Received: 19 January 2019 | Revised: 26 July 2019 | Accepted: 11 September 2019

DOI: 10.1111/joor.12885

SPECIAL ISSUE - REGENERATIVE MEDICINE AND ORAL REHABILITATION

JOURNAL OF ORAL REHABILITATION WILEY

Extracellular vesicles as a novel therapeutic tool for cell-free regenerative medicine in oral rehabilitation

Longwei Lv | Chunhui Sheng | Yongsheng Zhou

Department of Prosthodontics, National Clinical Research Center for Oral Disease, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing, China

Correspondence
Yongsheng Zhou, Department of Prosthodontics, Peking University School and Hospital of Stomatology, 22 Zhongguancun Avenue South, Haidian District, Beijing 100081, China.
Email: kqzhouysh@hsc.pku.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31600787; Beijing Natural Science Foundation, Grant/Award Number: 7192228, L182006; Young Elite Scientist Sponsorship Program by CAST, Grant/Award Number: 2015QNRC001; PKU School of Stomatology for Talented Young Investigators, Grant/Award Number: PKUSS20150107

Abstract

Oral maxillofacial defects may always lead to complicated hard and soft tissue loss, including bone, nerve, blood vessels, teeth and skin, which are difficult to restore and severely influence the life quality of patients. Extracellular vesicles (EVs), including exosomes, microvesicles and apoptotic bodies, are emerging as potential solutions for complex tissue regeneration through cell-free therapies. In this review, we highlight the functional roles of EVs in the regenerative medicine for oral maxillofacial rehabilitation, specifically bone, skin, blood vessels, peripheral nerve and tooth-related tissue regeneration. Publications were reviewed by two researchers independently basing on three databases (PubMed, MEDLINE and Web of Science), until 31 December 2018. Basing on current researches, we classified the origin of EVs for regenerative medicine into four categories: related cells in the regenerative niche, mesenchymal stem cells, immune cells and body fluids. The secretome of different cells are distinct, while the same cells secrete different EVs under varied conditions; therefore, the content profiles of EVs and regulatory mechanisms on target cells are compared and emphasised. By unravelling the regulatory mechanisms of EVs in tissue regeneration, modified cells and tailored EVs with specific target may be produced for precision medicine with high efficacy.

KEYWORDS
angiogenesis, bone, extracellular vesicles, nerve, tissue regeneration, wound healing

1 | BACKGROUND

Oral maxillofacial defect is one of the most tackle problems that clinicians should be facing, which always renders to complicated hard and soft tissue defect, such as bone, soft tissue, nerve, blood vessels and teeth, thus severely influencing the life quality of patients. Tissue engineering, based on stem cells, has brought hope to the sufferings by realising the regeneration of a wide range of tissue lost caused by trauma or diseases. However, with accumulating number of approved clinical trials on stem-cell therapies, some serious complications happened and considerable limitations have restricted a wider application of stem-cell therapy.[1] The first restriction is the low availability of stem cells harvested from donor tissues, as well as loss of potency and probable contamination after in vitro expansion. The second consideration is the low efficacy of injected cells, with growing evidences suggesting that only 1%-3% stem cells finally maintain or reach the target sites, and a majority may instead be trapped in the lungs, liver and spleen, while also a large number of cells ending to apoptosis in a short time after injection.[2-4] Most importantly, some of the injected stem cells lead to tumour formation, the most severe complication that made us reconsider the safety of stem-cell therapy.[5,6] Basing on the above unneglectable limitations of stem-cell therapy, the notion of cell-free therapy has attracted attentions. We are presently witnessing the emergence of a novel

paradigm that stem cell contributes to their therapeutic effects mainly through paracrine activity depending on their secretome, rather than engraftment or differentiation.[7]

The cellular secretome is a group of factors, including soluble proteins, growth factors, cytokines, free nucleic acids, lipids and extracellular vesicles, secreted to the extracellular space, which plays a critical role in biological regulation and cell-cell communication. Conditioned medium (CM), the culture medium containing biologically active components secreted from previously cultured cells or tissues that have released into the media substances, represents the complete milieu of cell-sourced secretomic and vesicular elements. Extracellular vesicles (EVs), the vesicular elements of the secretome, are nanoparticles (30-2000 nm) enclosed by phospholipid and contain complex and variable cargo of nucleic acids, proteins and lipids. Extracellular vesicles can be separated from the soluble factors in CM by ultracentrifugation, filtration, ion exchange chromatography, size-exclusion chromatography and polymer precipitation–based methodologies.[8-12] The soluble factors and free nucleic acids in CM are vulnerable and easy to be degraded; on the other hand, the membrane of EVs protects the component from a fast degradation. Therefore, EVs are more suitable for storage and transportation for clinical application compared with conditioned medium, with less dosage,[13] smaller volume and longer storage time. Researches on CM have been started in 1950s, due to their easy isolation methods. However, EVs had been considered as by-products of cellular metabolism till their biological roles were recognised. The latest decade has witnessed a booming number of researches on EVs, not only on their important biological roles, but also improved isolation methods and elevated yields.

Up till now, EVs have been identified as three different subtypes, exosomes, microvesicles and apoptotic bodies, classified by their diameters and biogenesis.[14] Exosomes, 30-150 nm in diameter, are generated within the endoplasmic reticulum and are released when multivesicular bodies (MVBs) fuse with the plasma membrane. Exosomes are usually cup-shaped and contain cytokines and growth factors, signalling lipids, as well as mRNA, miRNA and non-coding RNAs.[14,15] Microvesicles (MVs, also known as shedding vesicles, microparticles and ectosomes) are around 50-1000 nm in diameter. Microvesicles, formed by outward budding of the plasma membrane, shuttle local cytosolic proteins and nucleic acids.[16] Apoptotic bodies are larger vesicles (50-5000 nm) released as fragments of dying cells in the late stage of apoptosis, which contain cell debris, organelles and nuclear particulates as a result of karyorrhexis.[14,15,17] EVs not only play important roles in physiological conditions, but also are effective factors for tissue repair and regeneration.

2 | OBJECTIVES

In this review, we aim to summarise the functional roles of cellular secretome, including CM and EVs, in the regenerative medicine for oral maxillofacial rehabilitation, specifically bone, skin, blood vessels, peripheral nerve and tooth-related tissue regeneration, reassuring the feasibility and efficacy of EVs in tissue regeneration. In addition, the content profiles and regulatory mechanisms of EVs from various origins and distinct usages were highlighted, in the service of safety evaluation and modified EV production.

3 | METHODS

Publications were reviewed by two researchers independently basing on three databases (PubMed, MEDLINE and Web of Science), until 31 December 2018. The following keywords were used: (exosome OR (extracellular vesicles) OR microvesicles OR (conditioned medium)) AND (bone OR (blood vessels) OR angiogenesis OR neovascularization OR (skin healing) OR (wound healing) OR nerve OR (neural regeneration) OR (tooth regeneration) OR (dental pulp regeneration)). Titles and abstracts were reviewed by two researchers, respectively. And then, the full texts of the selected articles were further checked to make sure for inclusion. Reference tracking of the above included articles was also performed, and then, related reference articles were included.

4 | RESULTS

By summarising the functions of EVs in different tissue regeneration, we found the origin of EVs for regenerative medicine mainly fall into four categories (Figure 1): (a) related cells in the regenerative niche, such as bone marrow–derived stem cells (BMMSCs), osteoblast for bone regeneration; fibroblasts, keratinocytes and fibrocytes for skin regeneration; endothelial cells (ECs) and endothelial progenitor cells (EPCs) for angiogenesis; Schwann cells, neural stem cells for nerve regeneration; (b) mesenchymal stem cells(MSCs), such as bone marrow mesenchymal stem cells (BMMSCs), adipose-derived stem cells (ASCs), induced pluripotent stem cell (iPSC)–derived mesenchymal stem cells (iPSC-MSCs) and umbilical cord MSCs (ucMSCs); (c) immune cells, such as dendritic cells, monocytes and macrophages; (d) body fluids, such as platelet-rich plasma (PRP), and human umbilical cord blood (UCB). The secretome of different cells are distinct, while the same cells secrete different EVs under varied conditions. By unravelling the regulatory mechanisms of EVs in different and complex tissue regeneration, modified cells and EVs can be produced for precision medicine with high efficacy.

4.1 | EVs in bone regeneration

Oral and maxillofacial bone loss is a challenging clinical problem due to the complex morphology of maxilla and mandible, difficulties in functional bone regeneration for teeth prosthesis or implantation, and challenges in vascularisation and neurotisation of regenerated bone. New bone formation or osteogenesis can be divided into two processes, intramembranous ossification and endochondral ossification. The former one depends on differentiation

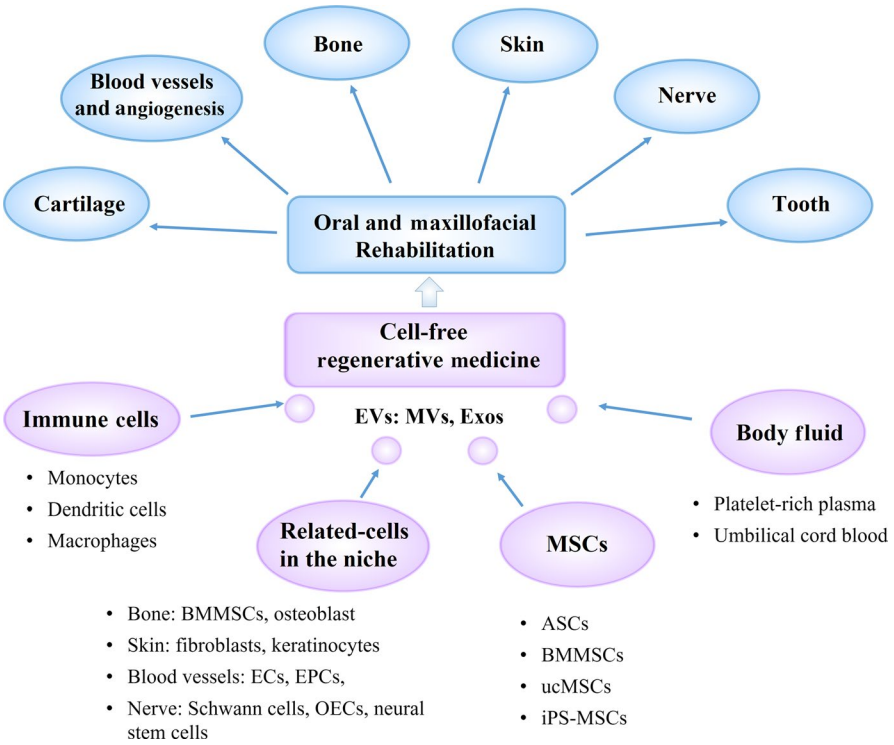
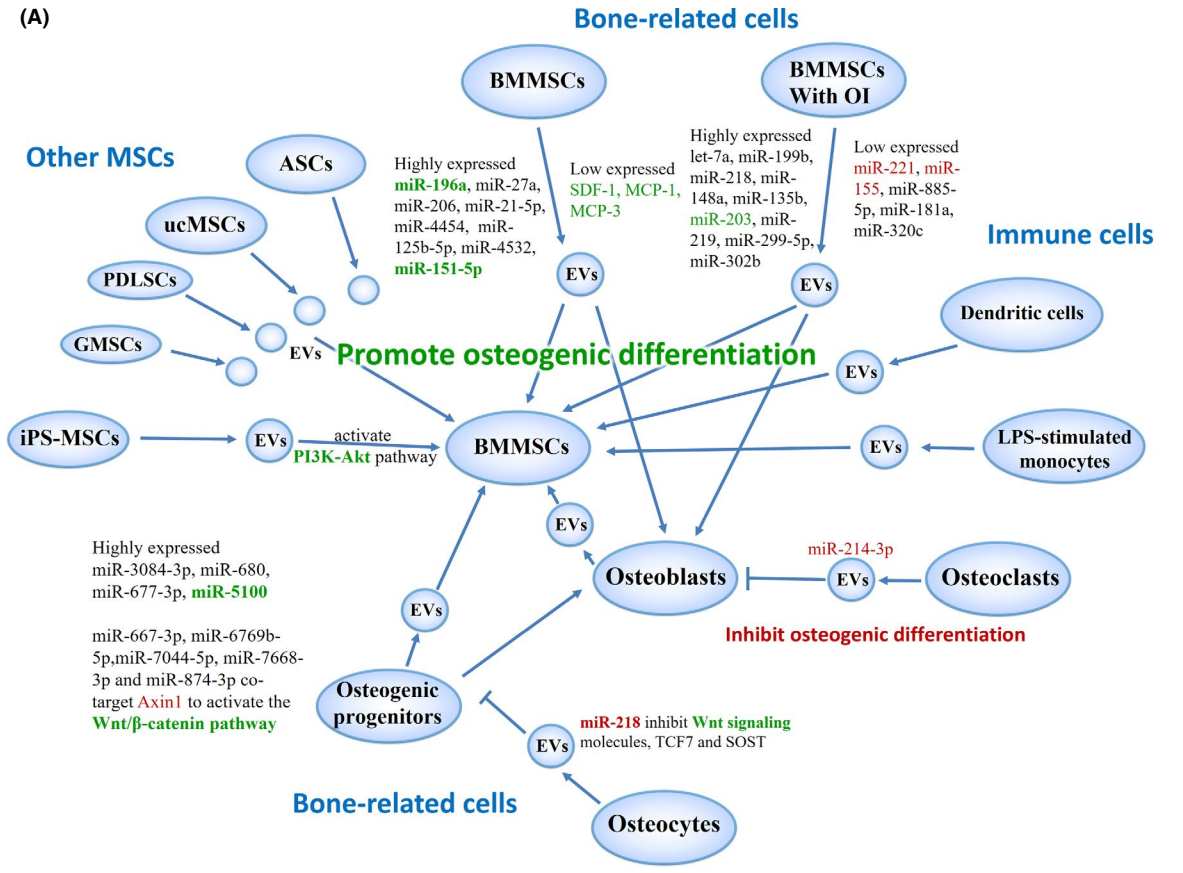


FIGURE 1 Overview of EV origins for different tissue regeneration in oral maxillofacial region. ASCs, adipose-derived stem cells; BMMSCs, bone marrow mesenchymal stem cells; ECs, endothelial cells; EPCs, endothelial progenitor cells; EVs, extracellular vesicles; Exos, exosomes; iPSC-MSCs, induced pluripotent stem cell (iPSC)–derived mesenchymal stem cells; MVs, microvesicles; OECs, olfactory ensheathing cells; ucMSCs, umbilical cord MSCs [Colour figure can be viewed at wileyonlinelibrary.com]

of MSCs into osteoblasts, which is the case in oral maxillofacial region, and the latter one leads to the longitudinal growth of long bones. Bone regeneration involves in complex processes of bone remodelling, which requires cooperation among BMMSCs, osteoblasts, osteoclasts, osteocytes and chondrocytes to maintain the balance of bone metabolism. Apart from bone-related cells, immune cells and endothelial cells (ECs) also contribute to bone remodelling. For example, ECs are involved in osteoblast maturation and angiogenesis. Immune cells, such as dendritic cells (DCs), T cells, monocytes, and macrophages, also participate in the process of bone remodelling.

