

REVIEW

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Extracellular vesicles as therapeutic tools in regenerative dentistry

Evelyn Jingwen Xia^{1†}, Shasha Zou^{2†}, Xiu Zhao³, Wei Liu³, Yang Zhang^{1,4*}  and Irene Shuping Zhao^{1*}

Abstract

Dental and maxillofacial diseases are always accompanied by complicated hard and soft tissue defects, involving bone, teeth, blood vessels and nerves, which are difficult to repair and severely affect the life quality of patients. Recently, extracellular vesicles (EVs) secreted by all types of cells and extracted from body fluids have gained more attention as potential solutions for tissue regeneration due to their special physiological characteristics and intrinsic signaling molecules. Compared to stem cells, EVs present lower immunogenicity and tumorigenicity, cause fewer ethical problems, and have higher stability. Thus, EV therapy may have a broad clinical application in regenerative dentistry. Herein, we reviewed the currently available literature regarding the functional roles of EVs in oral and maxillofacial tissue regeneration, including in maxilla and mandible bone, periodontal tissues, temporomandibular joint cartilage, dental hard tissues, peripheral nerves and soft tissues. We also summarized the underlying mechanisms of actions of EVs and their delivery strategies for dental tissue regeneration. This review would provide helpful guidelines and valuable insights into the emerging potential of EVs in future research and clinical applications in regenerative dentistry.

Keywords Extracellular vesicles, Exosomes, Dentistry, Dental tissue, Tissue regeneration

Introduction

The high prevalence of damage or loss in dental and maxillofacial tissue has garnered global attention, as it greatly impacts the quality of life of patients and imposes a substantial financial burden on society [1]. Exogenous transplantation or the use of maxillofacial prostheses are currently the most common treatments for repairing

dental and maxillofacial tissue [2]. However, these therapies only serve to halt disease progression and are unable to fully restore the normal physiological structure and function [3]. As a result, there is a pressing need for new treatments that can achieve genuine regeneration of dental and maxillofacial tissues.

Regenerative medicine has emerged as a promising approach to replace repaired tissue to restore normal biological functions and reduce the reliance on transplantation [4]. In particular, mesenchymal stem cells (MSCs) have shown significant potential in this field by animal and clinical studies [5]. MSCs possess remarkable abilities for self-renewal, multilineage differentiation, and robust immunomodulation, making them pivotal players in tissue regeneration [6]. However, their use has been restricted in the clinic due to concerns regarding uncontrollability and potential transformation risks, underscoring the need for alternative cell-free therapies [7]. Recent findings have shed light on the fact that MSCs primarily exert their effects through the secretion of cytokines or

[†]Evelyn Jingwen Xia and Shasha Zou contributed equally to this article.

*Correspondence:

Yang Zhang
yang.zhang@szu.edu.cn
Irene Shuping Zhao
zhao110@szu.edu.cn

¹ School of Dentistry, Shenzhen University Medical School, 1088 Xueyuan Ave, Shenzhen 518015, China

² Longgang Center for Chronic Disease Control, Shenzhen 518172, China

³ Department of Stomatology, Shenzhen University General Hospital, Shenzhen 518015, China

⁴ School of Biomedical Engineering, Shenzhen University Medical School, Shenzhen 518015, China



membranous vesicles. These secreted substances regulate the microenvironment surrounding damaged tissues and orchestrate subsequent regeneration processes via paracrine signaling [8].

Extracellular vesicles (EVs) were recently revealed as the primary component of paracrine signals of cells [9]. They constitute a heterogeneous group of cell secretomes and are secreted by almost all cell types. Based on the size, EVs can be classified into three subtypes: microvesicles, exosomes, and apoptotic bodies [10]. The International Society for Extracellular Vesicles has collectively termed these subtypes as “EVs” [11]. Compared to cells, EVs are non-replicable and exhibit lower immunogenicity and improved biocompatibility [12]. They play a crucial role in promoting the proliferation and differentiation of targeted cells and regulating the entire process of tissue regeneration [13, 14]. Previous studies have demonstrated that EVs can aid their parent cells in performing physiological functions [15]. Subsequent investigations have further explored the functions and underlying mechanisms of EVs [16], and shown that EVs can activate specific signaling pathways to facilitate cellular communication through their unique contents, including proteins, nucleic acids, and signaling peptides [17].

Compared to other organs, the oral cavity has direct communication with the external environment, which provides a favorable condition for the implantation of EVs. This also avoids the issue of EVs traversing the circulatory system, thereby reducing any residual or cumulative effects in non-treated areas. Consequently, the application of exogenous EVs in dental regenerative medicine has been extensively studied and has shown promising treatment effects [18]. Therefore, EVs, with their non-mutating and non-duplicating characteristics, are considered promising tools for dental tissue regeneration [19].

In this review, we examine the current literature (Fig. 1), regarding the functional roles of EVs in oral and maxillofacial tissue regeneration, including their impact on maxilla and mandible bone, periodontal tissues, temporomandibular joint cartilage, dental hard tissues, peripheral nerves, and soft tissues. Additionally, we summarize the underlying mode of actions of EVs (Fig. 2) and discuss their delivery strategies in the applications of regenerative dentistry. The existing challenges and the prospect of the future for EVs in dentistry regeneration are also discussed. This literature search was conducted in three databases (PubMed, Scopus and Google Scholar). English publications were searched using the keywords ((extracellular vesicles) OR (EVs) OR (exosomes) AND (dentistry regeneration) OR (dental tissue regeneration) OR (oral tissue regeneration)). After reviewing the titles and abstracts, 60 selected publications with full texts

were selected for detailed analysis. 3 research articles were duplicated in the part of mechanism of EV-mediated dentistry regeneration.

Origin of EVs and their roles

EVs are nanoscale membrane vesicles and secreted by nearly all types of cells [20]. They are formed through the inward budding of multivesicular bodies that originate from late endosomal membrane invagination. These vesicles are subsequently released into the extracellular microenvironment by fusing with the plasma membrane [21]. During the process of EV formation, specific proteins, lipids, and nucleic acids are selectively recruited and encapsulated, granting EVs the ability to mediate paracrine crosstalk [22]. These proteins include adapter protein ALIX, endosome-related protein TSG101, and the transmembrane proteins CD9, CD63, and CD81 [23, 24]. The lipid bilayer of EVs typically comprises cholesterol, sphingomyelin, and phosphatidylserine, which significantly contribute to EV formation and their interaction with target cells [25, 26].

EVs interact with target cells through three main mechanisms, offering various avenues for studying signal pathways and therapeutic targets in different diseases [27]. Firstly, EVs engage in information transmission through receptor-ligand interactions, even without direct cell contact [15]. Secondly, EVs enhance cell adhesion properties by binding to the target cell membranes [28]. Lastly, EVs can fuse with the target cell membrane, delivering their contents into the cytoplasm and exerting biological effects [29]. The specific mechanisms of interaction depend on the composition and properties of EVs, as well as the characteristics of their parent cells [30]. Once released into the microenvironment, EVs transport their bioactive cargoes to specific cells, triggering a cascade of signaling pathways. The majority of EV components consist of proteins and nucleic acids, including DNA, mRNA, miRNA, tRNA, and non-coding RNA. While miRNA has been the focus of significant EV research due to its functional roles, more recent studies have indicated that proteins in EVs, rather than miRNA, play more critical roles in cell-cell communication.

Dental tissue-derived EVs

The oral cavity constitutes a multifaceted environment encompassing diverse tissues, including jaws, periodontium, gingiva, teeth, oral mucosa, and glands. Saliva and gingival crevicular fluid create the fluid milieu within the oral cavity. In addition, various coatings envelop these tissues, each harboring an array of bacteria, collectively forming the bacterial biofilm [31]. All cells from these tissues and bacteria can secrete EVs to participate in the dental tissue development. More importantly, these EVs

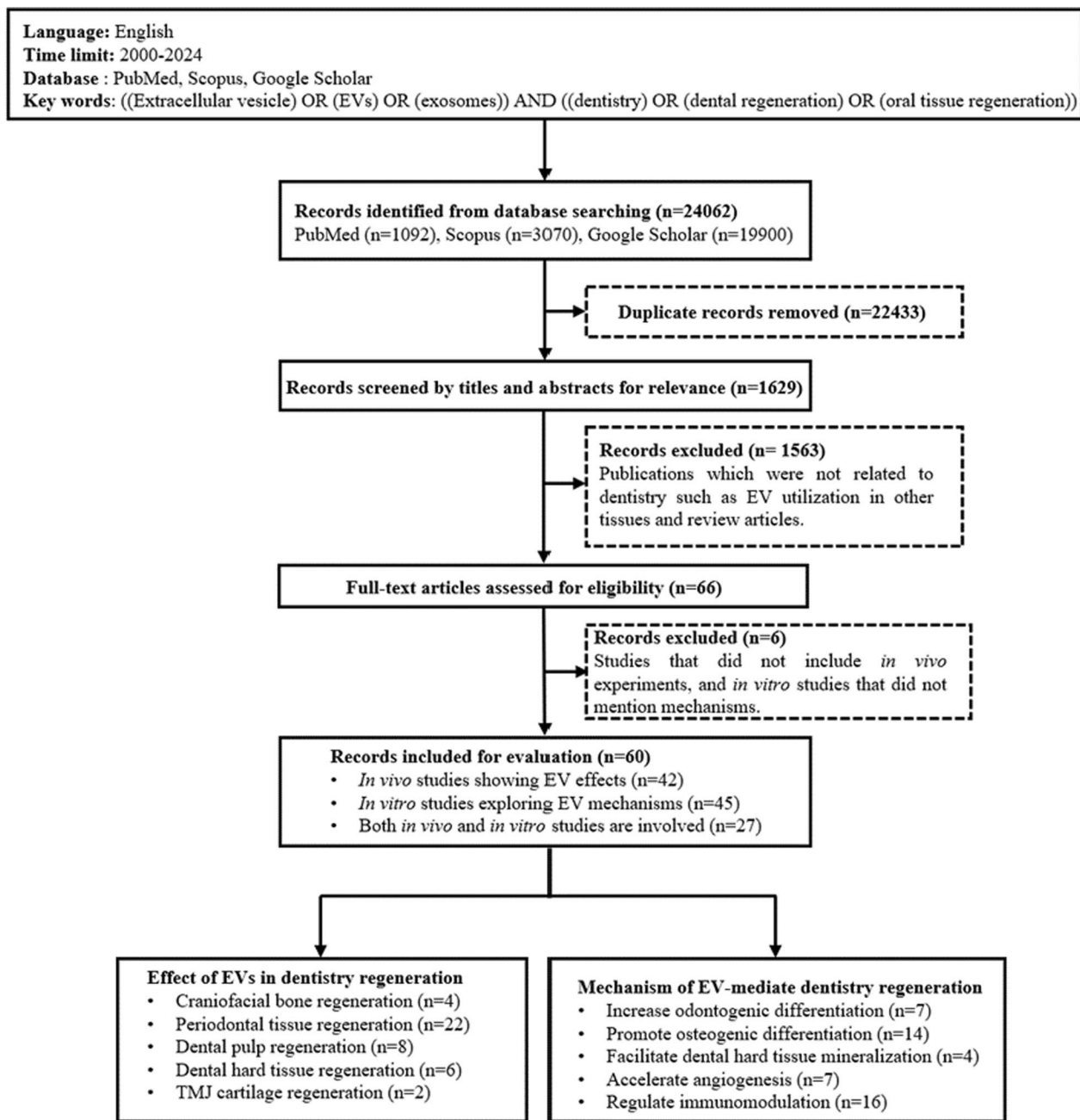


Fig. 1 Flow chart of literature search for EVs in regenerative dentistry

shape the ecological environment of the oral cavity and oral environment in turn affect the stability and bioactivity of these EVs. However, although all EVs play certain roles in the dental tissue development, EVs from stem cells derived from different dental tissues are mostly studied and utilized for dental tissue regeneration due to their multi-lineage differentiation and reproductive activity. These stem cells include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), dental follicle

progenitor cells (DFCs), gingival mesenchymal stem cells (GMSCs), stem cells from the apical papilla (SCAPs), alveolar bone-derived mesenchymal stem cells (ABMSCs) and stem cells from exfoliated deciduous teeth (SHEDs) (Fig. 3). They were utilized in different oral tissue regeneration according to their specific regenerative characteristics.

