3 fold in the high-concentration Exo-group
Liu et al. (2022)[33]	Mouse RAW264.7 macrophages-Exos	Mouse BMSCs CM formed by the M0, M1, and M2 macrophages. To the osteogenic media employed for culturing BMSC, M0-Exos, M1-Exos, or M2-Exos were added at a concentration of 1 μg/mL and investigated at 7 days for ALP and real-time PCR and 14 days for Alizarin Red staining	Results showed that bone marrow-derived mesenchymal stem cells (BMSCs) might benefit from exosomes produced by both M1 and M2 polarized macrophages when it came to enhancing bone production. The M1 derived exosomes seemed to favor the best osteogenic differentiation as evidenced by an up to 6 fold increase in ALP, BMP2, OPN, and Runx2 gene expression profiles
Novello et al. (2022)[34]	hPDLC-Exos	Saos-2 cells are a kind of human osteoblast cell line CM was collected from hPDLCs after 48 h of culture. The CM was then centrifuged and utilized as the Exo group	The introduction of PDLSC-Exos to Saos-2 cells resulted in a stimulation of their growth and upregulation of certain osteoblastic differentiation markers. Specifically IL-6 and OPN were upregulated most-significantly (2 fold increase) when compared to all genes analyzed
Song et al. (2022)[35]	Mouse RAW 264.7 macrophage-Exos induced with *P. gingivalis*	Mouse BMSCs four groups: ordinary exosome group, inflammatory exosome group, CM group, and CM + GW4869 group. 48 h after transfection, cells were collected for subsequent experiments	Macrophages may stimulate inflammation and block the exosome pathway that bone marrow mesenchymal stem cells use to differentiate into osteogenic cells. Inflammatory situations may benefit from inhibiting exosome production as a potential approach for bone tissue repair

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Zhao et al. (2022)[36]	hPDLSC-Exos	Rat BMSCs Centrifugation was carried out at 300 *g* for 10 min, 2000 *g* for 10 min, and 10 000 *g* for 30 min to eliminate dead cells and cellular debris. Subsequently, these samples were filtered through a 0.22 μm filter and centrifuged at 100 000 *g* for 70 min. After the supernatant was removed, pellets were resuspended in PBS and centrifuged again at 100 000 *g* for 70 min	Exosomes isolated from periodontal ligament stem cells significantly increased the osteogenic differentiation and proliferation of BMSCs
Chang et al. (2023)[37]	hPDLC-Exos	hBMSCs Culture medium was changed to basal medium with exosomes (100 μg/mL) and examined after 1, 2, and 6 h of incubation. Cells were treated with exosomes derived from cyclic tension stretched PDLCs (Exos_CTS, 30 μg/mL), unstretched PDLCs (Exos_US, 30 μg/mL) or PBS	Exosomes obtained from PDLCs subjected to cyclic tension stretching have the ability to enhance the migration of BMSCs by over 2 fold. The variation in microRNA profiles serves as a foundation for further investigation into the regulatory role of exosomal miRNAs in PDLCs
He et al. (2023)[38]	Rat BMSC-Exos	Rat BMSCs Cultured with 5% CO_2 in the incubator for 12 h	The in vitro experiment showed that CTNNB1 was successfully delivered to BMSCs by the PF127 hydrogel that was loaded with BMSC-Exos. The result was a rise in RUNX2 and OCN expression, mineralization of the extracellular matrix, and stronger and more active ALP staining, all of which pointed to the stimulation of osteogenic differentiation of BMSCs. Both ALP activity and alizarin red staining were increased 2 fold in the Exo-group
Lai et al. (2023)[39]	Rat BMSCs-Exos	Rat BMSCs BMSCs were incubated with Exos for 6, 12, 24, and 48 h	Exosomes containing miR-26a may promote growth, movement, and formation of bone tissue in BMSCs. After 48 h in vitro wound closure was doubled. The relative mRNA expression of BMP2 was increased 3 fold, ALP over 6 fold, and collagen type 1 was doubled
Lee et al. (2023)[40]	hDPSC-Exos	Human bone marrow-derived stromal cells 20 μg/mL DPSC-EVs for 72 h	Human bone marrow-derived mesenchymal stem cells (hBMSCs) and exosomes produced by dental pulp stem cells (DPSC-Exos) were both significantly improved after administration, leading to an increased expression of osteogenic genes such RUNX2, ALP, and OCN. The researchers discovered that the pro-osteogenic impact seen in laboratory conditions caused by DPSC-Exos that was produced was comparable to that of BMP-2. Both ALP and OCN mRNA levels and alizarin red staining were increased over 2 fold when compared to controls and to similar levels (or better than) rhBMP2
Seo et al. (2023)[41]	Ginseng-derived extracellular nanovesicles (GDNs)	Mouse bone marrow-derived macrophages (BMDMs) BMDMs were seeded into 48-well plates (2.5 × 104 per well) along with differing GDNs and cultured for 1, 3, and 7 days	The administration of GDNs preserved the high vitality and proliferation of BMDMs while inhibiting the formation of osteoclasts. Analysis using tartrate-resistant acid phosphatase (TRAP) and F-Actin staining demonstrated that concentrations of GDNs greater than 1 μg mL^{-1} significantly impeded the process of osteoclast development by as much as 75%. In addition, they significantly inhibited the RANKL-induced IκBα, JNK (c-JUN n-terminal kinase), and extracellular signal-regulated kinase signaling pathways, as well as the genes that control osteoclast formation

(Continues)

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Wang et al. (2023)[42]	hPDLC-Exos	hBMSCs Extraction was performed by centrifuging at 3000 *g* for 15 min to remove dead cells and debris and then filtering through a 0.22 μm filter to remove bacteria and impurities with a diameter greater than 200 nm. The filtrate was filtered with an Ultra15 centrifugal filter unit	Following 14 days, the results showed that the OPG/RANKL ratio gene expression levels of Osteocalcin (OCN), RUNX2, and osterix were all increased favorably
Xu et al. (2023)[43]	Rat BMSCs-Exos	Rat BMSCs Exosomes were loaded with photothermal material layered black phosphorus (BP) modified poly(N-isopropylacrylamide) (PNIPAAm) thermosensitive hydrogels	Experiments conducted in a laboratory setting showed that BP hydrogels containing BMSC-derived exosomes exhibited excellent compatibility with living organisms and were able to enhance the growth and specialized development (ALP activity) of MSCs. ALP was increased over 8 fold and mRNA levels was increased 6 fold fold ALP, 5 fold for Runx2, 4 fold for Col1, and 3 fold for OPN

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Bone regeneration—in vivo			
Li et al. (2018)[18]	hADSC-Exos	Mice calvarial defects For the immobilization of exosomes, PLGA/pDA, or PLGA only, substrates were immersed in 1 μg/μL exosome solution (250 μL/scaffold) for 12 h at 4°C. A 4 mm diameter critical-sized defect was made at the calvarium with a trephine bur and implanted with. (1) PLGA scaffold only (PLGA group); (2) PLGA scaffold coated with pDA (PLGA/pDA group); (3) PLGA/pDA scaffold + exosomes (PLGA/pDA-Exo group). Bone formation investigated 6 weeks post-implantation	According to in vivo data, this cell-free system greatly accelerated bone regeneration, at least in part because of its osteoinductive properties and ability to encourage the migration and homing of mesenchymal stem cells in the developing bone tissue. A marked 125% increase in new bone volume was observed in the exosome group
Takeuchi et al. (2019)[44]	hBMSCs-Exos	Rat model of calvaria bone Two defects of 5 mm in diameter were carefully made in the calvarial bone using a trephine drill. Atelocollagen sponges were used as a scaffold, and were soaked with MSC-Exo (30 μg), MSC-Exo (30 μg) + anti-VEGFA antibody (1 μg), MSC-CM, or PBS. Micro-CT and histological evaluation was performed at 1, 2, 3, and 4 weeks post-op	hBMSC-Exos promoted early bone regeneration in addition to increased angiogenesis. The exosome group doubled the percentage of new bone formation area at all time points when compared to controls. Furthermore, an anti-VEGF molecule was used in the exosome group demonstrating much lower new bone formation indicating that many of the effects of exosomes are related to angiogenesis
Huang et al. (2020)[22]	hBMSC-Exos	Rat calvarial bone defect Two 5 mm calvarial defects were created bilaterally in the calvarium without dura perforation using a trephine burr. A clinical grade collagen tape was placed on the wound with/without EVs (5×10^8 EVs per defect). Collagen tape alone served as control and rhBMP2 as positive control. Animals were euthanized at 4, 8, and 12 weeks post surgery	In rat calvarial abnormalities, genetically engineered hBMSCs (BMP2 overexpression) generated greater bone repair than control EVs. Therefore, EVs generated from hBMSCs may be modified for a variety of uses in regenerative medicine including the incorporation of BMP2 over-expression models

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Kang et al. (2020)[24]	Mouse macrophage Line J774A.1 (M0), M1 and M2 polarized macrophage-derived EVs	Rat calvaria defects Bilateral transcortical defects were created in male 8-week-old Sprague–Dawley rats using a 5.0 mm trephine dental drill. Defects were filled with collagen scaffolds containing PBS, or EVs (8 Å ~ 109/defect) from M0, M1, or M2 macrophages Healing was evaluated at 3 and 6 weeks post-op	M1 EVs impeded bone repair, whereas M0 and M2 EVs encouraged regeneration and repair. The M2 macrophage improved new bone formation by roughly 50% more than controls
Li et al. (2020)[21]	hBMSC-Exos from the jaw bone	Mouse calvarial defect model nonhealing, critical-sized calvarial defects (4 mm in diameter) were created using a sterile dental drill 12 week healing period	The in vivo stimulation of BMSC-Exos bone formation was shown in this mouse model of calvarial insufficiency by exosomes produced by BMSCs-J. BV/TV was increased over 4 fold in the Exo group, particularly from the Jaw bone
Swanson et al. (2020)[26]	hDPSC-Exos	Mouse calvarial defect. Exosome-releasing spheres (EXO-MS) were physically attached to the surface of nanofibrous, microporous tissue engineering scaffolds by a post-seeding method. A 2.7 mm craniotomy was performed using a trephine burr centered on the parietal calvarial bone and suture New bone formation evaluated at 8 weeks	At 8 weeks, the calvaria underwent microcomputed tomography (μCT) imaging, which showed that the hDPSC-Exos functionalized scaffold had more bone regeneration than both the control and blank MS-loaded scaffolds. The bone volume was increased by over 4 fold in calvarial defects when compared to controls
Wu et al. (2020)[28]	Rat Schwann cells-Exos (SC-Exos)	White rabbits—lateral femoral bone defect filled with titanium scaffold with/without exosomes. A cylindrical defect 5 mm was made scaffold in the lateral femoral epicondyle and then filled with Ti6Al4V scaffolds in the Exos L and Exos H groups were filled with Matrigel containing 107 particles/mL and 108 particles/mL exosomes. Animals were euthanized at 6 and 12 weeks	When coupled with porous titanium alloy, SC-derived exosomes may significantly increase the effectiveness of titanium alloy scaffolds used in bone healing. BV/TV was increased over 2 fold and bone mineralization was increased 3 fold
He et al. (2023)[38]	Rat BMSC-Exos	Rat models of alveolar bone defects. The model of alveolar bone defects was established in the second mandible molar (length: 3 mm; width: 1.5 mm; depth 1.5 mm) Rats were then implanted with scaffolds of hydrogel with various Exo groups	Rat BMSC-Exos promoted the growth of new bone, raised the BV/TV ratio, and improved BMD in the alveolar bone of the rats. BV/TV was increased by 20% and bone mineral density was increased by 3 fold
Lee et al. (2023)[40]	hDPSC-Exos	Rat mandibular ramus defect Collagen membrane + DPSC-EVs 100 μg/rat Euthanized 6 weeks post-surgery	Faster wound healing and increased bone density in the mandibular defects were seen in animals that underwent local administration of DPSC-EVs loaded with a collagen membrane that is commercially available. The authors provided evidence that DPSC-EVs had osteogenic and osteoinductive effects on jawbone regeneration. The additional use of exosomes to collagen membranes improved bone density

(Continues)

TABLE 2 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Seo et al. (2023)[41]	Ginseng-derived-Exos (GDNs)	On days 1 and 7, the mice were given subcutaneous injections of PBS, LPS, or GDNs into the tissue above the calvarial surface periosteum. Euthanized 7 days after the first injection and evaluated histologically and via micro-CT	MicroCT evaluation revealed that measures of the bone volume, and bone mineral density demonstrated that GDNs inhibited osteoclast development in a model of lipopolysaccharide-induced bone resorption in mice, as shown by BV/TV. The findings of this study indicate that GDNs possess anti-osteoporotic properties by impeding the process of osteoclast development. Consequently, GDNs have great potential for being used in the therapeutic management and prevention of bone loss disorders
Xu et al. (2023)[43]	Rat BMSCs-Exos	Rat calvarial defect models. Using a drill, two symmetrical 5 mm-diameter circular holes were created bilaterally on each rat and transplanted with hydrogels with/without Exos. Rats were euthanized at 4 and 8 weeks	The use of BMSC-derived exosomes in a cell-free environment shows significant promise for enhancing bone tissue regeneration when combined with BP. Bone volume ratio was more than doubled at both 4 and 8 weeks in the Exo group

Abbreviations: ADSC, Adipose derived stem cell; AKT1, set of three serine/threonine-specific protein kinases; ALP, Alkaline phosphatase; AMPK, adenosine monophosphate-activated protein kinase; ARS, Autonomous replication sequence; BMD, Bone Mineral Density; BMDM, Bone Marrow derived Macrophage; BMM, Bone marrow-derived Macrophages; BMP, Bone morphogenetic protein; BMSC-I, BMSC from the Ilium; BMSC-J, BMSC from the Jaw bone; BMSCs, Bone Marrow Mesenchymal Stem Cells; BP, black phosphorous; BV/TV, Bone volume/Total Volume, Bone volume fraction; COL1, Collagen Type 1; DPSCs, Dental Pulp Stem Cells; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinases; EV, Extracellular Vesicles; Exo, Exosome; FGF, Fibroblast growth Factor; GDN, Ginsend-derived extracellular nanovesicles; H202, Hydrogen Peroxide; hADSC, human ADSC; hBMSCs, human BMSCs; hDPSCs, human DPSCS; hPDLSC, human PDLSC; IGF, Insulin-like Growth Factor; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; JNK, c-Jun N-terminal Kinase; MEK, MAPK, Mitogen-activated protein kinase; miRNA, microRNA; MSC, Mesenchymal Stem Cell; NTA, Nanoparticle Tracking Analysis; OPG, Osteoprotegerin; OPN, Osteopontin; P13K, Phosphoinositide 3-kinase; PBS, Phosphate-buffered saline; PDGF, Platelet-derived growth factor; PDLSCs, Periodontal Ligament Stem Cells; P-gingivalis, Porphyromonas gingivalis, OCN, Osteocalcin; RANKL, receptor activator of nuclear factor kappa beta ligand; RUNX2, Runt-related transcription factor 2; SC, Schwann cells; SHED, Stem cells from Human Exfoliated Deciduous teeth; TGF-β, Transforming Growth Factor-β; μCT, Micro-computed tomography.

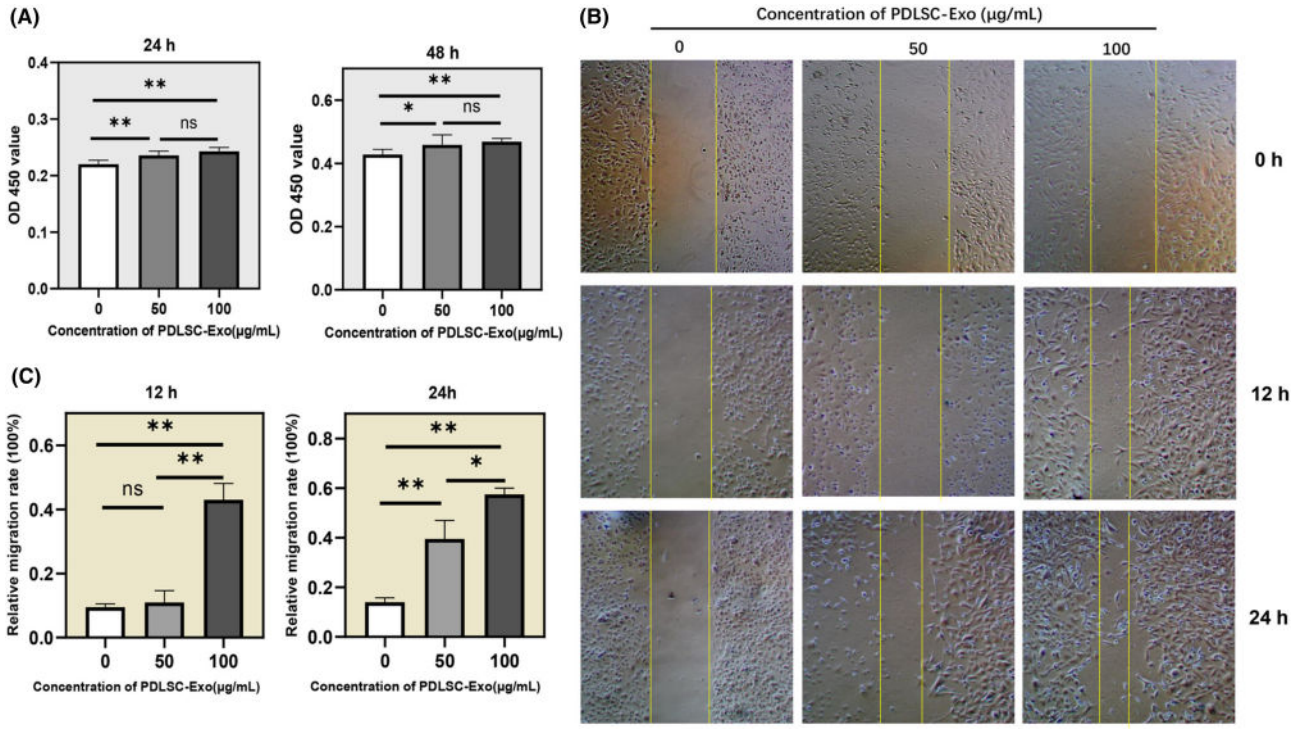

FIGURE 3 The migration and proliferation of hFOB1 are facilitated by PDLSC-Exos. The hFOB1 CCK-8 test used 19 cells (A). After being exposed to PDLSC-Exo (0, 50, and 100 μg/mL) during both 24 and 48 h, 19 cells were carefully examined. (B and C) Evaluation of hFOB1's migration potential using a scratch wound experiment. Twenty-nine cells were exposed to PDLSC-Exo (0.50 and 100 μg/mL) over 12 and 24 h, respectively. $^{*}p < 0.05$: $^{**}p < 0.01$. Adapted with permission from Lan et al.[32]

2.4 | Cementum regeneration

Only two in vitro studies have investigated the effects of exosomes on cementoblasts (Table 4). Both M2-derived macrophages and hPDLC-Exos were investigated in mouse OCCM-30 cementoblasts. It was found that exosomes promoted the migration, proliferation and mineralization of cementoblasts via the expression of p-PI3K and p-AKT pathways (Figure 6).

2.5 | Endodontic/Pulpal regeneration

A total of 23 studies investigated endodontic pulpal/odontogenic regeneration preclinically, of which 13 studies investigated the therapeutic potential of exosomes in vitro, while 10 tested exosomes in vivo. Based on the in vitro data, a total of 9 different derived exosomes were isolated. Seven studies isolated DPSC-Exos. One study isolated each of the following immortalized murine odontoblast cell line (MDPC-23)-Exos: Rat Hertwig's epithelial root sheath-Exos, SCAP-Exos, SHED-Exos, milk-derived-Exos, human umbilical cord mesenchymal stem cells (hUCMSCs)-Exos, human immortalized E1-MYC 16.3 embryonic stem cell (hESC)-Exos, and human thrombin-activated platelet-derived exosome (T-aPD-Exos) (Table 5). Twelve of the 15 cells utilized to produce exosomes were isolated from humans (80%), with 2 studies testing 2 different exosome cell sources. Exosomes were isolated from rats in 1 study, mice in 1 study and rabbits in 1 study. In 11 of 13 studies (85%), DPSCs were the target cell line investigated, with 1 study investigating rat BMSCs and one study investigating SHEDs. The cells tested were 69% from humans (9/13), 3 studies were derived from rats, and 1 study was derived from rabbits. All studies demonstrated favorable outcomes utilizing exosomes. In summary, exosomes were able to promote odontogenic differentiation by significantly upregulating TGFβ1, TGFR1, dentin sialophosphoprotein, p-Smad2/3, and Smad4 as well as increasing mineralization in vitro (Figure 7).[59] DPSCs-sEVs and encapsulated miR-125a-3p were shown to stimulate the release of BMP2 in macrophages, which in turn activated the BMP2 pathway and promoted odontogenesis in DPSCs. Exosomes have the ability to counteract the inhibitory/inflammatory impact of lipopolysaccharide on the proliferation of human dental pulp stem cells and to reduce lipopolysaccharide-induced apoptosis.[57]

Based on the in vivo data, a total of 6 different derived exosomes were isolated as follows: 5 studies isolated DPSC-Exos, 2 studies isolated SCAP-Exos, and 1 study each isolated Rat Hertwig's epithelial root sheath-Exos, SHED-Exos, and human immortalized E1-MYC 16.3 embryonic stem cell (hESC)-Exos (Table 5). In 7 of 11 studies (64%), exosomes were isolated from humans, whereas exosomes were isolated from mice in 1 study and rats in 3 studies. Of the 11 in vivo models (1 study had 2 in vivo models), 5 studies investigated subcutaneous transplantation of

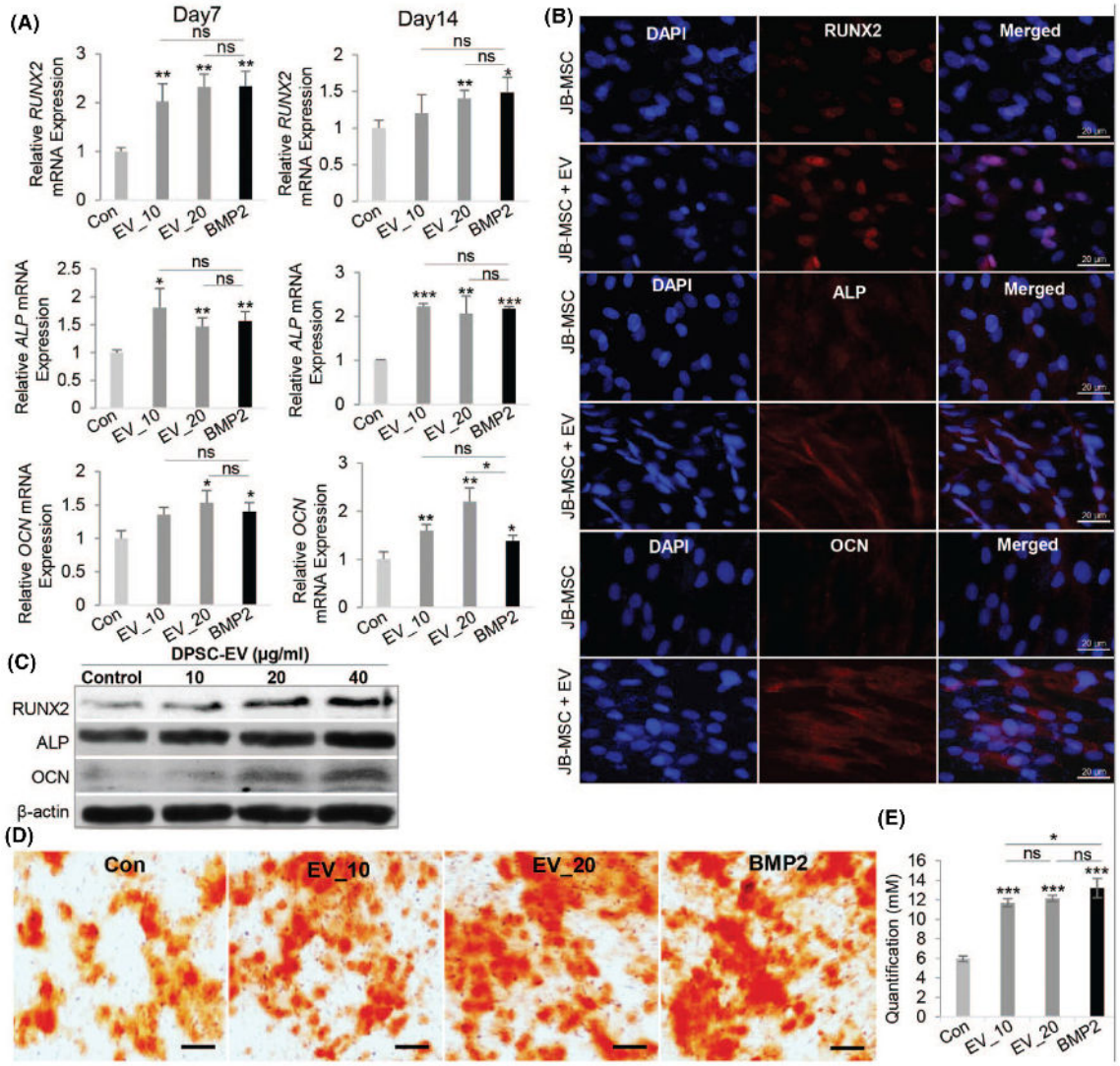

FIGURE 4 DPSC-EVs promote osteogenic differentiation of jaw bone (JB)-MSCs. (A) For 7 or 4 days, JB-MSCs were cultivated in an osteogenic induction medium with DPSC-EVs or recombinant human BMP-2 (250 ng/mL). RUNX2, ALP, and OCN gene mRNA expression was measured using a quantitative reverse transcriptase polymerase chain reaction on total RNA. B-actin was the internal control. Con, osteogenic induction control; EV_10, DPSC-EVs (10 g/mL); EV_20, DPSC-EVs (20 g/mL). Compared with the control: $^{*}p<0.05$. $^{**}p<0.01$. $^{**}p<0.001$. ns, no significance. (B) B-MSCs were grown in regular media. After reaching 80% confluence, the culture medium was changed with 2% fetal bovine serum and 20 g/mL DPSC-EVs for 72 h. After fixing cells in 4% paraformaldehyde, immunofluorescence was used to measure RUNX2, ALP, and OCN protein expression. (C) Regular culture media was used to cultivate JB-MSCs. After reaching 80% confluence, the culture media was changed with new medium containing 2% fetal bovine serum and various DeSC-Evs concentrations for 2 h. Western blot analysis was used to measure the RUNX2, ALP, and OC protein levels in collected cells (D). For 4 weeks, JB-MSCs were cultivated in an osteogenic induction medium with or without DPSC-EVs or BMP-2 (250 g/mL). A solution of alizarin red S stained the cells. Images were microscoped. Scale bars, 20 μm. (E) Quantification of mineralization using alizarin red S extraction solution OD405 values. Con, osteogenic induction control; EV_10, DPSC-EVs (10 μg, mL); EV 20, DPSC-EVs (20 μg/mL). Error bars indicate mean ‡ SD. Compared with the control: $^{*}p<0.05$. $^{**}p<0.01$. $^{***}p<0.001$. ns, no significance DPSC, dental pulp stem cell; EV, extracellular vesicle; JB-MSC, jawbone marrow-derived mesenchymal stem cell. Reprinted with permission from Lee et al.[40]

tooth fragments typically in dorsal tissues, 4 studies investigated some form of pulpitis often bur-induced, and 2 studies investigated pulpotomy. In total, 6 in vivo studies were performed on rats, and 5 were performed on mice. All studies found positive in vivo effects (Figure 8).

2.6 | Immune cells

In total, 19 studies investigated immune cells preclinically. All 19 of the studies conducted in vitro investigations and one of the studies also examined immune cell behavior in vivo. Considerable variability in both

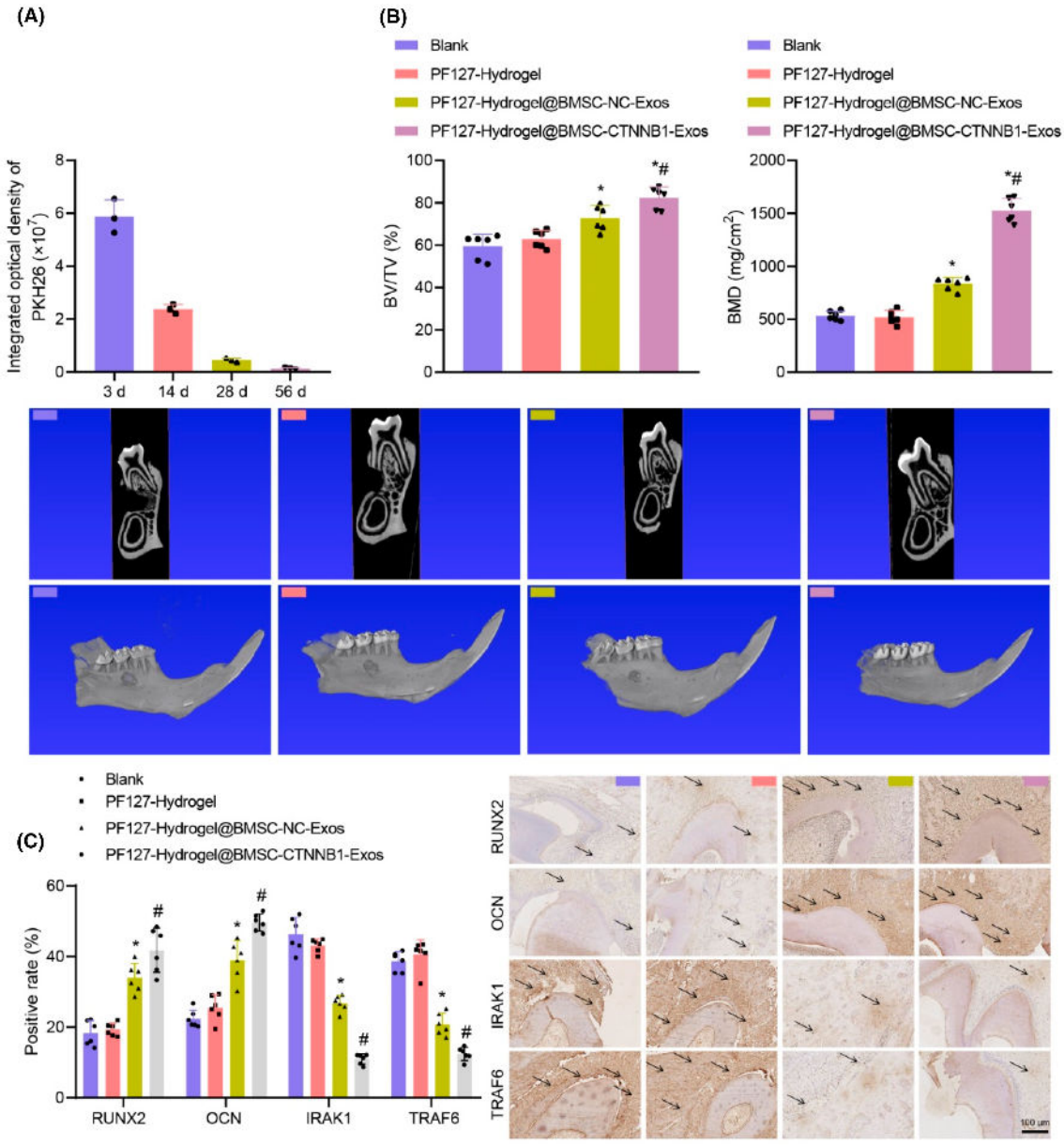

FIGURE 5 In rats, PF127 hydrogel containing BMSC-Exos stimulates the regeneration of alveolar bone. (A) Quantitative investigation and in vivo TPEF recording of the distribution of Exos implanted at various times that are tagged with PKH26. *n* is equal to 3. (B) Images captured by micro-CT scanning of the second mandibular molar (red frame) and quantitative findings of alveolar bone abnormalities in rats treated with PF127 hydrogel@BMSC-CTNNB1-Exos. Number of cases (*n*): 6 (C) New bones were stained with immunohistochemistry to reveal the presence of RUNX2, OCN, IRAK1, and TRAF6. Dark brown particles, shown by the black arrows, indicate protein-positive staining. comparative to the PF127 hydrogel group, with $n=6$ and $p<0.05$. Compare to the group that used PF127 hydrogel@BMSC-NC-Exos; $p<0.05$. $^{*}p<0.05$ vs. the PF127 hydrogel group. $^{\#}p<0.05$ vs. the PF127 hydrogel@BMSC-NC-Exos group. Reprinted with permission from He et al.[38]

exosome-derived cells and target cells was observed in these studies. Based on the in vitro data, a total of 11 different derived exosomes were isolated as follows: 6 studies isolated DPSC-Exos, 3 studies isolated BMSC-Exos, 3 studies isolated GMSC-Exos, 2 studies isolated PDLCs-Exos, 2 studies isolated dendritic cell-Exos, and one study each isolated SHED-Exos, ADSC-Exos, SCAP-Exos, embryo-kidney-Exos, and laboratory-derived miR-1260b-Exos (Table 6). Two studies investigated 2 different exosome sources, with DPSCs having been more commonly shown to be superior to other tissue types.[66] In almost all studies (18/21), human-derived exosomes were utilized, whereas one study utilized rat exosomes, 1 study utilized mice, and 1 study developed exosomes in a laboratory. Of the 19 studies, a wide variety of target cells were investigated in vitro. Five studies investigated human THP1 macrophages, 4 studies investigated CD4 monocytes, 4 studies investigated murine RAW264.7 cells, 2 studies investigated human CD14+ peripheral blood-derived monocytes, and 1 study each investigated human

TABLE 3 In vitro use of various exosomes derived from multiple sources for cancer therapy.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Cancer—in vitro			
Sjoqvist et al. (2019)[45]	1. HaCaT (ATCC)-Exos, human immortalized keratinocytes 2. NHDF, CC-2509 human dermal fibroblasts-Exos	Human carcinoma cell line TR146 The media was collected after 24 h and centrifuged at 300 ***g*** for 10 min at 4°C following by 3000 ***g*** for 10 min at 4°C. The media was then concentrated using 100 kDa filters and further concentrated using 10 kDa filters to reach a volume less than 500 μL	Exosomes produced from oral keratinocytes controlled the proliferation of fibroblasts and epithelial cells in the carcinoma cell line at comparable levels to dexamethasone, a medication that is often used to stop stricture development
Klimova et al. (2023)[46]	hDPSC-Exos (loaded with chemotherapeutic agents gemcitabine)	Human PANC1 and MiaPaca cell lines. Exos were isolated via concentration of CM through tangential flow filtration (TFF) sEVs concentrator 1. Pooled serum-free CM were filtered using sterile polysulfone hollow fibers with 5 nm cut-off pores to remove other cell components, free biomolecules and cell debris	The highly concentrated CM containing Exos significantly reduced the in vitro growth of pancreatic cancer cell types. Transgenic plasmids expressing a suicide gene fusion, yCD:UPRT, were introduced into the DPSCs using genetic engineering. Internally given to cancer cells, the innocuous prodrug 5-fluorocytosine (5-FC) is transformed into the powerful chemotherapeutic drug 5-fluorouracil (5-FU) by the use of the suicide gene. There was a further effect on the inhibition of cancer cell growth from 5-FU's conversion to 5-FC. By successfully transporting chemotherapeutic medications in conjunction with a prodrug suicide gene therapy system

Abbreviations: 5-FC, 5-fluorocystine; 5-FU, 5-fluorouracil; ATCC, American Type Culture Collection; DPSCs, Dental Pulp Stem Cells; Exo, Exosome; HaCat, human spontaneously immortalized keratinocyte cell; hDPSCs, human DPSCS; PANC-1, Pancreatic duct epithelioid carcinoma cell.