Bone-related cells, including BMMSCs, osteoblast progenitors, and osteoblasts, contribute to an effective group of secretome to promote bone regeneration (Figure 2A, Table 1). Exogenous BMMSC-EVs can be internalised by endogenous BMMSCs or osteoblasts into the Golgi apparatus,[18] and "turn on" the differentiation of endogenous BMMSCs[19,20] and osteoblasts[18] or rescue the function of BMMSCs in diseased states.[21-23] Exosomes from BMMSCs with or without osteogenic induction can both promote osteogenic differentiation of BMMSCs and osteoblasts.[20,24] There are no statistical difference between EVs and CM from BMMSCs without osteogenic induction, but classical osteoinduction medium (OM) is still better in promoting osteogenic differentiation.[18] Besides abundant evidence in vitro, BMMSC-derived EVs indeed facilitate angiogenesis and bone regeneration in vivo when used with bone scaffolds.[18,19,22,24-26] On the other hand, osteoblast progenitors or osteoblasts can, in turn, regulate the differentiation of BMMSCs via paracrine effects. For example, differentiated osteoblast progenitors (MC3T3-E1 cells) secrete exosomes to promote osteogenic differentiation of BMMSCs by activating Wnt signalling.[27]

MicroRNAs are important composition of EV cargos that contribute to their osteogenic capability.[17] MiR-196a, miR-27a and miR-206 are highly enriched in human bone marrow stromal cell (BMSC)–derived EVs, and miR-196a is verified to be a critical factor regulating osteogenic differentiation.[18] MiR-21-5p, miR-4454, miR-125b-5p and miR-4532 are also highly expressed in human BMSC-derived exosomes, but bone-repair–related proteins (SDF-1, MCP-1 and MCP-3) are lower in BMSC exosomes.[22] MiR-199b, miR-218, miR-148a, miR-135b and miR-221 are also reported to be enriched in hBMSC exosomes.[28] Mouse BMMSC exosomal miR-151-5p promotes osteogenic differentiation of BMMSCs in systematic sclerosis mice by inhibiting IL4Rα expression, thus down-regulating mTOR pathway.[23] MiR-3084-3p, miR-680, miR-677-3p, miR-5100 and miR-5100 are highly expressed in EVs from differentiated mouse osteogenic progenitors (MC3T3-E1 cells), and among the upregulated microRNAs, five upregulated miRNAs (miR-667-3p, miR-6769b-5p, miR-7044-5p, miR-7668-3p and miR-874-3p) co-target Axin1 to activate the Wnt signalling pathway by inhibiting Axin1 expression and increasing β-catenin expression.[27] To maintain the balance of bone remodelling, there are also EVs that carry inhibitors for osteogenesis. Osteoclast-derived exosomal miR-214-3p inhibits bone formation by targeting osteoblasts.[29] Another study showed that EVs from osteoclast precursors promote osteoclastogenesis of mouse marrow haematopoietic precursors, whereas EVs from osteoclasts reduced osteoclast formation instead.[30] Meanwhile, EVs derived from the bone marrow interstitial fluid of aged mice can inhibit the osteogenic differentiation of young BMSCs through miR-183-5p by targeting heme oxygenase-1 (Hmox1), a factor sensitive to oxidative stress and promoting osteoblastic differentiation of BMSCs.[31] Osteocyte-derived exosomal miR-218, which is related


(A)
Bone-related cells
BMMSCs
BMMSCs With OI
Other MSCs
ASCs
ucMSCs
PDLSCs
GMSCs
Highly expressed miR-196a, miR-27a, miR-206, miR-21-5p, miR-4454, miR-125b-5p, miR-4532, miR-151-5p
Low expressed SDF-1, MCP-1, MCP-3
Highly expressed let-7a, miR-199b, miR-218, miR-148a, miR-135b, miR-203, miR-219, miR-299-5p, miR-302b
Low expressed miR-221, miR-155, miR-885-5p, miR-181a, miR-320c
Immune cells
EVs
Promote osteogenic differentiation
Dendritic cells
iPS-MSCs
activate PI3K-Akt pathway
LPS-stimulated monocytes
Highly expressed miR-3084-3p, miR-680, miR-677-3p, miR-5100
miR-214-3p
Osteoblasts
Osteoclasts
Inhibit osteogenic differentiation
miR-667-3p, miR-6769b-5p,miR-7044-5p, miR-7668-3p and miR-874-3p co-target Axin1 to activate the Wnt/β-catenin pathway
Osteogenic progenitors
miR-218 inhibit Wnt signaling molecules, TCF7 and SOST
Bone-related cells
Osteocytes
(B)
MSCs
iPS / iPS-MSCs
ASCs
Body fluids
BMMSCs
lncRNA MALAT1
PRP
ucMSCs
activate PI3K/Akt signaling pathway
EPCs
UCB
Wnt4 miR-21, miR-23a, miR-125b, miR-145
YAP activation
miR-21-3p
α2M
AGWJ of HUC
TGFβ/SMAD2
Fibroblasts
Endothelial cells
Myofibroblasts
ANXA1
expressing α-SMA
Wound healing
Angiogenesis
Scar formation
activate ERK1/2, JNK, Smad, p38 signaling pathways
HSP-90α
STAT3
proangiogenic (miR-126, miR-130a, miR-132)
anti-inflammatory (miR124a, miR-125b)
regulating collagen deposition (miR-21).
keratinocytes
epithelial cells
fibrocytes
Skin-related cells

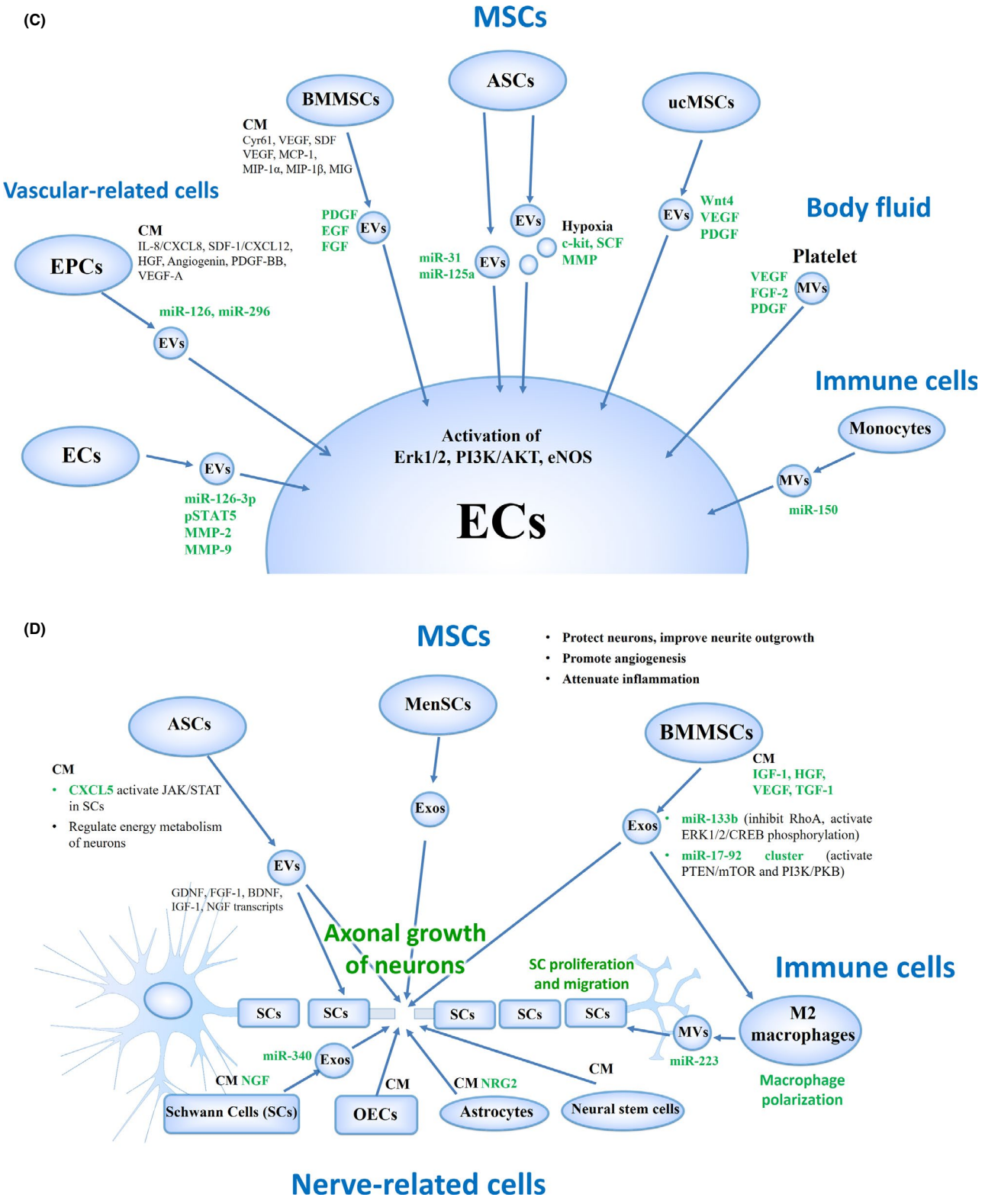


FIGURE 2 Network of interactions via EVs in bone regeneration, skin and wound healing, neovascularisation and nerve regeneration. A, Network of crosstalk among bone-related cells, mesenchymal stem cells and immune cells in bone regeneration; B, network of interactions among skin-related cells, mesenchymal stem cells and body fluids for skin and wound healing; C, network of interactions among vascular-related cells, mesenchymal stem cells, immune cells and body fluids for neovascularisation; D, crosstalk among nerve-related cells, mesenchymal stem cells and immune cells for nerve repair and regeneration. AGWJ of HUC, acellular Wharton's jelly (AGWJ) of the human umbilical cord (HUC); ASCs, adipose-derived stem cells; BMMSCs, bone marrow mesenchymal stem cells; EVs, extracellular vesicles; GMSCs, gingival mesenchymal stem cells; iPSC-MSCs, induced pluripotent stem cell (iPSC)–derived mesenchymal stem cells; OI, osteogenic induction; PDLSCs, periodontal-ligament stem cells; PRP, platelet-rich plasma; UCB, umbilical cord blood; ucMSCs, umbilical cord MSCs [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of researches on extracellular vesicles in bone regeneration

Cell origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
Mouse BMMSCs	Exos	20 μg/mL	—	—	Mesenchymal stem cell transplantation (MSCT) rescued BMMSC function and ameliorated osteopenia in Fas-deficient-MRL/lpr mice (a mouse model of SLE)	Normal BMMSC-Exos transfered Fas to recipient BMMSCs to reduce miR-29b, leading to recovery of Dnmt1-mediated Notch1 promoter hypomethylation	Liu[21]
hBMSCs	EVs CM	5 μg/mL EVs	miR-196a, miR-27a, miR-206	BMSC-EVs promoted osteogenic function of osteoblast. No statistical difference between EVs and CM, while OM was better	BMSC-EVs led to more bone formation in the 5mm critical-sized calvarial bone defects in SD rats	miR-196a played an essential role in osteoblastic differentiation. Exogenous EVs directly entered the Golgi apparatus rather than the lysosomes	Qin[18]
hBMMSCs	Exos	local injection of 100 μl of CM, Exo-depleted CM, or Exos	miR-21-5p, miR-4454, miR-125b-5p, miR-4532	—	BMMSC-Exos rescued delayed fracture healing in CD9-/- mice, a strain with reduced levels of Exos	Bone repair was related to miRNAs. Bone-repair-related cytokines (MCP-1, MCP-3, SDF-1) in Exos were lower compared with CM and Exo-depleted CM	Furuta[22]
hBMMSCs with OM	EVs	—		Promoted osteogenic differentiation of uncommitted hBMMSCs	—	—	Martins[20]
hBMSCs with or without OM	Exos	—	—	Induced osteogenic differentiation of hBMSCs under both 2D and 3D cultural environment	Subcutaneous implantation of hydrogels on the back of nude mice showed the bone regenerative potential of hBMSC-Exos incorporated into type I collagen hydrogels	—	Narayanan[24]
hBMMSCs	MVs	In vitro: 1, 20, and 50 μg/mL In vivo: 1 μg/μL	—	Promoted EC proliferation, migration and tube formation	Enhanced vessel formation and tissue-engineered bone regeneration in a nude mouse subcutaneous bone formation model	—	Xie [25]
Mouse BMMSCs	EVs	In vitro: 20 μg/mL In vivo: 100 μg EVs in 200 μl PBS	miR-151-5p	—	Systemic infusion of Exos rescued osteopenia in Tsk/$^{+}$ systematic sclerosis mice through miR-151-5p transfer	MSCT transferred miR-151-5p through Exos into Tsk^{+} BMMSCs to regulate IL4Rα/mTOR signalling	Chen[23]
Rat BMMSCs	EVs	1, 20, and 50 μg/mL for in vivo	—	Promoted osteogenic differentiation of rat BMMSCs	EVs coated decalcified bone matrix (DBM) promoted bone formation and vascularisation; **MSCs + EVs better than MSCs; MSCs better than EVs**	—	Xie[19]
Rat BMMSCs	Exos	—	—	Promoted the proliferation of osteoblasts	—	MSC-Exo promoted the proliferation of osteoblasts through **MAPK** pathway	Zhao[138]