Notably, EVs sourced from DPSCs (DPSC-EVs) have garnered significant attention in the field of dentistry

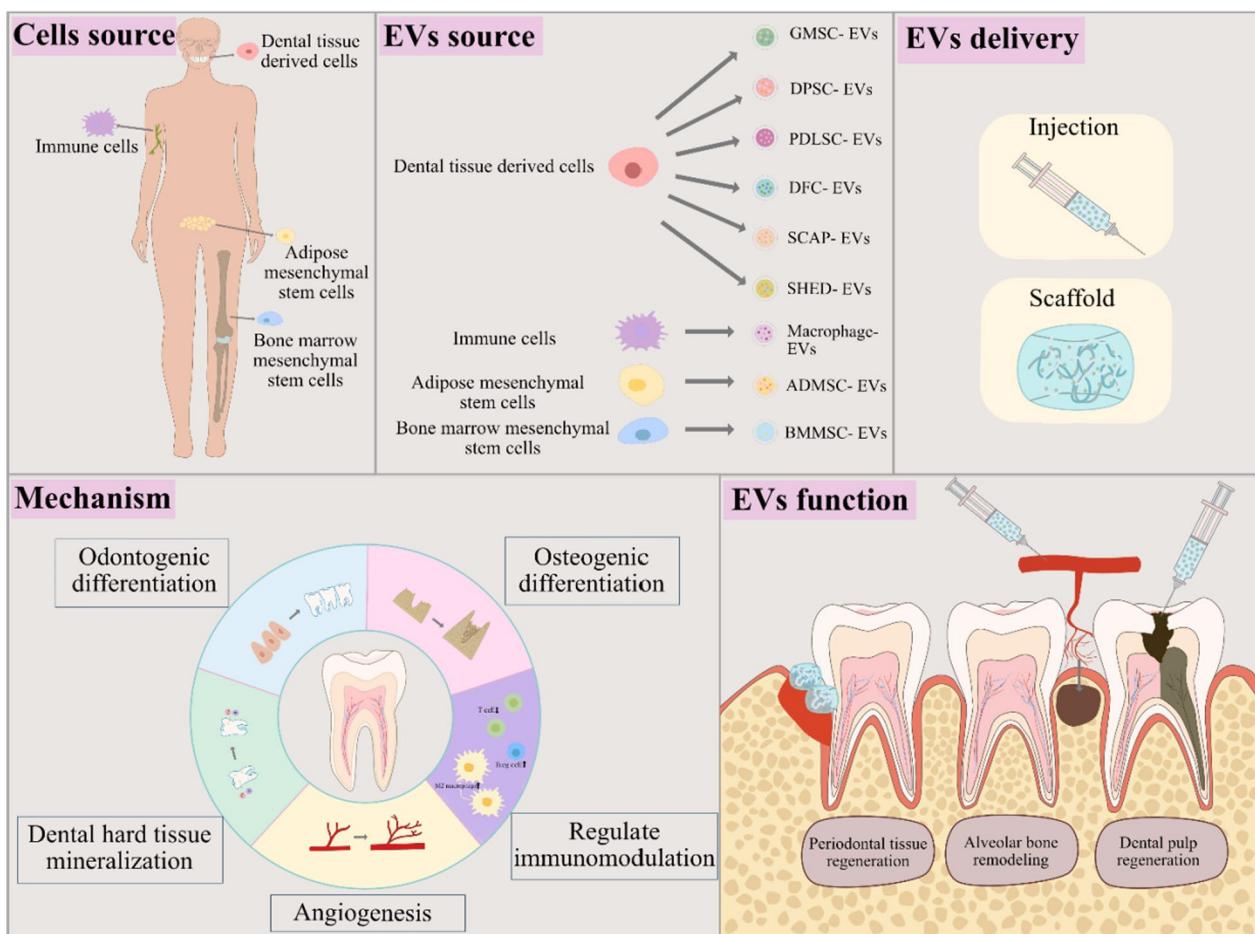


Fig. 2 EVs parent cells source, EVs source, delivery strategies, functions and mechanisms of EVs in dental tissue regeneration. Abbreviations: EVs, extracellular vesicles; GMSC-EVs, gingival mesenchymal stem cell-derived extracellular vesicles; DPSC-EVs, dental pulp stem cell-derived extracellular vesicles; PDLSC-EVs, periodontal ligament stem cell-derived extracellular vesicles; DFC-EVs, dental follicle cell-derived extracellular vesicles; SCAP-EVs, stem cells from apical papilla-derived extracellular vesicles; SHED-EVs, human exfoliated deciduous teeth stem cell-derived extracellular vesicles; Macrophage-EVs, macrophage derived extracellular vesicles; ADMSC-EVs, adipose mesenchymal stem cell-derived extracellular vesicles; BMMSC-EVs, bone marrow mesenchymal stem cell-derived extracellular vesicles

regeneration owing to their remarkable osteo/odonto-inductive capabilities [32, 33]. Furthermore, DPSC-EVs have exhibited enhanced anti-necrotic, immunomodulatory, and anti-apoptotic properties compared to EVs derived from bone marrow mesenchymal stem cells (BMMSC-EVs) [34]. On the other hand, EVs originating from PDLSCs (PDLSC-EVs) have been demonstrated to upregulate the expression of CD31 and VEGFA to promote angiogenesis. Additionally, they fortify osteogenesis through the regulation of insulin, AMPK, and MAPK signaling pathways, while also modulating the Th17/Treg balance to bolster anti-inflammatory capabilities [35–38]. GMSC-EVs and ABMSC-EVs have also emerged as significant contributors to bone regeneration. They exhibit anti-osteoclastogenic activity and convey miR-1260 to inhibit inflammatory bone loss [39, 40]. Furthermore,

when combined with a small intestinal submucosa-extracellular matrix, GMSC-EVs facilitate tongue lingual papillae repair and promote the recovery of taste buds [41]. Moreover, EVs derived from SCAPs (SCAP-EVs) hold great promise for dentistry regeneration. They enhance dentinogenesis of BMMSCs and are considered potential candidates for dentin-pulp regeneration [35]. Meanwhile, EVs sourced from SHEDs (SHED-EVs) effectively mitigate inflammation in temporomandibular joint diseases.

Effect of EVs in regenerative dentistry

Compared to tissues such as liver, skin, and muscle, oral tissues are generally constantly exposed to microorganisms from food, drink, and the oral microbiome and have limited blood supply during the regeneration process.

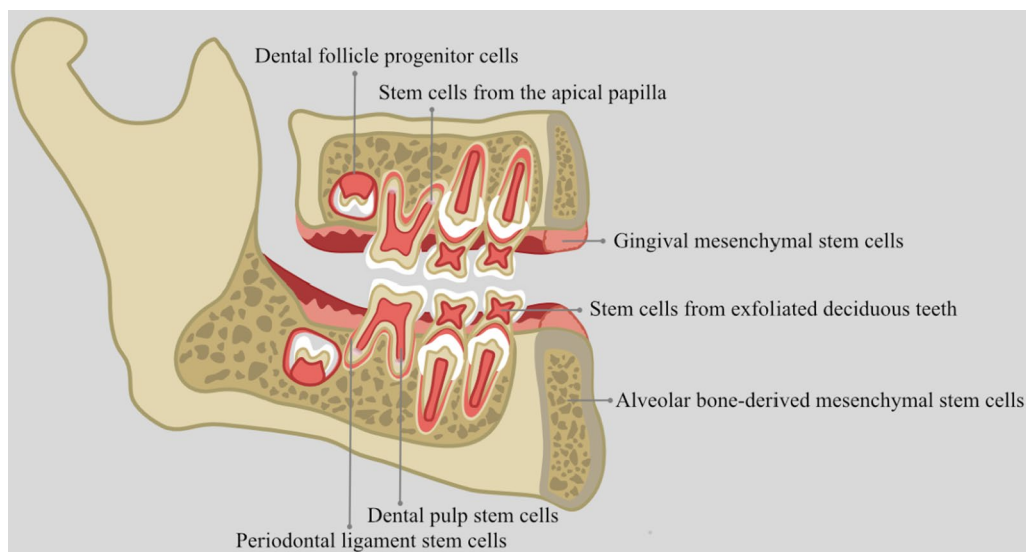


Fig. 3 EVs from different dental tissue-derived stem cells used for dental tissue regeneration. EVs from different dental tissue-derived stem cells are mostly studied and utilized for dental tissue regeneration due to their multi-lineage differentiation and reproductive activity. EVs from different dental tissue-derived stem cells are mostly studied and utilized for dental tissue regeneration due to their multi-lineage differentiation and reproductive activity. Dental follicle progenitor cells are sourced from the connective tissue surrounding the developing tooth germ. Stem cells from the apical papilla are obtained from the apical papilla of incompletely developed teeth. Gingival mesenchymal stem cells are found within the gingiva. Stem cells from exfoliated deciduous teeth are harvested from the dental pulp of exfoliated primary teeth. Alveolar bone-derived mesenchymal stem cells can be extracted from the alveolar bone. Dental pulp stem cells are isolated from the dental pulp of permanent teeth. Periodontal ligament stem cells are sourced from the periodontal ligament of permanent teeth

This greatly affect their ability to heal efficiently and makes them more vulnerable to infections and inflammation. Numerous studies have highlighted that EVs derived from various cells, particularly stem cells, exhibit beneficial effects such as pro-regenerative, pro-vascularization, anti-inflammatory, and anti-apoptotic properties, irrespective of their distinctiveness from different sources [42]. These minuscule vesicles have exhibited the capacity to regenerate bone, dental tissues, and cartilage, rendering them promising therapeutic agents in the field of dental tissue regeneration [43] (Table 1)(Fig. 4).