TABLE 4 In vitro therapeutic regenerative potential of various exosomes derived from multiple sources on cementoblast regeneration.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Cementoblast—In Vitro			
Zhao et al. (2022)[47]	Mouse RAW264.7 monocyte cell line (M0, M1 and M2 macrophage polarization)	Murine cementoblasts cell line OCCM-30 Cells were removed by centrifugation at 300 ***g*** for 10 min. Then, dead cells were removed by centrifugation at 2000 ***g*** for 10 min, followed by cell debris removal by centrifugation at 10 000 ***g*** for 30 min. The exosomes were initially collected by ultracentrifugation at 100 000 ***g*** for 70 min, washed in PBS and finally collected by ultracentrifugation under the same condition again. M0/M1/M2 exosomes were added to the culture medium of cementoblasts at a dose of 150 μg/mL every day	Using a Transwell assay and a CM-based coculture system, the authors found that M1-polarized macrophages inhibited cementoblast mineralization, whereas M2-polarized macrophages increased it
Li et al. (2023)[48]	hPDLC-Exos	Mice OCCM-30 Cementoblasts Cells were centrifuged at 1500 ***g*** for 15 min. The supernatant obtained was filteredvusing a 0.22 μm syringe-driven filter unit to remove particles larger than 200 nm. The prefiltered fluid was centrifuged at 4000 ***g*** for 30 min, using a 100 kDa centrifugal ultrafiltration tube. Finally, exosomes were isolated from the concentrated solution using an ExoQuick-TC kit	Exosomes enhanced the movement, reproduction, and formation of minerals in OCCM-30 cells. The group treated with exosomes exhibited a substantial upregulation in the expression of genes and proteins relevant to cementogenesis. In addition, exosome treatment cells increased the expression of p-PI3K and p-AKT. Runx2, osterix, osteocalcin and collagen 1 were all upregulated between 2 and 4 fold in the exosome group

Abbreviations: AKT, set of three serine/threonine-specific protein kinases; Exo, Exosome; hPDLSC; human PDLSC; OCCM-30, Cementoblast cells of murine origin; P13K, Phosphoinositide 3-kinase; PDLSC, Periodontal Ligament Stem Cells.

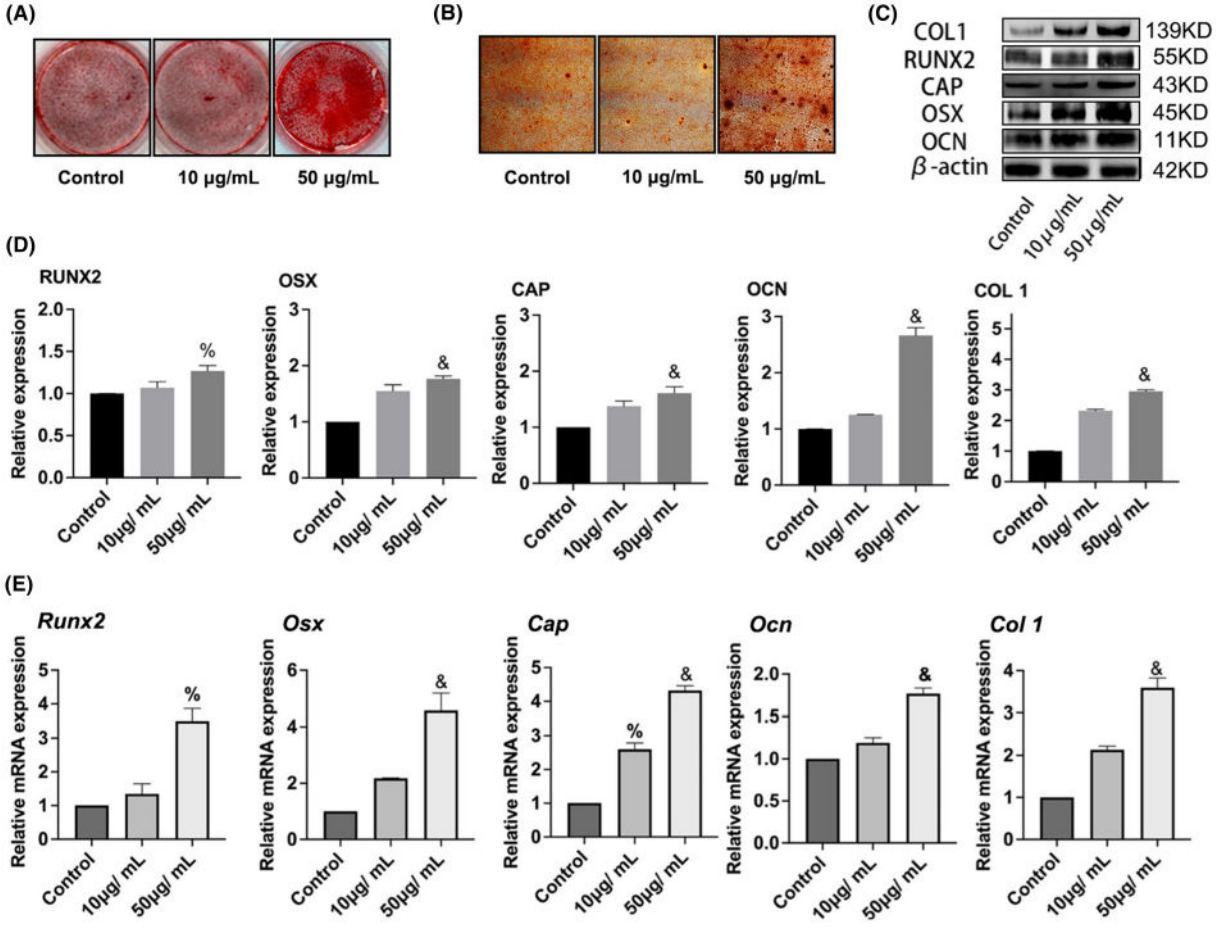

FIGURE 6 By injecting hPDLSC-Exos, OCCM-30's cementogenic activity enhanced. (A) Using ARS, the biomineralization of OCCM-30 induced for 10 days was envisioned. (B) ARS found calcium nodules, which were then photographed using an inverted microscope. (C) The western blotting bands showed that RUNX2, OSX, CAP, ON, and COL1 were all expressed at higher levels. (D) The quantitative examination of cementogenic-associated protein by western blotting. (E) The qRT-PCR analysis of cementogenic-associated gene mRNA expression in OCCM-30 activated with exosomes. $^{*}p<0.05$, $^{\#}p<0.01$, $^{\%}p<0.001$, $^{\&}p<0.0001$ versus control. Reprinted with permission from Li et al.[48]

dopaminergic neurons, a mouse hippocampal cell line (H3), human SCs from odontoblasts, and mouse Tregs (Table 6). Only one in vivo study investigated rat SCAP-Exos on rat dental pulp exposures.[63]

The most commonly reported findings were that exosomes derived from multiple sources were able to shift macrophage polarization from an M1 pro-inflammatory state into an M2 pro-resolution/regeneration state.[80] Furthermore, many key studies have associated the behavior of immune cell responses with in vivo clinical benefits. For instance, Elsayed et al.[78] concluded human DC-Exos were shown to affect the immunological responses of human DC and T-cells. This suggests that they might be used as a potential immunotherapeutic agent to regulate the immune response in gingival tissue and prevent bone loss in periodontal disease. A study by Zheng et al.[69] found similar conclusions where PDLSC-Exos alleviated an inflammatory microenvironment through the Th17/Treg/miR-155-5p/SIRT1 regulatory network, improving periodontitis. Two basic research studies also found that exosomes derived from separate sources were able to significantly decrease immune cell-driven osteoclastogenesis and prevent bone loss.[70,74] Interestingly, Jarmalavičiūtė et al.[64] found that exosomes specifically (but not microvesicles) derived from SHEDs improved dopaminergic neurons by approximately 80%, suggesting that exosomes derived from SHEDs should be considered a new potential therapeutic tool in the treatment of Parkinson's disease. As found in Part II of this 3-part review, exosomes derived from multiple sources (including dental sources) may pose many therapeutic advantages when compared to currently offered therapies.

2.7 | Mesenchymal cell function

Only 2 studies investigated the use of exosomes on mesenchymal cell function (Table 7). In the study by Li and Ge,[61] rat DPSC-expos were isolated and investigated in rat MSCs. It was found that when the exosomes were cultured with osteogenic differentiation media, osteoblastic differentiation markers, including OCN, OPN and RUNX2, were all upregulated.[61] The study by Han et al.[81] investigated 3 sources of exosomes on buccal fat pad-derived mesenchymal stromal cells (BFP-MSCs). It was concluded

TABLE 5 In vitro and in vivo therapeutic regenerative potential of various exosomes derived from multiple sources on pulpal regeneration.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Pulpal regeneration—in vitro			
Hu et al. (2019)[49]	The hDPSC-Exos, which were cultivated in the undergrowth and odontogenic differentiation settings, are referred to as UN-Exo and OD-Exo	hDPSCs Media was supplemented with the odontogenic media cocktail of 100 nmol dexamethasone, 10 mmol β- glycerophosphate, and 0.2 mmol L-ascorbic acid. The exosomes from the culture medium were isolated using the Exo-spin exosome isolation reagent as per the manufacturer's protocol	Conditions specific to tooth development allowed for the isolation of OD-Exos, which improved the differentiation of DPSCs. The expression of TGFβ1, TGFR1, p-Smad2/3, and Smad4 was significantly upregulated in the dental pulp stem cells (DPSCs) after they underwent odontogenic differentiation
Swanson et al. (2020)[50]	1. hDPSC-Exos 2. MDPC-23-2Exos, an immortalized mouse odontoblast cell line	hDPSCs The CM was centrifuged at 300 *g* × 10 min and 2000 *g* for 10 min to remove dead cells and debris. Then the supernatant was filtered through a 0.22-μm filter to remove residual debris. The supernatant was centrifuged at 4000 *g* to about 200 μL in a 15 mL Amicon Ultra-15 Centrifugal Filter Unit (Millipore) for 10 min. The ultra-filtrated liquid was washed twice and centrifuged at 100 000 *g* × 70 min. Then the pellet was re-suspended and centrifuged at 100 000 *g* for another 70 min	In vitro, exosomes obtained from both hDPSCs and MDPCs enhanced the expression of odontogenic genes and promoted their mineralization by over two fold compared to the odontogic media and growth media groups
Xie et al. (2020)[51]	hDPSC-Exos	hDPSCs DPSCs Cocultured with exosomes secreted by these osteogenic-induced DPSCs at days 0, 5, and 7 were extracted and marked as EX0, EX5 and EX7	The EX7 group showed as much osteogenic differentiation potential as cells cultured in osteogenic media
Zhang et al. (2020)[52]	Rat Hertwig's epithelial root sheath-Exos	Rat DPSCs First, the culture medium was centrifuged at 2000 *g* for 30 min, and then the supernatant was introduced into Amicon Ultra-15 Centrifugal Filter Units with Ultracel-100K and centrifuged at 5000 *g* for 30 min. Subsequently, ELVs from the culture medium were isolated using the Total Exosome Isolation TM reagent following the manufacturer's protocol	EVs-H1 stimulated odontogenic differentiation, activated Wnt/β-catenin signaling, and facilitated the migration and proliferation of DPSCs. Additionally, in vitro, EVs-H1 aided in capillary formation and neuronal development
Zheng et al. (2020)[53]	hDPSCs-Exos	hDPSCs A serum-free a-MEM was supplemented with the odontogenic media cocktail of 100 nmol dexamethasone, 10 mmol β- glycerophosphate, and 0.2 mmol L-ascorbic acid. The exosomes from the culture medium were isolated using the Exo-spin exosome isolation reagent as per the manufacturer's protocol	DPSC-Exos and the enclosed miR-125a-3p have the intriguing effect of increasing the release of BMP2 in macrophages. This, in turn, stimulates odontogenesis in DPSCs by activating the BMP2 signaling pathway
Zhuang et al. (2020)[54]	SCAP-Exos	Rat BMSCs SCAP were cultured for 48 h, then the supernatant was centrifuged sequentially at 4°C 3000 *g* for 20 min, 20 000 *g* for 30 min, and 120 000 *g* for 2 h. Finally, the exosome pellets were resuspended in 200 μL of PBS. 20 μL SCAP-Exo was added to 30 μL lysis buffer for 1 h on ice	It was discovered that BMSCs treated with SCAP-Exos showed a substantial increase in dentin sialophosphoprotein as well as mineralized nodule formation. There was about a 10-fold thickness of the new dentin and a nearly 4-fold increase in the number of odontoblasts in the SCAP-Exo group when compared to the control
Wu et al. (2021)[11]	SHED aggregate-Exos	SHEDs Supernatant was firstly centrifuged at 2000 *g* for 10 min. Next, the supernatant was centrifuged at 10 000 *g* for 30 min. The supernatant was then ultracentrifuged at 100 000 *g* for 70 min. Finally, the pellet was washed using phosphate-buffered saline by ultracentrifugation at 100 000 *g* for 70 min again	The presence of SHED aggregate-Exos enhanced the ability of SHED endothelial cells to promote angiogenesis and facilitated their proliferation. On a molecular level, the abundant miR-26a in SHED-Exos improved angiogenesis in SHED via modulating TGF-β/SMAD2/3 signaling

TABLE 5 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Bagio et al. (2022)[55]	Transforming thrombin into platelet-derived exosomes (T-PDE) in humans	hDPSCs Platelet-rich plasma was prepared from 3 donors by centrifuging blood for 400 ***g*** ×10 min. 10% of total plasma volume was transferred to a new tube and 0.5 mL of PBS was added. Next, 3 μL of total exosome isolation (TEI) reagent was added to the tube and mixed until homogeneous. The mixture was incubated at room temperature for 10 min, after which immediately centrifuged for 10 000 ***g*** for 5 min at room temperature. After the supernatant was disposed, it was centrifuged again at 10 000 ***g*** for 30 s to remove debris	Compared to the control and PRP-T, the T-aPDE groups demonstrated a greater capacity to stimulate dental pulp regeneration. VEGF-A expression increased significantly in the exosome groups by dose and time with greater expression showing after 72 h
Ganesh et al. (2022)[56]	Cultured rabbit DPSC-Exos in either a growth (Exo-G) or angiogenesis (Exo-A) medium	Rabbit DPSCs DPSC-Exos were isolated by a precipitation method using ExoQuick-TC TM. In brief, CM was centrifuged at 3000 ***g*** ×15 min to remove cells and cell debris. The supernatant was mixed with an ExoQuick-TC TM solution at 4°C overnight. After centrifugation, the exosome pellet was resuspended in PBS and stored at 80°C before use	After DPSC-Exos treatment, the expression of angiogenic markers such as VEGFA, FLT1, and PECAM1 significantly increased (Nearly two fold increase in FLT1, and PECAM1). Furthermore, the authors have discovered crucial exosomal microRNAs in Exo-A that are involved in cell homing and angiogenesis
Zeng et al. (2022)[57]	1. Human umbilical cord mesenchymal stem cell-derived exosomes ("hUCMSCs"). 2. Dental Pulp Stem Cell-Derived Exosomes from Humans	hDPSCs The supernatants were collected and filtered through a 22 μm sterilized filter. After centrifugation at 300 ***g*** ×10 min, 2000 ***g*** ×10 min, and 10 000 ***g*** ×30 min to eliminate cells and cellular debris, exosomes were isolated by ultra centrifugation at 110 000 ***g*** ×90 min at 4°C. Subsequently, the exosomes were washed with phosphate buffered saline and pelleted again by the second ultracentrifugation at 110 000 ***g*** ×90	Exosomes were able to mitigate lipopolysaccharide-induced cell death and neutralize lipopolysaccharide's inhibitory effect on human dental pulp stem cell development. Furthermore, anti-inflammatory cytokine expression was increased, while pro-inflammatory cytokine expression was lowered by exosomes
Azaryan et al. (2023)[58]	Human milk-Exos	hDPSCs Breast milk from 294 mothers were centrifuged to isolate the fat layer which was then centrifuged at 300 ***g*** for 45 min at 4°C. The supernatants were centrifuged at 2000 ***g*** for 1 h at 4°C to further remove deposited cells and then at 12 000 ***g*** for 1 h, and 14 000 ***g*** for 2 h at 4°C to pellet the vesicles. Ten exosomes were extracted from the remaining milk by the EXOCIB kit as directed by the manufacturer. A BCA protein assay kit was used to determine the total protein content of exosomes	Milk-Exos enhanced the migration and proliferation of LPS-exposed hDPSCs and decreased the expression of inflammatory cytokines. The authors suggested that they may be a suitable option for treating pulpitis
Shi et al. (2023)[59]	E1-MYC 16.3 embryonic stem cells, Originating from humans, have achieved immortality. (hESC)-Exos	Rat DPSCs For exosome preparation, the CM was prepared by growing 80% confluent cells in a chemically defined culture medium composed of DMEM supplemented with 1% non-essential amino acids, 1% glutamine, 1% insulin-transferrin-selenium-X, 1 mM sodium pyruvate, 0.05 mM β-mercaptoethanol, 5 ng/mL fibroblast growth factor (FGF)-2 and 5 ng/mL PDGF-AB for 3 days. The CM was size-fractionated by TFF and concentrated 50× using a membrane with a molecular weight cut-off of 100 kDa	DPSC migration, proliferation, and odontogenic differentiation were improved in the exosome group. This work establishes the foundation for the advancement of MSC exosomes as a cell-free therapeutic option for the regeneration of pulp and dentin
Wang et al. (2023)[60]	hDPSC-Exos	DPSCs The supernatant was centrifuged for 300 ***g*** ×10 min to remove dead cells, centrifuged again at 10 000 ***g*** ×10 min to remove the cell debris and centrifuged at 100 000 ***g*** ×70 min to discard the supernatant	Cell survival, proliferation, and the capacity of hydrogel-loaded hDPSC-Exos to induce odontogenesis and angiogenesis were dramatically enhanced as shown by in vitro cell tests

(Continues)

TABLE 5 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Pulpal regeneration—in vivo			
Swanson et al. (2020)[50]	1. hDPSC-Exos 2. Immortalized murine odontoblast cell line (MDPC-23)	Rat molar pulpotomy model 8–10 week old male mice were anesthetized and 2-inch dorsal incisions were made on the disinfected back where four pockets were made using blunt dissection, two on each side of the incision. One construct was placed in each pocket. Samples were harvested at 6 weeks	In vivo, the reparative dentin bridge construction produced by the regulated release of odontogenic exosomes was superior to that of glass-ionomer cement alone
Zhang et al. (2020)[52]	The root sheath-Exos of the rat Hertwig (HERS-Exos)	Subcutaneous transplanting of mice using the tooth root slice model DPSCs were mixed with collagen gel and combined with or without ELVs-H1 (2 mg/mL), and then used to fill the root canal spaces of a root slice. The treated dentin matrix canal was obtained from extracted incisors of pigs and formed to an internal diameter of 2 mm and a height of 3 mm. Samples were subcutaneously implanted on the right and left back of nude mice. Grafts were obtained 4 weeks postoperatively	Exos from immortalized HERS-H1 cells encouraged pulp-dentin tissue renewal, suggesting they may be used to create engineered dental pulp. A suitable environment was established by Exos-H1 to reproduce epithelial-mesenchymal interactions during tooth development. Additionally, it activated the Wnt/β-catenin pathway to regulate dental mesenchymal stem cell development
Zheng et al. (2020)[53]	hDPSCs	Rat—Bur induced exposure of pulp tissue of incisors. DPSCs-sEV utilized as a pulp capping biomaterial was injected into the exposed pulpal tissue	This research involving rats demonstrated that DPSC-Exos may be used as an effective biomimetic instruments to promote the formation of teeth by transforming macrophages into pro-healing M2 cells
Zhuang et al. (2020)[54]	SCAP-Exos	Mice: Subcutaneous transplantation of root pieces carrying BMSCs into immunodeficient mice. The mice were euthanized 12 weeks after transplantation	In the group that received exosomes made from stem cells derived from the apical papilla (SCAP), the authors found dental pulp-like structures and saw freshly created dentin deposited onto the existing dentin in the root canal
Wu et al. (2021)[11]	SHED-Exos	Cell aggregates with or without SA-Exo were introduced into the tooth fragments and transplanted subcutaneously into the backs of the mice. At 12 weeks post-implantation, the mice were sacrificed and the tooth fragments were collected and fixed in 4% paraformaldehyde for histological analysis	The authors discovered that SA-Exo had a considerable positive impact on the regeneration of pulp tissue and the formation of new blood vessels in living organisms
Li and Ge (2022)[61]	Rat DPSC-Exos	A pulpitis model was developed in six-week-old male Sprague–Dawley rats. The rats had their mandibular incisor labial pulp tissues resected by an electrosurgical generator following which the surgical wounds were tressed. The diameter of gingival defects was greater than 5 mm, deep to hard tissue. All pulp tissue was extracted on the 30th day after modeling	The current investigation showed that exosomes-derived from long non-coding RNA (lncRNA) Ankrd26 from dental pulp stem cells (DPSCs) enhanced the repair of the dental pulp via controlling the miR-150-TLR4 signaling pathway in mesenchymal stem cells (MSCs). TLR4 was significantly upregulated (over 2-fold increase) in the model group
Lan et al. (2022)[62]	hDPSCs combined with 1. stromal cell-derived factor-1 (SDF-1-Exos). 2. LPS-EXOs 3. NoLPS-Exos	For this investigation, we modeled root canals devoid of pulp using rat bilateral mandibular first molars	New pulp-like tissue had grown within the root canals of all three experimental groups, according to the histological analysis employing HE staining. Human dental pulp stem cells (hDPSCs)-Exos that, when combined with stromal cell-derived factor-1 (SDF-1), increased the density of blood vessels inside the tissue and stimulated the development of new tissue within the root canal

TABLE 5 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Yu et al. (2022)[63]	Rat SCAP-Exos	Rat exposure of the dental pulp of mandibular incisors. After anesthetizing the rats with 3% pentobarbital, the mandibular incisors were gently ground using a high-speed handpiece and round tungsten steel burs with a diameter of 0.8 mm under water cooling. The exposure of the dental pulp was confirmed using a sterile 40 K-file. Lipopolysaccharide (LPS, 5 pL at 10 mg/mL) from *Escherichia coli* 055:B5 (Sigma Chemical Co.) was injected into the cavity (Hong et al., 2020), and the cavity was sealed with GC Fuji IX glass ionomer cement (GC Dental Products Corporation). After 48 h, the glass ionomer cement was removed, and 5 μL of 1 pg/pL SCAP-Exos or PBS was injected into the cavity, and the cavity was sealed with GC Fuji IX glass ionomer cement. Seven days after the procedure, the animals were euthanized	SCAP-Exos converted Treg cells and reduced rat dental pulp inflammation. This study found that SCAP-Exos regulate the immunological milieu locally, supporting tissue regeneration. This shows that SCAP-Exos may be a potential cell-free therapy for early dental pulp inflammation in immature permanent teeth
Shi et al. (2023)[59]	Human immortalized E1-MYC 16.3 embryonic stem cell (hESC)-Exos	Study 1: Rat dental pulp defect model with carbide bur of maxillary first molars Under general anesthesia, class I defects (1.5 mm diameter × 1.5 mm depth) were surgically created on the occlusal surface of bilateral maxillary first molars using a 1/4 RA carbide bur and a dental handpiece. Study 2: The maxillary or mandibular premolars of individuals aged 11 to 30 who were receiving orthodontic treatment were extracted and inserted subcutaneously into immunodeficient (SCID) mice. At 5 weeks, animals were euthanized and specimens harvested for analysis.	1. In a model of rat pulp defect, MSC exosomes enhanced the production of dentin matrix proteins and bridge-like structures by stimulating the growth of dentin-like tissue. 2. MSC exosomes inserted subcutaneously in a mouse's dorsum regenerated pulp-dentin tissues in endodontically treated human premolar root canals
Wang et al. (2023)[60]	hDPSCs	Mice's rear subcutaneous space received root samples. The root samples were divided into four groups: (1) hDPSCs group (without hydrogel); (2) hDPSCs + HPCH group; (3) hDPSCs + HPCH/CW group; (4) hDPSCs + HPCH/CW/Exo group. After the hydrogel was injected into endodontic spaces via a syringe (21G), the coronal end of the root canal was sealed with mineral trioxide aggregate (MTA) at about 2 mm thickness to protect the underlying hydrogels. The roots were retrieved after 8 weeks	Showed that in an implanted tooth root model, new tissues resembling dental pulp was histologically demonstrated. Consequently, the suggested hydrogel offered a prospective substitute for conventional root canal treatment in dental offices

Abbreviations: Ankrd26, ankyrin repeat domain-containing protein-26; BMP, Bone morphogenetic protein; circRNA, circular RNA; DPSCs, Dental Pulp Stem Cells; ELV, Exosome-like extracellular vesicles; EV, Extracellular Vesicle; Exo, Exosome; Exo-A, exosomes cultured under angiogenic conditions; FLT1, Fms Related Receptor Tyrosine Kinase-1; hDPSCs, human DPSCS; exosomes cultured under angiogenic conditions; HE, Hematoxylin and Eosin; HERS, Hertwig's epithelial root sheath; hESC, Human Embryonic Stem Cell; hSCAPS, human SCAPS; hUSCMSCs, Human umbilical cord mesenchymal stem cells; LncRNA, Long noncoding RNA; LPS, Lipopolysaccharide; LPS-exo, exosome with LPS; LTBP, Latent transforming growth factor β binding proteins; MDPC, immortalized murine odontoblast cell; miRNA, microRNA; MSC, mesenchymal stem cell; NoLPS-exo, exosome without LPS; OD-Exo, exosomes cultured in odontogenic conditions; PECAM1, Platelet and Endothelial Cell Adhesion Molecule-1; SCAPs, Stem Cells from the Apical Papilla; SDF-1, Stromal cell-derived factor-1; SHED, Stem cells from Human Exfoliated Deciduous teeth; SMAD, Suppresor of Mothers against Decapentalegic; T-aPDE, thrombin-activated platelet-derived exosome; TGFR1, Transforming growth factor-1; TGF-β1, Transforming Growth Factor-β; TLR, Toll-like receptor; Un-Exo, exosomes cultured under growth; VEGFA, Vascular endothelial growth factor A; xo-G, exosomes cultured under growth.

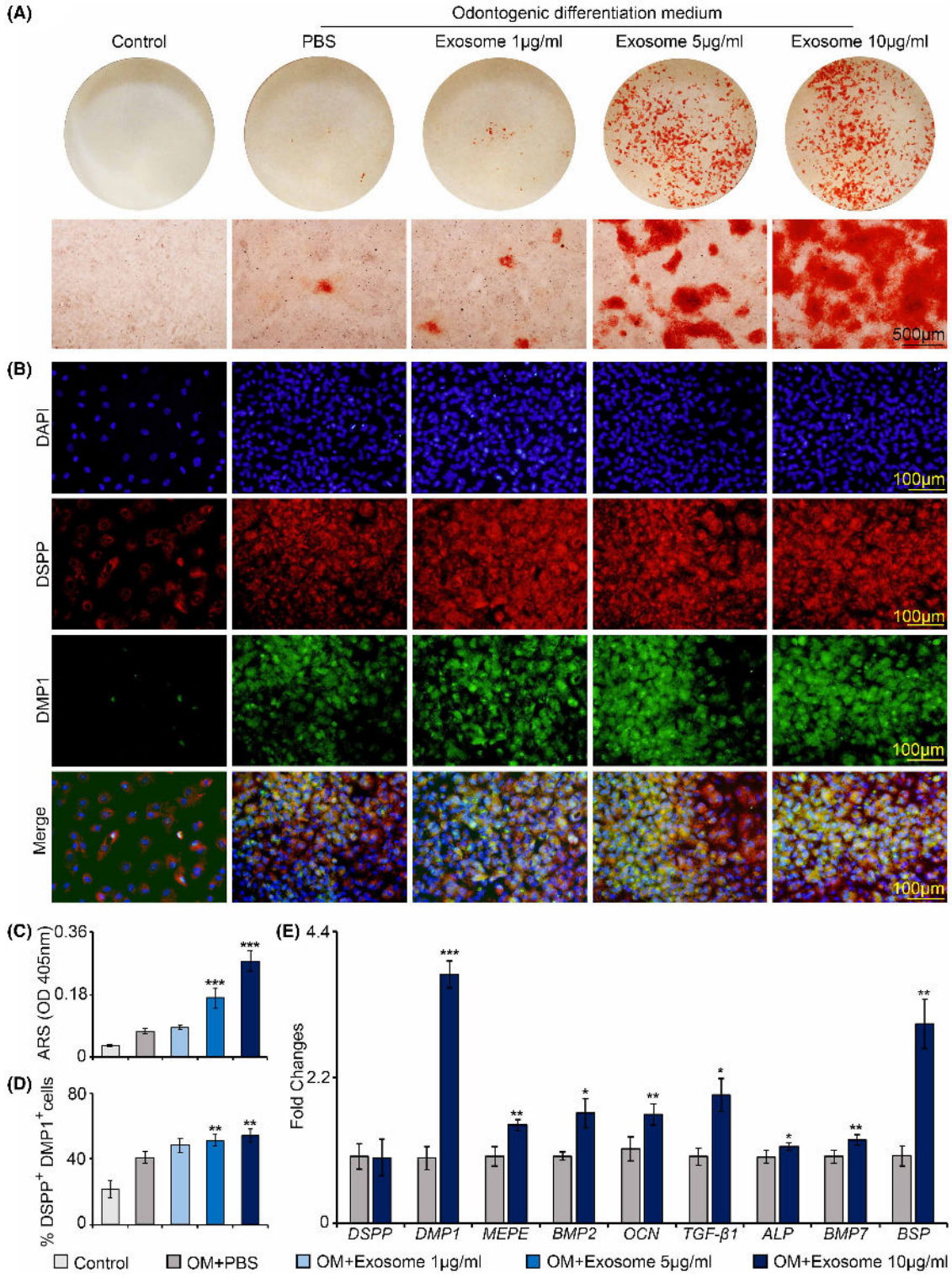

FIGURE 7 Effects of MSC-Exos on the odontogenic differentiation of DPCs. (A) ARS staining in addition to (C) When 5 and 10 μg/mL exosome treatment was applied, measurement of the eluted dye revealed increased odontogenic differentiation and mineralization. (B) Staining with immunofluorescence and (D) Measurements of DSPP and DMP1 showed significantly higher percentages of positively stained cells after treatment with 5 and 10 μg/mL exosomes. (E) Genes related to odontogenic differentiation were shown to be regulated by 10 μg/mL exosome treatment, as revealed by RT-qPCR analysis. Summary of findings from three separate studies. Data are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to PBS ($n = 3$). Reprinted with permission from Shi et al.[59]

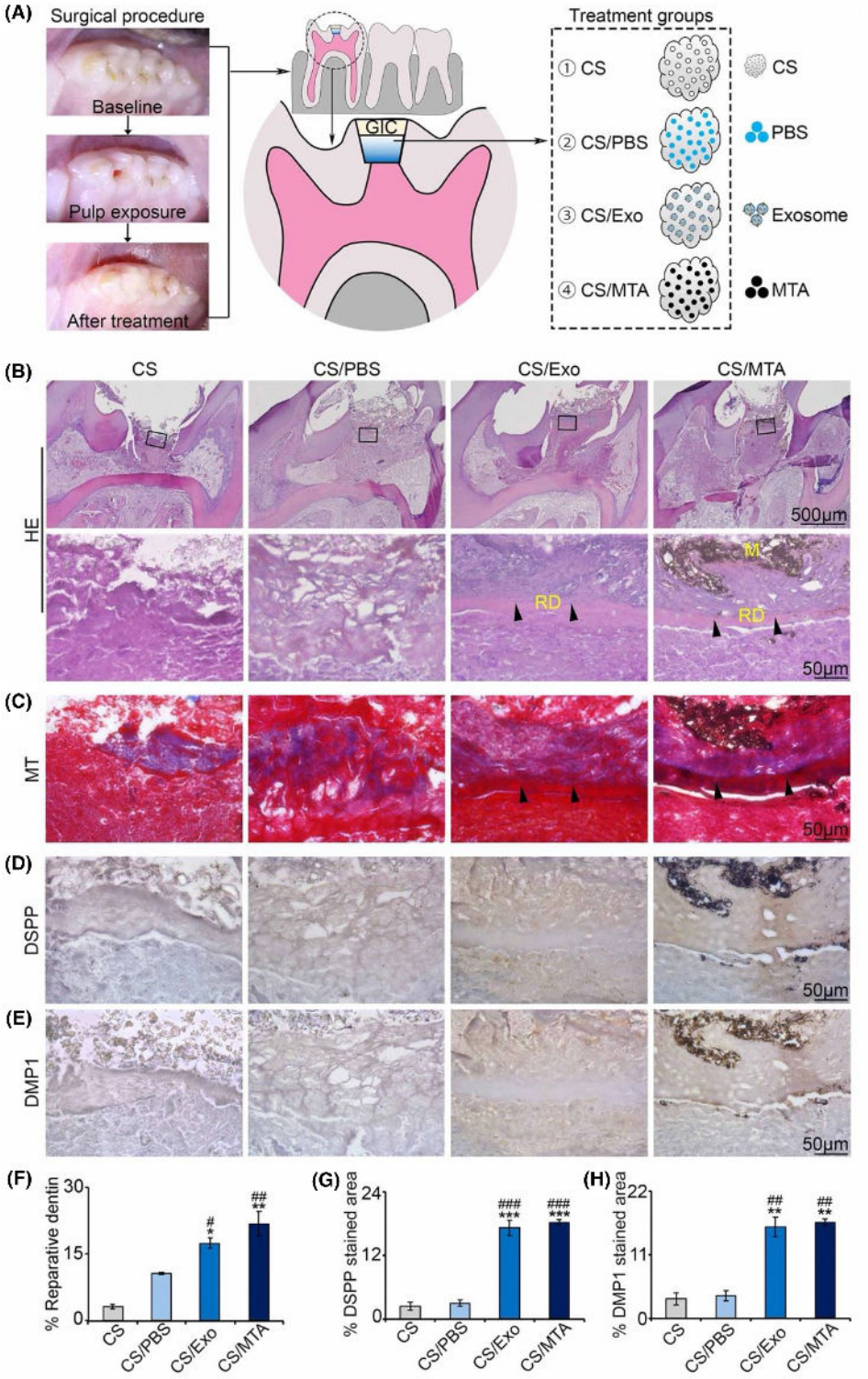

FIGURE 8 In a rat pulp damage model, MSC-Exos increase dentin-like tissue and odontogenesis. (A) Experimental design and animal model. Occlusal surface of maxillary first molars had 1.5 mm diameter and 1.5 mm depth dental pulp defect. The animals were randomly assigned to four groups ($n = 8$ facts/group) after pulp exposure and hemostasis: defects treated with collagen sponge (CS), CS/PBS, CS/Exo, and CS/MTA. Glass-ionomer cement fixed all faults. Animals were killed at 4 weeks for analysis. (B) HE staining. Eight representative photos. Scale bars: 500 or 50 μm. Magnified view is in black box. Arrowheads show dentin bridge repair. RD: reparative dentin, MTA. (C) MT staining. Representative images ($n = 8$). Scale bar: 50 μm. Arrowheads show dentin bridge repair. In CS/Exo and CS/MTA groups, immunohistochemical staining of (D) DSPP and (E) DMP1 exhibited favorably stained regions around reparative dentin bridges. Representative images ($n = 8$). Scale bar: 50 μm. (F) Per- centage area of reparative dentin. (G, H) Per cent-age area deposition of (G) DSPP and (H) DMP1. Data are expressed as mean ± _SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to CS, #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared to CS/PBS. Reprinted with permission from Shi et al.[59]