(Continues)

TABLE 1 (Continued)

Cell origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
DXM-stimulated Rat BMMSCs	MVs	10^7MVs/mL in vitro	—	DXM-MVs promoted the migration and osteogenesis of MC3T3	Injection of 5×10^7/mL MVs in a 2mm diameter and 1mm depth femur defect SD rat promoted bone healing	—	Zhao[26]
Mineralising MC3T3-E1 cells	Exos	3 μg of Exos in 2×10^5 cells	miR-3084-3p, miR-680, miR-677-3p, miR-5100	Promoted BMSCs (ST2) differentiation to osteoblasts	—	Five upregulated miRNAs (miR-667-3p, miR-6769b-5p, miR-7044-5p, miR-7668-3p and miR-874-3p) co-target Axin1 to activate the Wnt signalling pathway by inhibiting Axin1 expression and increasing β-catenin expression	Cui[27]
Osteoclast precursors & osteoclasts	EVs	1×10^7 or 1×10^8 EVs/mL	—	EVs from osteoclast precursors promote osteoclastogenesis, while EVs from osteoclasts reduced osteoclast formation	—	Rank-containing EVs inhibit osteoclastogenesis in $1,25(OH)_2D_3$-stimulated mouse marrow	Huynh[30]
hucMSCs	Exos	20 μg/mL		hucMSC-Exos promoted osteogenic differentiation of hBMSCs without OM	Exos with hydrogel and 1×10^5 rat BMSCs enhanced the bone repair of rat calvarial bone critical defect model		Wang[40]
PlaMSC	Exos	10 μg/well (24-well plate)	—	PlaMSC-exo influenced the differentiation competence of fibroblasts to both osteoblastic and adipocyte differentiation	—	PlaMSC-exo increased the expression of stemness-related genes' mRNA in fibroblasts, including OCT4 and NANOG	Tooi[39]
Senescent ECs	MVs		miR-31	MVs from senescent ECs or circulating MVs from elderly human donors reduced osteogenic differentiation of ASCs	—	Vesicular miR-31 reduced osteogenesis by knocking down its target Frizzled-3 (FZD3), a Wnt5A receptor	Weilner[139]
hiPSC-MSC	Exos	100, or 200 (better) μg/mL Exos	—	hiPSC-MSC-Exos promoted proliferation and osteogenic differentiation of OVX rat BMSC	Exos/β-TCP scaffolds promoted bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis in OVX rats	—	Qi[37]
hiPSC-MSC	Exos	5×10^{11} or 1×10^{12} particles/mL (100 μL)	—	hiPSC-MSC-Exos promoted osteogenic differentiation of hBMSCs	The Exos/β-TCP scaffolds enhanced osteogenesis compared with pure β-TCP scaffolds	Exos activated PI3K-Akt pathway, with increased expression of positive effector genes (PDGFA, FGF1/2, FGFR1, COL1A1/2, and BCL2L1), and a decrease in the negative effector genes (GSK3β, PTEN)	Zhang[38]
hASCs	Exos	10, 25, 50 μ g/mL	—	Promoted osteogenic differentiation of hBMMSCs	Exos with PLA promoted bone formation		Li[34]

(Continues)

TABLE 1 (Continued)

Cell origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hPDLSCs & PEI-EVs	EVs	—	—	More calcium deposition was observed on collagen membrane cultured in hPDLSCs with hPDLSCs EVs or PEI-EVs	Collagen membrane enriched with hPDLSCs or PEI-EVs promoted bone regeneration in rat calvarium defects	—	Diomede[35]
hGMSCs	EVs	—	—	hGMSCs cultured in 3D-PLA + EVs and 3D-PLA + PEI-EVs showed better osteogenic differentiation compared with EVs or PEI-EVs or 3D-PLA	3D-PLA + PEI-EVs + hGMSCs and 3D-PLA + PEI-EVs scaffolds improve bone healing of calvaria bone defect with a diameter of 5 mm		Diomede[36]
LPS-stimulated monocytes	Exos CM	—	—	Promoted osteogenic differentiation of MSCs	—	—	Ekstrom[44]
LPS-activated monocytes & osteoclasts	EVs	—	—	Upregulated the expression of MMPs, and promoted the secretion cytokines by hASCs	—	CXCL5, CXCL3, IL1RN, IL1B, MMP3 and MMP1 were upregulated in hASCs by monocyte EVs	Gebraad[140]

Abbreviations: CM, conditioned medium; ECs, endothelial cells; EV, extracellular vesicle; Exos, exosomes; hBMMSCs, human bone marrow mesenchymal stem cells; hBMSCs, human bone marrow–derived stromal cells; hGMSCs, human gingival mesenchymal stem cells; hiPSC-MSCs, mesenchymal stem cells derived from human induced pluripotent stem cells; hPDLSCs, human periodontal-ligament stem cells; hucMSCs, human umbilical cord–derived mesenchymal stem cells; MCP-1, monocyte chemotactic protein-1; MV, microvesicles; OM, osteoinduction medium; PEI-EVs, polyethyleneimine (PEI)-engineered EVs; TGF-β1, transforming growth factor-β1.

to muscle-bone communication, is found to inhibit osteogenic differentiation of osteoblast progenitors by targeting Wnt signalling molecules (TCF7 and SOST). [32]

Besides BMMSCs, other mesenchymal stem cells, such as ASCs, ucMSCs and iPSC-MSCs, can also secrete abundant EVs, providing wide choices for cell-free bone regeneration. Adipose-derived stem cells, obtained from discarded adipose tissue from liposuction, enjoy the advantages of full abundance and little side effects to donors and are promising cell sources for bone regeneration.[33] Exosomes from human adipose–derived stem cells (hASCs) promote osteogenic differentiation of hBMMSCs and facilitate cell-free bone regeneration in vivo.[34] Meanwhile, tooth-derived MSCs also consist a potential group of origins of EVs for osteogenesis. For example, EVs derived from human periodontal-ligament stem cells (hPDLSCs)[35] and human gingival mesenchymal stem cells (hGMSCs)[36] with cell-free scaffolds can also effectively improve healing of calvaria bone defects. Moreover, exosomes from hiPSC-MSCs accelerate osteogenic differentiation of BMSCs from ovariectomised (OVX) rat and facilitate bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis.[37] Mechanically, hiPSC-MSC exosomes activate PI3K-Akt pathway in BMSCs, leading to higher expression of pro-osteogenic genes (PDGFA, FGF1/2, FGFR1, COL1A1/2 and BCL2L1), and a decrease in the negative effector genes [glycogen synthase kinase 3β (GSK3β), phosphatase and tensin homolog (PTEN)].[38] Human placenta MSC (PlaMSC)–derived exosomes influence the differentiation competence of normal adult human dermal fibroblasts (NHDF) to both osteoblastic and adipocyte differentiation by increasing the expression of stemness-related genes mRNA, OCT4 and NANOG.[39] Exosomes from human umbilical cord–derived mesenchymal stem cells (hucMSCs) can promote osteogenic differentiation of BMSCs, but the in vivo bone-repair effect was examined under the existence of BMSCs.[40]

Inflammation is recognised as a double-edged sword for bone regeneration, with constant communications between immune cells and bone-related cells. Extracellular vesicles from BMMSCs may attenuate inflammatory responses by modulating maturation, apoptosis and proliferation of T cells.[41,42] In turn, EVs from immune cells, such as DCs, can be internalised by BMMSCs to promote their recruitment, homing and osteogenic differentiation.[43] Meanwhile, activated monocytes can also contribute to bone regeneration. Exosomes and CM from lipopolysaccharide (LPS)-stimulated human monocytes are found to promote osteogenic differentiation of BMMSCs.[44]

4.2 | EVs in skin and wound healing

Skin restoration without scar or little scar is important for oral maxillofacial region. How to accelerate wound healing and reduce scar formation are two major obstacles for soft tissue regeneration and skin healing. The process of wound healing includes four stages: coagulation or haemostasis, inflammation, cellular migration and proliferation, and tissue remodelling.[45-47] During cellular proliferative phase, fibroblasts differentiate into contractile myofibroblasts expressing α-smooth muscle actin (α-SMA) mediated by the TGF-β signalling pathway,[48] whereas myofibroblast aggregations lead to excessive scar formation. Therefore, factors that promote fibroblast migration and proliferation will accelerate wound healing, while methods to inhibit excessive myofibroblastic differentiation are potential to reduce scar formation.

In the first place, skin-and-epithelial-related cells are important origin of EVs for skin regeneration (Table 2, Figure 2B). Exosomes from fibrocytes, containing HSP-90α, total and activated STAT3, proangiogenic microRNAs (miR-126, miR-130a, miR-132), anti-inflammatory microRNAs (miR124a, miR-125b) and miR-21 (regulating collagen deposition), promote tube formation of endothelial cells (ECs) and the proliferation of diabetic dermal fibroblasts, thus accelerating wound closure in diabetic mice.[49] Microvesicles from human keratinocytes activate ERK1/2, JNK, Smad, and p38 signalling pathways in fibroblasts, decreasing cadherin-2 expression and reducing α-SMA mRNA expression, alleviating the process of myofibroblast differentiation.[50] Although excessive myofibroblasts are unexpected, they are also needed in the balanced process of wound healing, since MVs from myofibroblasts benefit angiogenesis in skin regeneration.[51] Exosomes from human amniotic epithelial cells (hAECs) are also reported to promote fibroblast proliferation and migration, and shorten the healing time and narrow the scars in full-thickness skin wounds of SD rats.[52] The RNA component of exosomes plays a critical role in this process, since exosomes with RNase A lose the ability to facilitate wound healing compared with exosomes or exosomes with proteinase K.[53]

Extracellular vesicles derived from MSCs are also promising candidates for skin regeneration. Exosomes from hucMSCs delivered Wnt4 to activate β-catenin nuclear trans-location and activity, and activated AKT pathway to enhance proliferation and migration of keratinocytes.[54] More importantly, exosomes from hucMSCs suppress myofibroblast aggregation via microRNAs (miR-21, miR-23a, miR-125b and miR-145) to inhibit excess αSMA and collagen deposition by regulating TGFβ/SMAD2 signalling pathway to minimise scar formation.[48] Extracellular vesicles from ASCs are considered better than EVs from BMMSCs in wound healing.[55] Extracellular vesicles from hASCs promote fibroblasts migration, proliferation and collagen synthesis [56] by increasing the level of p-Akt/Akt to activate PI3K/Akt signalling pathway.[57] hASC-derived exosomes contain lncRNA MALAT1, which is essential to stimulate fibroblast migration and angiogenesis involved in wound healing.[58] Meanwhile, MSCs can be stimulated or modified to secrete tailored EVs to meet certain clinical requirements, such as inflammatory status under diabetic conditions. For example, exosomes from hASCs overexpressing Nrf2 inhibited ROS and inflammatory cytokine expression and accelerate cutaneous wound healing of rat diabetic foot ulcers.[59] Synovium mesenchymal stem cells (SMSCs) overexpressing miR-126-3p accelerated re-epithelialisation, activated angiogenesis and promoted collagen maturity in full-thickness skin defect in a diabetic rat model.[60] Moreover, exosomes from hiPSCs[61] or hiPSC-MSCs[62] promote vessel formation, accelerate cutaneous wound healing and reduce scar widths in the process of skin and wound healing. These

TABLE 2 Summary of researches on extracellular vesicles in skin and wound healing

Cell Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
Fibrocytes	Exos	In vitro: 0.1, 1, and 10 μg/mL In vivo: 0.1, 1, and 10 μg/site	HSP-90α, STAT3, miR-126, miR-130a, miR-132, miR124a, miR-125b, miR-21	Promoted tube formation of ECs; enhanced migration and proliferation of diabetic keratinocytes, and proliferation of dermal fibroblasts	Accelerated wound closure in diabetic mice	—	Geiger[49]
Human keratinocytes (hKCs)	MVs	—	—	Regulated MMP-1, MMP-3, cadherin-2, THBS1 and IL-6 protein in fibroblasts; promoted fibroblast migration and fibroblast-mediated angiogenesis	—	Activated ERK1/2, as well as JNK, Smad and p38 signalling pathways in fibroblasts	Huang[50]
Intestinal ECs	EVs	—	Annexin A1 (ANXA1)	Resealed mucosal wounds in ex vivo cultures	Accelerated recovery of murine colitis	—	Leoni[141]
EPCs	Exos	2×10^{10} or 1×10^{11} particles/mL of Exos	—	Enhanced EC proliferation, migration and angiogenic tubule formation	Promoted wound healing in diabetic rats	Activated Erk1/2 signalling in ECs to promote angiogenesis thus facilitating cutaneous wound repair	Zhang[73]
EPCs	Exos	100 μg/mL	—	Enhanced EC proliferation, migration and tube formation; enhanced EC expression of angiogenic molecules (FGF-1, VEGFA, VEGFR-2, ANG-1, E-selectin, CXCL-16, eNOS, IL-8)	Accelerated cutaneous wound healing in diabetic rats	—	Li[74]
hAECs	Exos	In vitro: 25, 50, 100 μg/mL In vivo: 100 μL of 25, 50, 100 μg/mL Exos		Promoted fibroblasts proliferation and migration, while downregulated collagen expression	Shortened the healing time and **narrowed scars** in full-thickness skin wounds of SD rats	—	Zhao[52]
hAECs	Exos	in vivo: 100 μL of 50 μg/mL Exos	—	Promoted fibroblasts proliferation and migration, while downregulated collagen expression via RNA components	Accelerated wound healing in BALB/c mice	Exosomal miRNAs play an important role in wound healing	Zhao[52]
iPSCs	Exos	In vitro: 0, 10, 50, 100, or 200 μg/mL In vivo: 4 μg Exos/20 μl PBS	—	Promoted the migration and proliferation of diabetic fibroblasts	Promoted cutaneous wound healing, angiogenesis and peripheral nerve fibres regeneration in diabetic mice	—	Kobayashi[61]
hiPSC-MSCs	Exos	In vitro: 50, 100 μg/mL	—	Promoted the proliferation and migration of fibroblasts and ECs; increased type I, III collagen and elastin secretion and mRNA expression by fibroblasts and tube formation by ECs	Accelerated re-epithelialisation, reduced scar widths; promoted collagen maturity; promoted the generation of newly formed vessels	Promoted cutaneous wound healing by facilitating collagen synthesis and angiogenesis	Zhang[62]