Maxillofacial tissue regeneration

Maxillofacial diseases, such as congenital cleft palate, functional mandibular reconstruction, and conditions like odontogenic osteomyelitis or tumors, necessitate precise repair and functional restoration of the affected areas [44]. Noteworthy studies have demonstrated the efficacy of EVs in addressing these challenges [45]. For instance, in a model of bisphosphonate-related osteonecrosis of the jaw (BRONJ), the introduction of EVs derived from adipose mesenchymal stem cells (ADMSC-EVs) through tail vein injection in rats led to the formation of new jawbone and improvements in bone structure parameters [46]. BMMS-C-EVs showcased preventive properties against the spread of chronic inflammation associated with aging cells. They further promoted

osteogenesis and angiogenesis, effectively averting the occurrence of BRONJ [47]. DPSC-EVs implanted in a rat mandibular bone defect area also exhibited heightened jawbone density and facilitated the formation of new jawbone [48].

In addition to hard tissue regeneration, EVs have demonstrated promise in maxillofacial soft tissue regeneration. GMSC-EVs, when combined with small intestinal submucosa extracellular matrix, were implanted in a rat critical-sized tongue defect site, resulting in the regeneration of epithelial cells and the restoration of taste buds and lingual papilla [41]. Moreover, EVs derived from hair follicle epidermal neural crest stem cells, in conjunction with acellular nerve allografts, were employed to bridge facial nerve defects. This intervention led to thicker myelination and robust remyelination [49]. Additionally, SCAP-EVs enhanced angiogenesis and vascularization in a rat hard palate mucosa defect model [50].

Periodontal regeneration

The periodontium, encompassing the gingiva, periodontal ligament, and alveolar bone, serves as the structural support for teeth [51]. Periodontitis, a widespread global issue, is characterized by the progressive deterioration of the periodontium and inflammation [52]. The ultimate objective of periodontal tissue regeneration is to foster the development of new periodontal bone,

Table 1 Effect of different EVs on dental tissue regeneration

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
Maxillofacial tissue	ADMSCs	Intravenous injection	Rat BRONJ model	1 µg/kg	After treatment with ADMSC-EVs, bone parameters, including BV/TV, Tb-N, Tb-Th were increased, whereas Tb-Sp declined. Moreover, fewer necrotic bones but more osteoclasts were found in the EVs group. There were also more collagen and vessels in the EVs group.	[45]
	BMMSCs	Intravenous injection	Rat BRONJ model	30 µg	In a rat BRONJ model, some genes in maxillary, such as p21 and pRb were upregulated. Moreover, osteoclasts in the EVs group were not apoptosis, which showed opposite results in the control group.	[46]
	BMMSCs	Injection for each limb	Rat proximal tibial condyles	19.2 µg	In a rabbit proximal tibial condyles implanting EVs model for three days, the bone density was higher by 47.2% than that in control groups on average.	[47]
	DPSCs	Collagen	Rat jaw-bone defect model	100 µg	In a rat mandibula defect model, rats treated with DPSC-EVs showed an increase in bone mineral density, and the thickness of the buccal-lingual increased as well.	[48]
	SCAPs	Injection locally	Mouse palatal gingiva wound healing model	40 µg	In the defect area of mice gingiva, after injecting SCAP-EVs, it formed newly epidermis and connective tissues, with high expressions of COL1 and fibronectin. Furthermore, there formed new blood vessels in the defect area.	[50]
	GMSCs	SIS-ECM laden with EVs	Rat critical-sized tongue defect model	Not mentioned	In a rat critical-sized tongue defect model, rat treated with SIS-ECM laden with GMSC-EVs facilitate recovery of epithelial layers and the regeneration of taste buds.	[41]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
	EPH-NCSs	Not mentioned	Rat FNI model	Not mentioned	The FNI rat recovered eyelid-buccal synkinesis, and the group treated with EPH-NCSs showed higher amplitude during the blink reflex in the rats' left buccal territory.	[49]
Periodontal tissue Dental-pulp	ADMSCs	Injection locally	Rat periodontitis model	80–150 µg	In a rat periodontitis model, rat injected ADMSC-EVs showed cementum, periodontal fibers and alveolar bone formation. Moreover, newly formed alveolar bone showed various reversal lines and new osteoid tissue.	[57]
	ADMSCs	PLGA/pDA	Rat subcutaneous transplantation with the tooth root slice model	Not mentioned	Periodontitis rats treated with ADMSC-EVs showed a few high-density spots and bone nodules on the edge of the defect area. Meanwhile, osteogenesis related genes such as ALP, RUNX2, OCN were upregulated.	[58]
	BMMSCs	Collagen	Rat periodontitis model	40 mg EVs/sponge	Periodontitis rats treated with BMMSC-EVs showed oriented periodontal fibers span between the newly-formed bone and root surface, and higher BV/TV was seen compared with the other groups.	[54]
	BMMSCs	Hydrogel	Rat periodontitis model	500 µg/ml	In a rat periodontitis model, rats treated with BMMSC-EVs on hydrogel had fewer TRAP positive cells with lower OPG/RANKL ratio, and fibers destruction and alveolar bone loss were lower as well.	[55]
	DPSCs	Chitosan hydrogel	Mouse periodontitis model	50 µg	Mice treated with DPSC-EVs showed a lower alveolar bone loss in experiment groups. Additionally, it showed the thicker epithelial tissue layers on periodontal bone.	[61]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
	DPSCs	Injection locally	Mouse periodontitis model	1 million cells secretory EVs	In a mouse periodontitis model, injecting 3D-cultured EVs arose more alveolar bone and less inflammatory cells, which occurred less osteoclasts in the periodontium. In addition, proinflammatory genes including IL-1α, IL-1 β, Ccl12, TNF were downregulated.	[62]
	DPSCs	Injection locally	Mouse periodontitis model	75 million particles	In a mouse periodontitis model, EVs-treated group showed the least alveolar bone loss, while TRAP staining showed EVs inhibited osteoclast-like colonies.	[60]
	DPSCs	Gelatin	Mouse subcutaneous transplantation with the tooth root slice model	250 μl/scaffold	In contrast with the control group, more dentin and collagen were observed. Moreover, the account of DMP positive cells was higher.	[97]
	DFCs	Collagen	Rat periodontitis model	Not mentioned	New bone formation and newly bone showed denser outcomes compared to other groups. In addition, the higher thickness of trabecular was seen in the EVs-treatment group.	[64]
	DFCs (LPS-preconditioned)	Hydrogel	Rat periodontitis model	500 μg/ml	After treated DFC-EVs, the distance from the cementoamel junction to the alveolar bone crest was shortened significantly, with obviously alveolar bone formation and periodontal tissue arranged in a dense.	[72]
	DFCs	Collagen	Mouse periodontitis model	40 μg	Rats treated with DFC-EVs had increased BV/TV, with more trabecular bone continuously and completely. Moreover, more staining for OCN, OPN were found and more osteogenesis-gene expressed (such as ALP, MMP-2).	[65]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
	DFCs (LPS-preconditioned)	HA	Dog periodontitis model	200 µg	In a dog periodontitis model, HA carried DFC-EVs formed denser, more regular and wider periodontal ligaments, attaching new fibers to the cementum layer. Also significantly enhanced the bone parameters, including BV/TV and Tb-Sp.	[71]
	Dendritic cells	Injection locally/ intravenous injection	Mouse periodontitis model	200 million particles	Mice with periodontal diseases treated with EVs from dendritic cells by local injection promoted soft tissues covered on the alveolar bone better.	[59]
	GMSCs (TNF-α treated)	Injection locally	Mouse periodontitis model	200 µg	In a mouse periodontitis model, mice injected GMSC-EVs had less bone resorption and fewer TRAP-positive cells.	[39]
	GMSCs	PLGA	Rat periodontitis model	"full" EVs in PLGA	On the one hand, GMSC-EVs upregulated the expression of IL-10 and TGF-β significantly, whereas they downregulated the level of TNF-α. On the other hand, the defect area of rats' alveolar bone was significantly increased. Furthermore, osteogenic makers such as BMP2, RUNX2 and OCN were highly expressed.	[66]
	M2-Macrophages	Injection locally	Mouse periodontitis model	30 µl	In a mouse periodontitis model, after injecting EVs derived from M2-macrophage, less bone loss was found compared with the no EVs group.	[56]
	PDLSCs	β-TCP	Rat periodontitis model	150 µg/µl	In a rat periodontitis model, β-TCP carried EVs to the defect area had positive functions, in which the surface of the alveolar bone was more smooth, the periodontal fiber was more regular, also more collagen formation. The gene expression levels of OCN and RUNX2 were upregulated.	[68]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
PDLSCs		Gel-Alg Hydrogel	Rat alveolar bone defect model	2 µg/µl	Rats treated with PDLSC-EVs formed more new bones. Similarly, the bone parameter of BV/TV in EVs groups was higher than that in no-treatment groups.	[67]
SCAPs		Injection locally	Mouse periodontitis model	5 µg	Periodontitis mice injected with SCAP-EVs showed less bone resorption. In the periodontal tissue, the level of TNF-α and IL-8 also decreased.	[63]
SHEDs		β-TCP	Rat periodontal defect model	2 µg/µl	Periodontal rats treated with β-TCP/EVs had gained more newly-bone and more collagen accompanied by new blood vessels than the control group. EVs also enhanced COL1, and increased BV/TV.	[69]
Salivary		Injection locally	Mouse periodontitis model	Not mentioned	Exosomal miR-25-3p treatment group showed lower CD4 ⁺ and CD8 ⁺ T cells from TNF-α or IL-17, taking effect on developing diabetes-associated periodontitis.	[37]
DPSCs		Collagen	Mouse tooth root slice model	1.25 million cells' EVs in 1cm ² collagen membrane	In the root slice model, collagen membrane immobilized with DPSC-EVs increased odontogenic markers, such as DMP1, DPP on explant sections. The osteogenesis markers and angiogenesis markers such as vWF, BMP2, TGF-β, PDGF, RUNX2 were upregulated.	[76]
DPSCs (LPS-preconditioned)		Peptide-hydrogel	Rat dental pulp removed model	200 µg/ml	In a pulp-removing rat model, treated by DPSC-EVs facilitated more blood vessels and newly connective tissue formation.	[79]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
	DPSCs	1) Implantation; 2) Injection locally	(1) Mouse subcutaneous transplantation with the tooth root slice model; (2) Dog dental pulp removed model	Not mentioned	In the model of nude mice implanted tooth scaffold under dorsum, EV-treatment groups showed dentin-like tissue with higher vessel density; in the dog dental pulp removed model, after injecting EVs locally, it showed dental-pulp like tissue formation with high expression of DSPP and DMP-1.	[81]
	DPTs/DPSCs	Collagen with SCAPs	Mouse in vivo implant model based on TDM	160 µg/ml	Pre-dentin-like tissue appeared with a number of blood vessels, and formed some odontoblast-like cells and dense fibers. Furthermore, more polarizing odontoblast-like cells were shown after DPT-EVs were treated.	[80]
	Hertwig's epithelial root sheath cells	Collagen	Mouse subcutaneous transplantation with the tooth root slice model	2 mg/ml	It formed newly predentin-like tissue and odontoblast-like cells with collagen fibers, new blood and nerve nearby. Moreover, gene expressions of DSPP, DMP1 and β-catenin in the soft tissue around dentin also increased.	[78]
	Schwann cells	Collagen	Rat subcutaneous transplantation with the tooth root slice model	4 mg/ml	Mice treated with EVs from Schwann cells had significantly more blood vessels and pre-dentin-like tissues, and formed odontoblast-cells. Some proteins such as DSPP and DMP1 led to odontogenic differentiation were increased.	[77]
	SCAPs	Collagen	Mouse subcutaneous transplantation with the tooth root slice model	2 mg/ml	In a subcutaneous transplantation model with tooth root slice transplanted, EVs delivered by collagen formed newly regenerated tissues such as polarizing odontoblast-like cells, pre-dentin-like cells, collagen fibers with new blood vessels and nerves. Genes expression of DSPP, DMP1, β-catenin between dentin and soft fiber enhanced as well.	[35]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
Dental hard tissues	SHEDs	Implantation locally	Mouse subcutaneous transplantation with the tooth root slice model	Not mentioned	After implanting SHED-EVs, there appeared continuous dentin layers with new blood vessels, which showed the regeneration of the dentin-pulp complex.	[75]
	Ameloblasts	-	-	-	EVs secreted from ameloblasts mediated biomineralization of enamel, showing more enamel crystals formation. Compared with the control group, the width of the enamel was increased, and the distance of the interrod was decreased.	[84]
	DPSCs (in odontogenic differentiation media)	PEG-PLGA-PEG	Rat Pulpotomy Model	1.25 mg/scaffold	In a rat-pulpotomy model, DPSC-EVs implanted into the pulp interface induced more complete dentin formation while showing special dentin-tubes and mineral tissues stained with abundant collagen.	[86]
	DPSCs	Implantation locally	Mini-pig pulp repair model	1 mg/μl	The group treated with EVs from DPSCs cooperated with treated dentin matrix showing the thickest mineralized layers of dentin, and appeared dentin-bridge. Moreover, osteoid dentin also be observed nearby the pulp tissue.	[87]
	M2-Macrophages	Intravenous injection	Mouse mechanical force induced OTM model	100 μg	In a mechanical force-induced orthodontic tooth movement model, in comparison with other phenotypes macrophages, treated with M2-macrophage EVs induced cementoblast mineralization formation, which showed highly-expressions of BSP, OCN and OSX.	[88]
	Incisor epithelium and mesenchyme cells	-	-	-	In a knocked-down Rab27a/b CD1 mouse tooth organ reconstruction model, which reduces the secretion of EVs from epithelial and mesenchyme cells, weaker dentinogenesis compared with control groups was seen.	[89]