TABLE 6 The therapeutic regeneration potential of different immune cell exosomes in vitro and in vivo.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Immune cells—in vitro			
Jarmalavičiūtė et al. (2015)[64]	SHED-Exos	Human dopaminergic neurons Isolated using a 300 ***g*** for 10 min; 2000 ***g*** for 10 min; 20 000 ***g*** for 30 min. The final supernatants were ultracentrifuged at 100 000 ***g*** for 70 min. The pellets were then washed in 40 mL of PBS and ultracentrifuged again at 100 000 ***g*** for 70 min	Exosomes but not microvesicles derived from SHEDs grown on the laminin-coated three-dimensional alginate microcarriers suppressed 6-OHDA-induced apoptosis in dopaminergic neurons by approximately 80% throughout the culture period. Based on the results, SHED-produced exosomes are being investigated as a potential new treatment option for Parkinson's disease
Venugopal et al. (2018)[65]	hDPSC-Exos and hBMSC-Exos	Mice hippocampal cell line (H3) The collected condition media was subjected to centrifugation at 2000 ***g*** for 30 min at 4°C to eliminate debris and cells. The obtained supernatant was carefully transferred to a new tube. Total exosome isolation (cell culture media) reagent (Invitrogen) was added to the supernatant. The samples were mixed thoroughly and incubated at 2°C to 8°C overnight. Following that, samples were centrifuged at 10 000 ***g*** for 60 min at 4°C	The findings showed that BMSC-exosomes have neuroprotective efficacy comparable to that of BMSC-condition medium or neuron-BMSC coculture system and that using all three BMSC-based approaches to treat degenerating hippocampal neurons could increase the expression of endogenous growth factors in the host and prevent apoptosis by promoting the cell survival
Ji et al. (2019)[66]	hBMSC-Exos and hDPSC-Exos	The process begins with drawing whole blood from a human and continues with the separation of CD4+ T cells from the blood's periphery. After 48 h, the supernatant was collected and submitted to differential centrifugation to isolate exosomes. Briefly, the supernatant was centrifuged at 300 ***g*** for 10 min and then centrifuged at 16 500 ***g*** for 30 min at 4°C. The supernatant was next passed through a 0.2 μm filter. Next, the filter liquor was ultracentrifuged at 4°C at 100 000 ***g*** for 70 min, then the sediment was rinsed with PBS, and ultracentrifuged again at 4°C at 100 000 ***g*** for 70 min	The study's authors found that DPSC-Exos boosted Treg polarization and increased anti-inflammatory factors released from CD4 + T cells. It also suppressed Th17 differentiation and secretion of pro-inflammatory factors from CD4 + T cells and TNF-α. In comparison with BMSCs-Exos, DPSCs-Exos exhibited superior capabilities
Li et al. (2019)[67]	hDPSC-Exos	Human SCs from odontoblasts The CM was prepurified by centrifugation at 500 ***g*** for 10 min at 4°C to remove the floating cells, followed by a second centrifugation step at 2000 ***g*** for 10 min and a third step at 10 000 ***g*** for 1 h at 4°C and filtrated through a 0.22 μm filter to remove the apoptotic bodies and cell debris, respectively. Finally, the exosome pellet was collected by centrifugation at 100 000 ***g*** for 2 h and washed with PBS by ultracentrifugation at 100 000 ***g*** at 4°C for 70 min to eliminate protein contamination	Exosomes have the capacity to regulate SCs proliferation and migration. Dentin sialoprotein synthesis and SC mineralization were also activated by exosomes from both sets of cells; however, the capacity of LPS-generated Exos to influence SC migration and odontogenic differentiation was superior to that of exosomes
Wang et al. (2019)[68]	hPDLC-Exos with cyclic stretch	Primary monocytes and macrophages from humans, including the THP-1 human macrophage line Exosomes were isolated from cell culture supernatants using the PureExo exosome isolation kit. Briefly, cell culture supernatants were centrifuged at 3000 ***g*** for 15 min to remove cellular debris. Solutions A, B, and C were added and following several steps were then centrifuged at 5000 ***g*** for 3 min, forming a three-phase layer. T-final supernatant was processed on a PureExo column and centrifuged at 1000 ***g*** for 5 min. The flowthrough, which was the isolated pure exosome, was collected and stored at −80°C	Macrophages activated with LPS or nigericin were shown to have reduced NF-kB p65 DNA-binding activity, NF-kB nuclear translocation, and IL-1b production after exposure to isolated exosomes. These findings demonstrate that exosomes inhibit the NF-kB signaling pathway, which in turn reduces IL-1b production. The results imply that exosomes released by PDL cells in mechanical environments aid in regulating the immune response and inflammation inside the periodontal tissues

TABLE 6 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Zheng et al. (2019)[69]	hPDLC-Exos	human peripheral blood samples (CD4+ T lymphocytes) There were a series of unreported first centrifugation steps followed by a final at 120000 ***g*** to pellet the exosomes. The pellet was then washed in a large volume of PBS, to eliminate contaminating proteins, and centrifuged one last time at the same high speed	Exosomes from PDLSCs alleviated inflammatory microenvironment through Th17/Treg/miR-155-5p/SIRT1 regulatory network. This study aimed to find the "switching" factors that affected the further deterioration of periodontitis to maximally control the multiple downstream damage signaling factors to further understand periodontitis and find new targets for its treatment
Elashiry et al. (2020)[70]	Mice dendritic cell (DC)-exosome subtypes, including immunoregulatory (regDC-Exo), loaded with TGFB1 and IL10 after purification	Murine CD4T cells Exosomes were isolated using the following centrifugation techniques: 500 ***g*** for (5 min), 2000 ***g*** for (20 min), and 10000 ***g*** for (30 min) to eliminate cells and debris, followed by ultrafiltration with 0.2 μm and 3× with 100 kDA filters (to remove free proteins) and ultracentrifugation for 1.5 h at 120000 ***g***. To further remove excess free proteins, EXO pellets were washed with a large volume of PBS and ultra-centrifuged 2× at 120000 ***g*** for 1.5 h, and finally re-suspended in 100 μL of PBS	It was shown that T cells treated with regDC exo could suppress osteoclastogenesis in vitro, indicating their potential to prevent bone loss. Furthermore, the DC-Exo group suppressed LPS-induced inflammation and their associated cytokines in vitro
Wang et al. (2020)[71]	hGMSC-Exos	THP-1 is a cell line derived from human acute monocytic leukemia. The cell cultures were cultured for 48 h and centrifuged at 300 ***g*** for 10 min. The resulting precipitates were removed. The supernatants were collected, transferred to new test tubes, and centrifuged at 3000 ***g*** for 20 min. This precipitates also were removed. The supernatants from the second centrifugation were collected, transferred to new test tubes, and centrifuged at 10 000 ***g*** for 30 min. The supernatants from the third centrifugation were filtered through 0.22 μm filters. Finally, the supernatants from filtration were centrifuged twice in the ultracentrifuge machine at 100000 ***g*** for 70 min	In a coculture system with exosomes, the levels of the M1 markers tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), CD86, and interleukin-1β (IL-1β) were found to be significantly reduced. On the other hand, interleukin-10 (IL-10) levels, which are markers of M2 macrophage polarization were increased over 3 fold in the exosome group
Zheng et al. (2020)[53]	hDPSC-Exos	Mouse RAW264.7 cells Exosomes were isolated from the culture medium of DPSCs cultured in the presence of either growth (UN-Exo) or odontogenic differentiation media (OD-Exo) for a period of 10 days. 2 days prior to isolation, the cell cultures were washed in serum-free PBS and cultured for 48 h in serum-free a- MEM. When odontogenic media were used, the serum- free a-MEM was supplemented with the odontogenic media cocktail of 100 nmol dexamethasone, 10 mmol β- glycerophosphate, and 0.2 mmol L-ascorbic acid. The exosomes from the culture medium were isolated using the Exo-spin exosome isolation reagent as per the manufacturer's protocol	By blocking TLR and NFκB signaling, DPSC-Exos transformed macrophages into the pro-healing M2 phenotype
Nakao et al. (2021)[72]	hGMSC-Exos (treated with TNF- α)	Human peripheral blood-derived monocytes (PBMCs) positive for CD14 Medium was collected, centrifuged at 10000 ***g*** for 30 min to eliminate other large EVs. Cleared supernatants were passed through 0.22 mm filter membranes and concentrated using a Vivaspin-20 concentrator	In response to TNF-α activation, exosomes produced by GMSCs enhance M2 macrophage polarization and decrease Wnt5a-regulated production of receptor activator of NF-κB ligand (RANKL)

(Continues)

TABLE 6 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Zhang et al. (2021)[73]	hGMSC-Exos	Human THP1 monocyte cells The GMSC-CM was collected and centrifuged for 10 min at 300 *g* and 4°C to remove the cellular debris. The supernatant was transferred to a new tube and centrifuged for 20 min at 3000 *g* and 4°C. The subsequent supernatant was collected and centrifuged for 30 min at 10 000 *g* and 4°C to remove organelles and other impurities. The final supernatant was passed through 0.22 μm filters, placed in a Beckman ultracentrifuge tube, and centrifuged twice for 70 min at 100 000 *g* and 4°C in an ultracentrifuge	In a high-lipid milieu, GMSC-Exos decreased the production and release of inflammatory markers, prevented lipid buildup, and encouraged pro-inflammatory macrophages to polarize into anti-inflammatory phenotypes
Hayashi et al. (2022)[74]	miR-1260b	Human peripheral blood-derived monocytes (PBMCs) expressing CD14+ Transfection of miRNA mimics and siRNAs for PDL cells was performed using the Lipofectamine RNAiMAX Transfection Reagent for 24 h according to our reverse transfection protocol	Human CD14+ peripheral blood-derived monocytes showed a decrease in osteoclastogenesis when exposed to PDL cell secretomes driven by the miR-1260b/ATF6β-axis
Liu et al. (2022)[75]	hDPSCs	Mouse RAW 264.7 cells Exosomes were isolated from the supernatants of passage 4 DPSCs by differential centrifugation (300 *g* for 10 min; 2000 *g* for 10 min; 10 000 *g* for 30 min, 100 000 *g* for 70 min) and washing of the exosomes with PBS (100 000 *g* for 70 min). All centrifugations were performed at 4°C	Reducing macrophage M1 polarization, DPSC-Exos activated the ROS-MAPK-NFκB P65 signaling pathway while treating SCI
Liu et al. (2022)[76]	hADSC-Exos (under normal and inflammatory (IAE) conditions)	Human macrophage line (THP-1) Culture medium was harvested and centrifuged at 300 *g* for 10 min to remove cells, 2000 *g* for 10 min to remove dead cells, 10 000 *g* for 30 min to remove the cell debris, and 100 000 *g* for 70 min to collect sEVs and remove contaminating protein. The final pellet was washed in PBS at 100 000 *g* for 70 min to concentrate the sEVs and then resuspended in PBS. All the centrifugation steps were performed at 4°C	IAE markedly enhanced M2 macrophage differentiation with respect to the modulation of macrophage polarization. High levels of miR-27b-3p expression in IAE were shown to be associated with the regulation of macrophages via its targeting of macrophage colony-stimulating factor-1 (CSF-1). A 3–4 fold increase in cell migration as well as a 2 fold increase in the gene expression for Sox9, ACAN, Col II, OPN, Runx2, and BMP2 was observed in the IAE-Exo group
Yu et al. (2022)[63]	Rat SCAP-Exos	Mice Tregs Centrifugation was carried out at 3000 *g* ×20 min and 20 000 *g* ×30 min. After each of the mentioned centrifugations, pellets were discarded, and the supernatant was centrifuged at 120 000 *g* ×2 h. Then, pellets were resuspended in PBS and centrifuged at 120 000 *g* for 2 h. All centrifugations were performed at 4°C	Treg conversion was aided in vitro by SCAP-Exos. Technically, SCAP-Exos helped maintain Foxp3 demethylation via Tet2-mediated Foxp3 demethylation. The exosome group demonstrated a nearly 5-fold increase in number of Foxp3-positive cells
Yue et al. (2022)[77]	hBMSC-Exos	Mouse RAW 264.7 cells macrophages hBMSC culture medium was centrifuged at 500 *g* for 10 min, 2000 *g* for 10 min, and 10 000 g for 30 min to remove cell debris and large organelles. After that, exosomes were collected from supernatants by ultracentrifugation at 100 000 *g* for 70 min. The pellets were washed in 0.9% NaCl and centrifuged at 100 000 *g* for 70 min to further remove medium protein contaminants	In vitro, the periodontal keystone pathogen P. gingivalis-induced inflammatory response in macrophages was markedly attenuated by hBMSC-Exos. The addition of exosomes led to a 50% reduction of inflammatory markers such as IL-6
Elsayed et al. (2023)[78]	human monocyte-derived dendritic cell (DC)-Exos	Human Naïve CD4+ T-Cell Exosome uptake: Exosomes labeled with DiI (D282, Thermofisher Scientific) were cocultured with MoDCs or CD4T cells for 24 h. Cell culture: incubation with 108/mL MoDC Exo subtypes in MoDC culture medium on day 4. Cells were then harvested on day 6	An efficient delivery method for modifying human DC and T-cell immune responses in vitro is provided by human DC-Exos. Therefore, DC-Exos may provide a promising immunotherapeutic effect to regulate the gingival tissue's immune response and prevent bone loss in periodontal disease

TABLE 6 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Luo et al. (2023)[79]	Human embryo kidney tissue 293T/17 cells	Monocytic cell line from human leukemia (THP-1 cells) Centrifugation was carried out at 300 ***g*** for 10 min at 4°C to remove any cells or large cellular fragments. Differential centrifugation was used to isolate the exosomes: the supernatant was first centrifuged at 2000 ***g*** for 20 min at 4°C then 10 000 ***g*** for 30 min to remove microvesicles, and filtered through a 0.22 μm filter. The samples were carefully transferred to ultracentrifuge tubes and centrifuged at 110 000 ***g*** for 70 min at 4°C. The ultracentrifuge tubes were centrifuged again at 110 000 ***g*** for 70 min at 4°C	M1 macrophages demonstrated anti-inflammatory effects of CXCR4-miR126-Exo. Many cytokines related to inflammation IL-1a, IL-4, IL-6, IL-8, IL-10 and TNF-alpha were significantly upregulated in the periodontitis group
Qiao et al. (2023)[80]	hDPSC-Exos	Mouse RAW264.7 macrophages The exosomes labeled with PKH26 were cultured with RAW264.7 cells for 2 h	By blocking the IL-6/JAK2/STAT3 signaling pathway, DPSC-Exos were able to regulate the acute inflammatory stress. In addition, the results showed that DPSC-Exo might change macrophage phenotype from M1 to M2. Many genes related to anti-inflammation were upregulated including IL-10 (10 fold), TGF-beta (3 fold), whereas pro-inflammatory molecules were all significantly downregulated including TNF-alpha (60%), and IL-6 (50%)
Immune cells—in vivo			
Yu et al. (2022)[63]	Rat SCAP-Exos	Rat exposure of the dental pulp of mandibular incisors. The mandibular incisors were gently ground using a high-speed handpiece and round tungsten steel burs with a diameter of 0.8 mm under water cooling. The exposure of the dental pulp was confirmed using a sterile 40 K-file. Lipopolysaccharide (LPS), 5 μL at 10 mg/mL from *Escherichia coli* O55:B5 was injected into the cavity, and the cavity was sealed with GC Fuji IX glass ionomer cement. After 48 h, the glass ionomer cement was removed, and 5 μL of 1 μg/μL SCAP-Exos or PBS was injected into the cavity, and the cavity was sealed with GC Fuji IX glass ionomer cement. Seven days after the procedure, the animals were euthanized	The use of SCAP-Exos resulted in a decrease in inflammation and an increase in Treg conversion in the dental pulp of rats. This study provided new evidence that SCAP-Exos may regulate the local immune system to stimulate tissue regeneration, opening the door to a novel strategy for treating pulp inflammation in clinically immature permanent teeth without the use of cells

Abbreviations: 6-OHDA, 6-hydroxydopamine; AE, normal ADSC-derived EV; BMSCs, Bone Marrow Mesenchymal Stem Cells; CD4, Clusters of differentiation 4; CSF-1, Colony Stimulating Factor -1; CXCR4, C-X-C chemokine receptor type 4; DC, Dendritic Cell; DNA, Deoxyribonucleic acid; DPSCs, Dental Pulp Stem Cells; EV, Extracellular Vesicle; Exo, Exosome; FOXP3, Forkhead box P3; GMSC, gingival mesenchymal stem cells; hADSC, human Adipose-derived mesenchymal stem cell; hBMSCs, human BMSCs; hDPSCs, human DPSCS; hGMSC, human GMSC; hPDLSC, human PDLSc; hSCAPS, human SCAPS; IAE, inflammation-stimulated ADSC derived EV; IL, Interleukin; JAKF2, Janus kinase 2; LPS, Lipopolysaccharide; MAPK, mitogen-activated protein kinase; miRNA, microRNA; NF-kB, Nuclear factor kappa-B; PBMC, Peripheral blood-derived monocytes; PDL, Periodontal Ligament; PDL, periodontal ligament; PDLSCs, Periodontal Ligament Stem Cells; RANKL, receptor activator of nuclear factor kappa beta ligand; regDC, immuneregulatoryDC; RNA, Ribonucleic acid; ROS, Reactive Oxygen Species; SC, Schwann cells; SCAPs, Stem Cells from the Apical Papilla; SCI, Spinal Cord Injury; SHED, Stem cells from Human Exfoliated Deciduous teeth; SIRT1, Sirtuin-1; STAT3, signal transducer and activator of transcription; TET2, Tet methylcytosine dioxygenase-2; TGFB, Transforming Growth Factor-B; TGF-β, Transforming Growth Factor-β; TH17, T-helper 17 cell; THP-1, human leukemia monocytic cell; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-α; Treg, Regulatory T-cells.

TABLE 7 In vitro therapeutic regenerative potential of various exosomes derived from multiple sources on mesenchymal cell function.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Mesenchymal cell function—in vitro			
Li and Ge (2022)[61]	Rat DPSC-Exos	Mouse MSCs To remove cellular debris, medium from DPSCs was centrifuged at 2500 ***g*** for 15 min at 4°C and filtered with a 0.22 μm filter. The collected medium containing exosomes was laid on top of a 30% sucrose/D_2O cushion in a sterile UltracCear and ultracentrifuged at 100 000 ***g*** × 1 h at 4°C. The pellets were resuspended in 15 mL PBS and centrifuged at 4000 ***g*** at 4°C for 15 min until the volume was concentrated to ~200 μL	Markers of osteoblastic differentiation, such as OCN, OPN, and RUNX2, were seen after culturing in CM containing exosomes produced from DPSCs. Cell migration of MSCs in conditioned culture more than doubled the control group
Han et al. (2023)[81]	3 Sources: 1. hPDLC-Exos, 2. human alveolar bone-derived Osteoblasts-Exos 3. hGMSC—Exos	Buccal fat pad-derived mesenchymal stromal cells (BFP-MSCs). The CM was initially centrifuged at 300 ***g*** for 15 min to remove cell debris, then at 2600 ***g*** for 15 min at 4°C to pellet apoptotic bodies (termed "2.6 keV" in this study) in 500 μL of PBS. The resultant supernatant was concentrated to 500 μL using Amicon ultrafilters (100 kDa) and centrifuged at 16 000 ***g*** for 20 min at 4°C to pellet microvesicles (termed "16 keV"). The supernatant was subsequently subjected to the authors in-house size exclusion chromatography (SEC) columns to enrich sEV	BFP-MSCs showed cellular absorption of all three EV subtypes for as long as 7 days. Three periodontal cells' worth of sEV were encouraged. Cell multiplication, movement, and the activation of genes related to bone formation. hOBs-sEV exhibited higher levels of osteogenesis markers in comparison to hPDLCs-sEV and hGFs-sEV. Conversely, hOBs-16k EV stimulated adipogenic gene expression more than hPDLCs and hGFs

Abbreviations: BFP-MSCs, Buccal Fat Pad-derived mesenchymal Stromal Cells; DPSC, Dental Pulp Stem Cells; EVs, Extracellular Vesicles; Exo, Exosome; GFs, Gingival Fibroblasts; GMSC, gingival mesenchymal stem cells; hDPSCs, human DPSCS; hGFs, human Gingival Fibroblasts; hGMSC, human GMSC; hOBs, Human Osteoblasts; hPDLCs, human PDLCs; MSC, Mesenchymal Stem Cells; OCN, Osteocalcin; OPN, Osteopontin; PDLCs, Periodontal Ligament Stem Cells; RUNX2, Runt-related transcription factor 2.

TABLE 8 In vitro and in vivo therapeutic regenerative potential of exosomes in nerve regeneration.

Author–year	Source of exosomes	Target cell of exosomes and isolation method	Main findings
Nerve regeneration—in vitro			
Rao et al. (2019)[82]	hGMSCs-Exos	The in vitro assessment of peripheral nerve regeneration was conducted using the cocultivation of SCs and Dorsal Root Ganglion cells (DRGs). Supernatant was centrifuged at 3000 ***g*** for 10 min. The supernatant from the cells was then extracted, and 2.5 mL of the exosome extraction reagent (HieffTM Quick exosome isolation kit, 41201ES25) was added according to standard protocols	GMSC-Exos may strongly stimulate the development of DRG axons and SCs
Author–year	**Source of exosomes**	**Target tissue of exosomes**	**Main findings**
Nerve regeneration—in vivo			
Rao et al. (2019)[82]	hGMSCs-Exos	The effects of exosomes on the recovery of sciatic nerve function in vivo were tested using histology, electrophysiological, and gait analysis in rats with regeneration of sciatic nerves. Three groups as follows: The hollow chitin conduit group, chitin conduit plus GMSC-derived exosome group, and autograft group. The sciatic nerve was cut and exposed, and 10 μL of PBS was administered to the hollow chitin conduit group, retaining a 10 mm gap. The exosome group received 10 μL of PBS containing 10 μg exosomes	The in vivo research demonstrated that the chitin conduit and GMSC exosomes could greatly enhance nerve fiber quantity and diameter as well as stimulate the development of myelin. Furthermore, there was a clear recovery of motor function, nerve conduction function, and muscular function

Abbreviations: BFP-MSCs, Mesenchymal stromal cells originating from the buccal fat pad; DPSC, Dental Pulp Stem Cells; EVs, Extracellular Vesicles; Exo, Exosome; GFs, Gingival Fibroblasts; GMSC, gingival mesenchymal stem cells; hDPSCs, human DPSCS; hGFs, human Gingival Fibroblasts; hGMSC, human GMSC; hOBs, Human Osteoblasts; hPDLCs, human PDLCs; MSC, Mesenchymal Stem Cells; OCN, Osteocalcin; OPN, Osteopontin; PDLCs, Periodontal Ligament Stem Cells; Runt-related transcription factor 2, or RUNX2.

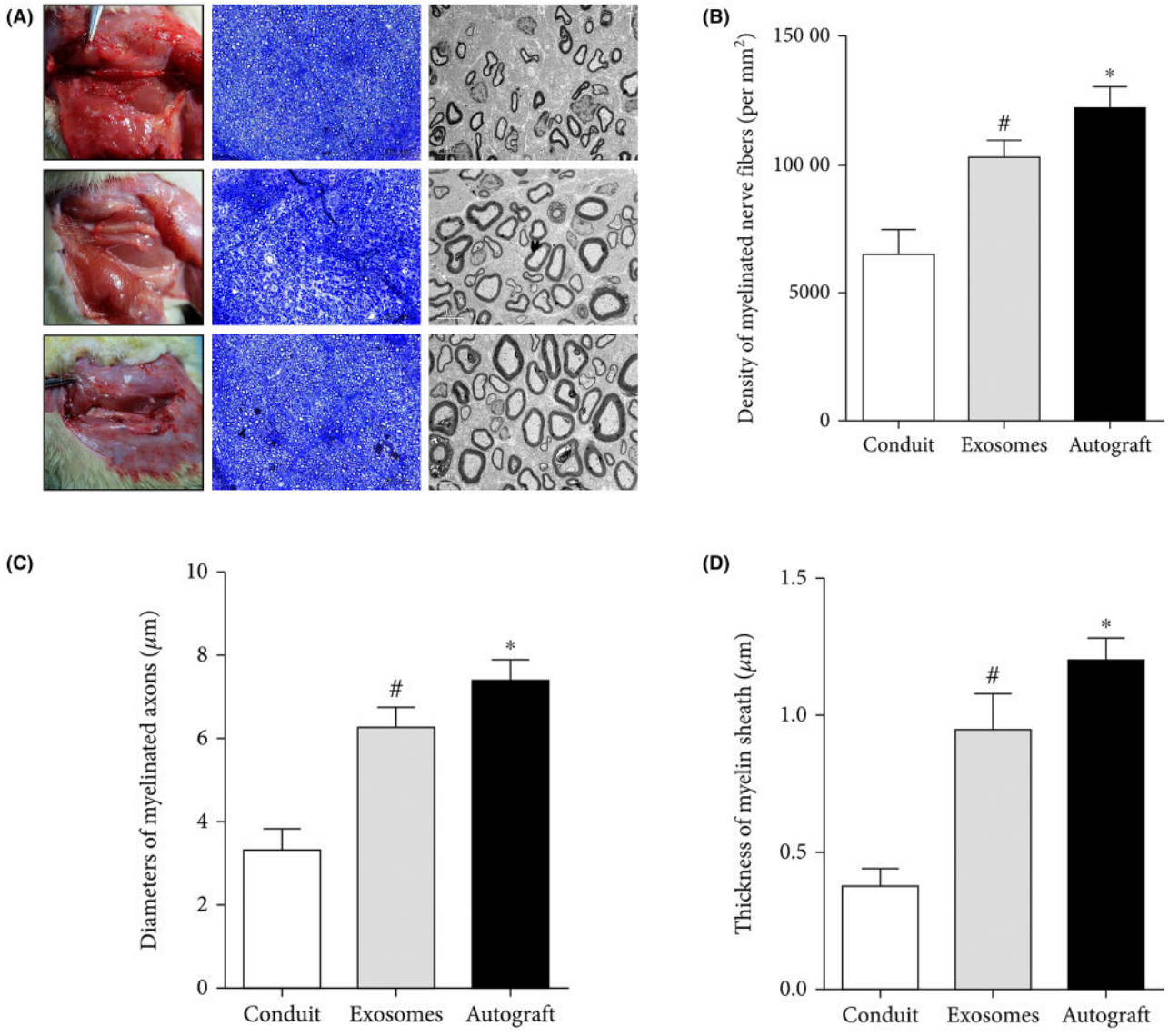

FIGURE 9 Analyzing the regeneration of nerve fibers 12 weeks after surgery. (A) TEM pictures, transverse slices stained with toluidine blue, and a gross view of the regenerated sciatic nerve. (B) Myelinated axon density. (C) the myelinated axons' diameters. (D) Sheath thickness of myelin. Data are expressed as means ± SEM ($n = 8$). A one-way ANOVA with Tukey's post hoc test was used to achieve statistical significance. *$p < 0\,05$, #$p < 0\,01$. Reprinted with permission from Rao et al.[82]

that hOBs-sEVs showed superior levels of osteogenesis markers compared to hPDLCs-sEVs and hGFs-sEVs, while hOBs-16 k EVs promoted adipogenic gene expression compared to hPDLCs and hGFs.[81] In summary, the interest in these studies was characterized by the study by Han et al., demonstrating that various exosomal subsets derived from multiple sources have the ability to impact various cell types differently.[81]

2.8 | Nerve regeneration

Only one study investigated the regenerative potential of exosomes in nerve regeneration (Table 8). A study by Rao et al.[82] found that GMSC-Exos could significantly promote SC proliferation and DRG axon growth (Figure 9). Furthermore, the in vivo component of the study also demonstrated an increase in the number and diameter of nerve fibers, leading to a promotion in myelin formation. Recovery was shown in muscle function, nerve conduction, and motor function, leading to the conclusion that exosomes could be utilized for nerve regeneration. Future studies are needed to decide which exosomes derived from which sources are most likely to lead to better clinical outcomes for nerve regeneration.

2.9 | Oral/Periodontal pathogens

Exosomes alone have been utilized to attenuate the effects of oral or periodontal pathogens (Table 9). A study by Sundaram et al.[83] demonstrated that plant-derived exosomes from ginger could attenuate

TABLE 9 In vitro therapeutic behavior of various exosomes on periodontal pathogens.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Oral/periodontal pathogens—in vitro			
Sundaram et al. (2019)[83]	Ginger exosome-like nanoparticles (GELNs)	Porphyromonas gingivalis P. gingivalis strains were grown to mid-log phase (OD600 = 0.8) and aliquots (200 μL) of samples were anaerobically incubated in the 25% saliva-coated wells with and without ginger exosomes like nano particles (4.0×10^8 particles/mL) for 24 h	*P. gingivalis* growth was inhibited by the selective uptake of Ginger exosome-like nanoparticles
Leiva-Sabadini et al. (2021)[84]	Exosomes derived from honey	Oral Streptococci Honey samples were initially diluted 1:20 in particle-free PBS and centrifuged at 500 ***g***, 1500 ***g***, 250 ***g*** for 15 min each. Then supernatants were filtered (0.2 μm) and ultra-centrifuged at 100 000 ***g*** for 60 min. The final EV-containing pellet was resuspended in PBS and stored at −80°C	Honey is a promising source of highly active EVs with exosomal origin, containing a number of antibacterial peptides as cargo molecules. Furthermore, the differential effect of Honey-Exos on *S. mutans* and *S. sanguinis* may serve as a novel biofilm-modulating strategy in dental caries

Abbreviations: Exo, Exosome; EV, extracellular vesicle; *P. gingivalis*, *Porphyromonas gingivalis*; *S. mutans*, *Streptococcus mutans*; *S. Sanguinis*, *Streptococcus sanguinis*.

TABLE 10 Effects of exosomes on orthodontic behavior and root resorption in living organisms.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Orthodontics—in vivo			
Liu et al. (2022)[85]	hPDLSC-Exos	Orthodontic nickel–titanium coiled springs were ligated between the maxillary left first molar and incisors. The springs were activated to deliver 50 g of orthodontic force, as measured by a dynamometer. The springs were removed to allow the left first molars to relapse after OTM for 2 weeks. Submucosal injection and intra-ligamentary injections were performed using an insulin needle delivering equal volumes of saline, simvastatin dissolved in saline, PDLSCs-Exo, and exosomal simvastatin	In the rat model of OTM, PDLSC-derived exosomes boosted simvastatin solubility and relapse inhibition. Recurrence after OTM was also prevented by local PDLC-Exos injection alone
Zhao et al. (2022)[47]	Mouse RAW264.7 monocyte cell line M0, M1 and M2 macrophage polarization	Mice: orthodontic tooth movement model: a nickel–titanium coil spring was positioned between the maxillary incisors and the right maxillary first molar to provide a force of around 30 g that was almost constant. Mice in the experimental group received injections of 10 mg/mL LPS with 5 μL on each side of the gingival sulcus around the maxillary first to third molars once every 2 days for a total of seven times. The control group was injected with normal saline with the same volume and frequency in the same anatomic regions. The in vivo exosome group had local injection on each side of the gingival sulcus around the maxillary first to third molars and caudal vein injection of exosomes once every 2 days for a total of 10 times. Mice were randomly divided into three groups, which were subsequently injected with exosomes from M0, M1, or M2, respectively, at the dose of 100 μg per mouse, at 100 μL in volume	In mice injected with M0, M1-, and M2-polarized macrophage exosomes, cementoblast mineralization was decreased in the M1-injected group and enhanced in the M2-injected group

Abbreviations: Exo, Exosome; OTM, orthodontic tooth movement; PDLCs, periodontal ligament stem cells; hPDLCs, human PDLCs.

