Exosomes and Regenerative Medicine: State of the art and Perspectives

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Exosomes have attracted the attention of the scientific community in recent years due to their widespread distribution, their possible functions as biomarkers of disease, and their great potential to be applied as therapeutic agents. Exosomes carry proteins and nucleic acids that can facilitate their uptake by distant target cells through endocytosis, such that exosomes could be targeted to a specific cell or cells to enhance or interfere with specific biological processes. This review will mainly focus on their roles in tissue repair and regenerative processes. Exosomal engineering and their potential applications in tissue regeneration are also reviewed here as an outlook for future research.

Introduction

Paracrine signaling is of utmost importance in maintaining cellular homeostasis, and it also plays a key role in the onset and dissemination of many diseases.[1, 2, 3, 4] In past decades, soluble factors secreted by cells, for instance cytokines and growth factors, were considered to be the principal form of paracrine communication between cells.[5, 6, 7] Recently, extracellular vesicles, particularly exosomes, have been identified as another vital mediator of paracrine communication.[8, 9, 10, 11, 12] A dramatic increase in the number of publications on exosomes highlights their growing importance in scientific research (Fig 1).

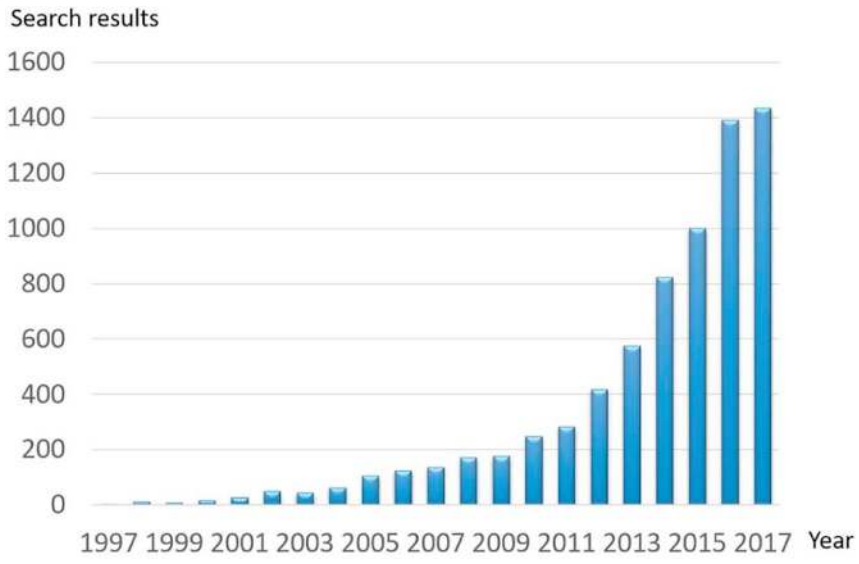

Fig 1

The dramatic increase in the number of publications on exosomes. The graph of "search results by year" is generated by searching "MeSH Terms: exosomes" in Pubmed, which indicates the growing importance of exosomes in scientific research the last decade.

Exosomes, nanovesicles sizing ranging from 40 to 150 nm, were first discovered in the supernatants of cultured sheep erythrocytes.[13, 14, 15] It was then discovered, through advancements in biological science and technology, that these nanovesicles are widely biologically distributed. Presently, exosomes have already been found in almost all types of bodily fluids, including saliva, milk, amniotic fluid, serum or plasma, and urine.[14, 16, 17, 18, 19, 20,21] The exosomes can be enriched from various fluids by differential centrifugation, density gradient isolation, and commercially available kits, and identified by specific biomarkers and particle diameters.[22, 23, 24, 25]

Exosomes originate from the endosomes that are generated by endocytosis of the cytoplasmic membrane.[26] After further processing, exosomes are released through membrane fusion.[27] They are enveloped by a lipid bilayer enriched in cholesterol, sphingomyelin, and ceramide.[28, 29] The membrane of exosomes is also abundant in some tetraspanins such as CD9, CD63, and CD81, which could be used as markers for identifying exosomes.[17, 20, 29,30, 31] The internal contents of exosomes are enriched in special biomolecules, functional proteins, and nucleic acids, including microRNAs (miRNAs), messenger RNAs (mRNAs), and even DNA.[19, 26, 32, 33]