Table 1 (continued)

Regeneration tissue	EVs source	Delivery strategy	Study model	EVs concentration/dose	Outcome in vivo	References
TMJOA	EBCs	Injection locally	Rat TMJOA model	100 µg	In a TMJOA model, EBC-EVs displayed significant functions in bone parameters, including BV/TV, Tb-Th. The height, thickness and cellularity of cartilage were also significantly improved.	[92]

EVs, extracellular vesicles; ADMSCs, adipose mesenchymal stem cells; BMMSCs, bone marrow mesenchymal stem cells; DPSCs, dental pulp stem cells; DFCs, dental follicle cells; GMSCs, gingival mesenchymal stem cells; PDLSCs, periodontal ligament stem cells; SCAPs, stem cells from apical papilla; EPI-NCSCs, hair follicle epidermal neural crest stem cells; SHEDs, human exfoliated deciduous teeth stem cells; CM, serum-free conditioned medium; DPTs, dental pulp tissues; DPCs, dental pulp cells; EBCs, embryonic stem cell; LPS, lipopolysaccharide; SIS-ECM, small intestinal submucosa–extracellular matrix; PLGA, poly (lactic-co-glycolic acid); PLA, poly (lactic acid); HA, hyaluronic acid; β-TCP, β-tricalcium phosphate; Gel-Alg Hydrogel, gelatin-sodium alginate hydrogel; PEG-PLGA-PEG, polyethylene glycol-poly (lactic-co-glycolic acid)-polyethylene glycol; BRONJ, bisphosphonate related osteonecrosis of the jaw; TDM, treated dental matrix; FNI, facial nerve injury; OTM, orthodontic tooth movement; TMJOA, temporomandibular joint osteoarthritis; BV/TV, bone volume/total volume; Tb-N, trabecular number; Tb-Th, trabecular thickness; Tb-Sp, trabecular separation; COL 1, collagen 1; ALP, alkaline phosphatase; RUNX2, runt-related transcription factor 2; OCN, osteocalcin; TRAP, tartrate resistant acid phosphatase; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand; IL-1, interleukin-1; TNF, tumor necrosis factor; DMP-1, dentin matrix protein-1; OPN, osteopontin; MMP-2, matrix metalloproteinase-2; HA, hydroxyapatite; TGF, transforming growth factor; DPP, dentin phosphoprotein; vWF, vonWillebrandfactor; PDGF, platelet-derived growth factor; DSPP, dentin sialoprophosphoprotein; BSP, bone sialoprotein; OSX, osterix.

complete with fresh periodontal ligaments, and the reattachment of the gingiva [53].

Numerous studies have unveiled the remarkable regenerative potential of EVs in periodontal tissue regeneration. For example, BMMSC-EVs have demonstrated the ability to stimulate alveolar bone formation and repair periodontal ligaments in models of periodontitis [54, 55]. Moreover, EVs released by M2 macrophages have proven effective in preventing alveolar bone loss [56]. The injection of EVs from ADMSCs into rat periodontal pockets has resulted in the formation of cellular periodontal tissue perpendicular to the cementum and alveolar bone [57, 58]. Moreover, EVs derived from dendritic cells have exhibited potential in treating degenerative alveolar bone diseases by promoting the coverage of soft tissues over the alveolar bone [59].

Emerging evidence suggests that EVs derived from dental tissues also contribute significantly to periodontal tissue regeneration. For instance, DPSC-EVs and SCAP-EVs have been reported to inhibit alveolar bone loss [60–63]. EVs derived from dental follicle progenitor cells (DFC-EVs) have been shown to enhance the formation of denser alveolar bone with increased trabecular thickness compared to control groups [64, 65]. Gingival mesenchymal stem cell-derived EVs (GMSC-EVs), PDLSC-EVs and SHED-EVs have proven efficient in repairing alveolar bone defects, accompanied by the development of new blood vessels [66–69].

Given that periodontal diseases often involve inflammation, evaluating the function of EVs under inflammatory conditions is essential [70]. Research has demonstrated that EVs released from GMSCs treated with TNF- α effectively prevent periodontal bone resorption [39]. Furthermore, studies have shown that lipopolysaccharide (LPS)-preconditioned DFC-EVs promote the proliferation of PDLSCs and macrophages [71]. Similarly, LPS-preconditioned DFC-EVs have been found to be beneficial for the formation of integrated periodontal tissue in PDLSCs compared to healthy DFC-EVs [72].

Dental pulp regeneration

The dental pulp, the sole soft tissue within a tooth, resides within the pulp cavity, encircled by dentin. It comprises connective tissue, blood vessels, and nerves, rendering it vascularized and innervated [73]. Consequently, endodontic regeneration is a multifaceted process encompassing not only dental pulp regeneration and dentin-pulp complex formation but also pulp revascularization and neurological recovery [74].

Numerous research studies have shed light on the role of EVs in fostering dentin-pulp regeneration. EVs derived from various sources, such as SHEDs, DPSCs, SCAPs, Hertwig's epithelial root sheath cells, and Schwann cells, have been subcutaneously implanted into mice, resulting in the promotion of dentin-pulp regeneration [35, 75–78]. Rats treated with LPS-preconditioned DPSC-EVs exhibited the formation of dental pulp-like tissue replete with new blood vessels in a model where dental pulp had been removed [79]. In another study, collagen containing SCAPs were placed at the root tip, and the cavity was filled with EVs derived from dental pulp tissue/stem cells, leading to the regeneration of dense pulp-like tissue and predentin-like tissue [80]. Intriguingly, Li et al. reported that apoptotic bodies, typically regarded as indicators of cellular end-of-life, spurred the formation of dental pulp-like tissue replete with abundant blood vessels [81]. As mentioned earlier, neurological recovery is also vital for dental pulp regeneration, with research highlighting the potential effects of EVs on neuroregeneration, thereby underscoring the promise of EVs in pulp regeneration [82].

Dental hard tissue regeneration and mineralization

Dental hard tissues encompass enamel, dentin, and cementum. Enamel is primarily composed of hydroxyapatite crystals, whereas dentin and cementum are a combination of hydroxyapatite and organic matrix [83]. Research has shown that EVs play a role in the formation and mineralization of dental hard tissues [84, 85].

In a rat pulpotomy model, DPSC-EVs prompted the creation of dentin tubes and reparative dentin bridges

(See figure on next page.)