TABLE 11 In vitro and in vivo therapeutic regenerative potential of various exosomes derived from multiple sources on periodontal regeneration.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Periodontal regeneration—in vitro			
Chew et al. (2019)[86]	hMSC-Exos	Rat PDL cells For the migration assay: Briefly, cells in 300 mL of low serum culture medium (DMEM-F12 supplemented with 0.5% FBS and 1% PS) were placed in the upper chamber and 1, 5, or 10 mg/mL exosomes or vehicle control (PBS) were added to the lower chambers Cell viability/proliferation: cells were then treated with 1, 5, and 10 mg/mL of exosomes or PBS as vehicle control. At 4, 24, 48, and 72 h, cell viability	MSC exosomes may drive PDL cell migration and proliferation by increasing pro-survival AKT and ERK signaling via CD73-mediated adenosine receptor activation. AKT/ERK phosphorylation hindered PDL cell migration and growth. Cell migration was improved over 250% and viability improved by 50%
Lv et al. (2020)[87]	Mouse osteocyte-like MLO-Y4 cell-Exos	hPDLCs HPDLSCs were cultured in a 96-well plate and subjected to the following treatments: (1) PBS, (2) Porphyromonas gingivalis lipopolysaccharide (P.g LPS) (1 μg/mL), (3) LPS + exosomes from normally cultured cells (Exosome); (4) exosomes from mechanical strain (MS)-treated cells (Exosome-MS), and (5) a combination of LPS + Exo-MS	Mechanical strain-generated exosomes induce HPDLSC osteogenic differentiation via BMP2/Runx2 and proliferation via miR-181b-5p/PTEN/AKT, suggesting a means to maintain periodontal homeostasis. Most markers analyzed for osteogenic differentiation significantly increased by ~50%
Shi et al. (2020)[88]	Human dental follicle cells (DFC-Exos) (LPS-preconditioned)	hPDLCs Cells were maintained in medium containing EVs with 10, 100, and 200 μg/mL and investigated at 1, 2, 3, 5, and 7 days for proliferation, and up to 14 days for alizarin red staining	Saturation- and dose-dependent enhancements to p-PDLC migration, differentiation, and proliferation were seen with both L-D-sEV and D-sEV. Furthermore, as compared to D-sEV, L-D-sEV was more effective in inducing p-PDLC differentiation. Most effectively, the EVs enhanced cell recruitment and alizarin red staining up to 4 fold greater when compared to controls
Wang et al. (2020)[89]	SHED-Exos	hPDLCs PDLSCs were treated with SHED-Exos (isolated from SHED treated with OM or non-OM for 3 days) for the indicated time, and the fresh medium containing re-isolated SHED-Exos was changed every 3 days during the osteogenic process up to 14 days	The CCK-8 test was used to determine if conditioned SHED-Exos had any cytotoxic impact on the viability of PDLSCs. These SHED-Exos promoted osteogenic growth in PDLSCs by increasing ALP activity, enhancing the expression of osteogenic genes (RUNX2, OPN, and OCN), and exhibiting high Alizarin red staining. In addition, the study demonstrated that the Wnt/β-catenin and BMP/Smad signaling pathways were activated by elevated nuclear β-catenin protein expression and Smad1/5/8 phosphorylation
Xu et al. (2020)[90]	hPDLC-Exos (including a P2X7R gene-modified cells)	hPDLCs Exosomes were extracted by using the Total Exosome Isolation Kit for Cell Culture Media (Invitrogen) from supernatants according to the manufacturer's protocol. In brief, the obtained supernatants were centrifuged at 2000 ***g*** for 30 min and then transferred to sterile tubes. Following the addition of the reagent to the supernatants and incubation overnight at 4°C, the mixture was centrifuged at 4°C and 10 000 ***g*** for 1 h	The use of exosomes significantly reduced inflammation-mediated changes in PDLCs in an inflammatory osteogenic milieu, as shown by quantitative real-time PCR (qRT-PCR), Western blot analysis, ALP staining/activity testing, and Alizarin red staining
Liu et al. (2021)[91]	Rat BMSC-Exos	hPDLCs sEVs were isolated from the supernatant by the Exosome Isolation reagent (Invitrogen)	hPDLC migration, proliferation, and osteogenic differentiation were all enhanced by BMSC-Exos. The expression level of osteopontin, in particular, was over 10 fold higher in the Exo group

(Continues)

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Nakao et al. (2021)[72]	hGMSC-Exos (TNF- α-treated)	hPDLCs Medium was then collected, centrifuged at 10000 ***g*** for 30 min to eliminate other large EVs. Cleared supernatants were passed through 0.22 mm filter membranes and concentrated using a Vivaspin-20 concentrator	M2 macrophage polarization was enhanced by GMSC exosomes that were activated by TNF-α. In PDLCs, the synthesis of RANKL (receptor activators of NF-κB ligand) was controlled by Wnt5a. In conclusion, a new mechanism for inhibiting the osteoclastogenic activity of the Wnt5a-mediated RANKL pathway has been identified: exosomal miR-1260b
Wang et al. (2021)[92]	SHED-Exos	hPDLCs The supernatant was harvested after centrifugation at 3000 ***g*** for 30 min. 500 μL of exosome extraction reagent was mixed with 1 mL of the supernatant and incubated at 4°C overnight with gentle agitation. The exosome was resuspended in sterile PBS (1×10^6 exosomes/100 μL) after centrifugation at 10000 g for 1 h and as the exosomes stock solution for subsequent experiments	In hPDLCs, SHED-Exos strongly increased Runx2 expression and mineralization and accelerated the cell cycle transition from G1 to the S phase. It also greatly increased the migration, apoptosis, and proliferation of PDLCs. Gene expression of OCN, Runx2 and ALP all significantly increased by over 2 fold in the SHED-Exo group
Dai et al. (2022)[93]	hPDLC-Exos	hPDLCs The supernatant was centrifuged at 300 ***g*** for 10 min at 4°C to transfer the supernatant and then centrifuged at 10000 ***g*** for 10 min at 4°C to remove dead cells and cell debris. The supernatant was carefully collected and transferred to a 50 mL open-ended ultracentrifugal tube for 4°C and 100000 ***g*** for 70 min, and the supernatant was discarded. The precipitate was suspended with 2 mL DPBS and then transferred to a 12.5 mL tube for centrifugation at 4°C for 70 min at 100000 ***g***	Following inflammatory-PDLC exosomes treated with gallic acid, a significant enhancement in osteodifferentiation was observed. It was revealed that the exosome group increased mRNA levels of OCN, Runx2, and Col1A and further increased alizarin red staining over 2 fold
Hayashi et al. (2022)[74]	miR-1260b	hPDLCs Transfection of miRNA mimics and siRNAs for PDL cells was performed using the Lipofectamine RNAiMAX Transfection Reagent for 24 h according to our reverse transfection protocol	Tunicamycin, an ER stress inducer, raised RANKL (receptor activator of NF-κB ligand) expression in PDL cells, but RANKL was suppressed by ATF6β downregulation mediated by miR-1260b
Lei et al. (2022)[106]	hPDLC-Exos	hPDLCs The proteins in exosomes samples were collected according to the instruction of a Cell Lysis Kit. 200 μL of exosome solution (the ratio of A: B was 50: 1) were added into the wells in the expiremental group.	Treatment of exosomes of h-PDLSCs led to an increase in the formation of mineralized nodules and the expressions of osteogenic genes and proteins in i-PDL SCs. Mechanistically, h-PDL SCs-exosomes suppressed the over-activation of canonical Wnt signaling to recover the osteogenic differentiation capacity of PDLCs
Liao et al. (2022)[94]	Human THP-1 cells M0, M1 and M2 macrophage-derived exosomes (M0-Exos, M1-Exos and M2-Exos)	hPDLCs The supernatants of M0, M1 and M2 macrophages were collected and centrifuged at 300 ***g*** for 10 min to remove cells, at 2000 ***g*** for 10 min to remove dead cells and at 10000 ***g*** for 30 min with 4°C to remove cell debris. Subsequently, the supernatant was transferred to an ultracentrifuge tube and centrifuged at 100000 ***g*** for 90 min with 4°C to precipitate exosomes. Finally, the exosomes were resuspended in pre-cooled PBS and centrifuged again at 100000 ***g*** for 90 min with 4°C to obtain exosomes	The findings of this work demonstrated the potential of M2-Exos to stimulate osteogenic differentiation of human PDLSCs and provided insight into the roles and processes behind the DE-miRNAs encoding M0- and M2-Exo as well as their downstream targets. In particular, the M2-Exo group showed up to a 4 fold increase in genes related to osteoblast differentiation including ALP and OCN
Shimizu et al. (2022)[95]	hDPSC-Exos	1. Mouse osteoblastic MC3T3-E1 cells 2. hDPSCs The medium was collected and filtered through 0.45 and 0.22 μm syringe filters (Minisart; Sartorius) before ultracentrifugation at 440000 ***g*** for 1.5 h at 4°C. The pellet was resuspended in PBS and stored at −80°C	Exosomes from DPSCs facilitated the migration of mice osteoblastic cells and human DPSCs. The MTT test had a favorable impact on human DPSC growth but not on mice osteoblastic cells. Overall the addition of exosomes led to roughly a 50% faster rate of in vitro wound closure

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Shuo et al. (2022)[96]	Human Umbilical Cord MSC-Exos	hPDLCs Cocultured with PDLSCs for 24 h. The following groups: (i) control group, cells treated with 5.6 mmol/L D-glucose; (ii) HG group, cells treated with 30 mmol/L D-glucose; (iii) HG + 25 μg/mL exo group, cells treated with 30 mmol/L D-glucose and 25 μg/mL exosomes; (iiii) HG + 50 μg/mL exo group, cells treated with 30 mmol/L D-glucose and 50 μg/mL exosomes	The CCK-8 test, ALP staining, ALP activity, and qRT-PCR assay findings demonstrated that hUCMSC-Exos substantially and dose-dependently increased osteogenic differentiation and cell proliferation
Sun et al. (2022)[97]	hGMSC-Exos	hPDLCs GMSCs achieved a confluence of 70%–80% and the mediums were replaced by a-MEM containing 10% exosome-depleted FBS and then cultured for 48 h prior to supernatant harvesting	The GMSC-Exo were isolated from the GMSC-encircling liquid by ultracentrifugation. Results showed that GMSC-Exos mitigated the LPS-induced inflammatory response in PDLSCs. The expression of NF-κB signaling and Wnt5a in LPS-induced PDLSCs was decreased by over 50% in response to GMSC-Exo therapy in comparison with LPS treatment
Cui et al. (2023)[98]	Mouse RAW264.7 M2-macrophage-Exos (loaded with melatonin)	hPDLCs under inflammatory conditions Interleukin-4 (100 ng mL^{-1}) was added to the medium to differentiate primary macrophages into M2 macrophages. The supernatant was collected in a centrifuge tube and centrifuged at 1000 ***g*** for 30 min to remove dead cells, then at 10000 ***g*** for 30 min to remove the cell debris. The resulting supernatant was ultracentrifuged at 100000 ***g*** for 90 min to pellet exosomes, which were suspended in PBS, and ultracentrifuged at 100000 ***g*** for 60 min to wash away impurities	The proper and timely reprogramming of macrophages from M1 to M2 type was induced by M2-Exos, leading to the resolution of chronic inflammation and an acceleration of periodontal repair. By lessening excessive ER stress, melatonin produced by melanin-loaded M2-Exos restored the osteogenic and cementogenic differentiation potential in inflammatory PDLCs
Hu et al. (2023)[99]	hGMSC-Exos	hPDLCs When the cells achieved 80% confluence, the osteogenic induction medium supplemented with either *Porphyromonas gingivalis*-derived oipopolysaccharide (P.g-LPS; 1 μg/mL) or GMSC-Exos (0, 1, 10 or 50 μg/mL) was substituted for the culture medium. The medium was exchanged at 2-day intervals	Osteogenic differentiation-related factors were upregulated in PDLCs treated with GMSC-Exo in both normal and inflammatory conditions. Subsequent research revealed that GMSC-Exos might also prevent the NF-κB pathway from being activated by P. gingivalis-LPS, which would cause the Wnt/β-catenin pathway to be upregulated
Liu et al. (2023)[100]	hPDLSC-Exos	hPDLCs After the CM was centrifuged at 300 ***g*** for 15 min, the supernatant was removed, and the samples were centrifuged again for 15 min at 3000 ***g***. The resulting supernatant was centrifuged twice at 100000 ***g*** for 70 min each	The results imply that PDLSC-Exos transferred miR-141-3p to activate the KEAP1-NRF2 signaling pathway, therefore mitigating the senescence of PDLSCs caused by high glucose. According to this study, PDLSC-Exos may exhibit characteristics of their parent PDLSCs and, by delivering their encapsulating bioactive compounds to target cells, may have a substantial influence on cellular senescence
Liu et al. (2023)[101]	hBMSC-Exos (exosome-derived miR-335-5p)	hPDLCs The binding site of DKK1 and miR-335-5p was punctured. A mutant DKK1 3′-UTR was inserted by PCR into a Dual-Luciferase carrier, and miR-335-5p mimic or mimic NC was transfected into 293T-cells. 48 h after transfection, a luciferase assay was used	Exosome treatment led to a significant reduction in IL-1β, IL-6, and IL-8 mRNA expressions in TNF-induced hPDLCs, an increase in RunX2, OCN, and BMP-2 mRNA expressions, and the promotion of calcium nodule growth. CD9 and CD81 relative gene expression was over 2 fold higher in the exosome group

(Continues)

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Qiao et al. (2023)[80]	hDPSC-Exos	hPDLCs Exosomes at different concentrations (0, 1, 5, 10 μg/mL) were used to evaluate at 1, 2, and 3 days	In vitro, DPSC-Exos enhanced the proliferation, migration, and osteogenesis of PDLSCs. DPSC-Exo exerted control over inflammation by suppressing the IL-6/JAK2/STAT3 signaling pathway in the context of acute inflammatory stress. Furthermore, the results demonstrated that macrophages may be polarized by DPSC-Exos from the M1 phenotype to the M2 phenotype. Exosomes at higher concentrations increased migration 2 fold, as well as their osteogenic differentiation
Yu et al. (2023)[102]	hPDLSC-Exos (treated with psoralen + Pso-Exos)	hPDLCs The blank control group was added with simple medium, and the experimental group was added with 10 μg/mL Pso-Exos, inhibitor +10 μg/mL Pso-Exos and inhibitor, respectively, for 7 days	Overall, psoralen promoted osteogenic differentiation in hPDLSCs by suppressing hsa-miR-125b-5p gene expression; this effect was replicated in exosomes. The exosome group Increased ALP, Runx2, and OCN expression by over 10 fold

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Periodontal regeneration—in vivo			
Mohammed et al. (2018)[103]	Mouse ADSC-Exos	Rat ligature-induced periodontitis 4/0 nonresorbable sterile silk threads were used to create a figure of eight ligature around the lower incisors for 14 days. The groups were divided as follows (1) SRP alone, (2) SRP + ADSCs and (3) SRP + ADSC exosomes. Both the cell and exosome group were injected locally into the pockets using a disposable plastic syringe The animals were evaluated at 2 days, 2 weeks, and 4 weeks	The exosome group revealed the best results in all intervals with significantly higher area % of newly formed tissues, followed by the stem cells alone (ADSCs) and, finally, SRP. At both 2 and 4 weeks, the exosome group performed much better than even the stem cell group (32% vs 23% of newly formed tissue at 4 weeks and 26.8% versus 18.2% at 2 weeks)
Chew et al. (2019)[86]	hMSC-Exos	Rat bur-induced periodontal defect Under general anesthesia, bilateral periodontal defects of size 2 × 2 × 1.5 mm ($W \times L \times D$) were created on the mesial surface of the first molar. The 3 groups are as follows: (1) Untreated, (2) treated with a collagen sponge containing 40 mg exosomes and (3) treated with control collagen sponge without exosome. Healing was evaluated at 2 and 4 weeks post-op	Exosome-treated rats had higher levels of periodontal regeneration (50%) when compared to controls. Histological findings found newly formed bone and PDL. Additionally, it was noted that there was an increase in cellular infiltration and proliferation of periodontal cells/tissues
Sundaram et al. (2019)[83]	Plant-Derived Exosomal Nanoparticles (from ginger) (GELN-Exos)	Mouse *P. gingivalis*-induced alveolar bone loss. The mice were divided into 4 groups: (1) control (uninfected) provided regular water, (2) *P. gingivalis* infected provided regular water, (3) *P. gingivalis* infected and provided GELNs containing water, control (uninfected) provided GELN-Exos in water, and 4) *P. gingivalis* suspended in 1 mL of 2% carboxymethylcellulose (CMC) and orally inoculated into mice at 2 day intervals over a ten-day period. Animals were euthanized 42 days after the final infection	The group of exosomes derived from plants effectively suppressed the bone loss produced by *P. gingivalis* in this live mice model. A significant decrease (3 fold) in inflammatory molecules such as IL1-beta, IL-6. and TNF-alpha was observed in the exosome group

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Wu et al. (2019)[7]	SHED-Exos	Rat bur-induced periodontal defect A bone defect of approximately $4 \times 2 \times 1.5\,mm^3$ was created at the buccal alveolar bone of the first to third mandibular molars. 100 μg of exosomes combined with 5 mg of β-TCP were used for each defect at 4°C overnight to enable complete loading of the scaffolds with exosomes. Animals were euthanized at 4 weeks	Bone regeneration was shown to be better in the exosomes/β-TCP group as compared to the β-TCP group and the control group, according to the Micro-CT research. In addition, the results of HE and Masson staining, as well as immunofluorescence staining, showed that the exosomes/β-TCP group had neovascularization and new bone growth. However the other two groups only showed limited new bone growth. BV/TV was increased by roughly 20% in the exosome + TCP group
Elashiry et al. (2020)[70]	Mice dendritic cell (DC-Exos) including subtypes of immunoregulatory (regDC EXO), which, upon purification, are loaded with TGFB1 and IL10	Mouse ligature-induced periodontitis Ligation of the upper right second molar with black silk suture to accumulate bacteria and induce inflammation induced alveolar bone loss Both intravenous and local injections of Exos into soft tissues were used. EXO were locally injected through palatal gingiva on day 3 relative to ligature placement. Animals were investigated up to 9 days post-op	When applied locally, regDC Exos exhibited a strong affinity for inflammatory areas and was absorbed in situ by DCs and T cells. Immunoregulatory cargo (TGFB1 and IL10) encased in RegDC Exos was shielded from proteolytic breakdown. Furthermore, regDC Exos inhibited recipient DC maturation and Th17 effector activation while promoting T-regulatory cell recruitment, which inhibited bone resorptive cytokines and decreased osteoclastic bone loss. This work served as the foundation for an innovative therapeutic approach and was the first to show the use of DC exosome-based therapy for a degenerative alveolar bone condition
Shen et al. (2020)[104]	hDPSC-Exos	Mice ligature-induced maxillary left second molar A 5-0 silk ligature was tied around the maxillary left second molar of the isoflurane-anesthetized mouse. The ligature was remained for 10 days to induce periodontitis. For exosome treatment, DPSC-Exo (50 μg), DPSCExo/CS or an equal volume of PBS/CS was injected locally after ligature removal. For antagomir treatment, the mixture of DPSC-Exo/CS and antagomir was injected locally after the ligature removal	In periodontitis-stricken animals, DPSC-Exo/CS induced a change in macrophage behavior, turning them anti-inflammatory instead of pro-inflammatory. It is possible that the presence of miR-1246 in DPSC-Exo is associated with this impact. Furthermore, the osteoclast numbers within the defects decreased over 5 fold in the Exo/CS group in vivo
Shi et al. (2020)[88]	Human dental follicle cells (hDFC-Exos)	Rat ligated in the right maxillary second molar a 3-0 silk thread was ligated in the rat's right maxillary second molar for 4 weeks. 4 groups were investigated (1) negative control, (2) PBS-loaded hydrogel (3) D-sEV-loaded hydrogel (4) L L-D-sEV-loaded hydrogel. Healing was evaluated after 4 and 8 weeks post-op	hDFC-Exos-loaded hydrogel applied in the treatment of periodontitis was beneficial for repairing lost alveolar bone in the early stage of treatment and to maintain the level of alveolar bone in the late stage of treatment in experimental periodontitis rats, which could partly by decreasing the expression of RANKL/OPG ratio
Wei et al. (2020)[27]	SHED-Exos	Mice—ligature induced periodontitis After removing the silk ligature, SHED (1×10^6), SHED-Exo (20 μg), or PBS were injected into the buccal and lingual sides of the first molar once per week. After 2 weeks, the mice were sacrificed	The administration of SHED-Exos was discovered to effectively reverse bone loss to a similar degree as the initial stem cells

(Continues)

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Nakao et al. (2021)[72]	hGMSC-Exos (also TNF- α-treated)	Mice—ligature-induced periodontitis model in mice 4 groups were used as follows: (1) Unligated + Placebo (PBS) group, (2) Ligated + Placebo (PBS) group, (3) Ligated + GMSC-derived exosomes (Exo-Ctrl) group, and (4) the TNF- α-preconditioned GMSC-derived exosomes (Exo-TNF) group. To induce periodontal bone loss in mice, a 5-0 silk ligature was tied around the left maxillary second molar. After ligation, either placebo or exosomes (20 μg) suspended in PBS (20 μL) were injected into the palatal gingiva of the ligated second maxillary molar using a 33-gauge needle Hamilton syringe. After 7 days, mice were euthanized, and the maxillae were removed for further analysis	Injecting exosomes originating from GMSCs locally resulted in a substantial decrease in bone loss in the periodontal area and a decrease in the number of osteoclasts that are positive for TRAP. These effects were even more pronounced when the GMSCs were pre-treated with TNF-α. It was revealed that osteoclast numbers within the defects decreased over 6 fold in the Exo group
Liu et al. (2021)[91]	Rat BMSC-Exos	Rat experimental ligature-induced periodontitis model Periodontitis model by ligaturing with 3.0 cotton threads and inoculating Porphyromonas gingivalis at the right upper jaw second molar for 4 weeks. After removal of the threads, the experimental group and control group were injected BMSC-sEV-hydrogels and PBS-hydrogels separately in the periodontal pocket once a week. Animals were sacrificed at the fourth week and eighth week	The number TRAP-positive cells and the expression ratio of OPG and RANKL in the BMSC-sEV-hydrogel group were lower than that in the PBS-hydrogel group and periodontitis group, while the expression of transforming growth factor-beta 1 (TGF-b1) and the ratio of macrophage type 2 and macrophage type 1 (M2/M1) were opposite
Zhang et al. (2021)[105]	hDPSC-Exos	Mouse experimental periodontitis model A ligature (5-0 silk) was placed around the maxillary left second molar from day 0 to day 14. After 14 days, PBS, 2D-exos, 3D-exos, NCI-3D-exos or miR1246l-3D-exos (50 μg per mouse) was injected into the palatal gingiva of the experimental mice over a period of 14 days (once every 7 days). The mice were sacrificed and analyzed 14 days after PBS or exosome treatment	3D-exos showed enhanced anti-inflammatory effects in a ligature-induced model of periodontitis by restoring the reactive T helper 17 (Th17) cell/Treg balance in inflamed periodontal tissues. Furthermore, the authors found that recovery of the Th17 cell/Treg balance in the inflamed periodontium by the local injection of 3D-exos also attenuated experimental colitis. The study provides a basis for treating inflammatory bowel disease (IBD) by restoring immune responses in the inflamed periodontium
Shimizu et al. (2022)[95]	hDPSC-Exos	Mouse experimental periodontitis model A 5-0 braided silk ligature was tied around the maxillary right second molar and the contralateral tooth was left unligated as the baseline control. Mice were divided into three groups: without treatment; treatment with physiological saline (PS); and treatment with HHH-DPC exosomes (DPC). Mice in the DPC and PS groups were treated, 0, 2, and 4 days after ligature, with 33G Hamilton micro-syringes. In these treatments, 5 μL of PS or 5 μL containing 7.5×10^8 particles HHH-DPC exosomes dissolved in PS were directly applied onto the silk ligature. Three days after the final treatment, animals were euthanized	μCT imaging demonstrated that the exosomes effectively inhibited the loss of alveolar bone in the animal model of periodontitis

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Chen et al. (2022)[31]	Mice M2-like macrophages (M2-Exos)	Mice with ligature-induce periodontitis A 5-0 silk ligature was immersed in P. gingivalis bacterial solution (1×10^9/CFU) for 1 h and then fixed to the maxillary left second molar of mice for 10 days. For exosomes treatment, 30 μL PBS or M2-Exos was injected locally when the ligature was removed on the left buccal mucosa on days 1, 4, and 7. Micro-CT and histological analysis was performed 2 weeks post-injections	Injections of exosomes into periodontal tissues showed that M2-Exos reduced alveolar bone resorption in mice with periodontitis via IL-10/IL-10R pathway
Hayashi et al. (2022)[74]	miR-1260b	Mice Ligature-induced periodontal model in mice Mice were randomly divided into the following three groups: (1) placebo (in vivo jetPEI only), (2) miR-control, and (3) miR-1260b mimic. To induce periodontal bone loss in mice, a 5-0 silk ligature was tied around the right maxillary second molar. After ligation, the placebo or miRPEI-NPs were injected into the palatal gingiva of the maxillary second molar using a 33-gauge needle	An experimental periodontal mouse model showed that increased ATF6β expression was associated with ER stress induction and that periodontal bone resorption was markedly inhibited by local delivery of miR-1260b and ATF6βsiRNA using polyethyleneimine nanoparticles (PEI-NPs)
Lei et al. (2022)[106]	hPDLC-Exos	Rat ligature-induced periodontitis. Bone defect of each rat ($L \times W \times H = 3 \times 1.5 \times 2$ mm) was constructed on the buccal alveolar bone of the right mandibular first molar by a low-speed drill. To imitate the bone loss in periodontitis, the alveolar bone and periodontal ligaments around the mesial roots, centro-buccal roots, and distal roots of the first molar were carefully removed during the surgery. One week later, silk threads were removed and the periodontitis model was established by injecting 1 μL of LPS (1 mg/mL) into the buccal mucosa and gingival sulcus of the right lower first molar with a microinjector every 3 days for 4 weeks. Defects left untreated (Untreated), defects treated with matrigel containing exosomes (Exo/matrigel) and defects treated with Matrigel without exosome (matrigel), defects treated with β-TCP containing exosomes (Exo/β-TCP) and defects treated with control β-TCP without exosome (β-TCP). Micro-CT and histological evaluation was performed 4 weeks post-op	hPDLC-Exo-treated rats resulted in more bone formation in the defect of alveolar bone. In conclusion, these results demonstrated that exosomes derived from healthy PDLSCs could rescue the osteogenesis capacity of endogenous stem cells under an inflammatory environment and promote regeneration of alveolar bone. It was reported that within a 4 week period, BV/TV was over 50% higher in the exosome group when compared to controls
Yue et al. (2022)[77]	hBMSC-Exos	Rat ligature-induced periodontitis model. Ligatures were placed around the left maxillary second molar (M2) for 3 weeks. Exosomes or saline were then injected to gingival tissues once a week	Weekly exosome injection into the gingival tissues reduced the tissue destruction and immune cell infiltration in rat ligature-induced periodontitis model. In vivo imaging revealed that DiR-labeled exosomes stayed in gingival tissues for at least a week

(Continues)

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Zhao et al. (2022)[36]	hPDLC-Exos	Rat bilateral maxillary first molar mesial root mesial alveolar bone. The alveolar bone defect of about 2 ×1 ×0.8 mm^3 was created in the mesial alveolar bone of the bilateral maxillary first molar mesial root. three groups: (1) PBS was administered to the control group; (2) a group received treatment with Gel-Alg hydrogel, and (3) a group treated with hydrogel combined with exosomes Four weeks after the surgery, the rats were euthanized via anesthetic overdose	More new bone growth was seen in alveolar periodontal bone deficiencies by the exosome group. Compared with the control and hydrogel groups, the hydrogel+exosome group had more new bone deposition when evaluated by Mason Staining
Cui et al. (2023)[98]	Mouse RAW264.7M2-macrophage-Exos (loaded with melatonin)	Rats suffering from periodontitis caused by ligation Periodontitis was induced by tying a 5-0 ligature wire in the cervical region of the maxillary bilateral first molars. GelMA hydrogel loading with 200 μLM2-exos andMel@M2-exos (or 0.9% NaCl solution as control and GelMA hydrogel groups) was injected into the palatal gingiva of the ligated molars every 2 days at the proximal, middle, and distal sites, respectively. Two weeks later, all rats were euthanized by overdose anesthesia	Exosomes produced from melatonin + M2 macrophages show promise as inflammatory periodontal tissue regeneration agents. Osteoclast numbers in the tissues were over 5 fold lower in the exosome group and BV/TV would doubled when compared to controls in the M2-Exo group
Kang et al. (2023)[107]	hPDLC-Exos (exosomal miR-205-5p)	Rat—LPS-induced periodontitis Rats were randomly divided into 3 groups, including the control, LPS, and LPS + ExomiR-205-5p groups. In the LPS group, 10 μL *Escherichia coli* LPS was injected into the gingival sulcus between the first and second molars every 2 days. Rats in the LPS + Exo-miR-205-5p group were injected with 10 μg Exo-miR-205-5p (exosomes isolated from miR-205-5p mimics-transfected PDLSCs) following consistent LPS treatment every 2 days. In addition, rats were injected with the same amount of normal saline in the control group. Histological evaluation was performed 4 weeks post-op	In chronic periodontitis, exosomal microRNA-205-5p from periodontal ligament stem cells (PDLSCs) targets XBP1 to reduce inflammation and reestablish a balance between T helper 17 (Th17) and regulatory effector 1 (Treg) cells. Inflammatory cytokines such as IL-1beta, TNF-alpha, IL-6 were all significantly downregulated in the exosome group by ~3 fold
Lai et al. (2023)[39]	Rat BMSC-Exos	Mice ligature-induced periodontal disease around the maxillary second molar. After 3 days of induction, 10 μL of exosome suspension was injected into the palatal gingiva. The injection was repeated every 3 days until the eleventh day	The exosome group demonstrated a suppressive effect on bone loss induced by periodontitis in live organisms. The bone volume around the ligature-induced periodontal defect was increased by 50% compared to controls
Luo et al. (2023)[79]	Human embryo kidney tissue 293T/17 cell-Exos	Rat periodontitis model SD rats were randomly divided into four groups: (1) CXCR4-miR126-Exo; (2) miR126-Exo; (3) Ctrl-Exo; and (4) Blank Ctrl. A 1 mm metal ligature was wrapped around the neck of the bilateral maxillary second molars for 4 weeks to establish chronic periodontitis. After 2 weeks, the rats were euthanized to harvest their maxillae and gingiva tissues	Exosome injections into periodontal tissues successfully slowed the development of periodontitis and decreased osteoclastogenesis and bone resorption. These findings provide fresh perspectives on the development of innovative immunomodulatory factor-targeted delivery methods for the treatment of biofilm-related illnesses, including periodontitis

TABLE 11 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Qiao et al. (2023)[80]	hDPSC-Exos	Rats with experimental periodontitis A stainless-steel wire with a diameter of 0.20 mm was placed around the bilateral maxillary first molars. LPS from p.g. was injected at a concentration of 10 μg/mL into the periodontal pocket every other day (5 times total). The Exosomes (50 μg/mL) were injected into the periodontal pocket every other day into the buccal mesial, buccal distal, palatal proximal, and palatal distal regions of the first molars (approximately 10 μL per site). Investigation was done 30 days post-op	DPSC-Exos have the potential to mitigate alveolar bone loss and facilitate periodontal epithelium repair. In the present model, nearly double the BV/TV was observed in the exosome group with a significant decrease (over double) in the number of labeled TRAP-positive cells

Abbreviations: 3.(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide, MTT 3-; ADSC, Adipose derived stem cell; AKT, set of three serine/PDLSCs, or periodontal ligament stem cells; AKT, set of three serine/threonine-specific protein kinases; ALP, Alkaline phosphatase; ATF, Activating transcription factor 6; BMP2, Bone morphogenetic Protein-2, whereas RUNX2 stands for Runt-related transcription factor 2; BMSCs, hBMSCs, Bone Marrow Mesenchymal Stem Cells; CCK-8, Cell Counting Kit 8; CS, chitosan hydrogel; DC, Dendritic Cell; DE-miRNAs, differentially expressed miRNAs; DFC, Dental Follicle Cells; DPSCs, Dental Pulp Stem Cells; D-sEV, DFC-derived sEV, and cell-derived sEV, human exfoliated deciduous teeth stem cells; ER, Endoplasmic Reticulum; ERK, or extracellular signal-regulated kinases; human PDLSc; EV, Extracellular Vesicles; Exo, Exosome; GMSC, gingival mesenchymal stem cells; hDFC, human DFC; hDPSCs, human DPSCS; HE, Hematoxylin and Eosin; hGMSC, human GMSC; hMSC, human MSC; hPDLSC, CD73, or cell surface ecto-5′-nucleotidase; I.V., Intravenous; IBD, Inflammatory Bowel Disease; IL, Interleukin; JAK2, Janus kinase 2; KEAP1, Kelch-like ECH-associated protein 1; L-D-sEV, LPS-preconditioned dental follicle SHED; LPS, Lipopolysaccharide; micro-CT, micro-computed tomography; miR, microRNA; MLO-Y4, Murine Osteocyte-like Cell Line; mRNA, Messenger RNA; MSC, mesenchymal stem cells; NF-kB, Nuclear factor kappa-B; NRF2, nuclear factor erythroid 2; OCN, Osteocalcin; OPN, Osteopontin; *P. gingivalis*, *Porphyromonas Gingivalis*; P.g-LPS, *Porphyromonas gingivalis*—LPS; PCR, Polymerase Chain Reaction; PDL, Periodontal Ligament; Pso-Edos, Exos treated with psoralen; PTEN, Phosphatase and TENsin homolog; qRT-PCR, Quantitative real-time reverse-transcription PCR; RANKL, or nuclear factor kappa beta ligand receptor activator; RegDC, immuneregulatory DC; RNA, Ribonucleic acid; SHED, Human Exfoliated Deciduous Tooth Stem Cells; SMAD, Suppresor of Mothers against Decapentalegic; SRP, Scaling and Root Planing; STAT3, signal transducer and transcription activator; TGFB1, Transforming Growth Factor-B1; THP-1, human leukemia monocytic cell; TNF-α, Tumor necrosis factor-α; TRAP, Translating Ribosome Affinity Purification; WNT, Wingless-related integration site; XBP-1, X-box-binding protein-1; β-TCP, β-Tricalcium phosphate.