(Continues)

TABLE 2 (Continued)

Cell Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hASCs	Exos	In vitro: 25, 50, 100 μg/mL In vivo: intravenous injection of 200 μg Exos	—	Promoted fibroblasts migration, proliferation and collagen synthesis in a dose-dependent manner, with increased genes expression of N-cadherin, cyclin-1, PCNA and collagen I, III	hASCs-Exos can be recruited to soft tissue wound area after systemic administration, and accelerated cutaneous wound healing	—	Hu[56]
hASCs	MVs	—	—	Enhanced wound healing ability of diabetic hASCs; rescued the expression of SDF-1, CXCR4, CXCR7, CCL2, and ANGPL4, genes that associated with cell migration, survival, inflammation, and angiogenesis, in diabetic hASCs	Normal hASCs MV-treated diabetic hASCs improve the wound healing ability in a mouse flap model	miR29c and miR150 was down-regulated in diabetic hASCs after treated by normal hASCs MVs	Trinh[142]
hASCs	CM Exos	10, 20 μg/mL Exos	lncRNA MALAT1	hASCs CM promoted cell migration of human dermal fibroblasts. Exosomes isolated from MALAT1-depleted CM failed to enhance cell migration	CM promoted the closure of ischaemic wounds in a rat model	Exosomal lncRNA MALAT1 is essential to stimulate fibroblast migration and angiogenesis involved in wound healing	Cooper[58]
hASCs	Exos	In vitro: 0, 25, 50 (optimal), 100 μg/mL In vivo: 200 μg of Exos in 200 μl PBS	—	hASC-Exos promoted fibroblast proliferation and migration, optimised collagen deposition and **increased the level of p-Akt/Akt**	hASC-Exos accelerated wound healing (1 × 1cm^2) in Balb/c mice	hASC-Exos promoted wound healing via the **PI3K/Akt** signalling pathway	Zhang[57]
hASCs over-expressing Nrf2	Exos	50 μg/mL	—	hASC-Exos reduced glucose-induced EPCs senescence. Nrf2-overexpressing hASC-Exos inhibited ROS and inflammatory cytokine expression	EPCs combined with Nrf2-overexpressing hASC-Exos accelerated cutaneous wound healing of rat diabetic foot ulcers	Nrf2-overexpressing hASC-Exos improved levels of SMP30 and VEGF and increased VEGFR2 phosphorylation, whereas ROS and inflammatory cytokine levels were reduced	Li[59]
rabbit ASCs & rabbit BMMSCs	EVs	local injection of 10 × 10^6 cells, or EVs obtained from equal number of cells	—	—	**EVs were better than MSCs** in the treatment of rabbit cutaneous wound model; **ASC-EVs were better than BMMSC-EVs**	—	Pelizzo[55]
hBMMSCs	Exos	0.1, 1, and 10 μg/mL	—	Exos caused a dose-dependent enhancement of proliferation and migration of fibroblasts derived from normal donors and chronic wound patients	—	MSC-exosomes were found to activate signalling pathways important in wound healing (Akt, ERK, and STAT3) and induce the expression of a number of growth factors (HGF, IGF1, NGF and SDF1)	Shabbir[80]

(Continues)

TABLE 2 (Continued)

Cell Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
LPS- pre-treated hucMSCs	Exos	10 μg/mL	Let-7b	Activated M2 macrophages	Alleviated inflammation and benefited diabetic cutaneous wound healing	Regulated inflammation through TLR4/NF-κB/STAT3/AKT signal pathway	Ti[143]
hucMSCs & human lung fibroblasts (HFL1)	Exos	1×10^6 cells, or 200 mg Exos	Wnt4	HucMSC-Exos inhibited heat stress-induced apoptosis of keratinocytes and promoted their proliferation compared with HFL1-Exos	HucMSC-Exos accelerated the re-epithelialisation, with increased expression of CK19, PCNA, collagen I (compared to collagen III) of skin second-deep burn injury in rats	hucMSC-Exos delivered Wnt4 to activate β-catenin nuclear trans-location and activity, and activated AKT pathway to enhance proliferation and migration of skin cells	Zhang[54]
hucMSCs	Exos	100 μg/mL	miR-21, miR-23a, miR-125b, and miR-145	hucMSC-Exos suppressed TGF-β-induced myofibroblast formation, which mainly depended on the RNA components from Exos	hucMSC-Exos suppressed myofibroblast aggregation and scar formation in a full-thickness skin defect in mice	miRNAs played key roles in suppressing myofibroblast formation by inhibiting excess αSMA and collagen deposition associated with activity of the TGFβ/SMAD2 signalling pathway	Fang[48]
GMSCs	Exos	—	—	—	Chitosan/Silk hydrogel with Exos promoted healing of skin defects in diabetic rats, with more microvessel and nerve density	—	Shi[144]
SMSCs over-expressing miR-126-3p	Exos	—	—	Exos promoted proliferation, migration and tube formation human dermal microvascular endothelial cells (HMEC-1)	Chitosan wound dressings with Exos accelerated re-epithelialisation, activated angiogenesis and promoted collagen maturity in full-thickness skin defect in a diabetic rat model	—	Tao[60]
PRP	Exos	—	—	PRP-Exos promoted proliferation and migration of ECs and fibroblasts to improve angiogenesis and re-epithelialisation	PRP-Exos-loaded sodium alginate hydrogel promoted healing of chronic ulcers in diabetic rat model	PRP-Exos promoted fibroblast proliferation and migration through YAP activation	Guo[63]
PRP	Exos	—	—	—	PRP-Exos/ZWP combination accelerated wound healing in diabetic rats comparing with either PRP-Exos or ZWP	—	Xu[64]

(Continues)

TABLE 2 (Continued)

Cell Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
human umbilical cord blood (UCB)	Exos	100 μg/mL Exos (200 μg/well in a 6-well plate)	miR-21-3p	UCB-Exos could promote the proliferation and migration of fibroblasts, and enhance the angiogenic activities of endothelial cells	The local transplantation of UCB-Exos into mouse skin wounds resulted in accelerated re-epithelialisation, reduced scar widths, and enhanced angiogenesis	miR-21-3p, highly enriched in UCB-Exos, promoted angiogenesis and fibroblast function through inhibition of phosphatase and tensin homolog (PTEN) and sprouty homolog 1 (SPRY1)	Hu[65]
AGWJ of HUC	Exos	—	α2M	AGWJ-Exos promoted fibroblasts viability and cell migration	AGWJ-Exos with matrigel enhanced skin wound healing on the back of C57/BL6 mice	α2M was highly expressed in AGWJ-Exos, and enhanced cell proliferation, migration and cell viability	Bakhtyar[66]

Abbreviations: AGWJ of HUC, acellular Wharton's jelly (AGWJ) of the human umbilical cord (HUC); CM, conditioned medium; ECs, endothelial cells; EV, extracellular vesicle; Exo, exosome; GMSCs, gingival mesenchymal stem cells; hAECs, human amniotic epithelial cells; hASCs, human adipose–derived stem cells; hEPCs, human endothelial progenitor cells; HGF, hepatocyte growth factor; hiPSC-MSCs, human induced pluripotent stem cell–derived mesenchymal stem cells; HSP, heat shock protein; hucMSCs, human umbilical cord MSCs; IGF, insulin-like growth factor; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; NGF, nerve growth factor; PRP, platelet-rich plasma; SDF, stromal-derived growth factor; SMSCs, Synovium mesenchymal stem cells; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; α2M, alpha-2-macroglobulin; αSMA, α-smooth muscle actin.

findings suggest that EVs from MSCs could potentially minimise scar tissue formation and promote skin tissue regeneration by facilitating vascularisation in the wound sites.

Exosomes derived from various body fluids can also accelerate wound healing. PRP-derived exosomes not only accelerate proliferation and migration of fibroblasts to stimulate re-epithelialisation, but also promote proliferation and migration of ECs to improve angiogenesis, thus benefiting wound healing.[63,64] Mechanically, PRP exosomes promote fibroblast proliferation and migration through YAP activation.[63] In addition, human umbilical cord blood (UCB) exosomes can also promote the proliferation and migration of fibroblasts, as well as exerting proangiogenic effects on ECs through miR-21-3p which inhibit PTEN and sprouty homolog 1 (SPRY1).[65] Exosomes derived from acellular Wharton's jelly (AGWJ) of the human umbilical cord promote fibroblasts viability and cell migration and accelerate wound healing through alpha-2-macroglobulin (α2M).[66]

4.3 | EVs in neovascularisation

Angiogenesis is well recognised to be essential in various tissue regenerative processes, including bone regeneration, skin and wound healing, and nerve regeneration. Just as bone remodelling and wound healing, vascularisation is also a dynamic and balanced process basing on communications between vascular endothelial cells (ECs) and their surrounding environment. During this angiogenic process, EC proliferation, migration and tube formation are the basis of new blood vessel formation.

Extracellular vesicles released by ECs and endothelial progenitor cells (EPCs) have been documented as effective mediators of neovascularisation (Figure 2C, Table 3). Microvesicles (MVs) or shedding vesicles from ECs, containing proenzyme forms of matrix metalloproteinases, including matrix metalloproteinase-2 (MMP-2) and MMP-9, promote the formation of capillary-like structures by other ECs. Extracellular factors, such as serum, FGF2 and VEGF, help to stimulate the shedding of MMPs as vesicle components.[67] Extracellular vesicles generated by ECs under interleukin-3 (IL3) stimulation promote vascularisation via the delivery of miR-126-3p and pSTAT5 into the recipient ECs thus activating Erk1/2 signalling.[68] Meanwhile, EPCs, a group of progenitor cells that play in important role in postnatal neovascularisation, have higher angiogenic potential compared with mature ECs. Therefore, EVs derived from EPCs have been recognised as potent regulators for neovascularisation. Hypoxic EPC-conditioned medium (CM), highly enriched in angiogenetic factors (angiogenin, PDGF-BB and VEGF-A), is as effective as EPC-based cell therapy for tissue revascularisation and muscle function.[69] Microvesicles of EPCs were reported to contain microRNAs (miR-126, miR-296)[70,71] and mRNAs (MAPKAPK2, eNOS, BCL-XL, CTNNB1),[72] and can be internalised by ECs via integrins α4 and β1 on the surface of EPC-derived MVs. EPC-derived MVs stimulate EC proliferation and new blood vessel formation by activation of the PI3K-Akt and eNOS signalling pathways in ECs mainly depending on their RNA component.[70-72] Meanwhile, exosomes from EPCs

TABLE 3 Summary of researches on extracellular vesicles in neovascularisation

Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
HUVECs	MVs	—	MMP-2, MMP-9 proenzyme forms	Promoted the formation of capillary-like structures by HUVECs	—	Serum, FGF2 and VEGF stimulated the shedding of MMPs as vesicle components	Taraboletti[67]
HUVECs	EVs	—	miR-126-3p pSTAT5	IL-3 (Inflammatory stimuli) increased EV secretion and improved proangiogenic capability of EVs	—	Transferred miR-126-3p and pSTAT5 into recipient ECs thus activating Erk1/2 signalling	Lombardo[68]
HUVECs	CM	1.5×10^4 hASCs in 100 μL CM	—	EC-CM promoted hASCs proliferation, migration, invasion and angiogenesis	—	—	Luo[145]
hEPCs	MVs	10 μg/mL	mRNA of *BCL-XL*, *CFL1*, *CTNNB1*, *EDF1*, *MAPKAPK2*, *PTPRT*, *eNOS*, *POLR2B*	Integrins α4 and β1 on MVs' surfaces mediated the incorporation of MVs into ECs MVs promoted ECs survival, proliferation and organisation in capillary-like structures	MV-stimulated human ECs organised in patent vessels in SCID mice	RNAs in MVs played an important role in angiogenesis MVs induced the activation of the **PI3K-Akt** and **eNOS** signalling pathways in ECs	Deregibus[72]
hEPCs	CM	250 μl EPC-CM, or 1×10^6 EPCs	IL-8/CXCL8, SDF-1/CXCL12, HGF, Angiogenin, PDGF-BB, VEGF-A increased in CM after 72h of hypoxia	Secretion of growth factors by EPCs was increased by hypoxia EPC-CM inhibited apoptosis of mature ECs and promoted angiogenesis in a rat aortic ring assay	Intramuscular injection of **EPC-CM was as effective as EPC transplantation** for promoting tissue revascularisation and functional recovery	—	Di Santo[69]
hEPCs	MVs	10 μg/mL	miR-126, miR-296	EPC-MVs enhanced human islet vascularisation MVs pretreated with RNase or derived from Dicer knocked-down EPCs showed a reduced angiogenic effect	EPC-MVs favoured insulin secretion, survival and revascularisation of islets transplanted in SCID mice	MVs induced the activation of the **PI3K-Akt** and **eNOS** signalling pathways in islet endothelium	Cantaluppi[70]
hEPCs	MVs	intravenous injection of 50 μg MVs	miR-126 and miR-296	—	EPC-MVs improved neovascularisation and favoured regeneration in severe hindlimb ischaemia induced in SCID mice	RNAs in MVs played an important role in angiogenesis	Ranghino[71]
hEPCs	Exos	2×10^{10} or 1×10^{11} particles/mL of Exos	—	EPC-Exos enhanced ECs proliferation, migration and angiogenic tubule formation	EPC-Exos enhanced proangiogenic and wound healing effects in streptozotocin-induced diabetic rats	EPC-Exos promoted angiogenesis of ECs by activating **Erk1/2** signalling	Zhang[73]

(Continues)

TABLE 3 (Continued)

Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hEPCs	Exos	100 μg/mL	—	EPC-Exos enhanced the proliferation, migration and tube formation of vascular ECs	EPC-Exos transplantation accelerated cutaneous wound healing in diabetic rats	FGF-1, VEGFA, VEGFR-2, ANG-1, E-selectin, CXCL-16, eNOS and IL-8 were upregulated in ECs stimulated with EPC-Exos	Li[74]
hEPCs	Exos	In vitro: 100 μg/mL In vivo: 30 μg Exos intravenous injection	—	EPC-Exos enhanced the proliferation and migration of ECs	EPC-Exos accelerated the re-endothelialisation in the early phase after endothelial damage	-	Li[75]
hMSCs	CM	—	—	hMSCs-CM inhibited hypoxia-induced apoptosis and promoted tube formation of ECs	—	CM from hypoxic hMSCs activated the **PI3K-Akt** pathway and increased p-Akt; increased the levels of pERK, but had minimal effect on pSTAT3	Hung[76]
MSCs after 24h serum-free or hypoxia	CM	-	-	MSCs exhibited resistance to hypoxia which induced increased secretion of VEGF and decreased levels of other cytokines, including SDF-1	Factors secreted by mesenchymal stem cells and endothelial progenitor cells had complementary effects on angiogenesis in vitro	-	Burlacu[77]
Peripheral blood-derived MSCs	CM	—	Activin A, angiopoietin 1, ET1, IGFBP2, IL-8, PDGF-AA, uPA, VEGF	Promoted proliferation and tube-like formation of ECs	-	ET1, IL-8, PDGF-AA, and IGFBP2, but not uPA, stimulated tube formation of ECs	Bussche[146]
BMMSCs	CM	—	Cysteine-rich protein 61 (Cyr61)	MSC CM promoted morphogenesis of ECs	ECs with matrigel cultured in MSC-CM implanted subcutaneously in athymic mice induced neovascularisation	Cyr61 polypeptide in MSC secretome contributes to the angiogenesis-promoting activity	Estrada[147]
Mouse BMMSCs	CM	—	VEGF, MCP-1, MIP-1α, MIP-1β, MIG	Promoted angiogenesis; MCP-1 and MIP-1α increased MSCs migration while VEGF reduced it; increased p-Akt (Thr308) in rat neonatal H9c2 myoblasts	—	Both MCP-1 and PI3K-Akt were involved in the protective effect, independent of each other; MCP-1 displayed a protective effect by reducing caspase-3 activity	Boomsma[148]
hBMMSCs	MVs	In vitro: 1, 20, 50 μg/mL In vivo: 1 μg/μL	—	Promoted proliferation, migration and tube formation of HUVECs	MV-alginate-PCL constructs enhanced vessel formation and tissue-engineered bone regeneration	—	Xie[19]

(Continues)

TABLE 3 (Continued)

Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hBMMSCs	Exos	5, 10, 20 μg/mL	Proteomic analysis showed increased level of factors related to PDGF, EGF, FGF	Promoted tubule formation of HUVECs	—	NFκB signalling was a key mediator of MSC-Exos induced angiogenesis in ECs	Anderson[78]
rat BMMSCs	Exos	10 μg/mL	—	Enhanced the tube formation of ECs, and impaired T-cell function by inhibiting cell proliferation	Enhanced the density of new functional capillary and recovered blood flow in rat myocardial infarction model	—	Teng[79]
hASCs	EVs	10k, 100k, and total 100k fractions of b-EVs and PDGF-EVs	c-kit, SCF in PDGF stimulated the ASC-EVs	ASC-EVs induced vessel-like structure formation of ECs. PDGF stimulated the secretion of EVs, changed protein composition and enhanced the angiogenic potential	Promoted vessel formation of ECs when injected subcutaneously within Matrigel in SCID mice	—	Lopatina[82]
ASCs	MVs	—	—	Promoted EC proliferation, migration and tube formation	—	—	Pascucci[149]
hASCs	Exos	25, 50, 100, 200 μg/mL	miR-125a	Promoted ECs angiogenesis	—	Exosomal miR-125a repressed the angiogenic inhibitor DLL4	Liang[81]
hASCs	MVs	30 μg/mL	miR-31; more small RNA (77.8%) in MVs compared with ASCs (7.5%)	Promoted the migration and tube formation of ECs	—	miR-31 contributed to MV-triggered angiogenesis by targeting FIH1 in ECs	Kang[150]
hucMSCs after hypoxia	MVs	10 μg/mL	—	Promoted proliferation, tube formation of ECs	Promoted new vessel formation in a limb ischaemic model	—	Zhang[84]
hucMSCs	MVs	—	Angiogenin, VEGF, MCP-1, VEGF R2, UPAR, VEGF, IGF, Tie-2/TEK, IL-6	Under hypoxic condition, most cytokines were expressed in greater quantity than normoxic in MSCs, while in MVs there was no significant difference except UPAR, Angiogenin, VEGF, IGF, Tie-2/TEK and IL-6	—	—	Chen[85]
hucMSCs	Exos	80, 160 μg/mL in vitro; 200 μg in vivo	Wnt4	Promoted the proliferation, migration and tube formation of ECs	Promoted angiogenesis in a cutaneous burn model	Promoted angiogenesis by activating **Wnt4/β-Catenin** pathway	Zhang B[86]
hucMSCs	EVs	—	RNA VEGF	EVs delivered VEGF to renal tubular epithelial cells, and all beneficial effects of EVs were abrogated by RNase except for the delivery of VEGF	Improved renal function, up-regulated VEGF and down-regulated HIF-1α	-	Zou[151]

(Continues)

TABLE 3 (Continued)

Origin	Kind of secretome	Dose	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hucMSCs overexpressing Akt	Exos	100 μg/mL in vitro; 400 μg in vivo	PDGF-D	Accelerated EC proliferation and migration, tube formation	promoted blood vessel formation	PDGF-D, upregulated in Akt-Exo. was important for the angiogenesis	Ma[87]
PlaMSCs	Exos CM	–	–	Both CM and Exos promoted migration and tube formation of ECs	Both CM and Exos promoted angiogenesis in ischaemic injury model, but Exo-depleted CM did not	–	Komaki[152]
Platelet microvesicles	MVs	10, 20, 50 μg/mL	–	Promoted EC tube formation in a dose-dependent manner; promoted EC migration	–	Upregulated MMP-2 and MMP-9 in ECs	Sun[89]
Monocytes	MVs	–	miR-150	Promoted EC migration and tube formation	Promoted angiogenesis	miR-150 inhibited c-Myb in ECs	Li[88]

Abbreviations: ECs, endothelial cells; EGF, epidermal growth factor; EPCs, endothelial progenitor cells; ERK, extracellular signal–regulated kinase; FGF, fibroblast growth factor; hEPCs, human endothelial progenitor cells; hucMSCs, human umbilical cord mesenchymal stem cells; HUVECs, Human umbilical vein endothelial cells; MCP, monocyte chemoattractant protein; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; PlaMSCs, human placenta–derived mesenchymal stem cells; SCID mice, severe combined immunodeficient mice; SDF, stromal-derived factor; UPAR, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor.

mediate their angiogenic effects by activating Erk1/2 signalling in vascular ECs, leading to upregulation of VEGFA, VEGFR-2, ANG-1, FGF-1, E-selectin, CXCL-16, eNOS and IL-8 in ECs.[73-75] Therefore, CM, MVs and exosomes from EPCs all have the capacity to stimulate blood vessel formation, while the angiogenic effects of EVs mainly depend on their RNA component, as verified by EVs with RNAase showed significantly decreased activity in angiogenesis.

Mesenchymal stem cells (MSCs)also secrete EVs as important mediators to interact with ECs and EPCs to regulate angiogenesis. MSCs have potent proangiogenic properties attributed to their secretion of paracrine factors, and the functionality of the MSC secretome is strongly influenced by the microenvironment, such as hypoxia, inflammation or other chemical inducers. Conditioned medium from MSCs on hypoxia stimulation is angiogenic by increasing the level of p-Akt and pERK in ECs, activating PI3K-Akt pathway with minimal effect on pSTAT3.[76,77] Extracellular vesicles from BMMSCs carry proangiogenic proteins related to PDGF, EGF and FGF, and promote neovascularisation and restrain the inflammation response of ECs through NFκB signalling and the transfer of transcriptionally active STAT3.[25,78-80] Exosomes derived from ASCs are also shown to promote angiogenesis in vitro and in vivo, due to the delivery of miR-125a targeting δ-like 4 (DLL4), an angiogenic inhibitor.[81] PDGF treatment changes EV protein composition, with an enrichment of c-kit, SCF (stem-cell factor), and metalloprotease content to stimulate angiogenesis more effectively.[82] ASCs cultured in endothelial differentiation medium tend to release more MVs with proangiogenic properties by shuttling miR-31 to inhibit HIF-1α.[83] Small RNAs take the majority (77.8%) of the composition of EVs compared with ASCs (7.5%). EVs from ucMSCs are also proangiogenic. Similarly with BMMSCs and ASCs, hypoxia stimulates MV secretion of hucMSCs with greater quantity and increased level of proangiogenic factors, including angiogenin, VEGF and IGF.[84,85] Exosomes released from hucMSCs promote angiogenesis by transferring Wnt4 and activating Wnt4/β-Catenin pathway in ECs.[86] Akt-overexpressing hucMSCs secrete exosomes carrying platelet-derived growth factor-D (PDGF-D), another key protein composition in EVs to stimulate angiogenesis.[87]

Extracellular vesicles derived from immune cells also actively participate in vascular homeostasis. Microvesicles from monocytes carry miR-150 to inhibit c-Myb in ECs, thus facilitating the proliferation and migration of ECs in vitro and angiogenesis in vivo.[88] Moreover, platelet MVs isolated from healthy donors are proangiogenic via delivery of cytokines, such as VEGF, FGF-2 and PDGF, to activate PI3 kinase, src kinase and ERK.[89]

4.4 | EVs in peripheral nerve regeneration

Peripheral nerve injury in oral maxillofacial region as results of tumour or trauma, leading to partial or complete sensory and motor nerve dysfunction, severely influences the life quality of patients. However, current therapies have remained limited and unsatisfied because of poor functional recovery.[90] Usually, larger neurologic defects are extremely difficult to repair, because the axons of neurons can only outgrow about 1 mm per day after injury.[91] Although tissue

TABLE 4 Summary of researches on cell secretome in nerve regeneration

Origin	Kind of secretome	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
rat Schwann cells	CM	nerve growth factor (NGF)	Both SC-CM and noncontact co-culture of SCs promoted dorsal root ganglia (DRG) neurite outgrowth	—	—	Hu[92]
rat Schwann cells	Exos	—	Promoted DRG axonal regeneration after axotomy, and promoted growth cone extension	Enhanced axonal regeneration after local injection into crushed sciatic nerves	—	Lopez-Verrilli[99]
OECs & B104 neuroblastoma cells	CM	—	Promoted neurogenic differentiation of ASCs	—	—	Lo Furno[94]
OECs	CM	—	—	BMMSCs induced by OEC-CM promoted nerve fibre regeneration following spinal cord injury	—	Feng[95]
OECs	CM	—	—	Improved the functional recovery and promoted the axonal regeneration around the injury epicentre	—	Gu[96]
Astrocytes	CM	NTAK (NRG2)	Promoted survival and neurite outgrowth of neurons	—	NTAK bound to ErbB3, stimulated phosphorylation of ErbB3 in neurons	Nakano[153]
Human foetal neural stem cells (hNSCs)	CM	—	—	Helped to set up a contour neural circuit via secretory factors after spinal cord injury	—	Liang[154]
Rat BMSCs	CM	IGF-1, HGF, VEGF, TGF-β1	Promoted survival and neurite outgrowth of neurons	—	—	Nakano[107]
Rat BMMSCs cultured in brain extracts after ischaemia	Exos	miR-133b, cel-miR-67	Increased the neurite branch number and total neurite length of neurons	—	—	Xin[115]
Rat BMMSCs	Exos	miR-133b	—	Improved neurite remodelling and functional recovery after stroke	miR-133b inhibited connective tissue growth factor (CTGF) and RhoA	Xin
Rat BMMSCs	CM	Anti-apoptotic factors: VEGF-A, TIMP-1, etc Pro-inflammatory: TLR4, CINC-3, etc Proangiogenic	Protected neurons from apoptosis; activated macrophages to secrete pro-inflammatory cytokines, IL-1β, IL-6, TNFα; proangiogenic effect	Improved motor recovery after spinal cord contusion	—	Cantinieaux[108]

(Continues)

TABLE 4 (Continued)

Origin	Kind of secretome	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
Rat BMMSCs from normal or cerebral ischaemia rats	CM	—	—	Improved recovery of neurological function by improving neurogenesis and attenuating microglia/macrophage infiltration	—	Tsai[109]
hBMMSCs	EVs	—	—	Induced long-term neuroprotection, enhanced angioneurogenesis and modulated peripheral post-stroke immune responses	—	Doeppner[110]
Human menstrual MSCs, chorion MSCs, hucMSCs, hBMMSCs	CM MVs Exos	—	The contact of MSCs with neurons inhibited neurite outgrowth, while CM enhanced it; Exos enhanced, but MVs inhibited neurite outgrowth; menstrual-MSC-Exos showed best effects on cortical neurons and had a comparable effect to BMMSC-Exos on DRG neurons	—	—	Lopez-Verrilli
Rat BMMSCs	Exos	miRNA-17-92 cluster	Promoted axonal growth of cortical neurons	Enhanced neuroplasticity and functional recovery	Activated the PTEN/mTOR signalling pathway	Zhang[113]
Rat BMMSCs	Exos	miRNA-17-92 cluster	—	Enhanced neuroplasticity and functional recovery after stroke	Activated PI3K/PKB signalling, inhibited GSK-3β activity	Xin[155]
BMMSCs overexpressing miR-133b	Exos	miR-133b	—	Improved neural plasticity and functional recovery after stroke with a contribution from a stimulated secondary release of neurite-promoting exosomes from astrocytes	—	Xin[114]
Rat BMMSCs overexpressing miR-133b	Exos	miR-133b	—	Decreased neuronal apoptosis and neurodegeneration in brain tissues after intracerebral haemorrhage (ICH)	Inhibited RhoA expression, promoted ERK1/2/CREB phosphorylation	Shen[116]
Rat BMSCs	CM		Promoted growth of DRG neurons	Improved functional recovery of spinal cord injury; decreased levels of IL-2, IL-6 and TNF in spinal cord extracts after localised intrathecal CM injection	—	Cizkova[111]
hASCs	CM	CXCL5	Promoted major pelvic ganglia (MPG) neurite growth	—	Activated JAK/STAT concentration-dependently in Schwann cells	Zhang[117]

(Continues)

TABLE 4 (Continued)

Origin	Kind of secretome	Content profile	In vitro effect	In vivo effect	Mechanisms	Ref.
hASCs	CM	–	Reduced damages of cortical neurons	–	Regulated energy metabolism by increasing the levels of GAP-43, ATP, NAD^+, NADH and the ratio of NAD^+/NADH expression	Hao[118]
Rat ASCs	MVs	–	–	Rest-MSC MVs and anti-inflammatory-MSC MVs improved the nerve regeneration compared with pro-inflammatory-MVs	–	Raisi[156]
Rat ASCs	Exos	GDNF, FGF-1, BDNF, IGF-1 and NGF transcripts	Increased neurite outgrowth	Enhanced regeneration after sciatic nerve injury	–	Bucan[119]
M2 Macrophage	MVs	miR-223	Improved SCs migration and proliferation; up-regulated NGF and laminin	Increased SCs infiltration and axon number in sciatic nerve injury rats	–	Zhan[97]

Abbreviations: BDNF, brain-derived neurotrophic factor; Ch-SCs, Chorion MSCs; FGF, fibroblast growth factor; GDNF, glial cell–derived neurotrophic factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGF, insulin-like growth factor; MenSCs, menstrual MSCs; NTAK, neural- and thymus-derived activator for ErbB kinases, also known as neuregulin-2 (NRG2); olfactory ensheathing cells (OECs); SCs, Schwann cells; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

engineering techniques for peripheral nerve regeneration are easier to be accepted for the patients compared with autologous nerve grafting, the outcomes are still suboptimal. Recently, it has been recognised that exosomes play critical regulatory functions during nerve repair, with accumulating evidences indicating that exosomal miRNAs are critical for nerve regeneration. Cellular secretome, including CM and exosomes, from Schwann cells,[92,93] olfactory ensheathing cells (OECs),[94-96] M2 macrophages[97] and MSCs,[98] has been considered to be beneficial for peripheral nerve regeneration (Figure 2D, Table 4).