Fig. 4 The therapeutic effects of extracellular vesicles on different dental tissue regeneration. **a** EVs derived from LPS-preconditioned DFCs laden on hydrogel applied in the treatment of periodontitis by repairing lost alveolar bone and promoting periodontal tissue regeneration. This figure is adapted and is freely accessible from reference [72], Licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0). **b** EVs derived from BMMSCs prevent BRONJ by preventing the spread of chronic inflammation and promoting angiogenesis and osteogenesis. This figure is adapted and is freely accessible from reference [46]. **c** TDM and EVs isolated from DPSCs promote reparative dentin formation. This figure is adapted and is freely accessible from reference [87], Licensed by Sage Publications and Copyright Clearance Center. **d** EVs derived from osteoclasts promote bone regeneration. This figure is adapted and is freely accessible from reference [14], Reprinted under the terms of the Creative Commons CC-BY license. Abbreviations: AB, alveolar bone; PL, periodontal ligament; D, cementum; ZOL, zoledronic acid; TDM, dentin matrix; D, dentin; P, pulp tissue; DB, dental bridge; BV/TV, bone volume/total volume; OCS-col, osteoclasts on collagen; OC-EVs-col, EVs derived from osteoclasts on collagen

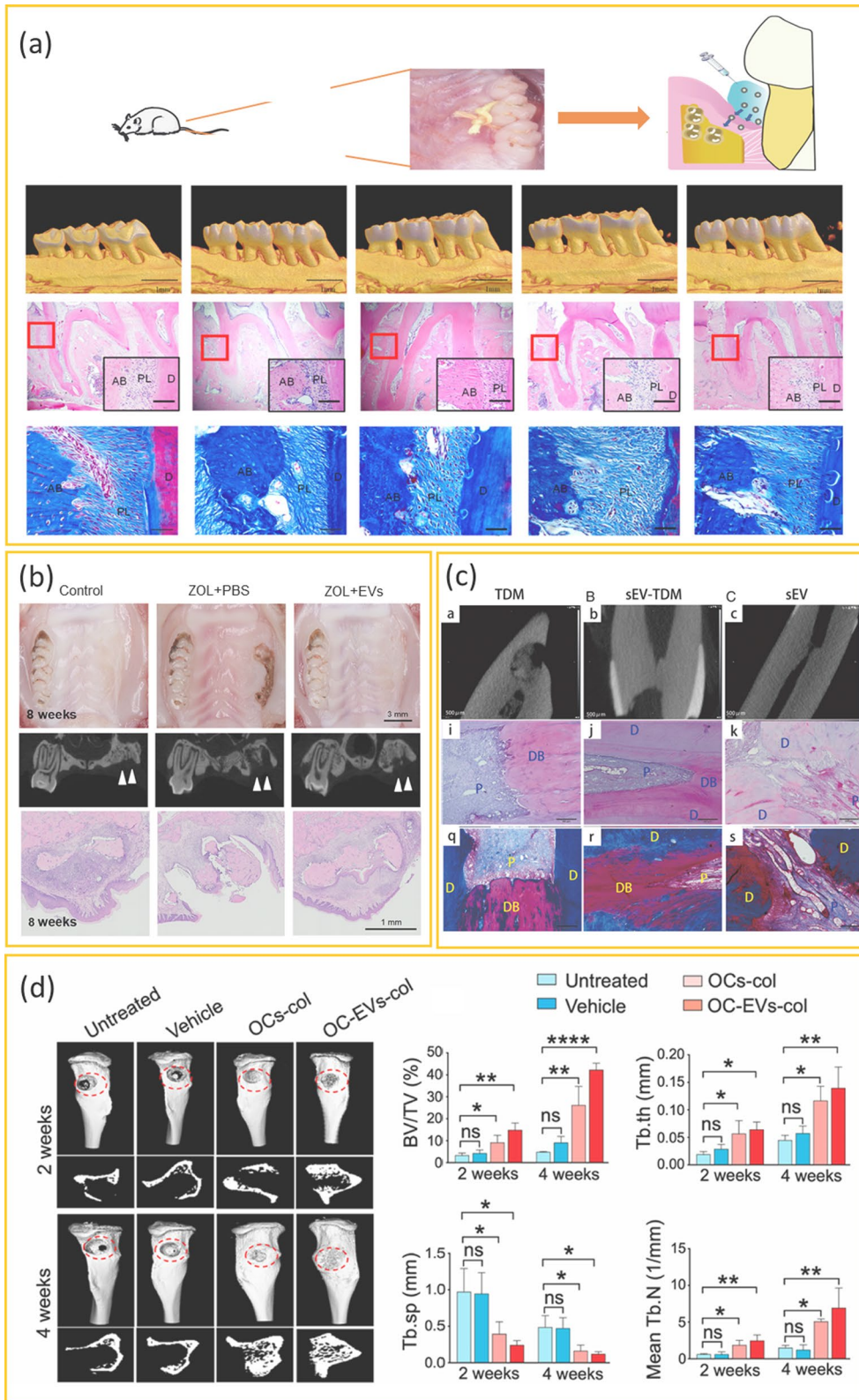


Fig. 4 (See legend on previous page.)

[86]. Another study illustrated how DPSC-EVs, in conjunction with dentin matrix, heightened the proliferation, migration, and odontogenesis of dental pulp cells, thus contributing to the continuous formation of reparative dentin [87]. Zhao et al. reported that EVs originating from macrophages with different polarization phenotypes had distinct effects on cementoblast mineralization. Specifically, EVs derived from M2 macrophages fostered cementum mineralization and curtailed root resorption [88]. Jiang et al. posited that EVs facilitated communication between epithelial and mesenchymal cells. Epithelial cell-derived EVs were found to stimulate mesenchymal cells to produce dentin sialoprotein (DSP) and partake in mineralization, while mesenchymal cell-derived EVs induced epithelial cells to generate ameloblastin and amelogenin [89]. Moreover, it was proposed that intracellular ameloblast secretory EVs played a role in enamel mineralization [84].

Temporomandibular cartilage regeneration

The temporomandibular joint (TMJ) is an intricate joint, comprising the mandibular condyle and the articular surfaces of the temporal bone, both covered with dense articular cartilage [90]. Temporomandibular joint osteoarthritis (TMJOA) is a degenerative disease characterized by an imbalance between the synthesis and degradation of the condylar matrix mediated by chondrocytes [91]. This imbalance leads to the breakdown of the condylar matrix, resulting in joint disorganization, biomechanical alterations, disruption of the microenvironmental homeostasis around cartilage cells, and inflammation [92]. Because the TMJ cavity is an enclosed joint space with well-defined boundaries, this enclosed space provides a contained environment for injected substances, preventing their immediate dispersion into the surrounding tissues. Therefore, it has great potential to employ therapeutic EVs for the treatment of TMJOA.

Shen et al. firstly demonstrated that extracellular vesicles derived from BMSCs under hypoxic conditions can enhance the proliferation, migration, and anabolic capacity of chondrocytes *in vitro*. Moreover, they exhibited pro-chondrogenic potential *in vivo* [93]. Similarly, SHED-EVs have shown the capability to down-regulate the expression of proinflammatory factors and matrix metalloproteinases, indicating their potential to mitigate inflammation in the temporomandibular joint and prevent further cartilage damage [94]. Other than dental tissue derived EVs, EVs derived from human embryonic mesenchymal stem cells have been observed to enhance chondrogenesis, leading to the formation of new hyaline cartilage closely resembling healthy tissue in a rat model of TMJOA [95].

Mechanisms of EV-mediate dentistry regeneration

Understanding these mechanisms is essential for harnessing the full regenerative potential of EVs. The mechanisms underlying extracellular vesicle-mediated dentistry regeneration are a complex and dynamic interplay of cellular and molecular processes. They facilitate key aspects of regeneration, including but not limited to osteogenesis, odontogenesis, mineralization of dental hard tissues, angiogenesis, immunomodulation (Table 2; Figs. 5 and 6). Through their ability to transfer these bioactive molecules, EVs modulate various signaling pathways, gene expression, and cellular behaviors, ultimately contributing to the repair and regeneration of dental tissues.

EVs increase odontogenic differentiation

Odontogenic differentiation constitutes a pivotal process in tooth development, and emerging research affirms the role of extracellular vesicles in inducing odontogenic differentiation and upregulating the expression of dental-related markers such as dentin sialophosphoprotein (DSPP) and dental matrix protein (DMP) [35, 96]. Of note, DPSC-EVs have been observed to undergo cellular endocytosis in a dose-dependent manner, consequently activating the p38/MAPK pathway and intensifying odontogenic differentiation [76]. Moreover, DPSC-EVs have demonstrated their potential to transport nuclear factor I/C (NFIC), a pivotal transcription factor central to tooth development. This transport, in turn, promotes the proliferation, migration, and dentinogenesis of SCAPs [97]. Studies utilizing miRNA sequencing have unveiled alterations in miRNA profiles following the uptake of DPSC-EVs, underscoring the role of EVs in orchestrating odontogenic differentiation through the TGF β 1/Smads signaling pathway [98]. In addition, miR-758-5p transported by DPSC-EVs under inflammatory conditions has the capacity to stimulate BMP signaling to ultimately govern odontogenic differentiation [99]. Meanwhile, EVs originating from Hertwig's Epithelial Root Sheath Cells have been shown to activate the Wnt/ β -catenin pathway, thereby establishing a conducive microenvironment for odontogenic differentiation by fostering the connection between epithelial cells and mesenchymal cells [78].

EVs promote osteogenic differentiation

Osteogenic differentiation plays a pivotal role in bone formation, including craniofacial and alveolar bone remodeling and repair [100]. Several studies have diligently explored the potential roles of EVs in osteogenesis. Reports indicate that PDLSC-EVs contributed to the alveolar bone regeneration by mitigating the over-activation of the Wnt signaling pathway and suppressing NF- κ B activity of osteoprogenitor cells [68, 101]. Meanwhile, DFC-EVs have been shown to activate the