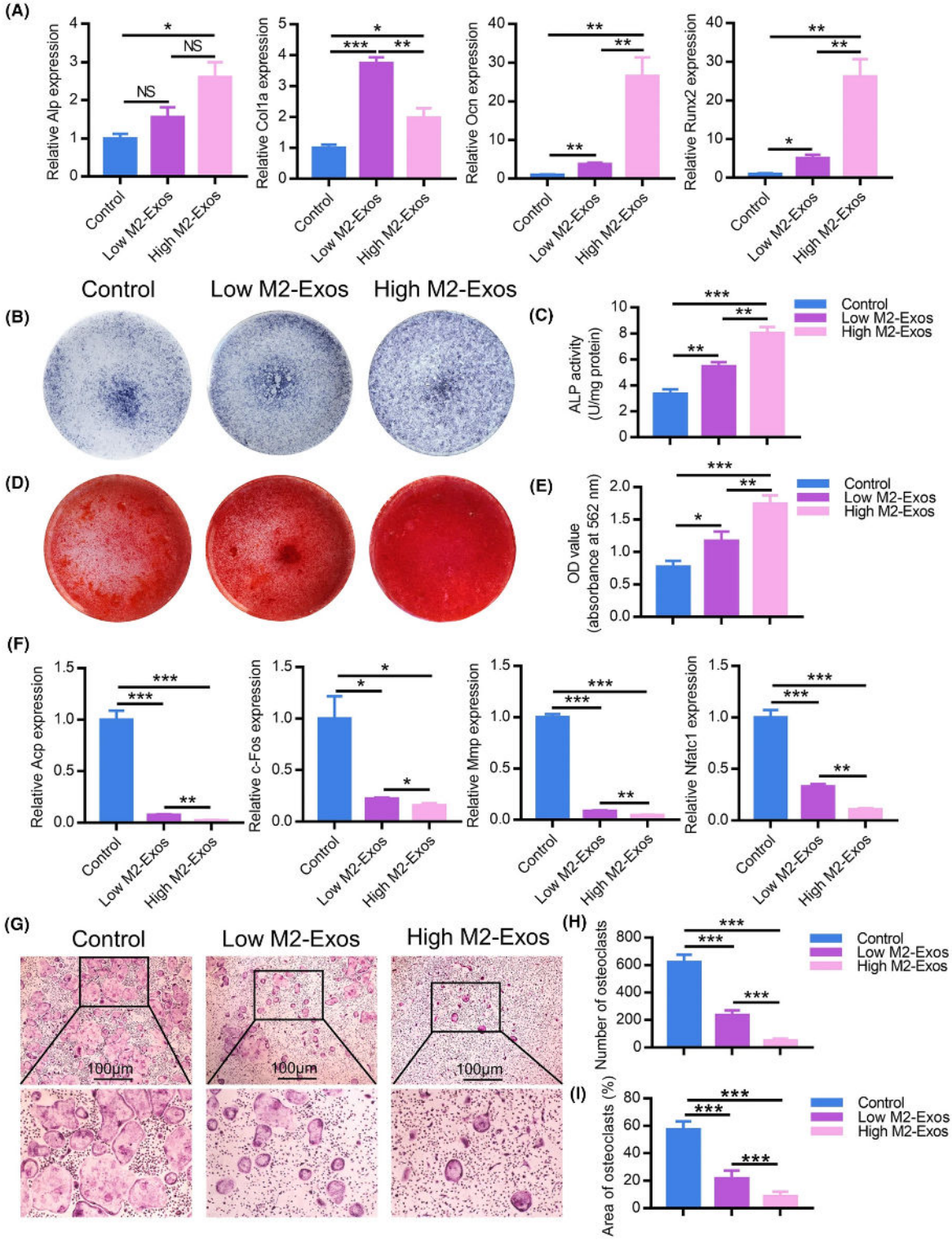

FIGURE 10 Through IL-10/IL-10R, M2-Exos stimulate osteogenesis and decrease osteoclastogenesis. (A) An RT-PCR study identified osteogenesis-related genes in BMSCs treated with PBS, M2-Exos, or anti-IL-10R antibody. (B) After 14 days of PBS, M2-Exos, or M2-Exos with anti-IL-10R antibody, BMSCs stained with ALP. (C) ALP test kit measured ALP activity. (D, E) BMSC alizarin red-mediated calcium staining after 21 days of PBS, M2-Exos, or M2-Exos plus anti-IL-10R antibody. The spectrophotometer measured alizarin red coloring extracted by cetylpyridinium chloride. (F) RT-PCR analysis assessed osteoclastogenesis-related genes in BMDM-induced osteoclasts treated with PBS, M2-Exos, or anti-IL-10R antibody. (G) BMDM-induced osteoclasts were treated with PBS, M2-Exos, or anti-IL-10R antibody for 7 days and stained with TRAP. The number (H) and area (I) of TRAP-positive cells were calculated. Data are mean Å} SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Reprinted with permission from Chen et al.[31]

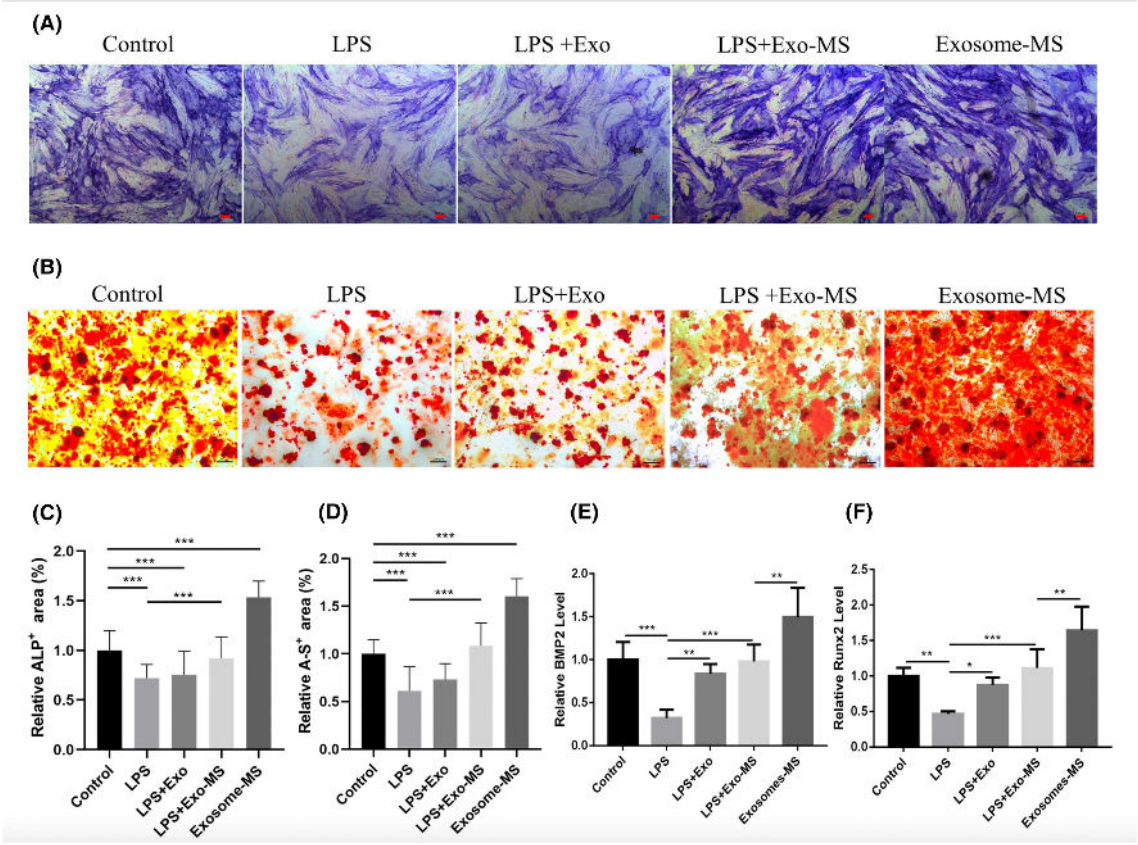

FIGURE 11 MSC-Exos effects on HPDLSC osteogenic differentiation. Alizarin red and ALP were used to evaluate HPDLSC osteogenic differentiation in different treatment groups. (A) ALP staining (scale bar 100 μm). (B) Alizarin red staining (scale bar: 200 μm). (C) ImageJ computed relative ALP-positive area. (D) ImageJ-calculated relative Alizarin red-positive area. (E, F) BMP2 and Runx2 levels were measured using real-time PCR in separate groups (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). Reprinted with permission from Lv et al.[87]

the negative effects of *P.gingivalis* in periodontal disease. Another study by Leiva-Sabadini et al.[84] found that honey-derived exosomes contained a number of antibacterial peptides as cargo molecules. Few studies have demonstrated the effects of exosomes on decreased periodontal pathogen counts, but future research may be needed in this field.

2.10 | Orthodontic tooth movement

Only 2 in vivo studies have investigated the role of exosomes in tooth movement and tooth resorption caused by tooth movement (Table 10). In the study by Zhao et al.,[47] the mouse RAW264.7 monocyte cell line was investigated, including their M0, M1 and M2 phenotypes. By isolating M0/M1/M2 macrophage exosomes and analyzing their impact on cementoblast mineralization, it was found that the effects of macrophages on cementoblast mineralization were at least partially exerted by exosomes.[47] A study by Liu et al.[85] found that encapsulating simvastatin into exosomes derived from PDLSCs improved simvastatin solubility and enhanced the inhibitory effect of relapse in a rat model of orthodontic tooth movement (OTM). Notably, local injection of PDLC-Exos alone could also block relapse after OTM.[85]

2.11 | Periodontal regeneration

In total, 34 studies investigated periodontal regeneration preclinically, of which 21 studies investigated the therapeutic potential of exosomes in vitro, while 22 of the studies used in vivo data (9 of the studies used both in vitro and in vivo investigations). A total of 11 differential exosome sources were isolated from the in vitro studies as follows: 4 studies investigated PDLC-Exos, 3 studies isolated GMSC-Exos, 2 studies investigated DPSC-Exos, 2 studies investigated BMSC-Exos, 2 studies isolated macrophage-Exos, 2 studies investigated SHED-Exos, and one study each investigated MSC-Exos, osteocyte-Exos, dental follicle-Exos, GMSC-Exos, umbilical cord-Exos and laboratory-derived miR-1260b (Table 11). The majority of studies isolated exosomes from humans (80% of studies), whereas 2 studies isolated exosomes from mice, 1 study isolated exosomes from rats, and 1 study isolated exosomes made in a laboratory (Table 11). The target cell line was most often human periodontal

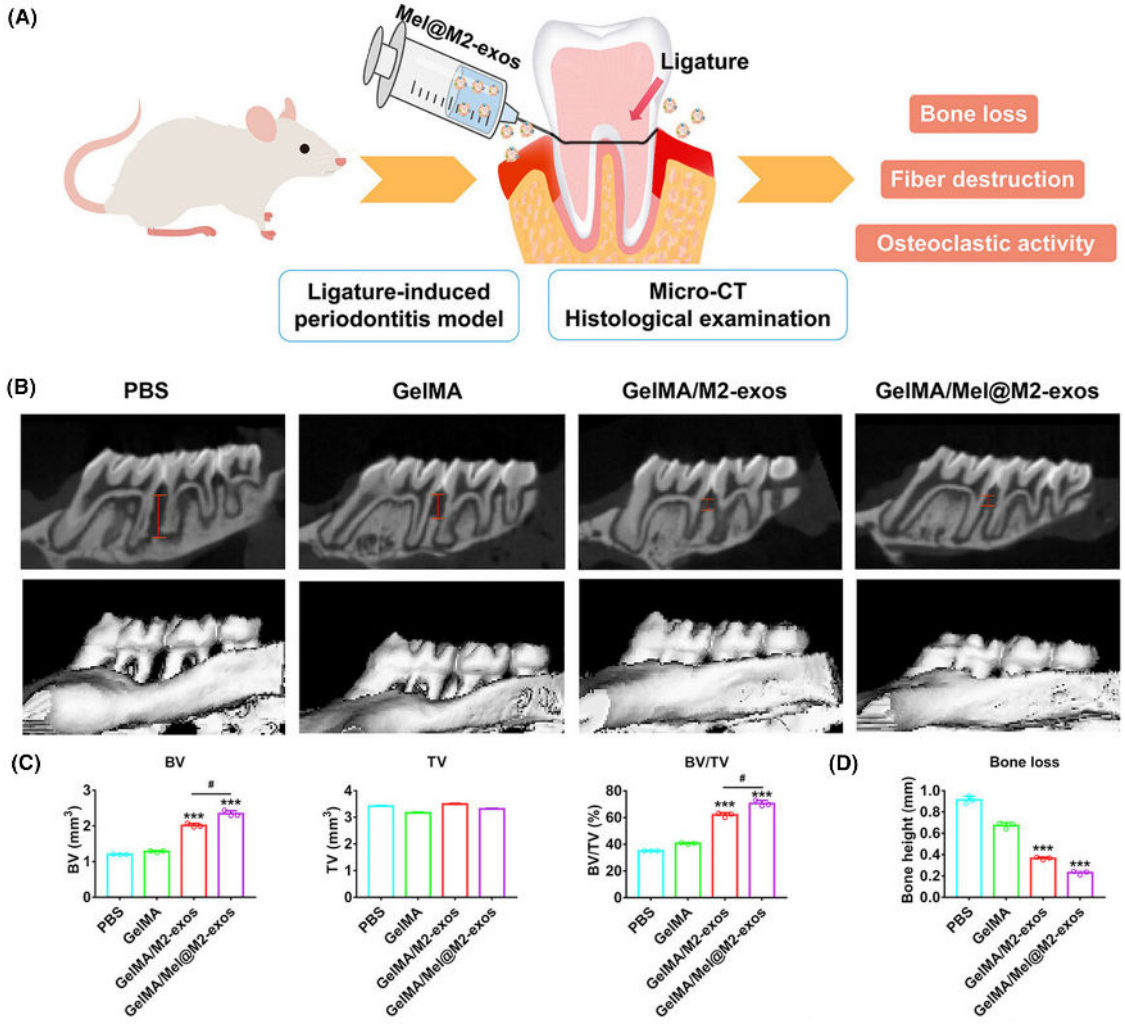

FIGURE 12 In rats with periodontitis brought on by ligatures, Mel-M2-expos reduced bone loss. (A) Diagrammatic representation of the rat experiment on periodontitis. (B) Micro-CT analysis of the maxillary first and second molars' 3D reconstructed digital pictures and mesiodistal section images. (C) Bone-related parameters (BV, TV, and BV/TV). (D) Bone loss data. The results are shown as mean SD for $n=3$. One-way ANOVA calculated data significance; $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ indicate statistically significant differences with the phosphate buffer saline (PBS) group. Reprinted with permission from Cui et al.[98]

ligament cells (19/20 studies), and one study investigated mouse preosteoblasts. Overall, exosomes derived mainly from human sources were found to have no cytotoxicity on PDLCs and to be able to stimulate osteogenesis, migration, and proliferation of cells with deep Alizarin red staining, high activity of ALP, and upregulated expression of osteogenic genes (RUNX2, OPN, and OCN). (Figure 10). Figure 11 demonstrates that exosomes are even capable of improving PDL cell differentiation under LPS-induced inflammatory conditions. Additional information was uncovered on the activation of BMP/Smad signaling and Wnt/β-catenin via higher phosphorylation of Smad1/5/8 and increased nuclear levels. β-catenin protein expression.[89]

A total of 13 differential exosome sources were isolated for the in vivo studies as follows: 4 studies investigated DPSC-Exos, 3 studies isolated PDLCs-Exos, 3 studies isolated BMSC-Exos, 2 studies isolated macrophage-Exos, 2 studies isolated SHED-Exos, and one study each isolated ADMSC-Exos, MSC-Exos, plant-based-Exos, dendritic cell-Exos, dental follicle-Exos, GMSC-Exos, embryo kidney-Exos and laboratory-derived miR-1260b (Table 11). In more than half of the studies (59% or 13/22 studies), exosomes were isolated from humans, whereas exosomes were isolated from rats in 4 studies, mice in 2 studies, plants in one study, and laboratory-derived exosomes in 1 study. All in vivo studies examined periodontitis models that were either ligature-induced, burr-induced, or LPS-induced. In total, 12 studies were conducted in rats, and 10 were conducted in mice. All studies found that the use of exosomes led to positive effects in vivo. Interestingly, several studies found that exosomes were capable of improving periodontitis in various animal models even via injection into the gum tissue surrounding the defects, as well as improving outcomes by a simple intravenous administration of the exosomes (as reviewed in Part II of this III part series; exosomes do possess an ability to target inflammation) (Figures 12 and

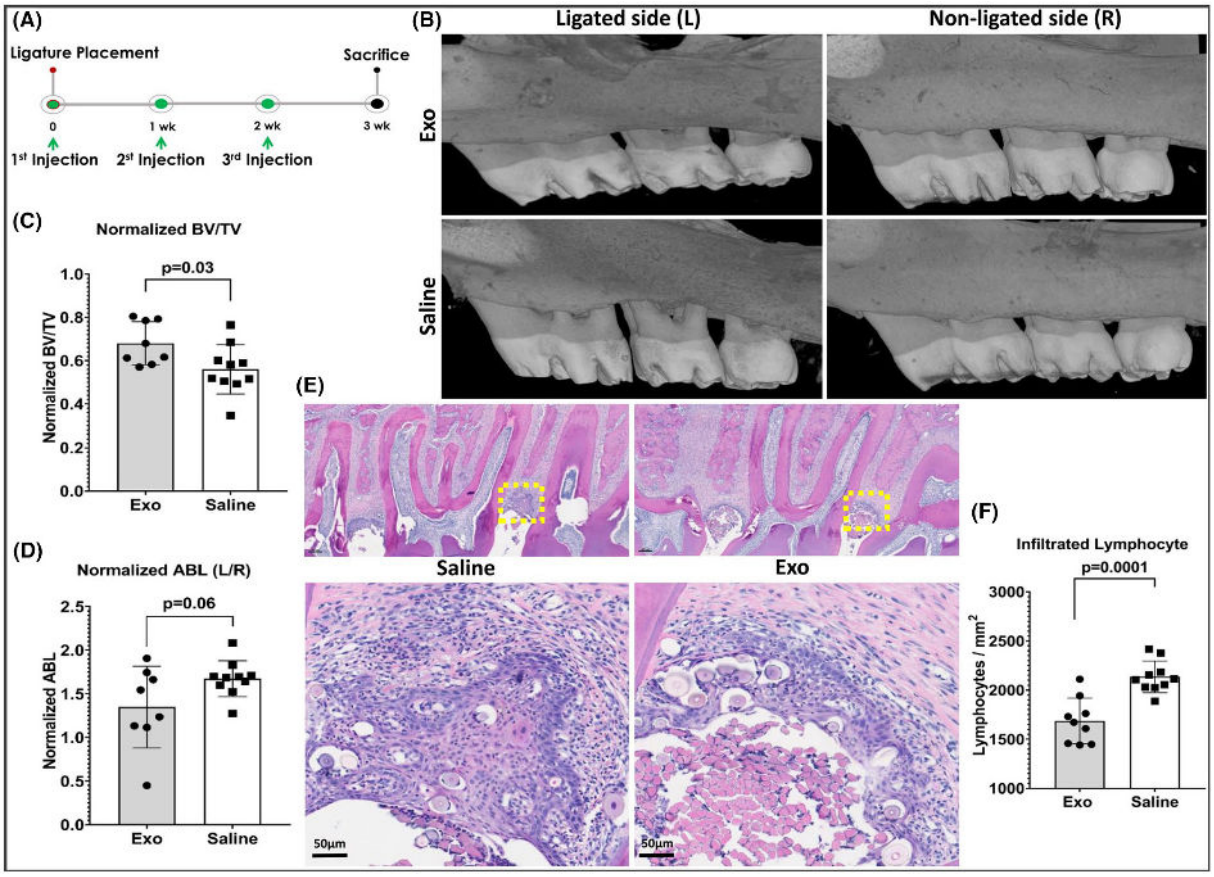

FIGURE 13 In vivo therapy with exosomes reduced periodontal bone loss. (A) Experiment timeline. Ligatures were placed around the left maxillary second molar (M2) for 3 week. Every week, gingival tissues were injected with either saline or exosomes. (B) Sample micro-computed tomography pictures of the maxillae, both ligated and unligated. (C) Normalized bone volume/tissue volume (BV/TV) comparisons between the treated (left) and healthy control (right) sides. (D) Left treatment-to-healthy-control ratio of normalized linear alveolar bone loss (ABL). (E) Hematoxylin–eosin. Upper panels: low magnification (200 μm scale bar). The lower panels show great magnification of the yellow boxes (scale bars: 50 μm). (F) Quantitative assessment of interproximal gingival lymphocyte infiltration. Reprinted with permission from Yue et al.[77]

13). Figure 14 demonstrates the added benefits in terms of defect filling when exosomes were added to a bone grafting material. A study by Elashiry et al.[70] found that one of the benefits of exosomes is that they are resistant to degradation by periodontal pathogens found routinely in a periodontitis environment.

Figure 15 overviews and highlights all the reported benefits to date of exosomes during periodontal healing/regeneration. Interestingly, two studies, one specifically by Mohammed et al.[103] found that the exosome group revealed better results in all intervals with a significantly higher area % of newly formed tissues when compared to their parent stem cells (Figure 16). Thus, when comparing exosomes to native parent stem cells, exosomes show either similar or better outcomes for the treatment and management of periodontitis.

2.12 | TMJ disorders

Four studies investigated the use of exosomes on TMJ healing (3 in vitro and 2 in vivo with 1 overlap). Regarding the in vitro studies, 1 study utilized SHED-Exos, 1 study utilized human embryonic MSC-Exos, and 1 study utilized rat condylar chondrocyte exosomes under resting and tensile stress (Table 12). All in vitro studies utilized condylar chondrosomes as their target tissue (2 rat studies and 1 human study). Luo et al.[108] found that administration of SHED-Exos resulted in the inhibition of the production of interleukin-6 (IL-6), IL-8, matrix metalloproteinase 1 (MMP1), MMP3, MMP9, MMP13, and disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) in chondrocytes. Zhang et al.[109] found that MSC-Exos enhanced s-GAG synthesis impeded by IL-1β and suppressed IL-1β-induced nitric oxide and MMP13 production. Shi et al.[110] found that Runx2 and Sox9 expression levels were considerably greater in the Force-Exo group, suggesting that exosomes produced in cells cultured under tensile stress may find use in the treatment of OA. Exosomes have been shown in all trials to reduce inflammation in TMJ chondrocytes, suggesting that they might be a potential therapeutic agent for TMJ inflammation.

Only 2 studies investigated TMJ disorders in animals. A study by Zhang et al.[109] utilized human embryonic MSC-Exos in rat TMJ-OA

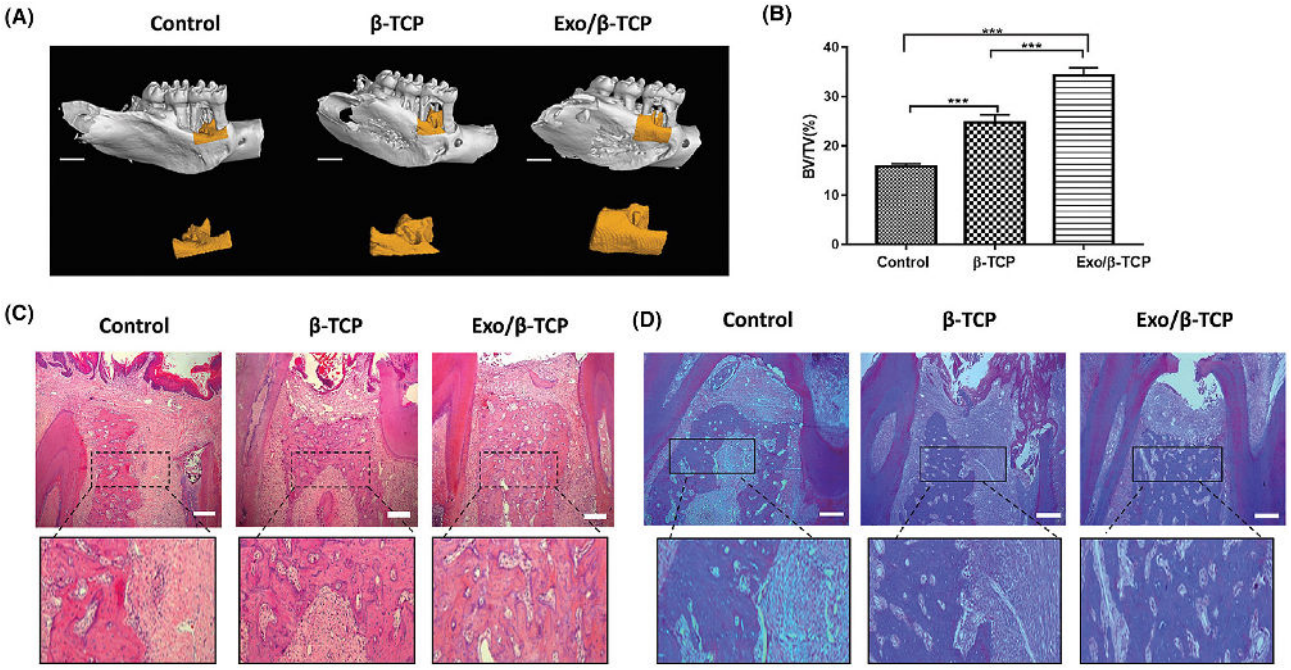

FIGURE 14 When periodontitis patients get treatment with hPDLSC-Exos and bone-inductive materials, the alveolar bone begins to regenerate. The periodontal bone abnormalities of rats with periodontitis were treated with a combination of A-TCP and β-TCP, together with h-PDLSCs exosomes (Exo/β-TCP). The control group consisted of the rats that did not undergo transplantation. Bones from the jaw were removed and studied. Four weeks subsequent to transplantation. (A) An example of fresh bone growth captured by microCT reconstruction pictures; (scale bar: 2 mm) (B) quantitative examination of images from microCT reconstruction; (C, D) Analysis of the freshly produced bone tissues using histomorphometrics. Hematoxylin and eosin (H&E) staining representative (C) and Masson staining (D) indicated the formation of new bone (Scale bar: 20 μm) (ns: no significance, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $n=3$). Reprinted with permission from Lei et al.[106]

induced by MIA injections. Exosome-mediated repair of osteoarthritic TMJs was shown to be typified by early suppression of pain and degeneration with decreased inflammation, which was followed by sustained proliferation and gradual improvements in subchondral bone architecture and matrix expression, which ultimately resulted in total joint restoration and regeneration.[109] A study by Liu et al. utilized human adipose-derived MSCs in rabbit TMJ condylar osteochondral defects and found that both inflammatory and normal Exos were able to enhance condylar regeneration and lower inflammation.[76]

Figure 17 depicts the ways in which exosomes are able to support the regeneration of the TMJ. Figures 18–20 demonstrate the profound regeneration capabilities of the joint space in various animal models where up to 4 times higher bone volume/total volume has been observed.

2.13 | Others

In total, 12 studies investigated dental-derived exosomes on "other" target tissues. In total, 5 in vitro studies and 12 animal studies were reported (with all 5 in vitro studies also having an in vivo component). Human oral mucosal epithelial cell-Exos were utilized in one study, whereas all other studies used hDPSCs. All studies derived exosomes from humans. In the in vivo portion, 8 studies used hPDSC-Exos, while 1 study used each of the following: SHED-Exos, oral epithelial mucosal-Exos, GMSC-Exos and PDLC-Exos. All exosomes were derived from humans (Table 13).

Zhang et al.[117] investigated GMSC-Exo constructions and discovered that they promoted taste bud regeneration and reinnervation, showing promise for use in patients with tongue cancer undergoing postoperative reconstruction of their tongues. Moreover, their findings demonstrate that GMSCs or their derived exosomes enhanced the innervation of taste buds that had undergone regeneration, as seen by increased neurofilament and P2X3 expression at the sites of damage. At every time point examined, exosomes dramatically decreased carrageenan-induced oedema (by 39.5, 41.6, and 25.6% at 6, 24, and 48 h after injection, respectively) to similar levels as the positive control (prednisolone).[116] Exosomes were introduced into deep skin wounds in rats and significantly reduced the wound size (Figure 21).[15,111] Shi et al. found that hDPSCs could enhance flap repair and revascularization and improve the survival and microvessel density of the flap, suppressing epithelial cell apoptosis (Figure 22).[17]

Two studies utilized exosomes derived from dental sources (hDPSCs) and found that they could substantially improve knee joint conditions in a rat model of OA.[112] Additionally, the findings demonstrated that exosomes produced from DPSCs efficiently restored aberrant subchondral bone remodeling, prevented osteophytes and

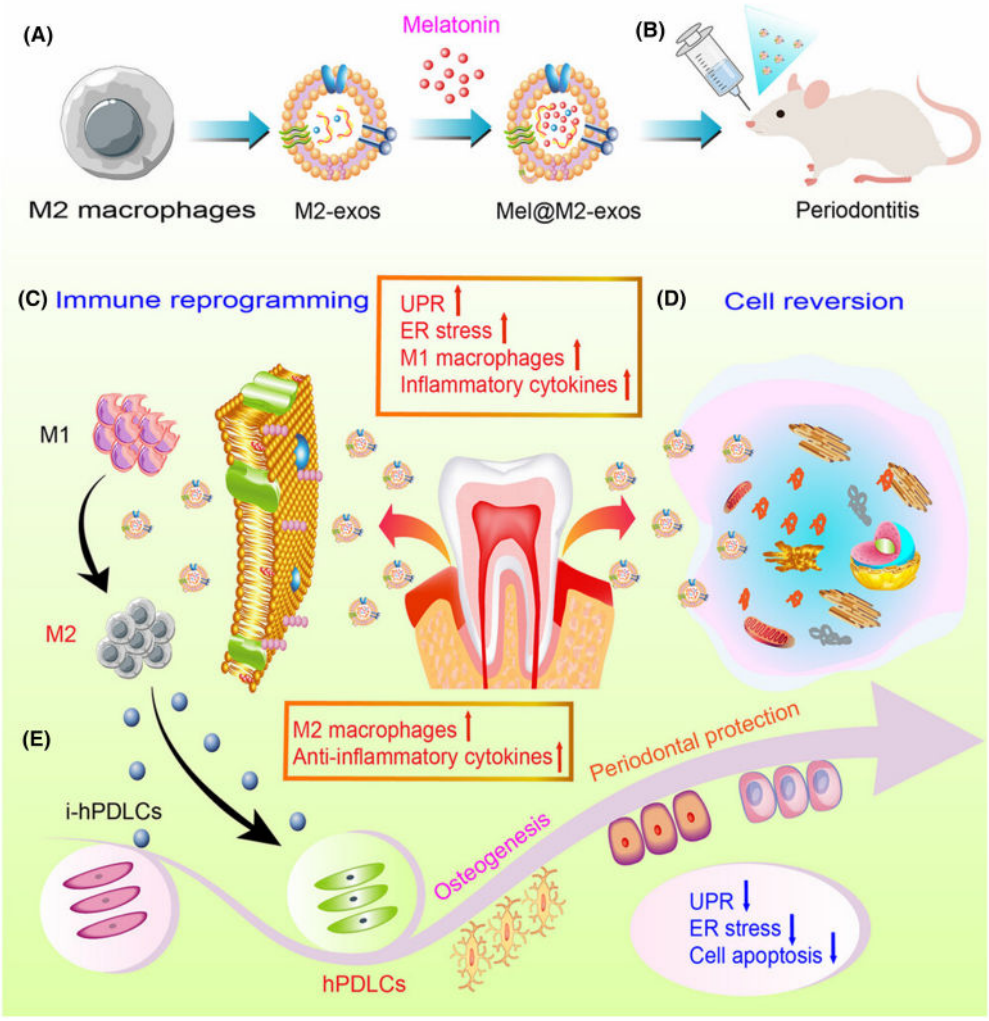

FIGURE 15 Illustration of modified exosomes produced from M2 macrophages used to treat periodontitis-related inflammatory bone loss by modulating endoplasmic reticulum stress and immunological reprogramming. (A) Making exosomes treated with melatonin using M2 macrophages as a source. (B) Mel@M2-exos was investigated in rats suffering from ligation-induced periodontitis for the purpose of treating inflammatory bone loss. (C) Mel@M2-exos triggers a repolarization of macrophages and immunological reprogramming. (D) Cellular states are reversed and the endoplasmic reticulum stress is mediated by Mel@M2-exos. (E) Mel@M2-exos revive hPDLCs' deficit performance in inflammatory settings. Reprinted with permission from Cui et al.[98]

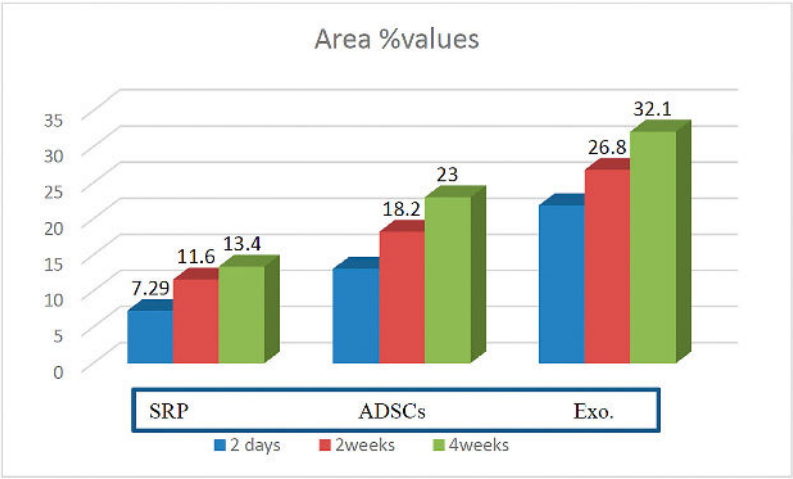

FIGURE 16 Bar graph demonstrating area percent values of newly regenerated tissue when comparing an animal model that received (1) SRP, (2) stem cells in the form of ADSCs, and (3) the derived exosomes from those ADSCs. Noteworthy, the exosome group performed superior than even the parent cells. Reprinted with permission from Mohammed et al.[103]

TABLE 12 The therapeutic regeneration potential of several exosomes produced from numerous sources on diseases of the TMJ in vitro and in vivo.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
TMJs—in vitro			
Luo et al. (2019)[108]	SHEDs	Human chondrocytes isolated from human condyles from patients with condylar fracture. All centrifugation steps were performed at 4°C. First, to eliminate dead cells and large cell debris, culture supernatants collected from SHEDs were centrifuged successively at increasing speeds (300 ***g*** for 10 min, 2000 ***g*** for 10 min, and 20 000 ***g*** for 30 min). The resultant supernatants were ultracentrifuged at 100 000 ***g*** for 70 min in an ultracentrifuge. Finally, the pellets were washed with 10 mL of PBS and centrifuged one last time at 100 000 ***g*** for 70 min to eliminate the contaminating proteins	Treatment with SHED-Exos suppressed the expression of interleukin-6 (IL-6), IL-8, matrix metalloproteinase 1 (MMP1), MMP3, MMP9, MMP13, and disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) in chondrocytes. This study demonstrated that SHED-Exos suppresses inflammation in TMJ chondrocytes and may thus be a novel therapeutic agent for TMJ pain and inflammation
Zhang et al. (2019)[109]	Human embryonic MSC-Exos	Rat condylar chondrocytes For exosome preparation, the cells were grown in a chemically defined medium for 3 days and exosomes were purified from the CM. The CM was size fractionated and concentrated 50× by TFF using a membrane with a molecular weight cut-off (MWCO) of 100 kDa (Sartorius, Gottingen, Germany), and assayed for protein concentration using a NanoOrange Protein Quantification Kit (Thermofisher Scientific)	Several physiological processes connected to exosome-mediated joint healing have been associated with adenosine-mediated activation of AKT, ERK, and AMPK signallng. Specifically, MSC exosomes enhanced the production of s-GAG, which IL-1β had previously suppressed, and decreased the production of nitric oxide and MMP13, which IL-1β had previously stimulated
Shi et al. (2022)[110]	Rat condylar chondrocytes exosomes under resting and tensile stress	Rat condylar Chondrocytes The collected medium was centrifuged in an ultra-high-speed refrigerated centrifuge at 300 ***g*** and 4°C for 10 min; the precipitated live cells were discarded to retain the upper liquid; this liquid was centrifuged at 2000 ***g*** and 4°C for 10 min and the precipitated dead cells were discarded to retain the upper liquid; this liquid was then centrifuged at 10 000 ***g*** and 4°C for 30 min and the precipitated tissue fragments were discarded to retain the upper liquid; the exosome precipitate was obtained after centrifugation at 100 000 ***g*** for 70 min and 4°C, resuspended, and washed in PBS for total protein extraction or addition to the cell culture medium	When comparing the Force-Exo group to the Control-Exo group, there was a statistically significant difference in the amounts of Runx2 and Sox9 protein expression. These results suggest that stressing rat condylar chondrocytes with tensile force may induce osteogenic differentiation by altering the expression of certain microRNAs in their exosomes. Consequently, exosomes that have been cultivated under tensile stress might potentially be used to treat osteoarthritis (OA)

TABLE 12 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
TMJs—in vivo			
Zhang et al. (2019)[109]	Human embryonic MSC-Exos	Sprague–Dawley (SD) female rats underwent TMJ-OA, which was induced by MIA injection. In this model, MIA induces sustained inflammation with increased expression of pro-inflammatory cytokines to result in OA-like pain and degenerative changes. Briefly, 0.5 mg of MIA dissolved in 50 μL of PBS was injected using a 30 gauge needle into the upper compartment of the bilateral TMJs in 32 rats in OA + PBS and OA + Exosome groups. Two weeks after OA induction, the rats received weekly intra-articular injections of either 100 μg of exosomes in 50 μL of PBS for the OA + exosome group or 50 μL of PBS for the OA + PBS group for 2, 4, or 8 weeks	Early pain and degeneration with decreased inflammation, sustained proliferation, and progressive improvements in matrix expression and subchondral bone architecture were the hallmarks of exosome-mediated repair of osteoarthritic TMJs, which resulted in total joint regeneration and restoration
Liu et al. (2022a)[76]	Human inflammation-stimulated sEV (IA-Exos) and normal sEV (A-Exos) generated from adipose-derived MSC (ADSC)	Rabbit condylar osteochondral defect model of the TMJ An osteochondral defect (diameter = 2 mm, depth = 2 mm) was made on the condyle. Scaffolds were implanted into the defects, and four groups were included in the experiment: nonimplanted group (blank), implanted scaffold only (scaffold), implanted scaffold with 5 μg of AE (AE), and implanted scaffold with 5 μg of IAE (IAE). Animals were euthanized at 4 and 8 weeks post-op	TMJ regeneration was aided by both A-Exos and IA-Exos, although IA-Exos had the greater therapeutic impact. Consequently, the authors attest that it is feasible to improve exosome functionalities by subjecting MSCs to an inflammatory milieu and that altered exosomes have superior therapeutic benefits. A 3–4 fold increase in collagen type 1 and 2 were observed particularly increased in the IAE-Exo group when compared to scaffolds alone

Abbreviations: ADAM, Separation and Metalloproteinase A; ADAMTS5, ADAM metallopeptidase with thrombospondin type 1 motif 5; ADSC, Adipose-derived stem cells; A-Exos, normal ADSC derived sEV; AMPK, AMP-activated protein kinase; ERK, Extracellular Signal-regulated Kinases; EV, Extracellular Vesicles; Exo, Exosome; IA-Exos, Inflammation stimulated ADSC derived sEV; IL, Interleukin; MIA, Monoiodoacetate; miRNA, microRNA; MMP, matrix metalloproteinase; MSC, Mesenchymal Stem Cells; OA, Osteoarthritis; RUNX2, Y-box 9's sex-determining region; SD, Sprague Dawley; s-GAGS, Sulfated glycosaminoglycans; SHED, Human Exfoliated Deciduous Tooth Stem Cells; SOX-9, runt-related transcription factor 2; The trio of serine/threonine-specific protein kinases known as AKT; TMJ, Temporomandibular joint.