Initially, exosomes were regarded as useless cellular metabolic waste, but it has since been recognized that they have many pivotal cellular functions. After release, exosomes can act upon special target cells in the vicinity of the parent cells in a paracrine manner, and they can also enter biological fluids, such as blood and urine, to be delivered to target cells far from the secreting cells, similar to the classical endocrine process.[19, 20, 21, 33] When exosomes are absorbed by specific target cells, the exosomal contents, especially miRNAs, will mediate numerous biological processes. The potential functions of the miRNAs contained within exosomes have already largely been explored.[8, 34, 35, 36] In detail, primary miRNAs are initially transcribed from genome of parental cells, processed by Drosha to pre-miRNA, and then transported into cytoplasm forming double-stranded mature miRNA. The mature miRNAs are integrated into late endosomes, and then miRNA-containing exosomes are released and captured by recipient cells. One strand of the exosomal miRNAs is integrated into Argonaute proteins which contain miRNA-induced silencing complex, and then interacts with target mRNA transcripts, which generally leads to the inhibition of corresponding gene expression.[26, 32, 37, 38] Thus, some biomolecules in exosomes might be applied as biomarkers for disease diagnosis, prognosis, and even injury conditions, because their levels or contents might change following the occurrence of some diseases or injuries.[32, 34, 39, 40, 41, 42]

Among the functions of exosomes reported thus far, their roles in cancer progression and immunoregulation have been predominantly studied.[32, 34, 43] In particular, cancer cell-derived exosomes have been found to promote tumor formation and metastasis in a variety of ways, for instance, by transferring tumorigenic factors to normal cells, remodeling the extracellular matrix, and mediating immune evasion.[19, 32, 35, 44, 45] Therefore, exosomes have been explored as potential biomarkers for cancer diagnoses and as special therapeutic vehicles for cancer treatments. In addition, immune cell-derived exosomes may function as proinflammatory or anti-inflammatory agents by transferring immunomodulatory cytokines, miRNAs, or other mediators between immune cells and other cell populations.[46, 47, 48]

Although exosomal functions have already been widely explored, the potential for regulating tissue repair and regeneration has not drawn nearly as much attention. Nevertheless, exosomes may be a promising substitute for many current cell and tissue engineering strategies.[49, 50, 51] The most striking evidence supporting this viewpoint originates from investigations focusing on mesenchymal stem cell (MSC) transplantation for tissue regeneration.[49, 51, 52, 53] Definitely, it has been shown through a series of elegant studies that MSCs induce cellular changes mainly through paracrine signaling, especially via the exosomes they produce.[51, 52, 53]Therefore, we could reasonably visualize a cell-free therapy utilizing paracrine factors, such as exosomes, to promote tissue repair and regeneration (Fig 2), which would avoid the risks associated with direct stem cell transplantation, such as teratomas, immune rejection, and the reduced regenerative capacity of engrafted cells.[54,55, 56]