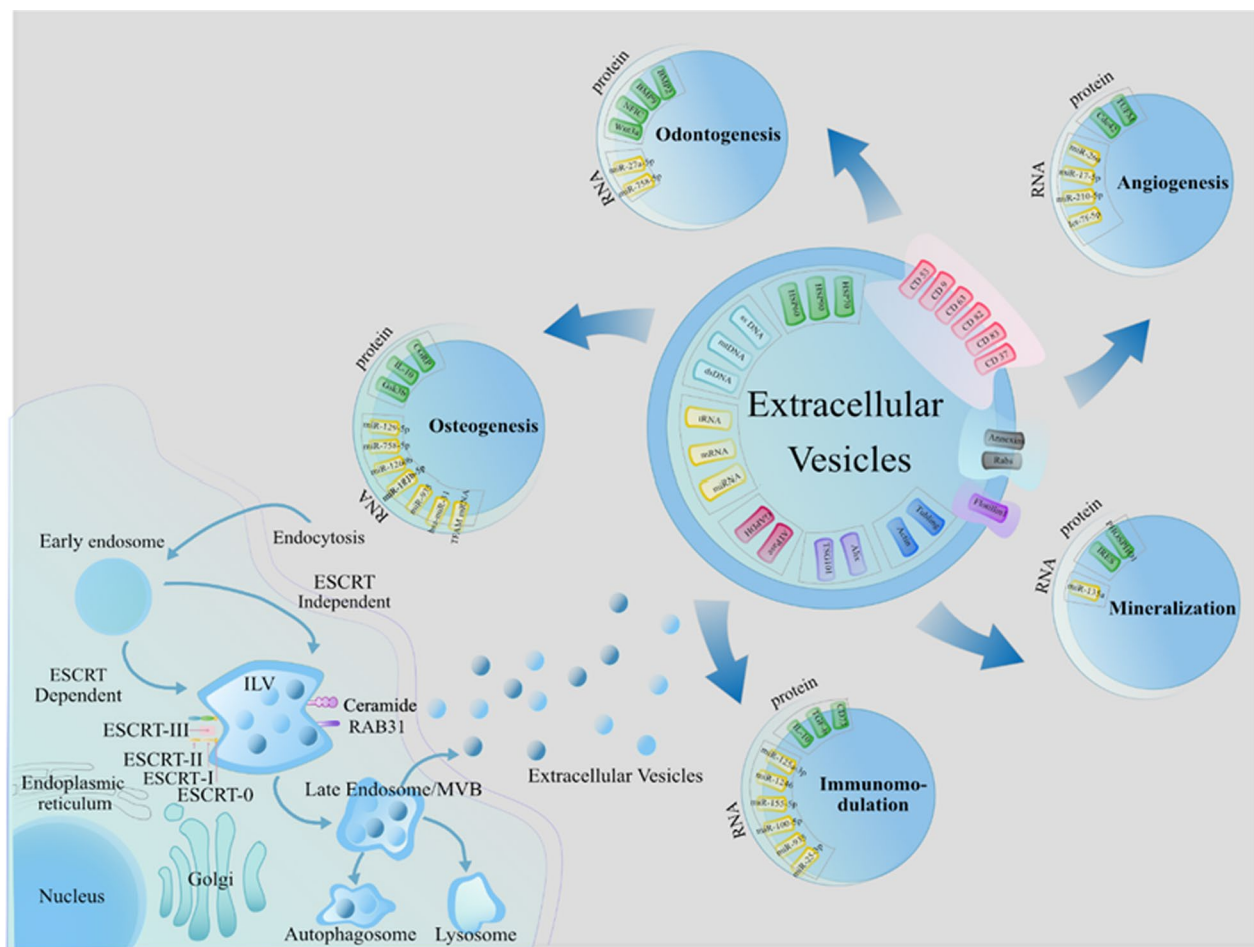


Fig. 5 The effective components and functions of EVs for dental tissue regeneration. EVs are released upon the fusion of multivesicular bodies with plasma membranes. They aid in dental tissue regeneration by promoting odontogenic differentiation, osteogenesis differentiation, dental hard tissue mineralization, angiogenesis and regulating immunomodulation through different cargos, including but not limited to protein, MicroRNA, and mRNA

MAPK pathway, aiding in the repair of alveolar bone defects [99, 100]. In contrast to DFC-EVs, SHED-EVs regulate osteogenesis through the AMPK pathway [69]. SHED-EVs transport mitochondrial transcription factor A mRNA, thereby instigating mitochondrial aerobic metabolism and consequently augmenting bone regeneration [102].

DPSC-EVs, GMSC-EVs, and SCAP-EVs are shown to promote the osteogenic differentiation of stem cells via the miRNAs they carry [39, 63, 99, 101]. For instance, miR-181b-5p found within osteocyte-derived EVs facilitates the osteogenic differentiation of PDLSCs through the PTEN/AKT pathway [103]. Conversely, miR-129-5p within plasma secretory EVs inhibits jawbone osteogenesis via the FZD4/ β -catenin pathway [104]. Except for miRNA, mRNA and proteins in EVs can also contribute to dental bone regeneration by upregulating the

osteogenic differentiations of stem cells. EVs derived from M2 macrophages transport IL-10 mRNA, activating the cellular IL-10/IL-10R pathway directly, thereby promoting osteogenesis and preserving bone homeostasis [56]. ADMSC-EVs expedite alveolar bone repair by transmitting calcitonin gene-related peptide (CGRP), a significant neuropeptide expressed during bone repair [58]. Meanwhile, umbilical cord mesenchymal stem cell-derived EVs (UMSC-EVs) were reported to enhance the osteoblastic differentiation capability of PDLSCs via the P13K/AKT pathway [105].

EVs facilitate dental hard tissue mineralization

The mineralization process of dental hard tissue is a multifaceted phenomenon characterized by intricate interactions among various organic compounds [106]. Within this context, EVs serve as reservoirs of numerous

Table 2 Underlying mechanisms of EVs in the dental tissue regeneration

Functional effects	EVs source	EVs cargos	Involved signaling pathway	Outcome in vitro	References
Odontogenesis	DPSCs	BMP-2, BMP-9	P38 MAPK	DSPP	[76]
	DPSCs	miR-27a-5p	TGFβ-1/Smads	DSP↑, DMP-1 ↑	[98]
	DPSCs	NFIC	–	DSPP↑, DMP-1 ↑, ALP↑, NFSC ↑	[97]
	DPSCs (under inflammatory environment)	miR-758-5p	BMP	DSPP↑, DMP-1 ↑	[99]
	Hertwig's epithelial root sheath cells	Wnt3a	Wnt/β-catenin	DSPP↑, DMP-1 ↑	[78]
Osteogenesis	ADMSCs	CGRP	-	RUNX2↑, ALP↑, OCN↑	[58]
	Plasma	miR-129-5p	FZD4/β-catenin	RUNX2↑, ALP↑, OPN↑	[104]
	DFCs	–	PLC/PKC/MAPK	ALP↑, OCN↑, OPN↑, MMP-2↑	[65]
	DFCs	–	P38 MAPK	RUNX2↑, ALP↑, BSP↑, COL1↑	[64]
	DPSCs (osteogenic-induced conditioned)	hsa-miR-31	–	RUNX2↑, COL1↑, OSX↑	[139]
	DPSCs (under inflammatory environment)	miR-758-5p	BMP	RUNX2↑, ALP↑, OCN↑	[99]
	GMSCs (TNF-α treated)	miR-1260b	NF-κB	RANKL/OPG↓	[39]
	M2-Macrophage	IL-10	IL-10/IL-10R	RUNX2↑, ALP↑, OCN↑, COL1a1↑	[56]
	Osteocyte	miR-181b-5p	PTEN/AKT	RUNX2↑, BMP-2↑, AKT1↑, P13KCA↑	[103]
	PDLSCs	Gsk3β	NF-κB	ALP↑, OCN↑, BMP-2↑	[101]
	PDLSCs	Gsk3β	Wnt	OCN↑, RUNX2↑	[68]
	SCAPs	miR-935	–	OCN↑, OPN↑	[63]
	SHEDs	-	AMPK	RUNX2↑, OPN↑, COL1↑	[69]
	SHEDs	TFAM mRNA	–	RUNX2↑, ALP↑, BMP-2↑	[102]
	UMSCs	–	P13k/AKT	RUNX2↑, ALP↑, OCN↑	[105]
Mineralization	17IIA11 cell lines	–	Erk1/2	Ca ²⁺ , Pi formation	[108]
	Ameloblast	PHOSPHO1	–	Enamel width↑, Interrod distance↓	[84]
	Incisor epithelial and mesenchymal cells	miR-135a	Wnt/β-catenin	COLIV↑, laminin↑, ALP↑, DSP↑, Bglap↑, Mineral nodule formation	[89]
Angiogenesis	T4-4 cell lines	IRES	–	DSPP↑, DMP-1↑, DPP↑	[109]
	DPSCs (from periodontally diseased teeth)	–	P38 MAPK	VEGF↑, MMP-9↑, KDR↑,	[114]
	DPSCs	TUFM	–	VEGF↑, ANG-2↑, MMP-9↑, HIF-1α↑	[81]
	PDLSCs	miR-17-5p	–	VEGFA↑	[111]
	SHEDs	–	AMPK	CD31↑, COL1↑	[69]
	SCAPs	Cdc42	–	CD31↑, vascular limen formation	[50]
	SHEDs	miR-26a	TGFβ/Smad2/3	VEGF↑, ANG-2↑, PDGF↑,	[75]
	SHEDs (hypoxic-preconditioned)	let-7f-5p, miR-210-5p	AGO/VEGF; miR-210-3p/ephrinA3	VEGF↑, MMP-9↑, ANGPT1↑	[113]

Table 2 (continued)

Functional effects	EVs source	EVs cargos	Involved signaling pathway	Outcome in vitro	References
Immunomodulation	BMMSCs	–	OPG-RANKL-RANK	Arginase↑, CD163↑, TGF-β1↑, iNOS↓, CD86↓	[55]
	DFCs (LPS-preconditioned)	–	ROS/JNK	RANKL/OPG↓	[71]
	DPSCs	miR-125a-3p	TLR, NF-κB	IL-1ra↑, IL-10↑, IL-1β↓, IL-6↓, TNF-α↓	[118]
	DPSCs	miR-1246	NF-κB p65, p38 MAPK	CD206↑, Arginase↑, CD163↑, IL-1β↓, IL-6↓, TNF-α↓, iNOS↓, CD86↓	[61]
	DPSCs	miR-1246	–	Th17/Treg↓	[62]
	Dendritic cells	TGF-β, IL-10	–	Th17/Treg↓	[59]
	EBCs	CD73	AKT/ERK, AMPK	MMP13↓, NO↓, s-GAG↑	[92]
	GMSCs	–	NF-κB p65, Wnt5a	TNF-α↓, IL-1β↓, IL-10↑	[140]
	PDLSCs	–	NF-κB	IL-1β↓	[116]
	PDLSCs	miR-155-5p	–	Th17/Treg↓, RORC↓, STRT1↓, FOXP3↑	[38]
	SHEDs	miR-100-5p	–	IL-6↓, IL-8↓, MMP-1↓, MMP-3↓, MMP-9↓, MMP-13↓	[94]
	SCAPs	miR-935	–	IL-6↓, IL-8↓	[63]
	Salivary	miR-25-3p	–	IL-17↓	[37]

factors that contribute to the formation of hydroxyapatite crystals and calcium phosphate [107]. Nevertheless, the precise mechanism through which EVs mediate mineralization remains the subject of debate and ongoing research. For instance, Chaudhary et al. demonstrated that EVs derived from the 17IIA11 cell line transport factors that induce enamel mineralization through the activation of the Erk1/2 pathway [108]. Another investigation proposed that miR-135a in EVs promote the

reciprocal interaction between epithelial and mesenchymal cells, thereby activating the Wnt/β-catenin signaling pathway and facilitating the production of dentin matrix proteins [89]. Furthermore, researchers have postulated that ameloblast secretory EVs engage in interactions with orphan phosphatase 1 (PHOSPHO1) and play a role in amelogenesis [84]. Similarly, EVs have been found to participate in the transport of Dentin Phosphophoryn (DPP) to the extracellular matrix, thereby contributing to the mineralization process [109].

(See figure on next page.)