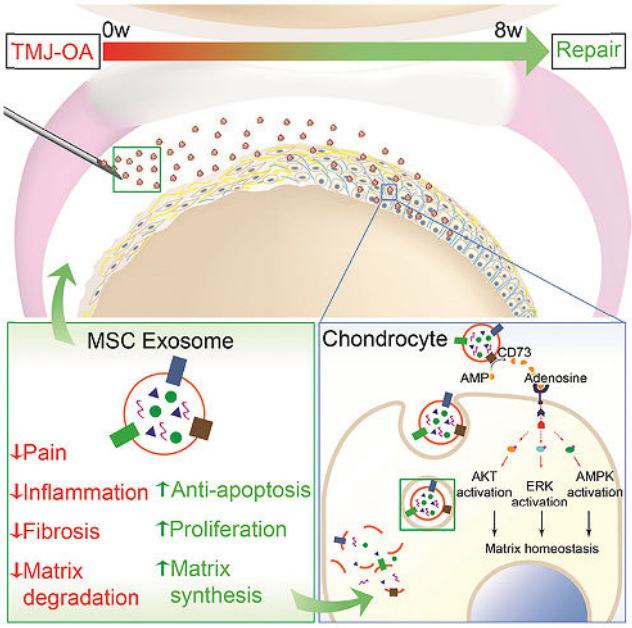

FIGURE 17 Possible MSC exosome pathways for TMJ regeneration and repair in OA. Reprinted with permission from Zhang et al.[109]

bone sclerosis, and reduced cartilage deterioration and synovial inflammation in vivo (Figure 23).[114]

An interesting study by Li et al.[113] used hDPSCs in a mouse stroke model that was exposed to temporary middle cerebral artery blockage for 2 h. They discovered that cerebral oedema was reduced by a single tail vein injection of hDPSC-Exos, cerebral infarction and neurological impairment in I/R mice.[113] Liu et al.[75] further found that hDPSCs could also stabilize spinal cord injury by inhibiting M1 macrophage polarization.

2.14 | Diagnostics

Interestingly, a number of studies have now pointed to not only the therapeutic uses of exosomes across many fields of medicine but entire research labs have been dedicated and focused on utilizing exosomes as small biomarkers for the detection of complex disorders, diseases and cancers.[166,167] These strategies have been discussed since cells communicate over longer distances via exosomes and remain more stable in body fluids. Although much research has mostly employed human blood and urine samples, it has more recently been shown that exosomes are found in high numbers in saliva as well as crevicular fluids, and many authors have reported specific diagnostic markers from either of these oral sources for the early diagnosis of various disorders (Table 14). In total, 47 studies have examined this topic, including the following 16 areas of focus: aging (1 study), birth defects (1 study), cardiovascular disease (1 study), diabetes (2 studies), gingival recession

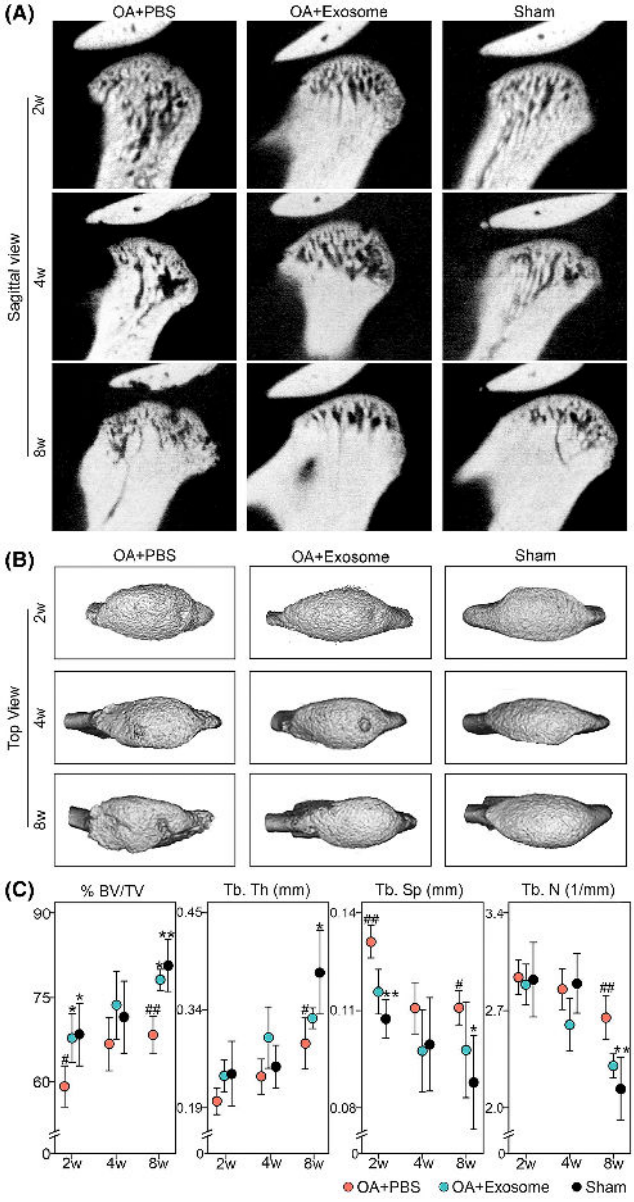

FIGURE 18 Subchondral bone volume and architecture are restored in OA by MSC-Exos. At 2, 4, and 8 weeks, rat tail metatarsal glands were collected for micro-CT imaging. (A) The condyles in sagittal (B) and top views, respectively. Representative images ($n = 6–8$) (C) Ratios of and trabecular number (Tb·N), trabecular separation (Tb. Sp), trabecular thickness (Tb·Th), bone volume (BV/TV). Data represent mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to OA + PBS group; $\#p < 0.05$, $\#\#p < 0.01$ compared to sham group. n = 6–8/group. Reprinted with permission from Zhang et al.[109]

detection (1 study), gingivitis (1 study), irritable bowel syndrome (1 study), neurodegenerative disease (1 study), oral lichen planus (1 study), oral squamous cell carcinoma (12 studies), oropharyngeal cancer detection (1 study), orthodontic root resorption (5 studies), pancreatic cancer (2 studies), periodontitis (14 studies), peri-implantitis (1 study), Sjogren syndrome (1 study) and systemic

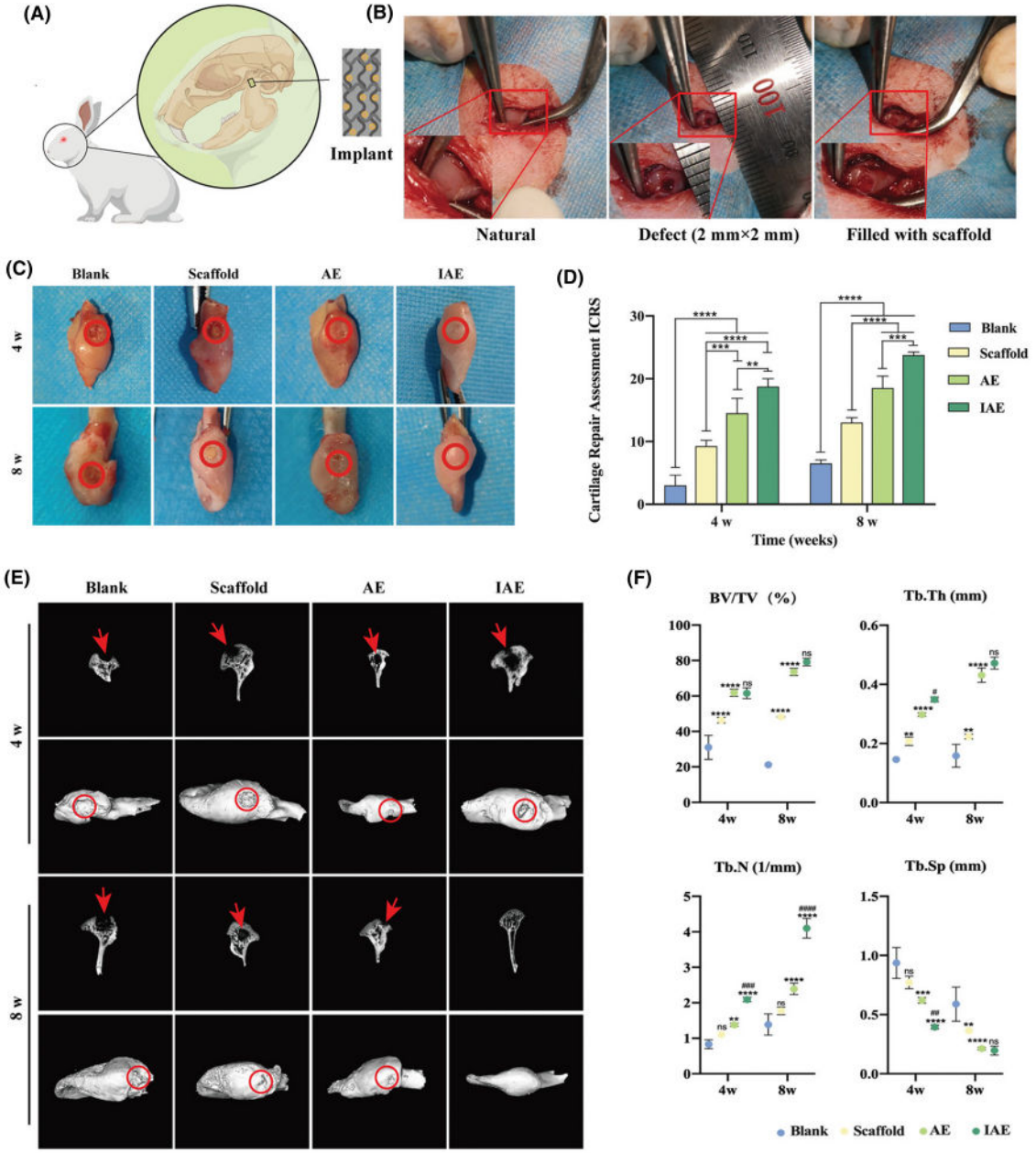

FIGURE 19 TMJ restoration after therapy with exosomes (EVs). (A) An illustration showing the surgical site's schematic (sEVs: yellow circle, scaffold: gray rectangle with wavy line). (B) The TMJ was captured during the procedure in its natural form, after the completion of the defect and the application of a functional scaffold to fill it. The defect was 2 mm Å ~ 2 mm. C) TMJ deformities photographed to illustrate gross healing rate at weeks 4 and 8 following sEV therapy ($n = 6$). 5 mm scale bar. (D) Posttreatment ICRS score of TMJ cartilage regeneration in each group at weeks 4 and 8. Data represent the mean Å} SD ($n = 3$). $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. (E) Micro-CT shows new TMJ subchondral bone. SUVs repaired TMJ subchondral bone structure. Micro-CT study of rabbit TMJs was done at 4 and 8 weeks. Condyle sagittal and top views. Representative images ($n = 3$). (F) trabecular number (Tb. N), trabecular separation (Tb. Sp), trabecular thickness (Tb. Th), and Bone volume ratio (BV/TV). Data represent the mean Å} SD ($n = 3$). $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, compared with the blank group; $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$, and $\#\#\#\#p < 0.0001$, ns, no significant difference, IAE group compared with AE group. Reprinted with permission from Liu et al.[76]

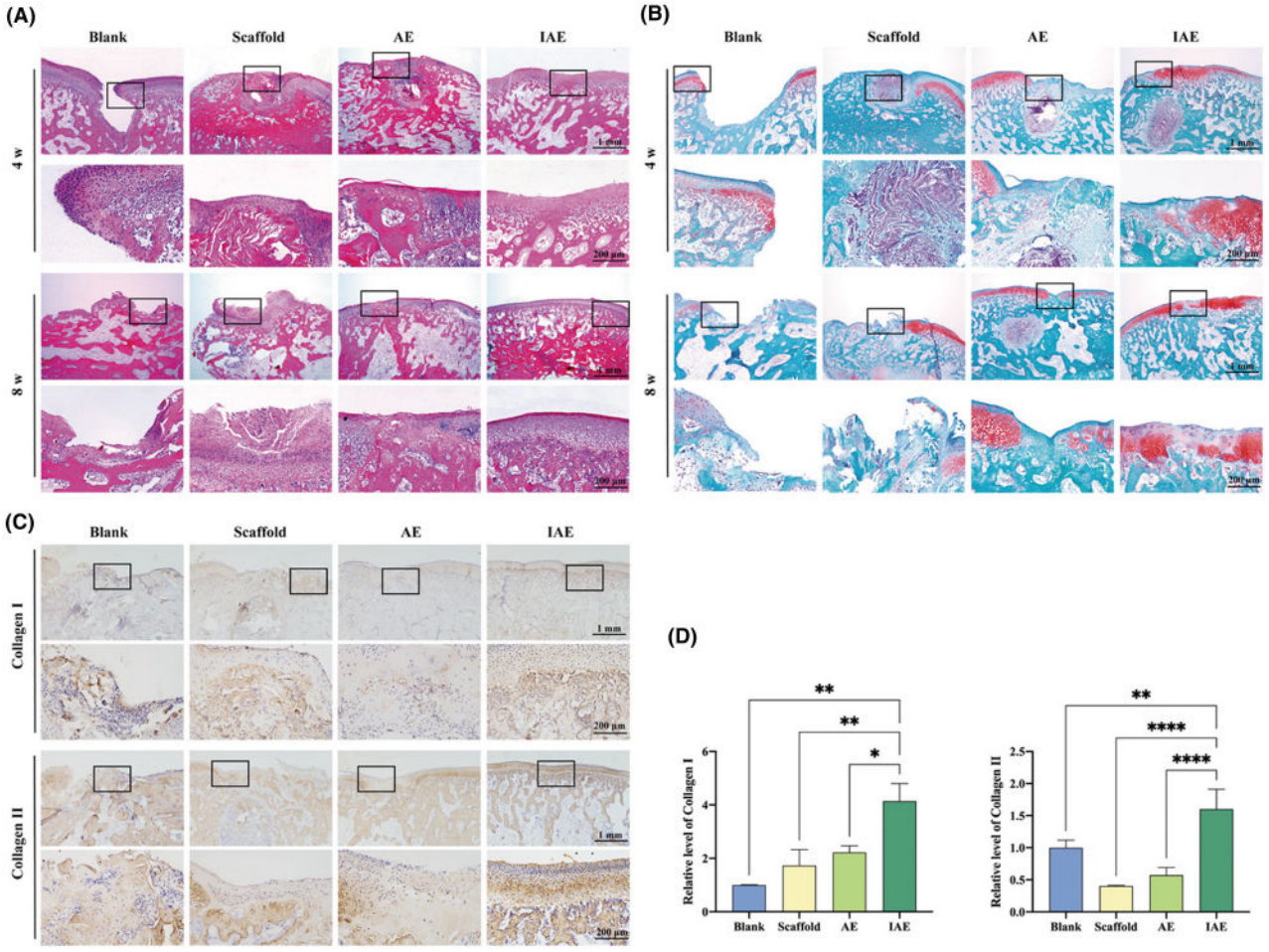

FIGURE 20 Hematological and immunohistochemical TMJ staining after exosome treatment. (A) TMJ sections stained with H&E showing enlarged defect areas at weeks 4 and 8 after therapy. The higher magnification panels' tiny area of vision, as shown below, is indicated by black boxes. (B) Safranin-O/Rapid TMJ parts stained with green dye. The enlarged microscopic fields of vision are shown by black boxes. (C) Collagen I and II immunohistochemical staining at week eight after therapy. The enlarged microscopic fields of vision are shown by black boxes. (D) Quantitative statistics for collagen I and II. Data represent the mean Å} SD ($n = 3$). Upper panel scale bar, 1 mm; lower panel scale bar, 200 µm. *$p < 0.01$, **$p < 0.001$, and ***$p < 0.0001$, compared with the blank group. Reprinted with permission from Liu et al.[76]

disease (1 study). Therefore, in the very near future, salivary exosomes may be used as early detection markers for complex diseases such as oral squamous cell carcinomas and periodontitis.

3 | DISCUSSIONS AND FUTURE PERSPECTIVES

The findings from this scoping review present the most comprehensive data to date on exosomes in dentistry, demonstrating their potential as biomarkers for diagnosis as well as treatment. The therapeutic potential of exosomes is quite prolific and has been used across several fields of dentistry with considerable success. Some of the most studied topics included the effects of exosomes on periodontitis, pulp regeneration, new bone formation and improvements in immune cell function. All of the studies included in this review showed positive outcomes using exosomes derived from various sources, and the majority of research mentioned exosomes' anti-inflammatory qualities. All studies investigating the impact of exosomes on immune cell function reported a transition in macrophage polarization from an M1 pro-inflammatory state to an M2 pro-resolution/regeneration phase.

3.1 | Sources/origins of exosomes

We were interested in investigating the main source of exosomes from dental-derived tissues. Tables 15 and 16 highlight all the different exosome sources from the 113 publications. Notably, exosomes were harvested from 25 different sources. Findings from

TABLE 13 In vitro and in vivo therapeutic regenerative potential of various exosomes derived from multiple sources in other categories.

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Other—in vitro			
Sjöqvist et al. (2019)[111]	Human oral mucosal epithelial cell-Exos	Human dermal fibroblasts Centrifugation at 300 ***g*** × 10 min at 4°C; afterwards the supernatant was filtered through a 0.22 μm syringe filter, concentrated using 100 kDa filters and stored in 80°C. The concentrated media was pooled and further concentrated using 10 kDa filter until the volume was less than 500 μL. Exosomes were isolated from bulk proteins using size exclusion chromatography	Exosome-exposed human skin fibroblasts demonstrated a significant upregulation of growth factor gene expression (HGF, VEGFA, FGF2, and CTGF) and a dose-dependent decrease in proliferation. Both groups' outcomes were comparable. However, there was a tendency for cExo to have a greater impact
Lin et al. (2020)[112]	hDPSCs-Exos (containing exosomal-miR-140-5p)	Human chondrocytes Culture supernatants of DPSCs were centrifuged at 3000 ***g*** × 30 min than 10 000 ***g*** for a further 30 min at room. Finally, exosomes were pelleted following centrifugation at 64 000 ***g*** × 110 min at 4°C using an SW28 rotor and washed once with 0.32 M sucrose	The delivery of exosomes produced from human dental pulp stem cells (hDPSC) enhanced the expression of mRNA linked to chondrocytes, including aggrecan, Col2α1, and Sox9, in human chondrocytes treated with interleukin (IL)-1β
Li et al. (2021)[113]	hDPSCs-Exos	BV2 cells were induced by human oxygen–glucose deprivation–reperfusion (OGD/R). An Exosome Isolation and Purification Kit (Umibio) was used to isolate the exosomes secreted by DPSCs in culture medium. To remove the cellular component, this 48-h-old culture medium was centrifuged at 3000 ***g*** for 10 min at 4°C. Then, exosome concentration solution was added to the supernatants (1:4) and refrigerated at 4°C for 2 h. Subsequently, the samples were centrifuged at 10 000 ***g*** (Beckman Coulter) for 1 h at 4°C	The ischemia/reperfusion (I/R)-induced expression of TLR4, MyD88, and NF-κB was significantly reduced by DPSC-Exos. In both in vitro and in vivo circumstances, DPSC-Exos demonstrated a reduction in the protein expression of IL-6, IL-1β, and TNF-α in comparison to the control group
Fu et al. (2023)[114]	hDPSC-Exos	Mouse RAW264.7 cells The culture supernatants were collected and centrifuged at 300 ***g*** for 10 min, 3000 ***g*** for 10 min, 20 000 ***g*** for 30 min, and 120 000 ***g*** for 70 min to collect the exosomes	In a model of OA in the knee, hDPSC-derived exosomes suppressed osteoclast activation by preventing TRPV4 activation
Hu et al. (2023)[115]	hDPSC-Exos	Human Salivary gland epithelial cells After DPSCs were cultured in serum-free media for 48 h, the supernatants were collected and centrifuged at 300 ***g*** for 20 min, 2000 ***g*** for 20 min, and 10 000 ***g*** for 30 min to remove residual cells and debris. Then the supernatants were ultracentrifuged at 100 000 ***g*** for 17 h at 4°C using ultracentrifugation	The findings demonstrated that, via the GPER-mediated cAMP/PKA/CREB pathway, DPSC-Exos restored salivary gland epithelial cell activity during SS, indicating the potential therapeutic use of DPSC-Exos in the management of Sjogren's syndrome

(Continues)

TABLE 13 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
OTHER—in vivo			
Pivoraitė et al. (2015)[116]	SHED-Exos	Intraplantar injection to BALB/c mice of exosomes vs. prednisolone vs. PBS To induce inflammation, a 20 μL quantity of 1% carrageenan was administered by intra-plantar (subcutaneous) injection into the right hindpaw. The point of injection was premarked for subsequent edema measurement. The contralateral paw was injected with 20 μL of PBS and developed non-measurable edema. At 1 h post-carrageenan injection, 20 μL exosomes (2 AU/mouse) in PBS was injected intraplantar to the right hind paw (exosome group); 30 mg/kg of prednisolone 21-hemisuccinate was injected intraperitoneally 1 h after intra-plantar injection of carrageenan (positive control). The negative control group was administered with PBS alone 1 h post-carrageenan injection. Edema was measured exactly at the point of injection at 6, 24, and 48 h after treatment with carrageenan using a digital caliper	Exosomes significantly reduced the carrageenan-induced edema at all the time points studied (by 39.5, 41.6, and 25.6% at 6, 24, and 48 h after injection, respectively), to similar levels seen with the positive control (prednisolone)
Sjöqvist et al. (2019)[111]	Human oral mucosal epithelial cell	Rat full-thickness skin wound model Four full-thickness wounds were made using a 5 mm biopsy punch. Exosomes labeled using fluorescent dye. To adjust for any anatomical differences that could affect the wound healing, the substances were rotated between each animal. After adding the substances, they were left for 30 min to allow adhesion, after which the wounds were covered with TegaDerm (3 M) and gauze. Two administration protocols: either 7.6 μg of exosomes spread over day 0 and day 1, or 12.5 μg of exosomes on day 0. The animals were observed daily and sacrificed on day 6 or 17	Rats with full-thickness skin wounds received exosomes, and up to 5 days following administration, a signal was seen. On days 6 and 17, the exosomes considerably reduced the size of the wound. To summarize, exosomes derived from OMEC sheets show pro-regenerative properties in the healing of skin wounds. According to the authors, this was the first investigation of the oral mucosa's ability to repair from an exosome standpoint
Zhang et al. (2019)[117]	hGMSC-Exos	Rat critical size tongue defects All rats underwent a left-sided anterior defect by way of a lateral approach. A tongue wound was created using a 6-mm biopsy punch in the left side of anterior dorsal tongue surface at the depth (3 mm) of the muscle layers to damage both the epithelium and the stroma. The constructs were patched over the wound, with the cell-seeded side oriented toward the wounded muscle layers. On day 14 and 28 post surgery, rats from each group were sacrificed and the tongues were harvested by transection at the circumvallate papillae and subsequently prepared for histological and immunohistochemical analysis	GMSC-Exo constructs could facilitate taste bud regeneration and reinnervation with promising potential application in postsurgery tongue reconstruction of patients with tongue cancer. Moreover, the data showed that GMSCs or their derivative exosomes promoted innervation of regenerated taste buds, as evidenced by elevated expressions of neurofilament and P2X3 at the injury areas
Lin et al. (2020)[112]	hDPSC-Exos (loaded with exosomal-miR-140-5p)	Rat OA knee model A total of 24 SD rats were randomly divided into the blank, OA model, exosome treatment and improved-exosome treatment groups. Subsequently, 50 μL of 25 mg/mL iodoacetic acid was administered to the double knee joints to induce OA. The same amount of saline was administered into the knee joint cavities of the rats in the blank group. After 1 week, the exosome treatment group and improved-exosome treatment group were injected with 50 μL of exosomes and miR-140-enriched exosomes, respectively, in the knee joint cavity of both hind limbs once a week; a total of 4 such injections were administered. The rats in the blank group and OA model group were administered the same amount of saline. The rats were sacrificed by an overdose of pentobarbital (150 mg/kg) intraperitoneally at 1 week after the end of treatment	Multiple injections of exosomes enriched with miR-140-5p considerably ameliorated knee joint symptoms in an OA model in rats

TABLE 13 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Li et al. (2021)[113]	hDPSC-Exos	Mouse stroke models were subjected to 2 h of transient middle cerebral artery occlusion A 1 cm-long midline incision was made on the skin of anterior neck area after sterilization. Under an operating microscope, right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were exposed through blunt dissection. Permanent ligations were made at the origin of ECA and CCA with 6-0 silk sutures, and an arteriotomy was made in the CCA. To completely block the middle cerebral artery (MCA), a silicone- coated 6-0 nylon filament was inserted into the CCA and advanced over 9-10 mm to the carotid bifurcation along the ICA and to the origin of the MCA. Two hours later, reperfusion was performed by removing the filament carefully and the incision of CCA was permanently ligated Two hours after reperfusion, animals administrated with DPSC- Exo via tail vein were marked as the I/R + DPSC- Exos group; animals administrated with 100 μL PBS were marked as the I/R + PBS group; animals went through surgical procedures but avoided the placement of filament at MCA were marked as the sham group. Mice were randomly assigned to the study groups	A single injection of DPSC-Exos was administered into the tail vein. Brain edoema, cerebral infarction, and neurological dysfunction were all ameliorated in I/R mice by DPSC-Exos
Zhang et al. (2021)[105]	hDPSC-Exos	Mouse experimental periodontitis and colitis model After the ligature had remained in place for 14 days, mice received 1.5% dextran sulfate sodium in their drinking water for 14 days, after which they received normal water for a 2-day recovery period. The severity of colitis was scored as follows (0, none; 1, 1%–5%; 2, 5%–10%; 3, 10%–20%; 4, >20%), stool consistency (2, loose stools; 4, diarrhea), and bleeding (2, positive haemoccult; 4, gross Bleeding). Mice were sacrificed on day 28. Colons were collected, and their lengths were measured	In a ligature-induced model of periodontitis, 3D-expos demonstrated increased anti-inflammatory properties that reduced experimental colitis. The findings established a framework for reestablishing immunological responses in the inflamed periodontium as a means of treating IBD
Liu et al. (2022)[75]	hDPSC-Exos	A laminectomy was performed at the T11–12 level to create spinal cord damage. A 10-g rod was dropped to prevent damage to the chord after laminectomy, which exposed the dorsal side of the cord. At 1, 3, 5, 7, 14, 21, and 28 days post-SCI, the recovery of spinal cord function was evaluated through Basso Mouse Scale (BMS) scoring	It has been shown that DPSC-derived exosomes shield the damaged spinal cord by preventing M1 macrophage polarization
Yin et al. (2022)[118]	hPDLC-Exos	A delayed tooth replantation model was established by extracting the right maxillary first molar. The dislocated teeth were implanted back into the alveolar fossa after 30 min. 40 μL of Hanks' balanced salt solution (HBSS) were injected into the periodontal tissue, and the experimental group was injected with 40 μL of HBSS containing exosomes. The rats were sacrificed at 1, 2, and 4 weeks. Hematoxylin–eosin (H-E) staining was used to observe tooth resorption. TRAP staining was used to observe the number of osteoclasts	Exosomes produced from PDLCs decrease osteoclast counts during delayed tooth replantation, increase OPG expression in the periodontal ligament, and lessen tooth root resorption following replantation
Zhou et al. (2022)[15]	hDPSC-Exos	Using surgical scissors, a 1 × 1 cm full-thickness excision skin incision was made on the mouse's backs. To observe the wound healing process, images of the wounds were captured using a digital camera on days 0, 3, 5, 7, 9, 12, and 14	Exosomes derived from dental pulp stem cells accelerate cutaneous wound healing

(Continues)

TABLE 13 (Continued)

Author–year	Source of exosomes	Target tissue of exosomes	Main findings
Fu et al. (2023)[114]	hDPSC-Exos	In a mouse knee OA model, a single intra-articular injection of exosomes is produced from DPSC. Intraperitoneal injection, and 0.1 mg of sodium mono-iodoacetate (MIA) dissolved in 5 μL of natural saline (NS) was intra-articularly injected into the left knee joint by a microsyringe. Two weeks after MIA administration, 10 μL of PBS containing 2×10^8 exosomes was administrated to the OA-Exo group by intra articular injection, and the equivalent PBS was injected into the NS and OA-NS groups	The findings showed that exosomes produced by DPSCs effectively decreased in vivo cartilage degradation and synovial inflammation, inhibited osteophytes and bone sclerosis, and improved abnormal subchondral bone repair
Hu et al. (2023)[115]	hDPSC-Exos	Mice: DPSC-Exos were intravenously given to non-obese diabetic female mice with Sjogren's syndrome. Female NOD/Ltj mice served as SS animal models which were randomly divided into a treatment group, a positive control group (hydroxy-chlorquine-treated), and a disease group. For the treatment group, the mice were injected with DPSC-Exos (25 mg/kg) into the tail vein once a week for 10 weeks. Hydroxychloroquine (HCQ) gastric-infused (60 mg/kg) mice served as positive controls, and the NOD/Itj mice injected with an equal volume of PBS served as a negative control. The saliva secretion flow rates were recorded weekly after the 1st injection. After 10 weeks of drug administration, mice were sacrificed	Intravenous injection of DPSC-Exos in NOD/Itj mice treated Sjogren's syndrome as indicated by the increased salivary flow rate, attenuated glandular inflammation, and increased AQP5 expression
Shi et al. (2023)[17]	hDPSC-Exos	The experiment included injecting 200 μL of a PBS solution containing 100 μg of hDPSC-Exos subcutaneously into rats with a superficial inferior epigastric artery flap in order to induce ischemia/reperfusion (I/R) damage	hDPSC-Exos can enhance flap repair after I/R injury and improved the survival and microvessel density of the flap and suppressed epithelial cell apoptosis

Abbreviations: AQP5, Aquaporin-5; BALB/C, albino, laboratory-bred strain of mouse; BV2, microglial cell derived from C57/BL6 murine; cAMP, cyclic adenosine monophosphate; cExo, Conditioned Exo; CREB, cAMP response element-binding protein; CTGF, development factor for connective tissues; DPSC, Dental pulp stem cells, also known as hDPSCs or human DPSCs; Exo, Exosome; FGF2, Fibroblast Growth Factor-2; GMSC, gingival mesenchymal stem cells; HGF, Growth factor for hepatocytes; hGMSC, human GMSC; hPDLCs, human PDLCs; I/R, Ischemia/reperfusion; IBD, Inflammatory Bowel Disease; IL, Interleukin; miR, microRNA; mRNA, messenger RNA; MyD88, Myeloid differentiation main response 88; NF-kB, Nuclear factor kappa-B; NOD/LtJ, non-obese diabetic; OA, Osteoarthritis; OGD/R, Oxygen glucose deprivation/re-oxygenation; OMEC, Oral Mucosal Epithelial Cell; OPG, Osteoprotegerin; P2XR, P2X purinoceptor 3; PBS, Phosphate buffered saline; PDLCs, Periodontal Ligament Stem Cells; PKA, protein kinase A; TLR4, Toll-like Receptor-4; TNF-α, Tumor necrosis factor-α; TRPV4 is an ion channel belonging to the Transient Receptor Potential Cation Channel Subfamily V Member 4; VEGFA, Vitamin A for vascular endothelial development.