Schwann cells (SCs), divided into myelinating type and non-myelinating type, dedifferentiate to a progenitor-like state, provide nutrition to support axon outgrowth, and guide axons to their original target tissues after peripheral nerve injury. Direct cell contact and soluble factors from paracrine effect contribute to the interactions between SCs and neurons during axonal regeneration.[99] Schwann cell proliferation, migration and axon myelination post-injury can be regulated by microRNAs, and microRNA Let-7,[100] miR-sc3,[101] miR-1[102] and miR-340[103] were reported to benefit this process. Meanwhile, SCs themselves can also deliver microRNAs by EVs to other SCs and neurons to improve the peripheral nerve repair. Schwann cell microRNA expression is drastically altered after peripheral nerve injury, and many injury-regulated SC microRNAs, such as miR-221 and miR-222 cluster (miR-221/222), facilitate proliferation and migration of SCs.[104] Conditioned medium or exosomes from SCs and noncontact co-culture of SCs with neurons promote dorsal root ganglia (DRG) neurite outgrowth by secretion of nerve growth factor (NGF),[92,93] and local injection of SC exosomes effectively drives axon growth after nerve crush injury.[93,105] Together, the content profile of SCs changed after injury, and microRNAs delivered by SC EVs are important mediators of SCs' regenerative response following nerve injury. However, existing researches are based on rat SCs. To obtain human SC exosomes, the necessity of harvesting SCs via sacrifice of normal nerve tissue has remained to be a major disadvantage for their clinical application.

Besides low regenerative rate of axons, inflammatory reactions and degenerative debris after peripheral nerve injury turn to be another obstacle for nerve regeneration, which always block the elongation of regenerating axons to reach their target sites. Macrophages play a critical role in modulating the inflammatory responses. The moment nerve damage occurs, damaged myelin will activate the macrophages to release pro-inflammation cytokines, thus mediating the removal of debris from apoptotic axons and myelins through macrophage phagocytosis, and also accelerate the recruitment and migration of other macrophages at the injury site.[106] Meanwhile, M2 macrophages were reported to secrete MVs highly expressing miR-223 to improve migration and proliferation of SCs and increase the expression level of NGF and laminin, thus promoting peripheral nerve repair in sciatic nerve injury rats.[97]

MSCs and their secretome are well recognised to improve functional recovery following nerve injury.[91,98] MSCs from various origins, such as menstrual MSCs (MenSCs), BMMSCs, ASCs and ucMSCs, have been reported to benefit the survival and neurite outgrowth of

neurons. BMMSCs secretome have been attracted the most attentions in the field of nerve regeneration. CM from BMMSCs contains various trophic factors, such as transforming growth factors β1 (TGF-β1), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and hepatocyte growth factor (HGF). [107,108] BMMSC CM and EVs protect neurons from apoptosis, promote neurite outgrowth, attenuate inflammations and exert proangiogenic effect, leading to improved recovery of neurological function.[108-112] Exosomes derived from BMMSCs delivered miRNA-17-92 cluster to promote axonal growth of cortical neurons and facilitate functional recovery after stroke by activating the PTEN/mTOR[113] and PI3K/PKB[114] signalling. MiR-133b is also highly expressed in BMMSC-derived exosomes, and miR-133b target and inhibit connective tissue growth factor (CTGF) and RhoA to improve neurite remodelling and functional recovery.[115] Tailored exosomes from BMMSCs overexpressing miR-133b decrease neuronal apoptosis and neurodegeneration, as well as exert a secondary release of neurite-promoting exosomes from astrocytes, through inhibition of RhoA expression and enhancement of ERK1/2/CREB phosphorylation.[114,116] In addition, CM from ASCs, enriched in CXCL5, can also promote neurite growth, reduced neuron damage by regulating energy metabolism, and activate JAK/STAT signalling in a concentration-dependent manner in Schwann cells.[117,118] Exosomes from ASCs are found to shuttle transcripts of glial cell–derived neurotrophic factor (GDNF), fibroblast growth factor-1(FGF-1), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and NGF to promote nerve regeneration after sciatic nerve injury.[119] When comparing different MSCs on nerve regeneration, menstrual-MSC exosomes show best effects on cortical neurons and have a comparable effect to BMMSC exosomes on DRG neurons compared with chorion MSCs and hucMSCs. Interestingly, the direct contact of MSCs with neurons inhibits neurite outgrowth, but CM from MSCs promotes growth of neurons. Exosomes from menstrual MSCs enhance neurite outgrowth, but MVs inhibit this effect on the contrary.[105] Therefore, the paracrine factors from MSCs may provide a neurogenic niche more importantly than the cells themselves, and trophic factors in CM and microRNAs in exosomes play critical roles in nerve regeneration by protecting neurons from degradation, attenuating inflammatory responses, facilitating angiogenesis and promoting axonal growth.

4.5 | EVs in tooth-related tissue regeneration

Tooth itself is such a complex organ which consists of hard tissues (enamel, dentin and cementum) and soft tissues (dental pulp with blood vessels and nerves, and periodontal ligament), that genuine tooth regeneration is considerably challenging. The process of tooth development may provide us information for realising tooth regeneration. Tooth development is precisely controlled by the crosstalk between epithelium and mesenchyme, and exosomes have been documented to play an important role in this process.[120,121] Extracellular vesicles derived from dental-related cells are preferential choices for tooth regeneration. Exosomes from hDPSCs cultured under odontogenic differentiation conditions promote odontogenic differentiation of recipient hDPSCs and hBMSCs through activation of P38 mitogen-activated protein kinase (MAPK) pathway.[122] Conditioned medium from odontoblast promotes differentiation and mineralisation of cementoblasts.[123] Schwann cells in dental pulp secrete EVs to maintain the multipotency of hDPSCs,[124,125] suggesting that nervous system may also play an important role in tooth regeneration. In turn, exosomes from human exfoliated deciduous teeth (SHEDs) protect neurons from apoptosis and have neurogenic capabilities.[126] Furthermore, MSCs from other tissue may also be potential candidates for tooth regeneration. Adipose-derived stem cells could be induced to cementoblasts,[127] while CM from BMSCs promotes periodontal tissue regeneration.[128]

4.6 | Perspectives and challenges

In the first place, the safety of EV local injection and systematic administration should be systematically evaluated. Therefore, EV biodistribution and clearance dynamics, content profiles and regulatory mechanisms are two essential indexes for safety evaluation. Extracellular vesicle labelling and tracking techniques are the bases for studies on biodistribution of EVs, and fluorescent reporter systems,[129-131] magnetic labelling observed by MRI[132,133] and radiolabelling observed by SPECT/CT[134] have been reported. Basing on existing researches, EV biodistribution depends on the cellular origin of EV, as well as dose, method and location of administration.[131,135] Meanwhile, the half-life time for exogenous EVs in vivo is around 30 minutes.[129] After intravenous injection via tail vein in the mice, EVs are detected to undergo a rapid distribution phase in the spleen, followed by the liver, and then the lungs and kidneys. Extracellular vesicles are eliminated through liver and kidney within six hours, albeit a very small percentage (~0.01% of total dose) of EVs are detected in brain, heart and muscles.[129] Moreover, intraperitoneal and subcutaneous injection significantly lowers the amount of EVs in liver and spleen, whereas increases the accumulation in pancreas and gastrointestinal tract.[131] Therefore, a criterion for the optimal route of EV delivery targeting different regenerative or therapeutic goals is urgently to be established in order to enhance the therapeutic efficacy of EVs. Moreover, no matter which kind of reporter system we choose, there is a risk that the signals originate from the free label itself, rather than the labelled EVs; therefore, control experiments using the label itself should also be analysed and tracking methods with less false-positive signals are expected.

Also basing on safety considerations, the content profiles and regulatory mechanisms of EVs should be systematically evaluated before its clinical application. The horizontal transfer of genetic information by EVs within and even across species boundaries[136,137] may risk uncontrolled transfer of genetic information among individuals or across species. Therefore, a rigorous, overall and genome-wide detection of all genetic elements (non-coding RNAs, mRNAs, etc) shuttled within candidate exosomes has become a prerequisite before clinical application.

In addition, the effectiveness of EV-based therapy is expected to be studied systematically. Although accumulating evidences have supported the efficacy and advantages of EV therapies, the

comparison between EV and traditionally well-recognised efficient methods should be conducted. For example, in the field of tissue engineering, the regenerative effects of EVs and stem cells are expected to be systematically compared, but difficult to realise due to lack of studies and the heterogeneity between researches.

Finally, tailored EVs, either bioengineered or generated from modified cells, are promising selections for precision medicine. To realise this goal, unravelling the composition of EVs and their underlying regulatory mechanisms is indispensable. Moreover, development in biomaterial science and nanotechnology may provide us a bright future for bioengineered EVs.

5 | CONCLUSION

Extracellular vesicles are effective and beneficial for various tissue regeneration, including bone, skin, blood vessels and nerve, thus contributing to their promising roles in oral rehabilitation. Whether EVs can be a total surrogate for stem-cell therapy should be evaluated carefully by more systematic studies for comparison. Tailored or bioengineered EVs with higher safety and efficiency are expected in the foreseeable clinical application.

ACKNOWLEDGMENTS

This work is supported by the grant (31600787) from the National Natural Science Foundation of China, the grant (7192228, L182006) from the Beijing Natural Science Foundation, the Project for Culturing Leading Talents in Scientific and Technological Innovation of Beijing (Z171100001117169), the grant of Young Elite Scientist Sponsorship Program by CAST (China Association for Science and Technology) (2015QNRC001), and the grant from the PKU School of Stomatology for Talented Young Investigators (PKUSS20150107).

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

ORCID

Longwei Lv https://orcid.org/0000-0002-2912-1530

Yongsheng Zhou https://orcid.org/0000-0002-4332-0878

REFERENCES

1. Soteriou D, Fuchs Y. A matter of life and death: stem cell survival in tissue regeneration and tumour formation. *Nat Rev Cancer*. 2018;18(3):187-201.
2. Fischer UM, Harting MT, Jimenez F, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev*. 2009;18(5):683-692.
3. Kraitchman DL, Tatsumi M, Gilson WD, et al. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. *Circulation*. 2005;112(10):1451-1461.
4. Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. *Circ Res*. 2009;104(3):398-402.
5. Volarevic V, Markovic BS, Gazdic M, et al. Ethical and safety issues of stem cell-based therapy. *Int J Med Sci*. 2018;15(1):36-45.
6. Prokhorova TA, Harkness LM, Frandsen U, et al. Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. *Stem Cells Dev*. 2009;18(1):47-54.
7. Fontaine MJ, Shih H, Schafer R, Pittenger MF. Unraveling the mesenchymal stromal cells' paracrine immunomodulatory effects. *Transfus Med Rev*. 2016;30(1):37-43.
8. Kim DK, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ. Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. *Proc Natl Acad Sci USA*. 2016;113(1):170-175.
9. Kenigsberg S, Wyse BA, Librach CL, da Silveira JC. Protocol for exosome isolation from small volume of ovarian follicular fluid: evaluation of ultracentrifugation and commercial kits. *Methods Mol Biol*. 2017;1660:321-341.
10. Marques-Garcia F, Isidoro-Garcia M. Protocols for exosome isolation and RNA profiling. *Methods Mol Biol*. 2016;1434:153-167.
11. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics*. 2017;7(3):789-804.
12. Greening DW, Xu R, Ji H, Tauro BJ, Simpson RJ. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. *Methods Mol Biol*. 2015;1295:179-209.
13. Lai RC, Arslan F, Lee MM, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem cell Res*. 2010;4(3):214-222.
14. Azoidis I, Cox SC, Davies OG. The role of extracellular vesicles in biomineralisation: current perspective and application in regenerative medicine. *J Tissue Eng*. 2018;9:2041731418810130.
15. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018;19(4):213-228.
16. Camussi G, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. *Biochem Soc Trans*. 2013;41(1):283-287.
17. Liu M, Sun Y, Zhang Q. Emerging role of extracellular vesicles in bone remodeling. *J Dent Res*. 2018;97(8):859-868.
18. Qin Y, Wang L, Gao Z, Chen G, Zhang C. Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo. *Sci Rep*. 2016;6:21961.
19. Xie H, Wang Z, Zhang L, et al. Extracellular vesicle-functionalized decalcified bone matrix scaffolds with enhanced pro-angiogenic and pro-bone regeneration activities. *Sci Rep*. 2017;7:45622.
20. Martins M, Ribeiro D, Martins A, Reis RL, Neves NM. Extracellular vesicles derived from osteogenically induced human bone marrow mesenchymal stem cells can modulate lineage commitment. *Stem Cell Rep*. 2016;6(3):284-291.
21. Liu S, Liu D, Chen C, et al. MSC transplantation improves osteopenia via epigenetic regulation of notch signaling in lupus. *Cell Metab*. 2015;22(4):606-618.
22. Furuta T, Miyaki S, Ishitobi H, et al. Mesenchymal stem cell-derived exosomes promote fracture healing in a mouse model. *Stem Cells Transl Med*. 2016;5(12):1620-1630.
23. Chen C, Wang D, Moshaverinia A, et al. Mesenchymal stem cell transplantation in tight-skin mice identifies miR-151-5p as a therapeutic target for systemic sclerosis. *Cell Res*. 2017;27(4):559-577.
24. Narayanan R, Huang CC, Ravindran S. Hijacking the cellular mail: exosome mediated differentiation of mesenchymal stem cells. *Stem Cells Int*. 2016;2016:3808674.