Fig. 6 The mode of action of extracellular vesicles in promoting different dental tissue regeneration. **a** EVs derived from DPSCs specifically activate endogenous EC autophagy by transferring TUFM, thereby causing angiogenesis. The acceleration of vascular reconstruction promotes dental pulp regeneration. This figure is adapted and is freely accessible from reference [81], Licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0). **b** EVs derived from DPSCs under an inflammatory microenvironment participate in the regulating of odontogenic and osteogenic differentiation by miR-758-5p/LMBR1/BMP2/4 axis. This figure is adapted and is freely accessible from reference [99], Licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0). **c** EVs derived from GMSCs under inflammation microenvironment enhance M2-type macrophage polarization and prevent periodontal bone loss. This figure is adapted and is freely accessible from reference [39], Licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0). Abbreviations: EC, endothelial cells; hDPSC, human dental pulp stem cells; TUFM, Tu translation elongation factor, mitochondrial; TFEB, transcription factor EB; VEGF, vascular endothelial growth factor; ANG2, angiotensin 2; hDPSC-apoVs, apoptotic vesicles from human dental pulp stem cells; BMP, bone morphogenetic protein; LMBR1, limb development membrane protein 1; TNF-α, tumor necrosis factor α; DPSC-EV, EVs from dental pulp stem cells; iDPSC-EVs, EVs from dental pulp stem cells under inflammatory environment; PDLSC, periodontal ligament stem cells

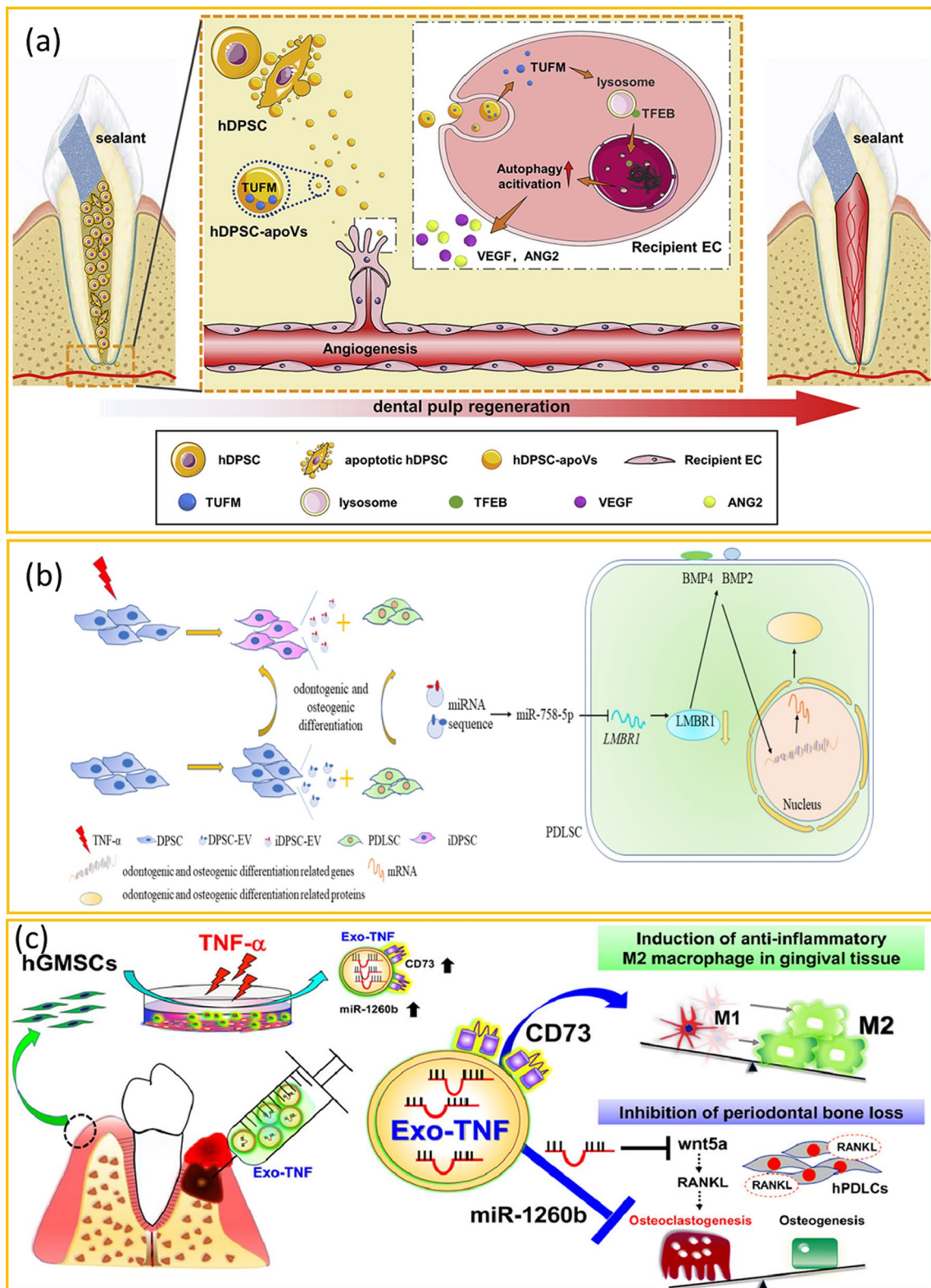


Fig. 6 (See legend on previous page.)

EVs accelerate angiogenesis

Blood vessels play a pivotal role in delivering vital bioactive elements, encompassing growth factors, nutrients, and progenitor cells, to sites of regeneration, thereby contributing significantly to the maintenance of homeostasis [110]. Accumulating evidence suggests that EVs exhibit the capacity to expedite angiogenesis in the context of dental regeneration [111, 112]. This facilitation primarily hinges on the transfer of microRNA (miRNA) payloads encapsulated within EVs. For instance, PDLSC-EVs augmented the vascularization of dental periodontal ligaments through the transmission of vascular endothelial growth factor (VEGF) via miR-17-5p [111]. Similarly, SHED-EVs transferred miR-26a to initiate the TGF- β /Smad signaling pathway, thereby fostering angiogenesis [75]. Another investigation illustrated that SHED-EVs regulated angiogenesis via the activation of the AMPK signal pathway [69]. Moreover, SHED-EVs were reported to enhance angiogenesis even under hypoxic conditions, accomplished by the transfer of let-7f-5p and miR-210-3p, which respectively modulate the AGO1/VEGF and miR-210-3p/ephrinA3 signaling pathways [113].

In addition to miRNAs, proteins transported by EVs significantly contribute to the expediting of angiogenesis. SCAP-EVs mediated the action of Cdc42, thereby promoting vascularization and aiding in the repair of craniofacial soft tissue [50]. DPSC-EVs regulated the activation of angiogenesis by modulating the translation elongation factor Tu via the transcription factor EB (TFEB)-autophagy pathway [81]. Additionally, DPSC-EVs demonstrated the capacity to promote angiogenesis and activate the p38 MAPK pathway, showcasing substantial angiogenic potential for pulp regeneration [114].

EVs regulate immunomodulation

Extracellular vesicles play a pivotal role in orchestrating immunoregulatory processes, with a significant contribution stemming from the encapsulated miRNAs. For instance, miR-100-5p within SHED-EVs [94], miR-935 in SCAP-EVs [63], and miR-25-3p found in salivary secretory EVs [37] have demonstrated the capacity to modulate immune responses. Notably, specific miRNAs encapsulated within EVs play immunomodulatory roles through different signaling pathways. For example, EVs derived from embryonic stem cells (EBC-EVs) have been shown to suppress inflammation through the activation of adenosine receptor-dependent AMPK and AKT/ERK signaling pathways [92]. The NF- κ B transcription factor, known for its pivotal role in regulating inflammatory responses, is proved to be another key response element in the immunomodulation process of EVs [115]. Numerous studies have established that EVs exert influence over immune responses in the regenerative dentistry by

modulating the NF- κ B signaling pathway [61, 116–118]. In the realm of osteoimmunology, the RANKL (NF- κ B ligand) and osteoprotegerin (OPG) system bear significance [71, 119]. EVs have demonstrated their ability to regulate the RANKL-RANK-OPG signaling within the context of osteoimmunology in dental bone regeneration [55]. Furthermore, the equilibrium between Th17 and Treg cells is revealed of importance in modulating inflammation to aid in the dental tissue regeneration [120]. EVs contribute significantly to this balance by virtue of various factors, including specific miRNAs like miR-1246 and miR-155-5p, as well as cytokines such as TGF- β and IL-10 [38, 59, 62].

Delivery strategy of EVs

The delivery of extracellular vesicles represents a critical aspect of their utilization in various therapeutic contexts. Effective delivery approaches for EVs are essential to harness their regenerative and therapeutic potential. Various strategies have been developed to facilitate the precise and targeted delivery of EVs to specific tissues or cells, ranging from direct injection to more sophisticated engineered delivery systems. In the context of regenerative dentistry, delivering EVs to oral tissues has their own particularity due to the special oral anatomical and physiological characteristics. To be specific, the oral cavity is rich in saliva containing enzymes and chemicals and oral tissues are subject to constant mechanical stress due to activities such as chewing and speaking. All these factors might affect the stability and function of extracellular vesicles which have to be taken into consideration in the application EVs in regenerative dentistry.

EVs delivery by injection

Due to the nano-size of EVs and well-established clinical procedure of intravenous injection, EVs have been initially and widely utilized through intravenous injection in various biomedical and therapeutic applications. In the field of regenerative dentistry, intravenous injection of EVs is proved to be feasible and effective. For example, in a BRONJ rat model, intravenously administered EVs were found to effectively modulate genes associated with osteogenesis and inflammation in the maxilla to promote bone regeneration [116]. However, EVs delivered systemically can become diluted in the bloodstream, which may reduce their concentration at the oral sites, potentially reducing the specificity of the treatment. Given the special oral anatomical structure, there is a preference for employing extracellular vesicles through direct injection into the target site in the context of regenerative dentistry regeneration. Research studies have provided evidence of the effectiveness of locally injected EVs in promoting the formation of new epidermal tissue and enhancing

vascularization, as demonstrated in a mouse model of palatal gingiva wound healing [50]. Moreover, in an experimental model of alveolar bone loss, locally injected EVs exhibited slower clearance and demonstrated higher affinity compared to systemic injection [59]. However, it is crucial to acknowledge a significant limitation associated with the use of injected EVs, namely the stability and retention of EVs post-administration, particularly in the oral cavity. The special oral environment and the salivary flow can result in a notable loss of EVs, which significantly affect the therapeutic effectiveness and potential applications of injected EVs for the treatment of dental diseases.

EVs delivery by carriers

The direct injection of EVs into target sites in the oral cavity presents challenges related to their stability and retention *in vivo*. In contrast, the utilization of carrier-based delivery systems, such as hydrogels and ceramics, offers notable advantages, primarily concerning controlled release and prolonged retention duration. This carrier-based EV delivery exhibits significant potential for augmenting the therapeutic effectiveness of EVs in the field of dental regenerative medicine [121, 122]. Given the superior biocompatibility and tunability, hydrogel materials are widely used to deliver cells and EVs via minimally invasive procedures for tissue regeneration. Collagen, a natural hydrogel derived from most tissues, stands out as a popular choice for delivering EVs in the realm of regenerative dentistry. When incorporated into collagen hydrogels, EVs have demonstrated their capacity to enhance osteogenesis, odontogenesis, and the regeneration of bone and dentin-like tissues across a spectrum of oral disease conditions [35, 48, 54, 55, 72]. Given that hydrogels have a texture similar to soft tissue, it is of great advantage that apply collagen hydrogels in the regeneration of dental pulp. Several studies have demonstrated that collagen hydrogels help promote angiogenesis, thus speeding up the rate of dental pulp regeneration [76–78, 80]. Gelatin, a collagen derivative, also exhibits controlled-release properties for EVs, amplifying their effectiveness in promoting dentin formation [97]. Furthermore, alternative hydrogels like chitosan and alginate hydrogel have found utility as carriers for EVs in dental regeneration endeavors [55, 61, 67, 101]. Additionally, synthetic polymers such as PLGA/pDA, PLGA, and PEG-PLGA-PEG, engineered with precision to control their physical and chemical attributes, offer a means of achieving more predictable and sustained EV release for dental tissue regeneration [58, 66, 86]. However, it's worth noting that the sustained release of EVs primarily relies on the physical encapsulation provided by these hydrogels, which typically spans several days. Consequently, there

is a growing interest in developing advanced materials capable of enabling long-term EV delivery in the context of regenerative dentistry, given the chronic nature of most oral diseases.