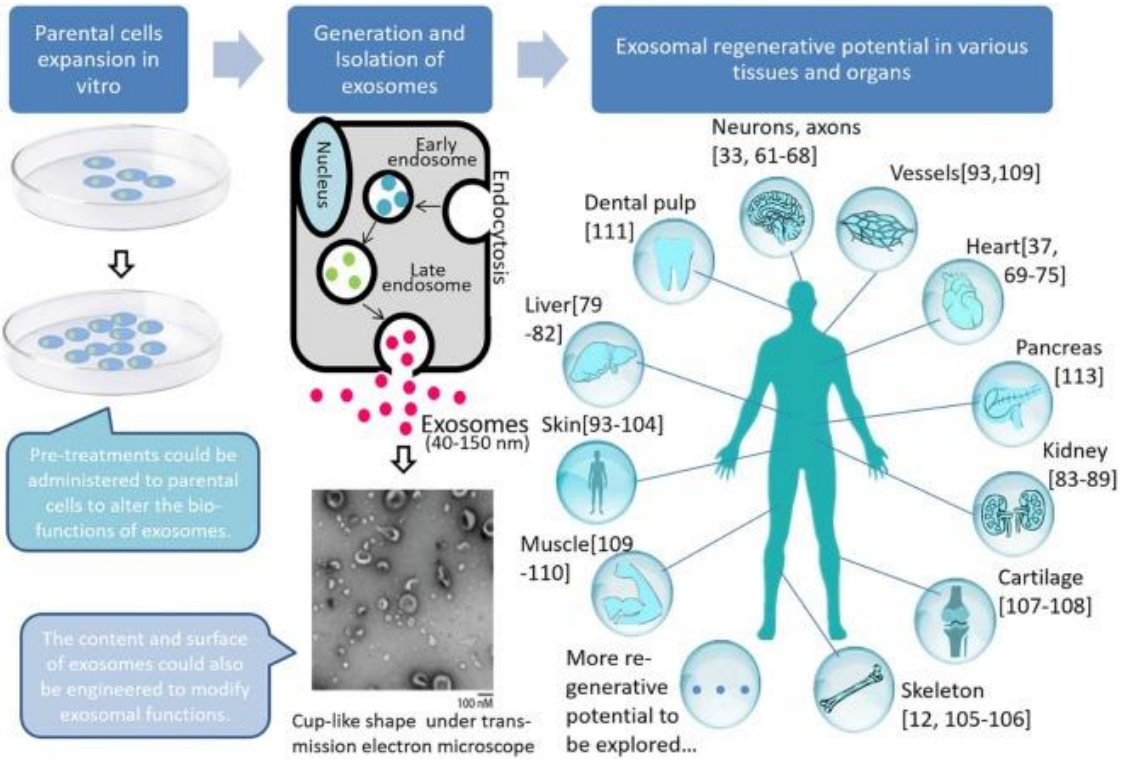

Fig 2. Exosomal generation and regenerative potential in various tissues and organs. Exosomes originate from the endosomes generated by endocytosis. After further processing, exosomes are released through membrane fusion. The regenerative potential of exosomes has been reported in many tissues and organs, such as nerve, heart, liver, kidney, skeleton, cartilage, muscle, pancreas, and dental pulp. It is reasonable to believe that more regenerative potential of exosomes will be discovered in the future.

Exosomal Regenerative Potential in Different Tissues and Organs

Taking into consideration the critical roles of MSCs and their products in tissue regeneration, MSC-derived exosomes are particularly promising candidates for developing cell-free therapies.[49, 51, 53] In addition, exosomes released from immune cells (monocytes, leukocytes, granulocytes, and lymphocytes) are implicated in several fundamental biological processes, such as the recruitment of inflammatory cells, neovascularization, and coagulation.[57, 58, 59] Thus, they are also of vital importance in ensuring the appropriate inflammatory reaction after injury, which would boost tissue repair and regeneration (Table I). Hence, existing evidence as to the potential uses of exosomes in promoting tissue repair and regeneration will be reviewed in the following section.

Neural Regeneration

It has been established that exosomes could be used as biomarkers for brain injuries. A proof of concept study conducted by Ji et al suggested that the serum exosomal miR-9 and miR-124 were promising biomarkers for diagnosing acute ischemic stroke (AIS) and evaluating the degree of damage caused by ischemic injury.[60]Furthermore, the regenerative effects of exosomes on neurons and nerves have been reported. Frohlich et al reported that exosomes derived from glutamate stimulated oligodendrocyte can promote survival in neurons deprived of oxygen and glucose.[61] In addition, Xin et al reported that exosomes extracted from multipotent mesenchymal stromal cells could deliver miRNA-133b to neural cells to boost neurite outgrowth, which was the first article revealing that communication occurs between MSCs and brain parenchymal cells.[62] Takeda et al found that treatment with exosomes derived from differentiating neuronal cells could induce neuronal differentiation in human MSCs.[63] This work also suggested that delivery of miR-125b via exosomes might be the possible underlying mechanism.

Recently, Zhang et al had investigated the regenerative potential of exosomes derived from human bone marrow mesenchymal stem cells (hBMSCs) on traumatic brain injury (TBI) in rats.[64] They found that compared with the negative control, endogenous angiogenesis and neurogenesis of rats with TBI systemically administered hBMSC-generated exosomes was enhanced, whereas neuroinflammation was attenuated. These results suggest that the exosomes released by hBMSCs significantly improve functional recovery in rats after TBI. The same group also found that native exosomes secreted by MSCs can promote axonal growth while tailored MSC-exosomes carrying the elevated miR-17-92 cluster could further boost this effect, because tailored exosomes can selectively deliver their cargo miRNAs to recipient neurons and activate their target signals.[65] Furthermore,

they also determined that neuronal internalization of MSC exosomes was accomplished mainly via the Soluble N-ethylmaleimide-Sensitive Factor Attachment Protein Receptor complex.

El Bassit et al also reported on the proregenerative effect of exosomes derived from human adipose-derived stem cells (hASCs) on HT22 neuronal cells post injury.[66] They found that exosomes derived from the hASCs boosted neuronal survival and proliferation by increasing expression of PKCδII in HT22 cells. They also found that MALAT1, a long noncoding RNA in hASCs-derived exosomes mediated splicing of PKCδII, thereby increasing its expression. Additional research by this group indicated that the regenerative effect of hASCs-derived exosomes could be further enhanced by insulin stimulation. More recently, it was reported by Mead et al that exosomes isolated from BMSCs could significantly promote the survival of retinal ganglion cells and regeneration of their axons; these beneficial effects might be correlated with argonaute-2, a key miRNA effector molecule.[33]