25. Xie H, Wang Z, Zhang L, et al. Development of an angiogenesis-promoting microvesicle-alginate-polycaprolactone composite graft for bone tissue engineering applications. *PeerJ*. 2016;4:e2040.
26. Zhao M, Li P, Xu H, et al. Dexamethasone-activated MSCs release MVs for stimulating osteogenic response. *Stem Cells Int*. 2018;2018:7231739.
27. Cui Y, Luan J, Li H, Zhou X, Han J. Exosomes derived from mineralizing osteoblasts promote ST2 cell osteogenic differentiation by alteration of microRNA expression. *FEBS Lett*. 2016;590(1):185-192.
28. Xu JF, Yang GH, Pan XH, et al. Altered microRNA expression profile in exosomes during osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. *PLoS ONE*. 2014;9(12):e114627.
29. Li D, Liu J, Guo B, et al. Osteoclast-derived exosomal miR-214-3p inhibits osteoblastic bone formation. *Nat Commun*. 2016;7:10872.
30. Huynh N, VonMoss L, Smith D, et al. Characterization of regulatory extracellular vesicles from osteoclasts. *J Dent Res*. 2016;95(6):673-679.
31. Davis C, Dukes A, Drewry M, et al. MicroRNA-183-5p increases with age in bone-derived extracellular vesicles, suppresses bone marrow stromal (stem) cell proliferation, and induces stem cell senescence. *Tissue Eng Part A*. 2017;23(21–22):1231-1240.
32. Qin Y, Peng Y, Zhao W, et al. Myostatin inhibits osteoblastic differentiation by suppressing osteocyte-derived exosomal microRNA-218: a novel mechanism in muscle-bone communication. *J Biol Chem*. 2017;292(26):11021-11033.
33. Lv L, Liu Y, Zhang P, et al. The nanoscale geometry of TiO_2 nanotubes influences the osteogenic differentiation of human adipose-derived stem cells by modulating H3K4 trimethylation. *Biomaterials*. 2015;39:193-205.
34. 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 Interf*. 2018;10(6):5240-5254.
35. Diomede F, D'Aurora M, Gugliandolo A, et al. A novel role in skeletal segment regeneration of extracellular vesicles released from periodontal-ligament stem cells. *Int J Nanomed*. 2018;13:3805-3825.
36. Diomede F, Gugliandolo A, Cardelli P, et al. Three-dimensional printed PLA scaffold and human gingival stem cell-derived extracellular vesicles: a new tool for bone defect repair. *Stem Cell Res Ther*. 2018;9(1):104.
37. Qi X, Zhang J, Yuan H, et al. Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells repair critical-sized bone defects through enhanced angiogenesis and osteogenesis in osteoporotic rats. *Int J Biol Sci*. 2016;12(7):836-849.
38. Zhang J, Liu X, Li H, et al. Exosomes/tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/Akt signaling pathway. *Stem Cell Res Ther*. 2016;7(1):136.
39. Tooi M, Komaki M, Morioka C, et al. Placenta mesenchymal stem cell derived exosomes confer plasticity on fibroblasts. *J Cell Biochem*. 2016;117(7):1658-1670.
40. Wang KX, Xu LL, Rui YF, et al. The effects of secretion factors from umbilical cord derived mesenchymal stem cells on osteogenic differentiation of mesenchymal stem cells. *PLoS ONE*. 2015;10(3):e0120593.
41. Del Fattore A, Luciano R, Pascucci L, et al. Immunoregulatory effects of mesenchymal stem cell-derived extracellular vesicles on T lymphocytes. *Cell Transplant*. 2015;24(12):2615-2627.
42. Chen W, Huang Y, Han J, et al. Immunomodulatory effects of mesenchymal stromal cells-derived exosome. *Immunol Res*. 2016;64(4):831-840.
43. Silva AM, Almeida MI, Teixeira JH, et al. Dendritic cell-derived extracellular vesicles mediate mesenchymal stem/stromal cell recruitment. *Sci Rep*. 2017;7(1):1667.
44. Ekstrom K, Omar O, Graneli C, Wang X, Vazirisani F, Thomsen P. Monocyte exosomes stimulate the osteogenic gene expression of mesenchymal stem cells. *PLoS ONE*. 2013;8(9):e75227.
45. Rani S, Ritter T. The exosome – a naturally secreted nanoparticle and its application to wound healing. *Adv Mater*. 2016;28(27):5542-5552.
46. Cabral J, Ryan AE, Griffin MD, Ritter T. Extracellular vesicles as modulators of wound healing. *Adv Drug Deliv Rev*. 2018;129:394-406.
47. Golchin A, Hosseinzadeh S, Ardeshirylajimi A. The exosomes released from different cell types and their effects in wound healing. *J Cell Biochem*. 2018;119(7):5043-5052.
48. Fang S, Xu C, Zhang Y, et al. Umbilical cord-derived mesenchymal stem cell-derived exosomal microRNAs suppress myofibroblast differentiation by inhibiting the transforming growth factor-beta/SMAD2 pathway during wound healing. *Stem Cells Transl Med*. 2016;5(10):1425-1439.
49. Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. *Biochem Biophys Res Comm*. 2015;467(2):303-309.
50. Huang P, Bi J, Owen GR, et al. Keratinocyte microvesicles regulate the expression of multiple genes in dermal fibroblasts. *J Invest Dermatol*. 2015;135(12):3051-3059.
51. Merjaneh M, Langlois A, Larochelle S, Cloutier CB, Ricard-Blum S, Moulin VJ. Pro-angiogenic capacities of microvesicles produced by skin wound myofibroblasts. *Angiogenesis*. 2017;20(3):385-398.
52. Zhao B, Zhang Y, Han S, et al. Exosomes derived from human amniotic epithelial cells accelerate wound healing and inhibit scar formation. *J Mol Histol*. 2017;48(2):121-132.
53. Zhao B, Li X, Shi X, et al. Exosomal microRNAs derived from human amniotic epithelial cells accelerate wound healing by promoting the proliferation and migration of fibroblasts. *Stem Cells Int*. 2018;2018:5420463.
54. Zhang B, Wang M, Gong A, et al. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. *Stem Cells*. 2015;33(7):2158-2168.
55. Pelizzo G, Avanzini MA, Icaro Cornaglia A, et al. Extracellular vesicles derived from mesenchymal cells: perspective treatment for cutaneous wound healing in pediatrics. *Regen Med*. 2018;13(4):385-394.
56. Hu L, Wang J, Zhou X, et al. Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep*. 2016;6:32993.
57. Zhang W, Bai X, Zhao B, et al. Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. *Exp Cell Res*. 2018;370(2):333-342.
58. Cooper DR, Wang C, Patel R, et al. Human adipose-derived stem cell conditioned media and exosomes containing MALAT1 promote human dermal fibroblast migration and ischemic wound healing. *Adv Wound Care*. 2018;7(9):299-308.
59. Li X, Xie X, Lian W, et al. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp Mol Med*. 2018;50(4):29.
60. Tao SC, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan wound dressings incorporating exosomes derived from microRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model. *Stem Cells Transl Med*. 2017;6(3):736-747.
61. Kobayashi H, Ebisawa K, Kambe M, et al. Editors' Choice Effects of exosomes derived from the induced pluripotent stem cells on skin wound healing. *Nagoya J Med Sci*. 2018;80(2):141-153.
62. Zhang J, Guan J, Niu X, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous

wound healing by promoting collagen synthesis and angiogenesis. *J Transl Med*. 2015;13:49.
63. Guo SC, Tao SC, Yin WJ, Qi X, Yuan T, Zhang CQ. Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. *Theranostics*. 2017;7(1):81-96.
64. Xu N, Wang L, Guan J, et al. Wound healing effects of a *Curcuma zedoaria* polysaccharide with platelet-rich plasma exosomes assembled on chitosan/silk hydrogel sponge in a diabetic rat model. *Int J Biol Macromol*. 2018;117:102-107.
65. Hu Y, Rao SS, Wang ZX, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. *Theranostics*. 2018;8(1):169-184.
66. Bakhtyar N, Jeschke MG, Herer E, Sheikholeslam M, Amini-Nik S. Exosomes from acellular Wharton's jelly of the human umbilical cord promotes skin wound healing. *Stem Cell Res Ther*. 2018;9(1):193.
67. Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol*. 2002;160(2):673-680.
68. Lombardo G, Dentelli P, Togliatto G, et al. Activated Stat5 trafficking via endothelial cell-derived extracellular vesicles controls IL-3 pro-angiogenic paracrine action. *Sci Rep*. 2016;6:25689.
69. Di Santo S, Yang Z, Wyler von Ballmoos M, et al. Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation. *PLoS ONE*. 2009;4(5):e5643.
70. Cantaluppi V, Biancone L, Figliolini F, et al. Microvesicles derived from endothelial progenitor cells enhance neoangiogenesis of human pancreatic islets. *Cell Transplant*. 2012;21(6):1305-1320.
71. Ranghino A, Cantaluppi V, Grange C, et al. Endothelial progenitor cell-derived microvesicles improve neovascularization in a murine model of hindlimb ischemia. *Int J Immunopathol Pharmacol*. 2012;25(1):75-85.
72. Deregibus MC, Cantaluppi V, Calogero R, et al. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*. 2007;110(7):2440-2448.
73. Zhang J, Chen C, Hu B, et al. Exosomes derived from human endothelial progenitor cells accelerate cutaneous wound healing by promoting angiogenesis through Erk1/2 signaling. *Int J Biol Sci*. 2016;12(12):1472-1487.
74. Li X, Jiang C, Zhao J. Human endothelial progenitor cells-derived exosomes accelerate cutaneous wound healing in diabetic rats by promoting endothelial function. *J Diabetes Complications*. 2016;30(6):986-992.
75. Li X, Chen C, Wei L, et al. Exosomes derived from endothelial progenitor cells attenuate vascular repair and accelerate reendothelialization by enhancing endothelial function. *Cytotherapy*. 2016;18(2):253-262.
76. Hung SC, Pochampally RR, Chen SC, Hsu SC, Prockop DJ. Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis. *Stem Cells*. 2007;25(9):2363-2370.
77. Burlacu A, Grigorescu G, Rosca AM, Preda MB, Simionescu M. Factors secreted by mesenchymal stem cells and endothelial progenitor cells have complementary effects on angiogenesis in vitro. *Stem Cells Dev*. 2013;22(4):643-653.
78. Anderson JD, Johansson HJ, Graham CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-kappaB signaling. *Stem Cells*. 2016;34(3):601-613.
79. Teng X, Chen L, Chen W, Yang J, Yang Z, Shen Z. Mesenchymal stem cell-derived exosomes improve the microenvironment of infarcted myocardium contributing to angiogenesis and anti-inflammation. *Cell Physiol Biochem: Int J Exp Cell Physiol Biochem Pharmacol*. 2015;37(6):2415-2424.
80. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. *Stem Cells Dev*. 2015;24(14):1635-1647.
81. Liang X, Zhang L, Wang S, Han Q, Zhao RC. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. *J Cell Sci*. 2016;129(11):2182-2189.
82. Lopatina T, Bruno S, Tetta C, Kalinina N, Porta M, Camussi G. Platelet-derived growth factor regulates the secretion of extracellular vesicles by adipose mesenchymal stem cells and enhances their angiogenic potential. *Cell Commun Signal: CCS*. 2014;12:26.
83. Kang T, Jones TM, Naddell C, et al. Adipose-derived stem cells induce angiogenesis via microvesicle transport of miRNA-31. *Stem Cells Transl Med*. 2016;5(4):440-450.
84. Zhang HC, Liu XB, Huang S, et al. Microvesicles derived from human umbilical cord mesenchymal stem cells stimulated by hypoxia promote angiogenesis both in vitro and in vivo. *Stem Cells Dev*. 2012;21(18):3289-3297.
85. Chen J, Liu Z, Hong MM, et al. Proangiogenic compositions of microvesicles derived from human umbilical cord mesenchymal stem cells. *PLoS ONE*. 2014;9(12):e115316.
86. Zhang B, Wu X, Zhang X, et al. Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/beta-catenin pathway. *Stem Cells Transl Med*. 2015;4(5):513-522.
87. Ma J, Zhao Y, Sun L, et al. Exosomes derived from Akt-modified human umbilical cord mesenchymal stem cells improve cardiac regeneration and promote angiogenesis via activating platelet-derived growth factor D. *Stem Cells Transl Med*. 2017;6(1):51-59.
88. Li J, Zhang Y, Liu Y, et al. Microvesicle-mediated transfer of microRNA-150 from monocytes to endothelial cells promotes angiogenesis. *J Biol Chem*. 2013;288(32):23586-23596.
89. Sun C, Feng SB, Cao ZW, et al. Up-regulated expression of matrix metalloproteinases in endothelial cells mediates platelet microvesicle-induced angiogenesis. *Cell Physiol Biochem: Int J Exp Cell Physiol Biochem Pharmacol*. 2017;41(6):2319-2332.
90. Parikh P, Hao Y, Hosseinkhani M, et al. Regeneration of axons in injured spinal cord by activation of bone morphogenetic protein/Smad1 signaling pathway in adult neurons. *Proc Natl Acad Sci USA*. 2011;108(19):E99-107.
91. Qing L, Chen H, Tang J, Jia X. Exosomes and their microRNA cargo: new players in peripheral nerve regeneration. *Neurorehabilitation Neural Repair*. 2018;32(9):765-776.
92. Hu J, Zhou J, Li X, Wang F, Lu H. Schwann cells promote neurite outgrowth of dorsal root ganglion neurons through secretion of nerve growth factor. *Indian J Exp Biol*. 2011;49(3):177-182.
93. Lopez-Verrilli MA, Picou F, Court FA. Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system. *Glia*. 2013;61(11):1795-1806.
94. Lo Furno D, Pellitteri R, Graziano AC, et al. Differentiation of human adipose stem cells into neural phenotype by neuroblastoma- or olfactory ensheathing cells-conditioned medium. *J Cell Physiol*. 2013;228(11):2109-2118.
95. Feng L, Gan H, Zhao W, Liu Y. Effect of transplantation of olfactory ensheathing cell conditioned medium induced bone marrow stromal cells on rats with spinal cord injury. *Mol Med Rep*. 2017;16(2):1661-1668.
96. Gu M, Gao Z, Li X, et al. Conditioned medium of olfactory ensheathing cells promotes the functional recovery and axonal regeneration after contusive spinal cord injury. *Brain Res*. 2017;1654(Pt A):43-54.