On the other hand, ceramics are frequently used to deliver EVs for hard tissue regeneration in oral diseases due to their mechanical properties such as hardness and corrosion resistance and chemical components that is calcium and phosphate ions. Hydroxyapatite (HA), which has been available on the market for clinical therapy since the 1970s, is considered the most conventional ceramics for regenerating dental hard tissues [123]. For instance, in an ectopic dentin regeneration model, the group utilizing EVs with HA exhibited significant formation of dentin-like tissue [71]. Another noteworthy ceramic material is beta-tricalcium phosphate (β -TCP) and it is known for its biodegradability. It promotes rapid bone tissue regeneration when used in conjunction with EVs in a periodontitis model. This structure facilitates angiogenesis and actively contributes to the formation of bone tissue [68, 69].

It is of note that a burst release frequently occurs when loading EVs in ceramic materials because EVs are mostly adsorbed on to the ceramic surfaces through hydrophilic action. The combination of exosome and hybrid scaffolds might exert better regenerative effects than organic or inorganic materials alone, which needs further investigations in the regenerative dentistry.

Limitations and future perspectives

While extracellular vesicles have demonstrated significant progress in advancing dentistry regeneration [124–127], their widespread implementation in clinical trials is contingent upon addressing several limitations and challenges. First, EV composition exhibits dependency on various factors, including the cell type, donor age, state, and the microenvironment in which parent cells reside, all of which influence their functional roles [128]. For instance, the immune profiles of mesenchymal stem cell-derived EVs (MSC-EVs) have been substantiated age-dependent variations [129]. Furthermore, EVs derived from samples of differing ages exhibit disparate effects during the osteogenesis process and display varying degrees of efficacy in bone repair [130]. Consequently, it is imperative to investigate EVs from diverse contextual sources to unravel the underlying mechanisms that intricately govern their therapeutic efficacy.

The other challenge that hampers the potential utilization of extracellular vesicles for dentistry regeneration is a dependable method for isolating and purification of EVs from cells or bodily fluids. Current isolation methods encompass ultracentrifugation, size-exclusion chromatography, asymmetrical flow field-flow fractionation, and immunoaffinity-based techniques [131]. Comparative

analyses of these methods have revealed variations in EV particle yield and purity [132]. For instance, one study compared the purification of serum-EVs using ultracentrifugation and Total Exosome Isolation reagent, with the latter displaying superior purity based on miRNA profiles [133]. Meanwhile, the conditions of the liquid for EV isolation, such as viscosity, preservation environment, and treatment methods, also influence EV purification outcomes. Nonetheless, a comprehensive comparison and optimization strategies for EV purification remains relatively unexplored. Moreover, these methods often incur high costs while yielding limited quantities of vesicles, thereby impeding their clinical applicability. Therefore, more advanced techniques for efficient and standardized EV isolation and purification are required for the future clinical application of EVs in regenerative dentistry. A relevant issue in this challenge is the standardization of EV usage, especially the determination of optimal dosages or concentrations. Notably, studies have reported a wide range of EV concentration/dosage across different investigations (Table 1). A general trend in the literature suggests that higher EV doses tend to yield relatively better tissue regeneration outcomes [134, 135], yet none of the studies provide definitive guidance regarding the optimal EV concentration for their respective animal models. To ensure consistency and efficacy, it is imperative to establish good manufacturing practices (isolation and purification) and comprehensive standards and guidelines for the clinical application of EVs [136].

The management of chronic dental diseases necessitates continuous engagement of extracellular vesicles. However, the sustained presence of EVs and their therapeutic effects at injury sites over extended periods remains a challenge. To address this, the development of a proficient delivery system for EVs offers distinct advantages in augmenting their therapeutic efficacy when integrated with modified scaffolds [137]. Consequently, forthcoming research endeavors may develop novel EV-loaded scaffolds, encompassing controlled release profiles, in vivo degradation characteristics, and loading efficiency. For instance, injectable microspheres with sustained release kinetics of EVs have been devised for addressing irregular tissue defects and for periodontitis [138]. More investigation on material-EV interaction would aid in optimizing the adaptability and plasticity of such scaffolds to ensure their effectiveness to deliver EVs.

Conclusion

Extracellular vesicles have emerged as pivotal elements in cellular interactions and hold the potential to revolutionize regenerative dentistry by facilitating tissue regeneration, encompassing the maxillofacial, periodontal, dental, and temporomandibular cartilage regions. These

vesicles, sourced from diverse origins, make substantial contributions to regenerative dentistry through various mechanisms, including the promotion of odontogenesis, osteogenesis, dental hard tissue mineralization, angiogenesis, and modulation of the immune response. Leveraging diverse delivery strategies has allowed for more effective utilization of EVs, enhancing their regenerative efficacy in the field of dental tissue regeneration. Nonetheless, the successful clinical translation of EV-based therapies hinges upon addressing several critical challenges. These include the optimization of EV yield, the establishment of a standardized definition for EVs, and the development of novel EV delivery strategies.

Abbreviations

MSCs	Mesenchymal stem cells
EVs	Extracellular vesicles
ADMSCs	Adipose mesenchymal stem cells
ABMSCs	Alveolar bone-derived mesenchymal stem cells
BMMSCs	Bone marrow mesenchymal stem cells
DPSCs	Dental pulp stem cells
DFCs	Dental follicle progenitor cells
GMSCs	Gingival mesenchymal stem cells
PDLSCs	Periodontal ligament stem cells
SCAPs	Stem cells from the apical papilla
SHEDs	Stem cells from exfoliated deciduous teeth
UMSCs	Umbilical cord mesenchymal stem cells
ADMSC-EVs	Adipose mesenchymal stem cell-derived extracellular vesicles
ABMSC-EVs	Alveolar bone-derived mesenchymal stem cell-derived extracellular vesicles
BMMSC-EVs	Bone marrow mesenchymal stem cell-derived extracellular vesicles
DPSC-EVs	Dental pulp stem cell-derived extracellular vesicles
DFC-EVs	Dental follicle cell-derived extracellular vesicles
GMSC-EVs	Gingival mesenchymal stem cell-derived extracellular vesicles
PDLSC-EVs	Periodontal ligament stem cell-derived extracellular vesicles
SCAP-EVs	Stem cells from apical papilla-derived extracellular vesicles
SHED-EVs	Human exfoliated deciduous teeth stem cell-derived extracellular vesicles
Macrophage-EVs	Macrophage derived extracellular vesicles
EBC-EVs	Embryonic stem cells
MSC-EVs	Mesenchymal stem cell-derived EVs
LPS	Lipopolysaccharide
BRONJ	Bisphosphonate-related osteonecrosis of the jaw
TMJ	Temporomandibular joint
TMJOA	Temporomandibular joint osteoarthritis
AB	Alveolar bone
PL	Periodontal ligament
ZOL	Zoledronic acid
TDM	Dentin matrix
DB	Dental bridge
DSPP	Dentin sialophosphoprotein
DMP	Dentin matrix protein
CGRP	Calcitonin gene-related peptide
PHOSPHO1	Orphan phosphatase 1
DPP	Dentin phosphoporyn
miRNA	MicroRNA
VEGF	Vascular endothelial growth factor
TFEB	Transcription factor EB
EC	Endothelial cells
OPG	Osteoprotegerin
HA	Hydroxyapatite

β -TCP	Beta-tricalcium phosphate
SIS-ECM	Small intestinal submucosa–extracellular matrix
PLGA	Poly (lactic-co-glycolic acid)
PLA	Poly (lactic acid)
Gel-Alg Hydrogel	Gelatin-sodium alginate hydrogel
PEG-PLGA-PEG	Polyethylene glycol-poly (lactic-co-glycolic acid)-polyethylene glycol
TDM	Treated dental matrix
FNI	Facial nerve injury
OTM	Orthodontic tooth movement
BV/TV	Bone volume/total volume
Tb-N	Trabecular number
Tb-Th	Trabecular thickness
Tb-Sp	Trabecular separation
COL1	Collagen 1
ALP	Alkaline phosphatase
RUNX2	Runt-related transcription factor 2
OCN	Osteocalcin
TRAP	Tartrate resistant acid phosphatase
RANKL	Receptor activator of nuclear factor- κ B ligand
IL-1	Interleukin-1
TNF	Tumor necrosis factor
OPN	Osteopontin
MMP-2	Matrix metalloproteinases-2
TGF	Transforming growth factor
DPP	Dentin phosphoprotein
vWF	VonWillebrandfactor
PDGF	Platelet-derived growth factor
BSP	Bone sialoprotein
OSX	Osterix
BMP	Bone morphogenetic protein
NFIC	Nuclear factor I C
IRES	Internal ribosome entry site
TUFM	Tu translation elongation factor, mitochondrial
MAPK	Mitogen-activated protein kinase
FZD4	Frizzled 4 gene
AMPK	A d e n o s i n e 5 ' - m o n o p h o s p h a t e (AMP)-activatedprotein kinase
AGO	Arginase
RANK	Receptor activator of nuclear factor- κ B
ROS	Reactive oxygen species
JNK	c-Jun N-terminal kinase
NFSC	Neuro-fuzzy signal classifier
OSX	Osterix
P13KCA	Phosphoinositide-3-kinase, catalytic, alpha gene
KDR	Kinase insert domain receptor
ANG-2	Angiopoietin-2
HIF-1	Hypoxia inducible factor-1
PDGF	Platelet-derived growth factor
ANGPT1	Angiopoietin-1 gene
TGF	Transforming growth factor
iNOS	Inducible nitric oxide synthase
IL	Interleukin
NO	Nitric oxide
s-GAG	Sulfated glycosaminoglycan
RORC	The nuclear receptor retinoic acid receptor-related orphan receptor-gamma
STR1	Sodium ion trehalose transporter 1

FOXP3 Forkhead box P3 Supplementary Information

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Supplementary material 1

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Author contributions

E. Xia and S. Zou contributed equally to this work. E. Xia, I. Zhao, and Y. Zhang gave the concept and designed the study. E. Xia, S. Zou, X. Zhao, W. Liu, I. Zhao, and Y. Zhang conducted the literature review. E. Xia, S. Zou, I. Zhao, and Y. Zhang wrote the manuscript. All authors commented on the manuscript.

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