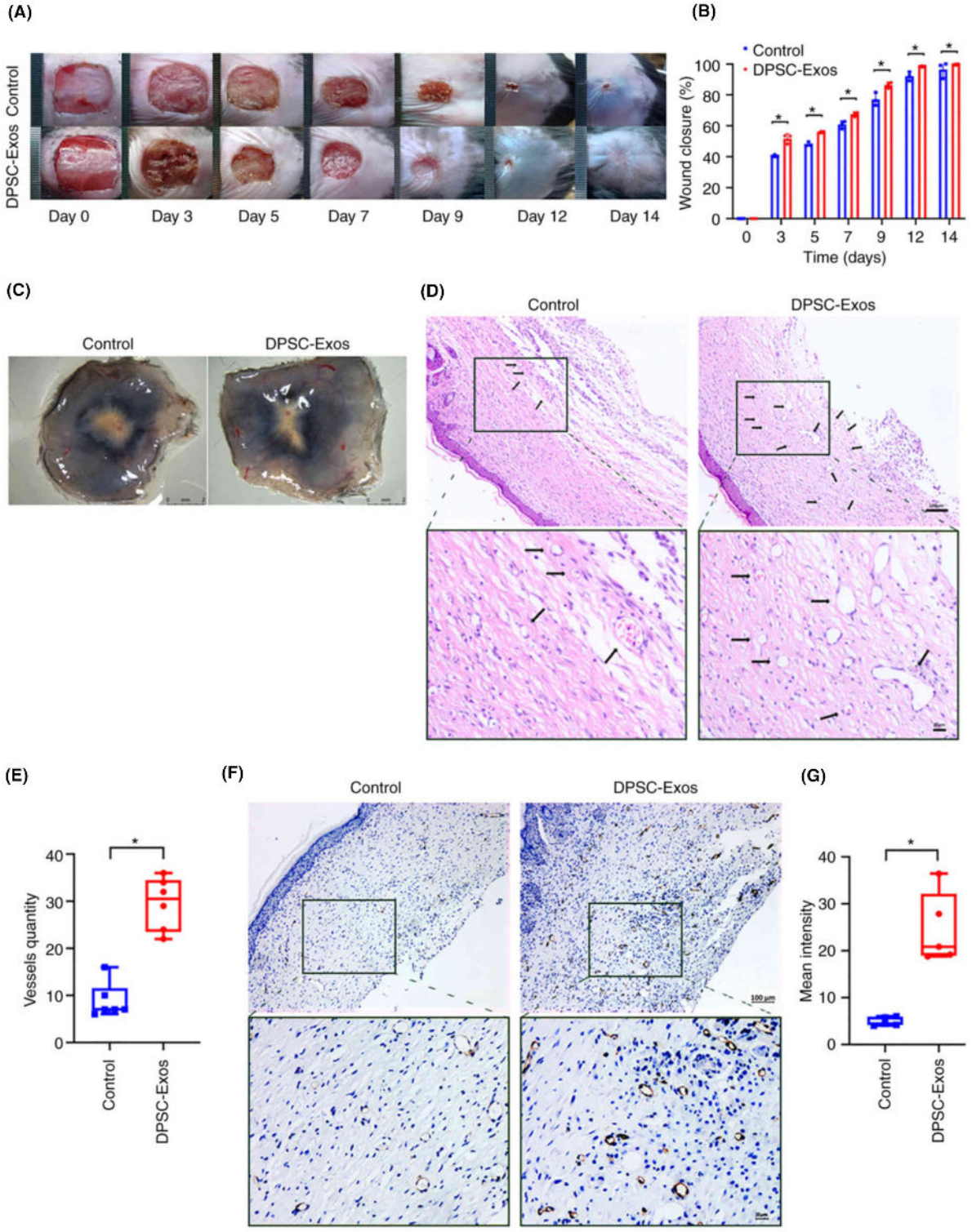

FIGURE 21 Through the promotion of angiogenesis, DPSC-Exos hasten the healing of cutaneous wounds in mice. (A) Gross observation and measurement of the wound area on days 3, 5, 7, 9, 12, and 14 after wounding in mice treated with PBS and DPSC-Exos. Scale bar, 1 cm. (B) At the given time points ($n = 3$), the rate of wound closure was considerably greater in wounds receiving DPSC-Exos treatments. (C) An enlarged picture of the mice's wounds from below on day 14 after they were treated with PBS and DPSC-Exos. The DPSC-Exo group had new blood vessels in their wounds. (D and E): Hematoxylin and eosin staining of PBS and DPSC-Exos-treated wound sections on day 14 following surgery. The black arrows show new blood vessels. The DPSC-Exo-treated group had more vessels than the control group ($n = 3$). Scale bar: 100 μm (F and G). Immunohistochemical CD31 staining in PBS and DPSC-Exos-treated mice wound sections 14 days after surgery. CD31 expression was greater in DPSC-Exo-treated ($n = 3$) groups. Scale bar, 100 μm. $^*p < 0.05$. DPSC, dental pulp stem cell; Exo, exosome; PBS, phosphate-buffered saline. Reprinted with permission from Zhou et al.[15]

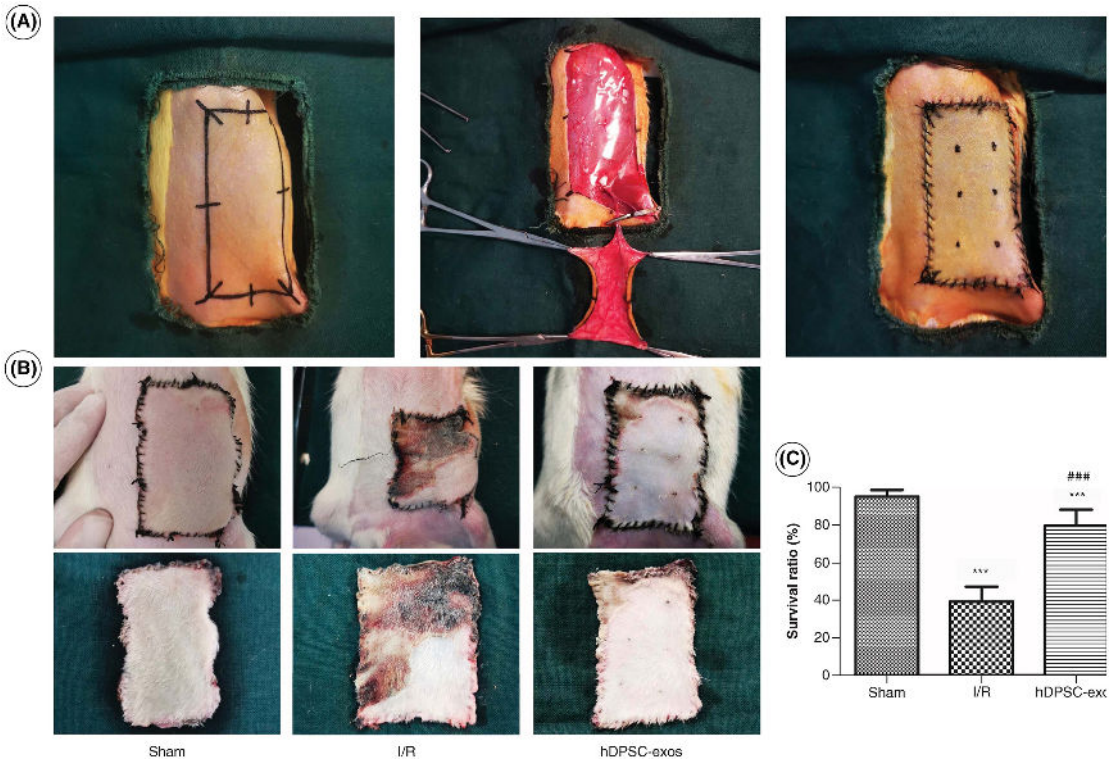

FIGURE 22 The survival of flaps was enhanced by exosomes produced from human dental pulp stem cells. (A) Creation of the rat model with I/R damage and a superficial inferior epigastric artery flap. (B) The exemplary photos of flaps on day five after surgery. (C) The hDPSC-Exos group's flap survival ratio was significantly greater than that of the I/R group. Data are represented as the mean Â} standard deviation. *versus sham; #versus I/R; ***$p < 0.0001$; ###$p < 0.0001$. hDPSC: Human dental pulp stem cells; hDPSC-Exos: Exosomes derived from human dental pulp stem cells; I/R: Ischemia–reperfusion. Reprinted with permission from Shi et al.[17]

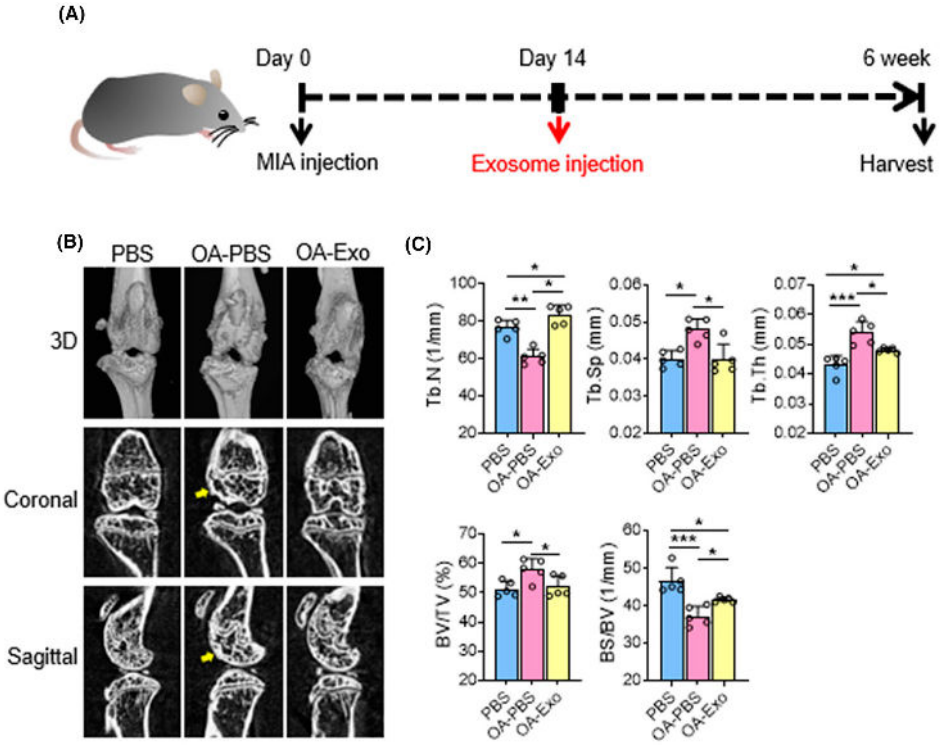

FIGURE 23 The local injection of DPSC-Exos successfully alleviated bone degradation in patients with progressing knee OA. (A) Timeline of mouse knee OA induction and therapy. (B) Representative sagittal and coronal views, as well as three-dimensional micro-CT reconstructions of the knees in the various groups. Yellow arrows: sclerosis or osteophytes. (C) Statistical analysis of bone deterioration-relative parameters. Data are presented as mean ‡SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $n = 5$. Reprinted with permission from Fu et al.[114]

TABLE 14 Use of exosomes found in saliva and crevicular fluids for the marker detection of various disorders and diseases.

Author	Main findings
Aging	
Machida et al. (2015)[119]	MiR-24-3p was discovered as a new potential aging biomarker after comparing each total RNA acquired by the 13 elderly and the 15 young people in order to verify the FC values using quantitative real-time PCR. According to this pilot investigation, salivary exosomal miRNAs may be shown to be potential aging indicators
Birth defect	
Seelan et al. (2022)[120]	Cells (including fetal and placental cells) release microRNAs (miRNAs) into the environment via exosomes, which are then carried by blood, urine, saliva, and cerebrospinal fluid. Because of this, they are able to interact with certain cells and serve as diagnostic and prognostic indicators for congenital malformations
Cardiovascular diseases	
Rehman et al. (2017)[121]	Saliva can be used as a liquid biopsy to diagnose cardiovascular diseases by analyzing biomarkers like Myoglobin (MYO), Cardiac troponin I (cTnI), Creatine phosphokinase MB (CK-MB), Myeloperoxidase (MPO), brain natriuretic peptide (NT-proBNP), Exosomal miRNA, CRP, MMP-8, MMP-9, TIMP-1, and leukotriene B4)
Diabetes	
Li et al. (2020)[122]	These findings indicate that osteocyte-derived exosomes carrying miR-124-3p may regulate Gal-3 expression of osteoblasts, especially under high-glucose conditions, suggesting a possible mechanism for DM-related alveolar bone pathologies
Monteiro et al. 2019[123]	The authors were able to isolate extracellular vesicles from gingival crevicular fluid using a method that is suitable to be applied in a clinical setting. The results provide an insight into the potential capacity of first trimester oral extracellular vesicles as early biomarkers for the prediction of gestational diabetes mellitus in presymptomatic women
Gingival recession	
Pei et al. (2022)[124]	A quantitative proteomic analysis, which encompasses the study of proteins in exosomes found in gingival crevicular fluids, has successfully identified new biomarkers associated with gingival recession in orthodontic patients. The ROC analysis findings revealed that 26 markers/proteins have the potential to be considered biomarker candidates for GR. The investigation of protein–protein interaction networks indicated that IQGAP1, ACTN1, TLN1, VASP, FN1, FERMT3, MYO1C, RALA, RPL35, SEC61G, KPNB1, and NPM1 may play a role in the development of GR via the control of the cytoskeleton
Gingivitis	
Han et al. (2020)[125]	Salivary small extracellular vesicles associated with gene methylation of inflammatory cytokines in gingivitis
Inflammatory bowel disease	
Zheng et al. (2017)[126]	The researchers determined that salivary exosomal PSMA7 was found in significant quantities in the salivary exosomes of individuals with IBD. It has the potential to serve as a very effective biomarker for alleviating the discomfort associated with colonoscopy in patients
Neurodegenerative disease	
Tatullo et al. (2019)[127]	Human periapical cyst-mesenchymal stem cells are unique MSCs that grow into functioning neurons and express significant neuronal markers. Human periapical cyst-derived mesenchymal stem cells may be an intelligent "lab-on-a-cell" for studying neurodegenerative diseases and exosome composition
Oral lichen planus	
Byun et al. (2015)[128]	miR-4484 was significantly upregulated in the salivary exosomes of patients with oral lichen planus. This study thus identified a potential miRNA biomarker for oral lichen planus and provided insight into the functions of miRNAs in the pathogenesis of oral inflammatory diseases
Oral squamous cell carcinoma	
Zlotogorski-Hurvitz et al. (2016)[129]	Nanoparticle tracking analysis revealed a markedly elevated quantity of nanoparticles per mL in oral cancer samples of oral fluids (OF) in comparison to those from healthy persons. The ELISA and WB assays revealed distinct expression patterns of exosomal markers in exosomes derived from oral cancer (OC) compared to healthy individuals (HI): The expression of CD81 and CD9 is lower compared to the expression of CD63 (~53 kDa). This work established a starting point for identifying exosomal biomarkers that may be used to detect early indicators of malignancy in high-risk individuals who do not have obvious clinical symptoms or lesions
Momen-Heravi et al. 2018[130]	The researchers in this study showed that oncomiR, namely miR-21 and the miR-27 family, are abundant in patients who have been diagnosed with oral cancer. Exosomes were extracted from individuals diagnosed with oral squamous cell cancer (OSCC). These results may have significance for the development of biomarkers for oral squamous cell carcinoma (OSCC) and for understanding and addressing the molecular and vesicular factors in the tumor microenvironment

(Continues)

TABLE 14 (Continued)

Author	Main findings
Hunsaker et al. (2019)[131]	This was one of the first studies to examine oral cancer microRNA expression and melatonin. The results suggest that miR-21, miR-133a, and miR-155 may be differently regulated and may explain melatonin's anti-tumor actions
Zlotogorski-Hurvitz et al. (2019)[132]	The authors demonstrated the unique infrared spectral signature of OC salivary exosomes, which allowed for the precise separation of these exosomes from HI exosomes by using tiny datasets to develop artificial neural networks for the detection of minute changes in protein, lipid, and nucleic acid conformations. More research should be done on this noninvasive technique for the detection of oral cancer in its very early stages or in lesions in the mouth that have the potential to become malignant
Coon et al. (2020)[133]	It was shown that miR-365 is not only expressed in oral cancer cell lines but is also exported into exosomes and extracellular vesicles. The possible relationship between this microRNA and the characteristics and actions of oral tumors that express it may be better understood in light of these studies. Future studies will look at these possible routes and processes to see whether miR-365 may be used as a biomarker for oral cancer in salivary or liquid biopsies
Jin et al. (2020)[134]	We selected three long noncoding RNAs (lncRNAs) (NR 026892.1, NR 126435.1, and NR 036586.1) as potential OSCC biomarkers. Expression levels of selected lncRNAs varied significantly ($p < 0.001$) in both whole cells (CAL 27 vs. HOECs) and exo (CAL 27 vs. HOEC exo). Quantitative PCR confirmed the sequencing results for the three lncRNAs' expression levels. Finally, lncRNAs expressed differentially in malignant and non-cancerous exosomes, suggesting they might be cancer biomarkers
Masaoka et al. (2021)[135]	Comparison with human normal keratinocytes detected 8 upregulated and 12 downregulated miRNAs in OSCC-secreted exosomes. The potential target mRNAs of these dysregulated miRNAs were suggested by Ingenuity Pathway Analysis, and 6 significant genetic networks were indicated by genetic network analysis. Furthermore, 4 crucial upstream miRNAs—miR-125b-5p, miR-17-5p, miR-200b-3p, and miR-23a-3p—were identified. miR-125b-5p was a central node in the most significant network. Gene ontology analysis showed significant enrichment of genes with cancer-related functions, such as molecular mechanisms of cancer, cell cycle, and regulation of the epithelial-mesenchymal transition
Faur et al. (2022)[136]	Oropharyngeal and oral squamous cell carcinoma patients exhibited an increase in salivary exosomal miR-486-5p and a decrease in miR-10b-5p compared to healthy controls. Furthermore, miR-486-5p expression was highest in stage II cancer compared to other cancer stages. Compared to the control samples, the cancer samples had a greater exosome dimension. Finally, miR-10b-5p and miR-486-5p expression in salivary exosomes has been changed by oral and oropharyngeal malignancies
Jayaseelan et al. (2022)[137]	The current study's findings showed three micro-RNAs that may have a significant impact on the TP53 gene's activities and cause dangerous consequences for patients with HNSCC. When it comes to HNSCC patients, exosomal-targeting microRNAs (miRNAs) of the TP53 gene might be a useful prognostic indicator. These miRNAs can also be employed as a treatment approach by developing inhibitors
Faur et al. (2023)[138]	Based on multivariate analysis of surface-increased Raman spectra of salivary exosomes, the authors developed a new approach to detect oral and oropharyngeal squamous cell cancer
Ludwig et al. (2023)[139]	Tumor tissues in HNSCC patients exhibited overexpression of TGFβ, Smad3, and TGFB1, which was correlated with elevated levels of soluble TGFβ. TGFβ expression in malignancies and soluble TGFβ levels did not show any correlation with clinicopathological information or overall survival. The only marker of tumor growth that corresponded with tumor size was TGFβ coupled with exosomes. The scientists came to the conclusion that circulating TGFβ+ exosomes in HNSCC patients' plasma may be a noninvasive indicator of the disease's progression
Wang et al. (2023)[140]	Cancer-associated fibroblasts (CAFs)-Exo was found to be involved in tumor immune regulation through hsa-miR-139-5p, ACTR2 and EIF6, while PIGR, CD81, UACA and PTTG1IP may be potentially effective targets for the treatment of oral squamous cell carcinoma in the future
Oropharyngeal cancer detection	
Wang et al. (2020)[141]	Compared with the current gold standard, differential centrifugation, droplet digital RT–PCR analysis showed that the average yield of salivary exosomal small RNA from the acoustofluidic platform is 15 times higher. With this high-yield exosome isolation platform, the authors showed that HPV16 DNA could be detected in isolated exosomes from the saliva of HPV-associated OPC patients at 80% concordance with tissues/biopsies positive for HPV16. Overall, these data demonstrated that the acoustofluidic platform can achieve high-purity and high-yield salivary exosome isolation for downstream salivary exosome-based liquid biopsy applications. Additionally, HPV16 DNA sequences in HPV-OPC patients are packaged in salivary exosomes and their isolation will enhance the detection of HPV16 DNA

TABLE 14 (Continued)

Author	Main findings
Orthodontic root resorption	
Rody Jr et al. (2014)[142]	The teeth with root resorption showed both significantly increased and downregulated proteins. Interestingly, exosomes are known to contain a large number of these proteins. Individually or as a panel, the authors discovered new proteins that might serve as helpful indicators of root resorption
Atsawasuwan et al. (2018)[143]	The researchers demonstrated that during 6 weeks of orthodontic tooth movement, the expression patterns of the miR-29 family increased. GCF contains secretory miRNAs, and human tooth movement is associated with increased expression patterns of the secretory miRNA-29 family. Potential periodontal remodeling indicators might be secretory miRNA-29 in GCF
Holliday et al. (2018)[144]	Signal amplification techniques, such as polymerase chain reaction (PCR), are able to enhance the sensitivity required for the use of exosomes from gingival crevicular fluid (GCF) as biomarkers. Research on the impact of extracellular vesicles (EVs) on orthodontic tooth movement (OTM) will provide innovative perspectives that might uncover methods for improving OTM techniques
Rody Jr et al. (2018)[145]	The findings provide evidence for the existence of molecular distinctions between odontoclasts and osteoclasts. The use of exosomes in GCF as a therapeutic tool to identify indicators of root resorption may be made possible by emerging technology
Rody Jr et al. (2022)[146]	The authors came to the conclusion that odontoclast EVs include a variety of compounds that are different from those identified in osteoclast EVs, suggesting that they might be biomarkers for the destruction of periodontal tissue and root resorption
Pancreatic cancer	
Lau et al. (2013)[147]	The prevalence of systemic diseases, including pancreatic, breast, lung, and ovarian cancers, is on the rise, but a new study suggests that distinguishing salivary biomarkers might be readily detected. This study provides further evidence that tumor-derived exosomes are the mechanism by which saliva and distant systemic diseases may produce discriminating biomarkers.
Machida et al. (2016)[148]	Based on quantitative real-time PCR (RT-qPCR) results, the cancer group exhibited significantly higher relative expression ratios of miR-1246 and miR-4644 in comparison to the control group. This preliminary study suggests that miR-1246 and miR-4644-containing salivary exosomes might be useful diagnostic tools for pancreaticobiliary tract cancer
Periodontitis	
Tomofuji et al. (2016)[149]	MiR-207, miR-495, and miR-376b-3p are three serum miRNAs that may be useful indicators for periodontitis
Huang et al. (2019)[150]	Twenty-six proteins were identified only in the SP group, and 58 proteins were identified only in the healthy group. Gene ontology analysis revealed that innate immune response, cytolysis and complement activation were highly enriched in the SP group. Interaction network analysis showed that the correlations among immune-related proteins (e.g., complement components and chemokine (C-C motif) ligand 28) were significant in the SP group. C6 proteins expressed only in the SP group were evaluated by Western blotting. The authors concluded that salivary exosomes from periodontitis patients are enriched immune-related proteins that might participate in the immune response during the development of periodontitis
Tobón-Arroyave et al. (2019)[151]	A lower level of CD9/CD81 exosomes in saliva may be significant for the pathophysiology of periodontal disease
Yu et al. (2019)[152]	According to the present research, there is a correlation between the severity and stage of periodontitis and the amounts of exosome-based PD-L1 mRNA in saliva, which may be used to identify periodontitis in healthy individuals
Chaparro Padilla et al. (2020)[153]	People with periodontitis had far higher levels of the CD63 exosome marker in their gingival crevicular fluid (GCF) ($p=0.00001$). Significant positive relationships were seen with bleeding on probing (rho = 0.63, $p=0.002$), periodontal probing depth (rho = 0.56, $p=0.009$), and clinical attachment level (rho = 0.48, $p=0.030$) in relation to the total content of extracellular vesicles (EVs) in gingival crevicular fluid (GCF)
Han et al. (2020)[154]	Three substantially increased miRNAs (hsa-miR-140-5p, 146a-5p, and 628-5p) were exclusively discovered in sEVs from periodontal patients compared to healthy controls and exhibited strong discriminating power (AUC = 0.96) for periodontitis diagnosis. Salivary sEVs provide noninvasive miRNAs for periodontitis diagnosis, according to this study. Three selectively increased miRNAs in sEVs but not total saliva may indicate periodontal disease
Nik Mohamed Kamal et al. (2020)[155]	Plasma-exosomal samples such as hsa-miR-let-7d, hsa-miR-126-3p, and hsamiR-199a-3p and salivary-exosomal samples such as hsa-miR-125a-3p are deserving of further investigation for detection of periodontitis. Due to their substantial correlation with the mean PPD, and considerable differential expression between samples with chronic periodontitis and healthy controls, these miRNAs are the most trustworthy candidates for the creation of a periodontitis biomarker panel

(Continues)

TABLE 14 (Continued)

Author	Main findings
Han et al. (2021)[156]	Notably, salivary sEVs with global 5mC hypermethylation had great sensitivity and specificity (AUC = 1) in differentiating individuals with periodontitis from both healthy controls and those with gingivitis. According to the study's results, measuring global sEV methylation may be a helpful biomarker for periodontitis
Xia et al. (2021)[157]	The authors concluded that salivary exosome miR-223-3p may control GSDMD-mediated pyroptosis by targeting NLRP3 in periodontitis. MiR-223-3p expression in salivary exosomes may be a promising noninvasive method for diagnosing and assessing periodontitis severity
Byun et al. (2022)[158]	These data suggest that salivary exosomal miR-25-3p contributes to diabetes-related periodontitis. Other miR-25-3p targets may be needed to develop drugs or diagnostics that modulate T-cell-mediated local inflammation and treat periodontitis
Bachtiar et al. (2023)[159]	In COVID-19-diagnosed periodontitis patients with and without diabetes, Porphyromonas gingivalis is associated with inflammatory markers and exosomal miRNA-155 in saliva
Han et al. (2023)[160]	The detection of sEVs mRNA for osterix and TNF-α was lower in periodontitis and higher in healthy controls, respectively, indicating good discrimination for periodontitis diagnosis. This pilot study suggested salivary sEVs mRNAs might be a noninvasive periodontitis biomarker
Kwon et al. (2023)[161]	Plasma-derived exosomal miRs (miR-1304-3p and miR-200c-3p) and SNORS (SNORD57 and SNODB1771) may be periodontitis biomarkers
Yamaguchi et al. (2023)[162]	The periodontal inflamed surface area, miR-142, and miR-144 were substantially greater in patients with elevated C6 expression after initial periodontal treatment (IPT) than in patients with reduced C6 expression following IPT. Salivary exosomes from individuals with periodontitis showed a substantial shift in the expression levels of proteins and miRNAs after intraoral phototherapy (IPT), indicating that exosome components may be used as biomarkers for the disease
Peri-implantitis	
Chaparro et al. (2021)[163]	A correlation between the development of periimplantitis and a rise in EV concentration and downregulated expression of miRNA-21-3p and miRNA-150-5p has been suggested
Sjogren's syndrome	
Yamashiro et al. (2022)[164]	The researchers found SS biomarkers in exosome-derived microRNAs utilizing noninvasive mouth rinse samples from SS patients and healthy patients. They identified 12 possible biomarkers in mouth rinse samples from the two groups using microarrays and real-time PCR to identify exosome-produced miRNAs. The SS group had significantly greater expression rates for four miRNAs than the control group. New, noninvasive SS diagnostics may employ these miRNAs. The first noninvasive SS screening and diagnosis using mouthwash is reported here
Systemic disease	
Han et al. (2018)[165]	Determining the roles of salivary exosomes and comprehending their unique processes will provide fresh perspectives on the potential uses of salivary exosomes in the detection and management of systemic disorders, given their constantly expanding importance

Abbreviations: ACTN1, Alpha-actinin-1; ACTR2, Actin-related protein 2; CAFs, Cancer-associated fibroblasts; CD, Cluster of Differentiation; CK-MB, Phosphokinase MB-Creatine; COVID-19, Coronavirus disease 2019; CRP, C-Reactive Protein; cTnI, Cardiac Troponin I; DM, Diabetes Mellitus; EIF6, Eukaryotic Translation initiation factor 6; EVs, Extracellular Vesicles; Exo, Exosomes; FC, Fold changes; FERMT3, FERM Domain Containing Kindlin 3; FN1, fibronectin-1; Gal-3, Galectin-3; GCF, Gender Crevicular Fluid; GR, Gingival Recession; GSDMD, gasdermin D; HI, Healthy Individuals; HNSCC, head and neck cancers caused by squamous cells; HOEC, Human Olfactory Ensheathing Cel; hPCy–MSCs, human periapical Mesenchymal stem cells with cysts; HPV, Human Papilloma Virus; IBD, inflammatory bowel disease; IPT, Initial Periodontal Therapy; IQGAP1, Ras GTPase-activating-like protein; IR, Infrared; KPNB1, Importin subunit beta-1; lncRNAs, long non-coding RNAs; miR, miRNA, microRNA; MMP, Matrix metalloproteinase; MPO, Myeloperoxidase; MYO, Myoglobin; MYO1C, Myosin-1; NLRP3, Three NOD-like receptor (NLR) pyrin domains; NPM1, nucleophosmin; NT-proBNP, peptide brain natriuretic; OC, Oral Cancer; OF, Oral Fluids; OPC, Oropharyngeal cancer; OSCC, Oral Squamos Cell Carcinoma; OTM, Orthodontic Tooth Movement; PCR, Polymerase Chain Reaction; PD-L1, Area under the curve, or AUC, for programmed death-ligand 1; PIGR, Polymeric immunoglobulin receptor; PPD, Periodontal Probing Depth; PPI, Protein–protein interaction; PSMA7, Proteasome 20S Subunit Alpha 7; PTTG1IP, Pituitary tumor-transforming gene 1 protein-interacting protein; RALA, R-Alpha Lipoic Acid; RNA, Ribonucleic acid; ROC, receiver operating characteristic; RPL35, ribosomal protein L35; RT-PCR, Real-Time PCR; SEC61G, SEC61 Translocon Subunit Gamma; SMAD3, Suppresor of Mothers against Decapentalegic-3; SNORS, SnoRNAs signature; SP, Severe Periodontitis; TGFβ, ransforming Growth Factor-β; TIMP, tissue-inhibitory protein; TLN1, Talin-1; TP53, tumor protein p53; UACA, Uveal autoantigen with ankyrin repeats and coiled-coil domains; VASP, vasodilator-stimulated phosphoprotein; Western Blot, Enzyme-Linked Immunosorbent Assay.

the in vitro publications demonstrated that DPSCs were the most common source of exosomes and were found in 29 of the 113 publications (26%). PDLCs were utilized in 15 studies (13%), BMSCs were utilized in 13 (1%), SHEDs were utilized in 10 (9%), monocytes/macrophages were utilized in 9, and GMSCs were utilized in 9 (both 8%). We grouped all other cell types, which were used 1 or 2 times, into the "Other" category (encompassing 22 studies). These included exosomes derived from MSCs, osteocytes, dental follicle cells, Herwig epithelial root sheath (HERS), odontoblasts, SCs, dendritic cells, umbilical cord, embryonic stem cells, platelets, keratinocytes,

TABLE 15 Comparative use of exosome sources from various in vitro studies.

	DPSCs	PDLSc	BMSC	SHEDs	Monocyte Macrophage	GMSC	SCAP	ADSC	Other
1. Angiogenesis	5	1	1	3			2		
2. Bone	3	6	6	3	5	1		2	2
3. Cancer	1								2
4. Cementoblasts		1							
5 Endo/Pulpal Cells	7			1			1		6
6 Immune Cell	6	2	3	1	1	3	1	1	4
7 Mesenchymal	1	1	1			1			
8 Nerve Regeneration						1			
9 Oral Pathogens									2
10 Orthodontics					1				
11 Periodontics	2	4	2	2	2	3			5
12 TMJ	4								1
13 Other									
Totals	29	15	13	10	9	9	4	3	22

TABLE 16 Comparative use of exosome sources from various in vivo studies.

	DPSCs	PDLSc	BMSC	SHEDs	Monocyte Macrophage	GMSC	SCAP	ADSC	Other
1 Angiogenesis				1					
2 Bone Regeneration	2		5		1			1	2
3 Cancer									
4 Cementoblasts									
5 Endo/Pulpal Cells	5			1			2		3
6 Immune Cells							1		
7 Mesenchymal									
8 Nerve regeneration						1			
9 Oral pathogens									
10 Orthodontics		1			1				
11 Periodontics	4	3	3	2	2	1		1	6
12 TMJ	8	1		1		1			1
13 Other									
Totals	19	5	8	5	4	3	3	2	12

oral epithelial cells, dermal fibroblasts, plant-based derived, honey-derived, milk-derived and laboratory-made cells. Thus, multiple options are available to clinicians.

One noteworthy aspect, however, is that few studies characterized their exosomes (internal signaling molecules) or compared exosomes to other sources. A couple of studies found that hDPSC-Exos exhibited superior regenerative properties when compared to hBMSCs, although limited research has been conducted on this subject. Future research aimed at enhancing our understanding and determining which exosomes are most effective for specific tissues is warranted. As depicted in part II of this three-part article, it is clear that different signaling molecules may impact various tissues differently. Future studies investigating differences between exosomes derived from periodontal tissues (Periosomes) may not be the ideal set of signaling factors (exosomes) for a joint/cartilage such as those injected within the TMJ. Further research clarifying which subset of exosomes performs better for each indication remains needed. Additionally, as discussed in Part I of this 3-part article, little information has been provided on the isolation methods for each of these 25 subsets of exosomes utilized in therapeutic dentistry, and a great deal of research should be dedicated to the optimization of exosome isolation.

One interesting finding was the fact that oral and dental-derived exosomes were able to have a significant impact on other tissues. Notably, exosomes from the dental pulp were found to greatly accelerate cutaneous wound healing, had a significant impact on retaining flap viability, and were used to greatly improve OA of knees, lower inflammation post spinal injury and improve both tongue and Sjogren's syndrome. The most astounding finding was that hDPSC-Exos might even cut dopaminergic neuron death by around 80%, and the authors considered exosomes as a potentially new therapeutic tool for the treatment of diseases such as Parkinson's disease.[64] Another animal study that purposefully caused an animal stroke found that a single intravenous injection of DPSC-Exos alleviated brain oedema, cerebral infarction and neurological impairment.[113] Last, one study found that exosomes were as efficient as prednisolone.[116] It was also interesting to learn that various nonhuman and nonanimal sources of exosomes, such as plant-derived, milk-derived or honey-derived exosomes, all had positive outcomes in therapeutic dentistry and/or had positive effects on oral/periodontal pathogen reduction, thus highlighting the vast potential for future research with nearly unlimited exosomal sources.

One area that remains to be further researched is the ability of exosomes to be utilized as diagnostic markers. As they have much longer half-lives when compared to proteins and hormones, these small biomarkers may, in fact, be utilized as detection markers for cancers much more effectively. Many research groups are currently investigating this topic in cancer diagnostics in medicine, but much less work is presently being done in dentistry. Since saliva is much more readily accessible, considerable work could be done in that space to develop specialized saliva kits focused on detecting disease or illness from saliva quite easily. This area of research is surely to grow in the coming years.

In summary, we have summarized the extensive data to date on exosomes in the dental field. Despite the research on the vast methods to produce and isolate exosomes (Part I), their widespread use in medicine (Part II) and their use in dentistry (Part III), there remain many open questions that should fill the upcoming years of research in this exciting field. Currently, while many human studies have been conducted on medicine using exosomes, no single study has yet investigated the regenerative potential of exosomes in humans in the dental field. These future studies may lead to significant breakthroughs, and ongoing investigations will surely take place in the coming years.

ACKNOWLEDGEMENTS

None. Open access funding provided by Universitat Bern.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Yufeng Zhang https://orcid.org/0000-0001-8702-5291

REFERENCES

1. Ghosh A, Davey M, Chute IC, et al. Rapid isolation of extracellular vesicles from cell culture and biological fluids using a synthetic peptide with specific affinity for heat shock proteins. *PLoS One*. 2014;9:e110443.
2. Tan A, Rajadas J, Seifalian AM. Exosomes as nano-theranostic delivery platforms for gene therapy. *Adv Drug Deliv Rev*. 2013;65:357-367.
3. Chen J-j, Zhao B, Zhao J, Li S. Potential roles of exosomal microRNAs as diagnostic biomarkers and therapeutic application in Alzheimer's disease. *Neural Plast*. 2017;2017:7027380.
4. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm Sin B*. 2016;6:287-296.
5. Zhuang X, Xiang X, Grizzle W, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther*. 2011;19:1769-1779.
6. Xian X, Gong Q, Li C, Guo B, Jiang H. Exosomes with highly angiogenic potential for possible use in pulp regeneration. *J Endod*. 2018;44:751-758.
7. Wu J, Chen L, Wang R, et al. Exosomes secreted by stem cells from human exfoliated deciduous teeth promote alveolar bone defect repair through the regulation of angiogenesis and osteogenesis. *ACS Biomater Sci Eng*. 2019;5:3561-3571.
8. Zhang Z, Shuai Y, Zhou F, et al. PDLSCs regulate angiogenesis of periodontal ligaments via VEGF transferred by exosomes in periodontitis. *Int J Med Sci*. 2020;17:558.
9. Gardin C, Ferroni L, Erdoğan YK, et al. Nanostructured modifications of titanium surfaces improve vascular regenerative properties of exosomes derived from mesenchymal stem cells: preliminary in vitro results. *Nano*. 2021;11:3452.
10. Huang X, Qiu W, Pan Y, et al. Exosomes from LPS-stimulated hDPSCs activated the angiogenic potential of HUVECs in vitro. *Stem Cells Int*. 2021;2021:1-15.
11. Wu M, Liu X, Li Z, et al. SHED aggregate exosomes shuttled miR-26a promote angiogenesis in pulp regeneration via TGF-β/SMAD2/3 signalling. *Cell Prolif*. 2021;54:e13074.
12. Li B, Xian X, Lin X, et al. Hypoxia alters the proteome profile and enhances the angiogenic potential of dental pulp stem cell-derived exosomes. *Biomol Ther*. 2022;12:575.
13. Lin X, Wang H, Wu T, Zhu Y, Jiang L. Exosomes derived from stem cells from apical papilla promote angiogenesis via miR-126 under hypoxia. *Oral Dis*. 2022;29:3408-3419.
14. Liu P, Qin L, Liu C, et al. Exosomes derived from hypoxia-conditioned stem cells of human deciduous exfoliated teeth enhance angiogenesis via the transfer of let-7f-5p and mir-210-3p. *Front Cell Dev Biol*. 2022;10:879877.
15. Zhou Z, Zheng J, Lin D, Xu R, Chen Y, Hu X. Exosomes derived from dental pulp stem cells accelerate cutaneous wound healing by enhancing angiogenesis via the Cdc42/p38 MAPK pathway. *Int J Mol Med*. 2022;50:1-15.
16. Liu D, Shi B, Zhou W, Tao G. Exosomes from hypoxia-conditioned apical papilla stem cells accelerate angiogenesis in vitro through Notch/JAG1/VEGF signaling. *Tissue Cell*. 2023;84:102197.
17. Shi X, Yang G, Liu M-y, Yuan M-t, Wang D, Wang X-f. Exosomes derived from human dental pulp stem cells increase flap survival with ischemia-reperfusion injuries. *Regen Med*. 2023;18:313-327.
18. Li W, Liu Y, Zhang P, et al. Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces*. 2018;10:5240-5254.
19. Du W, Su L, Zhang N, Wang H. Exosomes derived from preadipocytes improve osteogenic differentiation, potentially via reduced miR-223 expression. *Mol Med Rep*. 2019;19:951-958.