97. Zhan C, Ma CB, Yuan HM, Cao BY, Zhu JJ. Macrophage-derived microvesicles promote proliferation and migration of Schwann cell on peripheral nerve repair. *Biochem Biophys Res Commun*. 2015;468(1–2):343-348.
98. Salgado AJ, Sousa JC, Costa BM, et al. Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities. *Front Cell Neurosci*. 2015;9:249.
99. Lopez-Leal R, Court FA. Schwann cell exosomes mediate neuron-glia communication and enhance axonal regeneration. *Cell Mol Neurobiol*. 2016;36(3):429-436.
100. Li S, Wang X, Gu Y, et al. Let-7 microRNAs regenerate peripheral nerve regeneration by targeting nerve growth factor. *Mol Ther: J Am Soc Gene Ther*. 2015;23(3):423-433.
101. Yi S, Wang S, Zhao Q, et al. miR-sc3, a novel microRNA, promotes schwann cell proliferation and migration by targeting Astn1. *Cell Transplant*. 2016;25(5):973-982.
102. Yi S, Yuan Y, Chen Q, et al. Regulation of Schwann cell proliferation and migration by miR-1 targeting brain-derived neurotrophic factor after peripheral nerve injury. *Sci Rep*. 2016;6:29121.
103. Li S, Zhang R, Yuan Y, et al. MiR-340 regulates fibrinolysis and axon regrowth following sciatic nerve injury. *Mol Neurobiol*. 2017;54(6):4379-4389.
104. Yu B, Zhou S, Wang Y, et al. miR-221 and miR-222 promote Schwann cell proliferation and migration by targeting LASS2 after sciatic nerve injury. *J Cell Sci*. 2012;125(Pt 11):2675-2683.
105. Lopez-Verrilli MA, Caviedes A, Cabrera A, Sandoval S, Wyneken U, Khoury M. Mesenchymal stem cell-derived exosomes from different sources selectively promote neuritic outgrowth. *Neuroscience*. 2016;320:129-139.
106. Horie H, Kadoya T, Hikawa N, et al. Oxidized galectin-1 stimulates macrophages to promote axonal regeneration in peripheral nerves after axotomy. *J Neurosci: Off J Soc Neurosci*. 2004;24(8):1873-1880.
107. Nakano N, Nakai Y, Seo TB, et al. Characterization of conditioned medium of cultured bone marrow stromal cells. *Neurosci Lett*. 2010;483(1):57-61.
108. Cantinieaux D, Quertainmont R, Blacher S, et al. Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation. *PLoS ONE*. 2013;8(8):e69515.
109. Tsai MJ, Tsai SK, Hu BR, et al. Recovery of neurological function of ischemic stroke by application of conditioned medium of bone marrow mesenchymal stem cells derived from normal and cerebral ischemia rats. *J Biomed Sci*. 2014;21:5.
110. Doeppner TR, Herz J, Gorgens A, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. *Stem Cells Transl Med*. 2015;4(10):1131-1143.
111. Cizkova D, Cubinkova V, Smolek T, et al. Localized intrathecal delivery of mesenchymal stromal cells conditioned medium improves functional recovery in a rat model of spinal cord injury. *Int J Mol Sci*. 2018;19(3):870.
112. Kanekiyo K, Wakabayashi T, Nakano N, et al. Effects of intrathecal injection of the conditioned medium from bone marrow stromal cells on spinal cord injury in rats. *J Neurotrauma*. 2018;35(3):521-532.
113. Zhang Y, Chopp M, Liu XS, et al. Exosomes derived from mesenchymal stromal cells promote axonal growth of cortical neurons. *Mol Neurobiol*. 2017;54(4):2659-2673.
114. Xin H, Wang F, Li Y, et al. Secondary release of exosomes from astrocytes contributes to the increase in neural plasticity and improvement of functional recovery after stroke in rats treated with exosomes harvested from microRNA 133b-overexpressing multipotent mesenchymal stromal cells. *Cell Transplant*. 2017;26(2):243-257.
115. Xin H, Li Y, Liu Z, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. 2013;31(12):2737-2746.
116. Shen H, Yao X, Li H, et al. Role of exosomes derived from miR-133b modified MSCs in an experimental rat model of intracerebral hemorrhage. *J Mol Neurosci: MN*. 2018;64(3):421-430.
117. Zhang H, Yang R, Wang Z, Lin G, Lue TF, Lin CS. Adipose tissue-derived stem cells secrete CXCL5 cytokine with neurotrophic effects on cavernous nerve regeneration. *J Sexual Med*. 2011;8(2):437-446.
118. Hao P, Liang Z, Piao H, et al. Conditioned medium of human adipose-derived mesenchymal stem cells mediates protection in neurons following glutamate excitotoxicity by regulating energy metabolism and GAP-43 expression. *Metab Brain Dis*. 2014;29(1):193-205.
119. Bucan V, Vaslaitis D, Peck CT, Strauss S, Vogt PM, Radtke C. Effect of exosomes from rat adipose-derived mesenchymal stem cells on neurite outgrowth and sciatic nerve regeneration after crush injury. *Mol Neurobiol*. 2019;56(3):1812-1824.
120. Jiang N, Xiang L, He L, et al. Exosomes mediate epithelium-mesenchyme crosstalk in organ development. *ACS Nano*. 2017;11(8):7736-7746.
121. Zhu Y, Zhang P, Gu RL, Liu YS, Zhou YS. Origin and clinical applications of neural crest-derived dental stem cells. *Chin J Dent Res*. 2018;21(2):89-100.
122. Huang CC, Narayanan R, Alapati S, Ravindran S. Exosomes as biomimetic tools for stem cell differentiation: applications in dental pulp tissue regeneration. *Biomaterials*. 2016;111:103-115.
123. Kim HS, Lee DS, Lee JH, et al. The effect of odontoblast conditioned media and dentin non-collagenous proteins on the differentiation and mineralization of cementoblasts in vitro. *Arch Oral Biol*. 2009;54(1):71-79.
124. Couve E, Lovera M, Suzuki K, Schmachtenberg O. Schwann cell phenotype changes in aging human dental pulp. *J Dent Res*. 2018;97(3):347-355.
125. Li Z, Liang Y, Pan K, et al. Schwann cells secrete extracellular vesicles to promote and maintain the proliferation and multipotency of hDPCs. *Cell Prolif*. 2017;50(4):e12353.
126. Jarmalaviciute A, Tunaitis V, Pivoraite U, Venalis A, Pivoriunas A. Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine-induced apoptosis. *Cytotherapy*. 2015;17(7):932-939.
127. Wen X, Nie X, Zhang L, Liu L, Deng M. Adipose tissue-deprived stem cells acquire cementoblast features treated with dental follicle cell conditioned medium containing dentin non-collagenous proteins in vitro. *Biochem Biophys Res Commun*. 2011;409(3):583-589.
128. Kawai T, Katagiri W, Osugi M, Sugimura Y, Hibi H, Ueda M. Secretomes from bone marrow-derived mesenchymal stromal cells enhance periodontal tissue regeneration. *Cytotherapy*. 2015;17(4):369-381.
129. Lai CP, Mardini O, Ericsson M, et al. Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS Nano*. 2014;8(1):483-494.
130. Lai CP, Kim EY, Badr CE, et al. Visualization and tracking of tumour extracellular vesicle delivery and RNA translation using multiplexed reporters. *Nat Commun*. 2015;6:7029.
131. Wiklander OP, Nordin JZ, O'Loughlin A, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J Extracellular Vesicles*. 2015;4:26316.
132. Hu L, Wickline SA, Hood JL. Magnetic resonance imaging of melanoma exosomes in lymph nodes. *Magn Reson Med*. 2015;74(1):266-271.
133. Qi H, Liu C, Long L, et al. Blood exosomes endowed with magnetic and targeting properties for cancer therapy. *ACS Nano*. 2016;10(3):3323-3333.
134. Hwang DW, Choi H, Jang SC, et al. Noninvasive imaging of radiolabeled exosome-mimetic nanovesicle using (99m)Tc-HMPAO. *Sci Rep*. 2015;5:15636.

135. Yanez-Mo M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracellular Vesicles*. 2015;4:27066.
136. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9(6):654-659.
137. Buck AH, Coakley G, Simbari F, et al. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. *Nat Commun*. 2014;5:5488.
138. Zhao P, Xiao L, Peng J, Qian YQ, Huang CC. Exosomes derived from bone marrow mesenchymal stem cells improve osteoporosis through promoting osteoblast proliferation via MAPK pathway. *Eur Rev Med Pharmacol Sci*. 2018;22(12):3962-3970.
139. Weilner S, Schraml E, Wieser M, et al. Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. *Aging Cell*. 2016;15(4):744-754.
140. Gebraad A, Kornilov R, Kaur S, et al. Monocyte-derived extracellular vesicles stimulate cytokine secretion and gene expression of matrix metalloproteinases by mesenchymal stem/stromal cells. *FEBS J*. 2018;285(12):2337-2359.
141. Leoni G, Neumann PA, Kamaly N, et al. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. *J Clin Investig*. 2015;125(3):1215-1227.
142. Trinh NT, Yamashita T, Tu TC, et al. Microvesicles enhance the mobility of human diabetic adipose tissue-derived mesenchymal stem cells in vitro and improve wound healing in vivo. *Biochem Biophys Res Comm*. 2016;473(4):1111-1118.
143. Ti D, Hao H, Tong C, et al. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med*. 2015;13:308.
144. Shi Q, Qian Z, Liu D, et al. GMSC-derived exosomes combined with a chitosan/silk hydrogel sponge accelerates wound healing in a diabetic rat skin defect model. *Front Physiol*. 2017;8:904.
145. Luo ML, Liu XP, Wang F, et al. Conditioned medium from human umbilical vein endothelial cells promotes proliferation, migration, invasion and angiogenesis of adipose derived stem cells. *Curr Med Sci*. 2018;38(1):124-130.
146. Bussche L, Van de Walle GR. Peripheral blood-derived mesenchymal stromal cells promote angiogenesis via paracrine stimulation of vascular endothelial growth factor secretion in the equine model. *Stem Cells Transl Med*. 2014;3(12):1514-1525.
147. Estrada R, Li N, Sarojini H, An J, Lee MJ, Wang E. Secretome from mesenchymal stem cells induces angiogenesis via Cyr61. *J Cell Physiol*. 2009;219(3):563-571.
148. Boomsma RA, Geenen DL. Mesenchymal stem cells secrete multiple cytokines that promote angiogenesis and have contrasting effects on chemotaxis and apoptosis. *PLoS ONE*. 2012;7(4):e35685.
149. Pascucci L, Alessandri G, Dall'Aglio C, et al. Membrane vesicles mediate pro-angiogenic activity of equine adipose-derived mesenchymal stromal cells. *Vet J*. 2014;202(2):361-366.
150. Kim HK, Song KS, Chung JH, Lee KR, Lee SN. Platelet microparticles induce angiogenesis in vitro. *Br J Haematol*. 2004;124(3):376-384.
151. Zou X, Gu D, Xing X, et al. Human mesenchymal stromal cell-derived extracellular vesicles alleviate renal ischemic reperfusion injury and enhance angiogenesis in rats. *Am J Transl Res*. 2016;8(10):4289-4299.
152. Komaki M, Numata Y, Morioka C, et al. Exosomes of human placenta-derived mesenchymal stem cells stimulate angiogenesis. *Stem Cell Res Ther*. 2017;8(1):219.
153. Nakano N, Kanekiyo K, Nakagawa T, Asahi M, Ide C. NTAK/neuregulin-2 secreted by astrocytes promotes survival and neurite outgrowth of neurons via ErbB3. *Neurosci Lett*. 2016;622:88-94.
154. Liang P, Liu J, Xiong J, et al. Neural stem cell-conditioned medium protects neurons and promotes propriospinal neurons relay neural circuit reconnection after spinal cord injury. *Cell Transplant*. 2014;23(Suppl 1):S45-56.
155. Xin H, Katakowski M, Wang F, et al. MicroRNA cluster miR-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke*. 2017;48(3):747-753.
156. Raisi A, Azizi S, Delirezh N, Heshmatian B, Farshid AA, Amini K. The mesenchymal stem cell-derived microvesicles enhance sciatic nerve regeneration in rat: a novel approach in peripheral nerve cell therapy. *J Trauma Acute Care Surg*. 2014;76(4):991-997.

How to cite this article: Lv L, Sheng C, Zhou Y. Extracellular vesicles as a novel therapeutic tool for cell-free regenerative medicine in oral rehabilitation. *J Oral Rehabil*. 2020; 47(Suppl. 1):29–54. https://doi.org/10.1111/joor.12885