20. Ivica A, Ghayor C, Zehnder M, Valdec S, Weber FE. Pulp-derived exosomes in a fibrin-based regenerative root filling material. *J Clin Med*. 2020;9:491.
21. Li X, Zheng Y, Hou L, et al. Exosomes derived from maxillary BMSCs enhanced the osteogenesis in iliac BMSCs. *Oral Dis*. 2020;26:131-144.
22. Huang C-C, Kang M, Lu Y, et al. Functionally engineered extracellular vesicles improve bone regeneration. *Acta Biomater*. 2020;109:182-194.
23. Jiang S, Xu L. Exosomes from gingival mesenchymal stem cells enhance migration and osteogenic differentiation of pre-osteoblasts. *Die Pharmazie-an Int J Pharmaceut Sci*. 2020;75:576-580.
24. Kang M, Huang C-C, Lu Y, et al. Bone regeneration is mediated by macrophage extracellular vesicles. *Bone*. 2020;141:115627.
25. Liu T, Hu W, Zou X, et al. Human periodontal ligament stem cell-derived exosomes promote bone regeneration by altering microRNA profiles. *Stem Cells Int*. 2020;2020:8852307.
26. Swanson WB, Zhang Z, Xiu K, et al. Scaffolds with controlled release of pro-mineralization exosomes to promote craniofacial bone healing without cell transplantation. *Acta Biomater*. 2020;118:215-232.
27. Wei J, Song Y, Du Z, et al. Exosomes derived from human exfoliated deciduous teeth ameliorate adult bone loss in mice through promoting osteogenesis. *J Mol Histol*. 2020;51:455-466.
28. Wu Z, Pu P, Su Z, Zhang X, Nie L, Chang Y. Schwann cell-derived exosomes promote bone regeneration and repair by enhancing the biological activity of porous Ti6Al4V scaffolds. *Biochem Biophys Res Commun*. 2020;531:559-565.
29. Luo L, Avery SJ, Waddington RJ. Exploring a chemotactic role for EVs from progenitor cell populations of human exfoliated deciduous teeth for promoting migration of naive BMSCs in bone repair process. *Stem Cells Int*. 2021;2021:6681771.
30. Zhang T, Jiang M, Yin X, Yao P, Sun H. Mechanism of exosomes involved in osteoimmunity promoting osseointegration around titanium implants with small-scale topography. *Front Bioeng Biotechnol*. 2021;9:682384.
31. Chen X, Wan Z, Yang L, et al. Exosomes derived from reparative M2-like macrophages prevent bone loss in murine periodontitis models via IL-10 mRNA. *J Nanobiotechnol*. 2022;20:110.
32. Lan Q, Xiao X, Bi X, Gu Y, Ai Y. Effects of periodontal ligament stem cell-derived exosomes on osteoblastic proliferation, migration, differentiation, apoptosis, and signaling pathways. *Oral Dis*. 2022. https://doi.org/10.1111/odi.14375
33. Liu K, Luo X, Lv Z-Y, et al. Macrophage-derived exosomes promote bone mesenchymal stem cells towards osteoblastic fate through microRNA-21a-5p. *Front Bioeng Biotechnol*. 2022;9:801432.
34. Novello S, Tricot-Doleux S, Novella A, Pellen-Mussi P, Jeanne S. Influence of periodontal ligament stem cell-derived conditioned medium on osteoblasts. *Pharmaceutics*. 2022;14:729.
35. Song X, Xue Y, Fan S, Hao J, Deng R. Lipopolysaccharide-activated macrophages regulate the osteogenic differentiation of bone marrow mesenchymal stem cells through exosomes. *PeerJ*. 2022;10:e13442.
36. Zhao Y, Gong Y, Liu X, He J, Zheng B, Liu Y. The experimental study of periodontal ligament stem cells derived exosomes with hydrogel accelerating bone regeneration on alveolar bone defect. *Pharmaceutics*. 2022;14:2189.
37. Chang M, Chen Q, Wang B, Zhang Z, Han G. Exosomes from tension force-applied periodontal ligament cells promote mesenchymal stem cell recruitment by altering microRNA profiles. *Int J Stem Cells*. 2023;16:202-214.
38. He L, Zhou Q, Zhang H, Zhao N, Liao L. PF127 hydrogel-based delivery of exosomal CTNNB1 from mesenchymal stem cells induces osteogenic differentiation during the repair of alveolar bone defects. *Nano*. 2023;13:1083.
39. Lai S, Deng L, Liu C, et al. Bone marrow mesenchymal stem cell-derived exosomes loaded with miR-26a through the novel immunomodulatory peptide DP7-C can promote osteogenesis. *Biotechnol Lett*. 2023;45:905-919.
40. Lee A, Choi J, Shi S, He P, Zhang Q, Le A. DPSC-derived extracellular vesicles promote rat jawbone regeneration. *J Dent Res*. 2023;102:313-321.
41. Seo K, Yoo JH, Kim J, et al. Ginseng-derived exosome-like nanovesicles extracted by sucrose gradient ultracentrifugation to inhibit osteoclast differentiation. *Nanoscale*. 2023;15:5798-5808.
42. Wang J, Qiao Q, Sun Y, et al. Osteogenic differentiation effect of human periodontal ligament stem-cell initial cell density on autologous cells and human bone marrow stromal cells. *Int J Mol Sci*. 2023;24:7133.
43. Xu T, Hua Y, Mei P, Zeng D, Jiang S, Liao C. Black phosphorus thermosensitive hydrogels loaded with bone marrow mesenchymal stem cell-derived exosomes synergistically promote bone tissue defect repair. *J Mater Chem B*. 2023;11:4396-4407.
44. Takeuchi R, Katagiri W, Endo S, Kobayashi T. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. *PLoS One*. 2019;14:e0225472.
45. Sjoqvist S, Kasai Y, Shimura D, et al. Oral keratinocyte-derived exosomes regulate proliferation of fibroblasts and epithelial cells. *Biochem Biophys Res Commun*. 2019;514:706-712.
46. Klimova D, Jakubechova J, Altanerova U, et al. Extracellular vesicles derived from dental mesenchymal stem/stromal cells with gemcitabine as a cargo have an inhibitory effect on the growth of pancreatic carcinoma cell lines in vitro. *Mol Cell Probes*. 2023;67:101894.
47. Zhao Y, Huang Y, Liu H, et al. Macrophages with different polarization phenotypes influence cementoblast mineralization through exosomes. *Stem Cells Int*. 2022;2022:4185972.
48. Li S, Guan X, Yu W, Zhao Z, Sun Y, Bai Y. Effect of human periodontal ligament stem cell-derived exosomes on cementoblast activity. *Oral Dis*. 2023. https://doi.org/10.1111/odi.14671
49. Hu X, Zhong Y, Kong Y, Chen Y, Feng J, Zheng J. Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through TGFβ1/smads signaling pathway via transfer of microRNAs. *Stem Cell Res Ther*. 2019;10:1-14.
50. Swanson WB, Gong T, Zhang Z, et al. Controlled release of odontogenic exosomes from a biodegradable vehicle mediates dentinogenesis as a novel biomimetic pulp capping therapy. *J Control Release*. 2020;324:679-694.
51. Xie L, Guan Z, Zhang M, et al. Exosomal circLPAR1 promoted osteogenic differentiation of homotypic dental pulp stem cells by competitively binding to hsa-miR-31. *Biomed Res Int*. 2020;2020, 6319395.
52. Zhang S, Yang Y, Jia S, et al. Exosome-like vesicles derived from Hertwig's epithelial root sheath cells promote the regeneration of dentin-pulp tissue. *Theranostics*. 2020;10:5914.
53. Zheng J, Kong Y, Hu X, et al. MicroRNA-enriched small extracellular vesicles possess odonto-immunomodulatory properties for modulating the immune response of macrophages and promoting odontogenesis. *Stem Cell Res Ther*. 2020;11:1-14.
54. Zhuang X, Ji L, Jiang H, et al. Exosomes derived from stem cells from the apical papilla promote dentine-pulp complex regeneration by inducing specific dentinogenesis. *Stem Cells Int*. 2020;2020:5816723.
55. Bagio DA, Julianto I, Margono A, Suprastiwi E. Analysis of thrombin-activated platelet-derived exosome (T-Apde) potential for dental pulp regeneration: in-vitro study. *Eur J Dentistry*. 2022;17:173-182.
56. Ganesh V, Seol D, Gomez-Contreras PC, Keen HL, Shin K, Martin JA. Exosome-based cell homing and angiogenic differentiation for dental pulp regeneration. *Int J Mol Sci*. 2022;24:466.

57. Zeng J, He K, Mai R, et al. Exosomes from human umbilical cord mesenchymal stem cells and human dental pulp stem cells ameliorate lipopolysaccharide-induced inflammation in human dental pulp stem cells. *Arch Oral Biol*. 2022;138:105411.
58. Azaryan E, Karbasi S, Saharkhiz M, et al. Effect of HM-exos on the migration and inflammatory response of LPS-exposed dental pulp stem cells. *BMC Oral Health*. 2023;23:95.
59. Shi J, Teo KYW, Zhang S, et al. Mesenchymal stromal cell exosomes enhance dental pulp cell functions and promote pulp-dentin regeneration. *Biomater Biosyst*. 2023;11:100078.
60. Wang S, Xing X, Peng W, et al. Fabrication of an exosome-loaded thermosensitive chitin-based hydrogel for dental pulp regeneration. *J Mater Chem B*. 2023;11:1580-1590.
61. Liu P, Zhang Q, Mi J, et al. Exosomes derived from stem cells of human deciduous exfoliated teeth inhibit angiogenesis in vivo and in vitro via the transfer of miR-100-5p and miR-1246. *Stem Cell Res Ther*. 2022;13:1-17.
62. Lan B-Y, Lin X, Chen W-J, Xie J, Chen W-X. Effect of lipopolysaccharide-stimulated exosomes from human dental pulp stem cells combined with stromal cell-derived factor-1 on dental pulp regeneration. *Zhonghua Kou Qiang Yi Xue Za Zhi= Zhonghua Kouqiang Yixue Zazhi= Chinese J Stomatol*. 2022;57:60-67.
63. Yu S, Chen X, Liu Y, et al. Exosomes derived from stem cells from the apical papilla alleviate inflammation in rat pulpitis by upregulating regulatory T cells. *Int Endod J*. 2022;55:517-530.
64. Jarmalavičiūtė A, Tunaitis V, Pivoraitė U, Venalis A, Pivoriūnas A. Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine–induced apoptosis. *Cytotherapy*. 2015;17:932-939.
65. Venugopal C, Rai KS, Pinnelli VB, Kutty BM, Dhanushkodi A. Neuroprotection by human dental pulp mesenchymal stem cells: from billions to nano. *Curr Gene Ther*. 2018;18:307-323.
66. Ji L, Bao L, Gu Z, et al. Comparison of immunomodulatory properties of exosomes derived from bone marrow mesenchymal stem cells and dental pulp stem cells. *Immunol Res*. 2019;67:432-442.
67. Li J, Ju Y, Liu S, Fu Y, Zhao S. Exosomes derived from lipopolysaccharide-preconditioned human dental pulp stem cells regulate Schwann cell migration and differentiation. *Connect Tissue Res*. 2021;62:277-286.
68. Wang Z, Maruyama K, Sakisaka Y, et al. Cyclic stretch force induces periodontal ligament cells to secrete exosomes that suppress IL-1β production through the inhibition of the NF-κB signaling pathway in macrophages. *Front Immunol*. 2019;10:1310.
69. Zheng Y, Dong C, Yang J, et al. Exosomal microRNA-155-5p from PDLSCs regulated Th17/Treg balance by targeting sirtuin-1 in chronic periodontitis. *J Cell Physiol*. 2019;234:20662-20674.
70. Elashiry M, Elashiry MM, Elsayed R, et al. Dendritic cell derived exosomes loaded with immunoregulatory cargo reprogram local immune responses and inhibit degenerative bone disease in vivo. *J Extracellular Vesic*. 2020;9:1795362.
71. Wang R, Ji Q, Meng C, et al. Role of gingival mesenchymal stem cell exosomes in macrophage polarization under inflammatory conditions. *Int Immunopharmacol*. 2020;81:106030.
72. Nakao Y, Fukuda T, Zhang Q, et al. Exosomes from TNF-α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. *Acta Biomater*. 2021;122:306-324.
73. Zhang Y, Wang Z, Shi B, et al. Effect of gingival mesenchymal stem cell-derived exosomes on inflammatory macrophages in a high-lipid microenvironment. *Int Immunopharmacol*. 2021;94:107455.
74. Hayashi C, Fukuda T, Kawakami K, et al. miR-1260b inhibits periodontal bone loss by targeting ATF6β mediated regulation of ER stress. *Front Cell Dev Biol*. 2022;10:s.
75. Liu C, Hu F, Jiao G, et al. Dental pulp stem cell-derived exosomes suppress M1 macrophage polarization through the ROS-MAPK-NFκB P65 signaling pathway after spinal cord injury. *J Nanobiotechnol*. 2022;20:1-19.
76. Liu Y, Zhang Z, Wang B, et al. Inflammation-stimulated MSC-derived small extracellular vesicle miR-27b-3p regulates macrophages by targeting CSF-1 to promote temporomandibular joint condylar regeneration. *Small*. 2022;18:2107354.
77. Yue C, Cao J, Wong A, et al. Human bone marrow stromal cell exosomes ameliorate periodontitis. *J Dent Res*. 2022;101:1110-1118.
78. Elsayed R, Elashiry M, Tran C, et al. Engineered human dendritic cell exosomes as effective delivery system for immune modulation. *Int J Mol Sci*. 2023;24:11306.
79. Luo H, Chen D, Li R, et al. Genetically engineered CXCR4-modified exosomes for delivery of miR-126 mimics to macrophages alleviate periodontitis. *J Nanobiotechnol*. 2023;21:1-15.
80. Qiao X, Tang J, Dou L, et al. Dental pulp stem cell-derived exosomes regulate anti-inflammatory and osteogenesis in periodontal ligament stem cells and promote the repair of experimental periodontitis in rats. *Int J Nanomedicine*. 2023;31:4683-4703.
81. Han P, Johnson N, Abdal-hay A, Moran CS, Salomon C, Ivanovski S. Effects of periodontal cells-derived extracellular vesicles on mesenchymal stromal cell function. *J Periodontal Res*. 2023;58:1188-1200.
82. Rao F, Zhang D, Fang T, et al. Exosomes from human gingiva-derived mesenchymal stem cells combined with biodegradable chitin conduits promote rat sciatic nerve regeneration. *Stem Cells Int*. 2019;2019:2546367.
83. Sundaram K, Miller DP, Kumar A, et al. Plant-derived exosomal nanoparticles inhibit pathogenicity of Porphyromonas gingivalis. *Iscience*. 2019;21:308-327.
84. Leiva-Sabadini C, Alvarez S, Barrera NP, Schuh CM, Aguayo S. Antibacterial effect of honey-derived exosomes containing antimicrobial peptides against oral streptococci. *Int J Nanomedicine*. 2021;20:4891-4900.
85. Liu X, Muhammed FK, Liu Y. Simvastatin encapsulated in exosomes can enhance its inhibition of relapse after orthodontic tooth movement. *Am J Orthod Dentofacial Orthop*. 2022;162:881-889.
86. Chew JRJ, Chuah SJ, Teo KYW, et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. *Acta Biomater*. 2019;89:252-264.
87. Lv P-y, Gao P-f, Tian G-j, et al. Osteocyte-derived exosomes induced by mechanical strain promote human periodontal ligament stem cell proliferation and osteogenic differentiation via the miR-181b-5p/PTEN/AKT signaling pathway. *Stem Cell Res Ther*. 2020;11:1-15.
88. Shi W, Guo S, Liu L, et al. Small extracellular vesicles from lipopolysaccharide-preconditioned dental follicle cells promote periodontal regeneration in an inflammatory microenvironment. *ACS Biomater Sci Eng*. 2020;6:5797-5810.
89. Wang M, Li J, Ye Y, He S, Song J. SHED-derived conditioned exosomes enhance the osteogenic differentiation of PDLSCs via Wnt and BMP signaling in vitro. *Differentiation*. 2020;111:1-11.
90. Xu X-Y, Tian B-M, Xia Y, et al. Exosomes derived from P2X7 receptor gene-modified cells rescue inflammation-compromised periodontal ligament stem cells from dysfunction. *Stem Cells Transl Med*. 2020;9:1414-1430.
91. Liu L, Guo S, Shi W, et al. Bone marrow mesenchymal stem cell-derived small extracellular vesicles promote periodontal regeneration. *Tissue Eng Part A*. 2021;27:962-976.
92. Wang M, Li J, Ye Y, Chen D, Song J. SHED-derived exosomes improve the repair capacity and osteogenesis potential of hPDLCs. *Oral Dis*. 2023;29:1692-1705.
93. Dai Z, Li Z, Zheng W, et al. Gallic acid ameliorates the inflammatory state of periodontal ligament stem cells and promotes pro-Osteodifferentiation capabilities of inflammatory stem cell-derived exosomes. *Lifestyles*. 2022;12:1392.

94. Liao X-m, Guan Z, Yang Z-j, et al. Comprehensive analysis of M2 macrophage-derived exosomes facilitating osteogenic differentiation of human periodontal ligament stem cells. *BMC Oral Health*. 2022;22:647.
95. Shimizu Y, Takeda-Kawaguchi T, Kuroda I, et al. Exosomes from dental pulp cells attenuate bone loss in mouse experimental periodontitis. *J Periodontal Res*. 2022;57:162-172.
96. Shuo Y, Biao Z, Tian XY, et al. Exosomes derived from human umbilical cord mesenchymal stem cells enhance the osteoblastic differentiation of periodontal ligament stem cells under high glucose conditions through the PI3K/AKT signaling pathway. *Biomed Environ Sci*. 2022;35:811-820.
97. Sun J, Wang Z, Liu P, et al. Exosomes derived from human gingival mesenchymal stem cells attenuate the inflammatory response in periodontal ligament stem cells. *Front Chem*. 2022;10:863364.
98. Cui Y, Hong S, Xia Y, et al. Melatonin engineering M2 macrophage-derived exosomes mediate endoplasmic reticulum stress and immune reprogramming for periodontitis therapy. *Adv Sci*. 2023;10:e2302029.
99. Hu Y, Wang Z, Fan C, et al. Human gingival mesenchymal stem cell-derived exosomes cross-regulate the Wnt/β-catenin and NF-κB signalling pathways in the periodontal inflammation microenvironment. *J Clin Periodontol*. 2023;50:796-806.
100. Liu M, Chen R, Xu Y, Zheng J, Wang M, Wang P. Exosomal miR-141-3p from PDLSCs alleviates high glucose-induced senescence of PDLSCs by activating the KEAP1-NRF2 signaling pathway. *Stem Cells Int*. 2023;2023:7136819.
101. Liu Y, Zeng L, Wang W, et al. Human bone marrow mesenchymal stem cell exosome-derived miR-335-5p promotes osteogenic differentiation of human periodontal ligament stem cells to alleviate periodontitis by downregulating DKK1. *Nan Fang yi ke da xue xue bao = J Southern Med Univer*. 2023;43:420-427.
102. Yu J, Wu X, Zhang W, et al. Effect of psoralen on the regulation of osteogenic differentiation induced by periodontal stem cell-derived exosomes. *Hum Cell*. 2023;1-14.
103. Mohammed E, Khalil E, Sabry D. Effect of adipose-derived stem cells and their exo as adjunctive therapy to nonsurgical periodontal treatment: a histologic and histomorphometric study in rats. *Biomol Ther*. 2018;8:167.
104. Shen Z, Kuang S, Zhang Y, et al. Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice via a macrophage-dependent mechanism. *Bioactive Mater*. 2020;5:1113-1126.
105. Zhang Y, Chen J, Fu H, et al. Exosomes derived from 3D-cultured MSCs improve therapeutic effects in periodontitis and experimental colitis and restore the Th17 cell/Treg balance in inflamed periodontium. *Int J Oral Sci*. 2021;13:43.
106. Lei F, Li M, Lin T, Zhou H, Wang F, Su X. Treatment of inflammatory bone loss in periodontitis by stem cell-derived exosomes. *Acta Biomater*. 2022;141:333-343.
107. Kang L, Miao Y, Jin Y, Shen S, Lin X. Exosomal miR-205-5p derived from periodontal ligament stem cells attenuates the inflammation of chronic periodontitis via targeting XBP1. *Immunity, Inflamm Dis*. 2023;11:e743.
108. Luo P, Jiang C, Ji P, Wang M, Xu J. Exosomes of stem cells from human exfoliated deciduous teeth as an anti-inflammatory agent in temporomandibular joint chondrocytes via miR-100-5p/mTOR. *Stem Cell Res Ther*. 2019;10:1-12.
109. Zhang S, Teo KYW, Chuah SJ, Lai RC, Lim SK, Toh WS. MSC exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis. *Biomaterials*. 2019;200:35-47.
110. Shi Y, Shao J, Zhang Z, Zhang J, Lu H. Effect of condylar chondrocyte exosomes on condylar cartilage osteogenesis in rats under tensile stress. *Front Bioeng Biotechnol*. 2022;10:1061855.
111. Sjöqvist S, Ishikawa T, Shimura D, et al. Exosomes derived from clinical-grade oral mucosal epithelial cell sheets promote wound healing. *J Extracellular Vesicles*. 2019;8:1565264.
112. Lin T, Wu N, Wang L, Zhang R, Pan R, Chen Y-F. Inhibition of chondrocyte apoptosis in a rat model of osteoarthritis by exosomes derived from miR-140-5p-overexpressing human dental pulp stem cells. *Int J Mol Med*. 2021;47:1.
113. Li S, Luo L, He Y, et al. Dental pulp stem cell-derived exosomes alleviate cerebral ischaemia-reperfusion injury through suppressing inflammatory response. *Cell Prolif*. 2021;54:e13093.
114. Fu Y, Cui S, Zhou Y, Qiu L. Dental pulp stem cell-derived exosomes alleviate mice knee osteoarthritis by inhibiting TRPV4-mediated osteoclast activation. *Int J Mol Sci*. 2023;24:4926.
115. Hu S, Chen B, Zhou J, et al. Dental pulp stem cell-derived exosomes revitalize salivary gland epithelial cell function in NOD mice via the GPER-mediated cAMP/PKA/CREB signaling pathway. *J Transl Med*. 2023;21:1-16.
116. Pivoraitė U, Jarmalavičiūtė A, Tunaitis V, et al. Exosomes from human dental pulp stem cells suppress carrageenan-induced acute inflammation in mice. *Inflammation*. 2015;38:1933-1941.
117. Zhang Y, Shi S, Xu Q, Zhang Q, Shanti R, Le A. SIS-ECM laden with GMSC-derived exosomes promote taste bud regeneration. *J Dent Res*. 2019;98:225-233.
118. Yue Y, Shuo T. Effects of human periodontal ligament fibroblast-derived exosomes on reducing rat root resorption after delayed tooth replantation. *Shanghai J Stomatol*. 2022;31:232.
119. Machida T, Tomofuji T, Ekuni D, et al. MicroRNAs in salivary exosome as potential biomarkers of aging. *Int J Mol Sci*. 2015;16:21294-21309.
120. Seelan RS, Pisano MM, Greene RM. MicroRNAs as biomarkers for birth defects. *MicroRNA*. 2022;11:2-11.
121. Abdul Rehman S, Khurshid Z, Hussain Niazi F, et al. Role of salivary biomarkers in detection of cardiovascular diseases (CVD). *Proteome*. 2017;5:21.
122. Li J, Guo Y, Chen YY, et al. miR-124-3p increases in high glucose induced osteocyte-derived exosomes and regulates galectin-3 expression: a possible mechanism in bone remodeling alteration in diabetic periodontitis. *FASEB J*. 2020;34:14234-14249.
123. Monteiro LJ, Varas-Godoy M, Monckeberg M, et al. Oral extracellular vesicles in early pregnancy can identify patients at risk of developing gestational diabetes mellitus. *PLoS One*. 2019;14:e0218616.
124. Pei F, Wang M, Wang Y, et al. Quantitative proteomic analysis of gingival crevicular fluids to identify novel biomarkers of gingival recession in orthodontic patients. *J Proteome*. 2022;266:104647.
125. Han P, Lai A, Salomon C, Ivanovski S. Detection of salivary small extracellular vesicles associated inflammatory cytokines gene methylation in gingivitis. *Int J Mol Sci*. 2020;21:5273.
126. Zheng X, Chen F, Zhang Q, et al. Salivary exosomal PSMA7: a promising biomarker of inflammatory bowel disease. *Protein Cell*. 2017;8:686-695.
127. Tatullo M, Codispoti B, Spagnuolo G, Zavan B. Human periapical cyst-derived stem cells can be a smart "lab-on-a-cell" to investigate neurodegenerative diseases and the related alteration of the exosomes' content. *Brain Sci*. 2019;9:358.
128. Byun JS, Hong SH, Choi JK, Jung JK, Lee HJ. Diagnostic profiling of salivary exosomal micro RNA s in oral lichen planus patients. *Oral Dis*. 2015;21:987-993.
129. Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Salo T, Vered M. Morphological and molecular features of oral fluid-derived exosomes: oral cancer patients versus healthy individuals. *J Cancer Res Clin Oncol*. 2016;142:101-110.
130. Momen-Heravi F, Bala S. Extracellular vesicles in oral squamous carcinoma carry oncogenic miRNA profile and reprogram monocytes via NF-κB pathway. *Oncotarget*. 2018;9:34838.
131. Hunsaker M, Barba G, Kingsley K, Howard KM. Differential microRNA expression of miR-21 and miR-155 within oral cancer extracellular vesicles in response to melatonin. *Dentistry J*. 2019;7:48.

132. Zlotogorski-Hurvitz A, Dekel BZ, Malonek D, Yahalom R, Vered M. FTIR-based spectrum of salivary exosomes coupled with computational-aided discriminating analysis in the diagnosis of oral cancer. *J Cancer Res Clin Oncol*. 2019;145:685-694.
133. Coon J, Kingsley K, Howard KM. miR-365 (microRNA): potential biomarker in oral squamous cell carcinoma exosomes and extracellular vesicles. *Int J Mol Sci*. 2020;21:5317.
134. Jin J, Huang Z, Lu X, et al. Bioinformatics analysis of aberrantly expressed exosomal lncRNAs in oral squamous cell carcinoma (CAL-27 vs. oral epithelial) cells. *Oncol Lett*. 2020;20:2378-2386.
135. Masaoka T, Shinozuka K, Ohara K, Tsuda H, Imai K, Tonogi M. Bioinformatics analysis of dysregulated exosomal microRNAs derived from oral squamous cell carcinoma cells. *J Oral Sci*. 2021;63:174-178.
136. Faur CI, Roman RC, Jurj A, et al. Salivary exosomal MicroRNA-486-5p and MicroRNA-10b-5p in oral and oropharyngeal squamous cell carcinoma. *Medicina*. 2022;58:1478.
137. Jayaseelan VP, Arumugam P. Exosomal microRNAs targeting TP53 gene as promising prognostic markers for head and neck squamous cell carcinoma. *Global Med Genet*. 2022;9:277-286.
138. Faur CI, Dinu C, Toma V, et al. A new detection method of oral and oropharyngeal squamous cell carcinoma based on multivariate analysis of surface enhanced Raman spectra of salivary exosomes. *J Personalized Med*. 2023;13:762.
139. Ludwig N, Yerneni SS, Harasymczuk M, et al. TGFβ carrying exosomes in plasma: potential biomarkers of cancer progression in patients with head and neck squamous cell carcinoma. *Br J Cancer*. 2023;128:1733-1741.
140. Wang W-Z, Cao X, Bian L, et al. Analysis of mRNA-miRNA interaction network reveals the role of CAFs-derived exosomes in the immune regulation of oral squamous cell carcinoma. *BMC Cancer*. 2023;23:1-14.
141. Wang Z, Li F, Rufo J, et al. Acoustofluidic salivary exosome isolation: a liquid biopsy compatible approach for human papillomavirus–associated oropharyngeal cancer detection. *J Mol Diagn*. 2020;22:50-59.
142. Rody WJ Jr, Holliday LS, McHugh KP, Wallet SM, Spicer V, Krokhin O. Mass spectrometry analysis of gingival crevicular fluid in the presence of external root resorption. *Am J Orthod Dentofacial Orthop*. 2014;145:787-798.
143. Atsawasuwan P, Lazari P, Chen Y, Zhou X, Viana G, Evans CA. Secretory microRNA-29 expression in gingival crevicular fluid during orthodontic tooth movement. *PLoS One*. 2018;13:e0194238.
144. Holliday LS, Truzman E, Zuo J, Han G, Torres-Medina R, Rody WJ Jr. Extracellular vesicle identification in tooth movement models. *Orthod Craniofac Res*. 2019;22:101-106.
145. Rody WJ Jr, Truzman EL, Foster DT, et al. Clastic cells in orthodontic treatment: translational challenges and recent advances. *Orthod Craniofac Res*. 2019;22:180-185.
146. Rody WJ Jr, Reuter NG, Brooks SE, et al. Metabolomic signatures distinguish extracellular vesicles from osteoclasts and odontoclasts. *Orthod Craniofac Res*. 2023;26:632-641.
147. Lau C, Kim Y, Chia D, et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development. *J Biol Chem*. 2013;288:26888-26897.
148. Machida T, Tomofuji T, Maruyama T, et al. miR-1246 and miR-4644 in salivary exosome as potential biomarkers for pancreatobiliary tract cancer. *Oncol Rep*. 2016;36:2375-2381.
149. Tomofuji T, Yoneda T, Machida T, et al. Micro RNA s as serum biomarkers for periodontitis. *J Clin Periodontol*. 2016;43:418-425.
150. Huang X, Hu X, Zhao M, Zhang Q. Analysis of salivary exosomal proteins in young adults with severe periodontitis. *Oral Dis*. 2020;26:173-181.
151. Tobón-Arroyave SI, Celis-Mejía N, Córdoba-Hidalgo MP, Isaza-Guzmán DM. Decreased salivary concentration of CD 9 and CD 81 exosome-related tetraspanins may be associated with the periodontal clinical status. *J Clin Periodontol*. 2019;46:470-480.
152. Yu J, Lin Y, Xiong X, et al. Detection of exosomal PD-L1 RNA in saliva of patients with periodontitis. *Front Genet*. 2019;10:202.
153. Chaparro Padilla A, Weber Aracena L, Realini Fuentes O, et al. Molecular signatures of extracellular vesicles in oral fluids of periodontitis patients. *Oral Dis*. 2020;26:1318-1325.
154. Han P, Bartold PM, Salomon C, Ivanovski S. Salivary small extracellular vesicles associated miRNAs in periodontal status—a pilot study. *Int J Mol Sci*. 2020;21:2809.
155. Nik Mohamed Kamal NNS, Awang RAR, Mohamad S, Shahidan WNS. Plasma-and saliva exosome profile reveals a distinct MicroRNA signature in chronic periodontitis. *Front Physiol*. 2020;11:587381.
156. Han P, Bartold PM, Salomon C, Ivanovski S. Salivary outer membrane vesicles and DNA methylation of small extracellular vesicles as biomarkers for periodontal status: a pilot study. *Int J Mol Sci*. 2021;22:2423.
157. Xia Y, Zhou K, Sun M, Shu R, Qian J, Xie Y. The miR-223-3p regulates pyroptosis through NLRP3-caspase 1-GSDMD signal axis in periodontitis. *Inflammation*. 2021;44:2531-2542.
158. Byun J-S, Lee HY, Tian J, et al. Effect of salivary exosomal mir-25-3p on periodontitis with insulin resistance. *Front Immunol*. 2022;12:775046.
159. Bachtiar BM, Bachtiar EW, Kusumaningrum A, et al. Porphyromonas gingivalis association with inflammatory markers and exosomal miRNA-155 in saliva of periodontitis patients with and without diabetes diagnosed with COVID-19. *Saudi Dental J*. 2023;35:61-69.
160. Han P, Jiao K, Moran CS, et al. TNF-αand OSX mRNA of salivary small extracellular vesicles in periodontitis: a pilot study. *Tissue Eng*. 2023;298-306.
161. Kwon EJ, Kim HJ, Woo BH, Joo JY, Kim YH, Park HR. Profiling of plasma-derived exosomal RNA expression in patients with periodontitis: a pilot study. *Oral Dis*. 2023;29:1726-1737.
162. Yamaguchi A, Tsuruya Y, Igarashi K, et al. Changes in the components of salivary exosomes due to initial periodontal therapy. *J Periodontal Implant Sci*. 2023;53:347-361.
163. Chaparro A, Atria P, Realini O, et al. Diagnostic potential of periimplant crevicular fluid microRNA-21-3p and microRNA-150-5p and extracellular vesicles in peri-implant diseases. *J Periodontol*. 2021;92:e11-e21.
164. Yamashiro K, Hamada T, Mori K, et al. Exosome-derived microRNAs from mouthrinse have the potential to be novel biomarkers for sjögren syndrome. *J Personalized Med*. 2022;12:1483.
165. Han Y, Jia L, Zheng Y, Li W. Salivary exosomes: emerging roles in systemic disease. *Int J Biol Sci*. 2018;14:633.
166. Jalalian SH, Ramezani M, Jalalian SA, Abnous K, Taghdisi SM. Exosomes, new biomarkers in early cancer detection. *Anal Biochem*. 2019;571:1-13.
167. Soung YH, Ford S, Zhang V, Chung J. Exosomes in cancer diagnostics. *Cancer*. 2017;9:8.

How to cite this article: Miron RJ, Estrin NE, Sculean A, Zhang Y. Understanding exosomes: Part 3—therapeutic + diagnostic potential in dentistry. *Periodontol 2000*. 2024;94:415-482. doi:10.1111/prd.12557