Spinal cord injuries often result in permanent damage due to the failure of axonal regeneration. In the peripheral nervous system, axonal regeneration is mainly supported by Schwann cells (SCs). After nervous damage, SCs can dedifferentiate, proliferate, and efficiently guide axons to their original target tissues. Lopez-Verrilli et al reported that exosomes derived from dedifferentiated SCs could be specifically internalized by axons, markedly increasing axonal regeneration in vitro and enhancing regeneration after sciatic nerve injury in a Sprague Dawley (SD) rat model.[67] Their research also indicated that SCs-derived exosomes promoted axonal regeneration by inhibiting activity of RhoA, a GTPase that could inhibit axonal elongation and promote growth cone collapse, but they did not mention the underlying molecular mechanism in the article. Goncalves et al found that the retinoic acid receptor β (RARβ) agonist could promote locomotor and sensory recovery in rat cervical avulsion models.[68]Further mechanism research revealed that in RARβ-agonist-treated neurons, activity of PTEN (a major negative regulator of neuronal regeneration) was obviously decreased by cytoplasmic phosphorylation. Moreover, the exosomal secretion of RARβ-agonist-treated neurons also increased. After being taken up by astrocytes, these exosomes could reduce the proliferation of astrocytes and cause them to arrange around the regenerating axons, preventing scar formation. Finally, the neuronal and neuronal-glial regenerative effects of RARβ signaling result in axonal regeneration into the spinal cord.

Myocardial Regeneration

The potential protective effects of exosomes have already been explored in a series of myocardial ischemia reperfusion injury models. Ibrahim et al showed that exosomes isolated from cardiosphere-derived cells could inhibit apoptosis and promote the proliferation of cardiomyocytes when injected into mouse hearts suffering from ischemia injury.[69] They also found that these beneficial effects were closely related to the enrichment of miR-146a in exosomes. Teng et al reported that exosomes generated from BMSCs significantly enhanced tube formation of human umbilical vein endothelial cells and inhibited proliferation of T cell in vitro.[70] In addition, reduced infarct size and preserved cardiac function were also observed in SD rats with acute myocardial

infarction due to enhanced neovascularization and suppressed inflammation response. Zhang et al also found that preconditioning with MSC exosomes could boost the proliferation, migration, and angio-tube formation of cardiac stem cells in a dose-dependent manner.[71]

It was reported by Khan and colleagues that mouse embryonic stem cell-derived exosomes (mES-Ex) possessed the ability to promote endogenous repair and enhance cardiac function after myocardial infarction.[72] They found that after mES-Ex were intramyocardially administered in mice at the time of myocardial infarction, both neovascularization and cardiomyocyte survival was enhanced, and myocardial fibrosis post infarction was evidently suppressed concurrent with enhanced c-kit(+) cardiac progenitor cells (CPCs) survival and proliferation. This research group also investigated the underlying mechanisms of these beneficial effects via microRNA array analysis. Their results indicated that the regenerative potential of mES-Ex was tied to the delivery of embryonic stem cell-specific miR-294 to CPCs, which could promote cell survival and proliferation of the latter.

Zhao et al showed that exosomes derived from human umbilical cord mesenchymal stem cells (hUCMSCs) possessed a protective effect in an acute myocardial infarction rat model.[73] They found that exosomes might improve cardiac systolic function by protecting myocardial cells from apoptosis and promoting angiogenesis. These beneficial effects were potentially associated with modulating the expression of members of the Bcl-2 family. Vicencio et al also showed that a specific cardioprotective pathway, involving TLR4 and HSP27, could be activated by exosomes in plasma.[74]

Recently, Agarwal and coworkers evaluated the regenerative role of human CPCs-derived exosomes in a rat myocardial ischemia reperfusion injury model.[75] In their investigation, human CPCs obtained from children of different ages were isolated and cultured under hypoxic and normal conditions. Then, exosomes were isolated from the conditioned media and delivered to rats. Finally, their results indicated that exosomes released by neonate CPCs improved cardiac function by decreasing fibrosis and improving angiogenesis regardless of oxygen levels in culture conditions, whereas exosomes from older children could only gain reparative power when CPCs were subjected to hypoxic conditions. This is the first investigation demonstrating that donor age and hypoxia level can influence the therapeutic efficacy of human CPC-derived exosomes.

Interestingly, Beltrami et al found that the pericardial fluid (PF) also contained exosomes enriched with miRNAs co-expressed in the patient myocardium and vasculature vs peripheral plasma.[37] Amazingly, the specific exosomes in the PF could improve the survival, proliferation, and networking of endothelial cells (ECs) cultured in vitro and restore the angiogenic capacity of ECs depleted of endogenous miRNA profiles. Most importantly, the PF exosomes could improve blood flow recovery and angiogenesis after ischemic injury in the mouse model. Further investigation suggested that PF exosomes might orchestrate the vascular repair process by delivering miRNA let-7b-5p to ECs.

Hepatic Regeneration

Exosomes have already been used as specific biomarkers for hepatocyte damage and inflammation in acute liver injury.[41, 76, 77, 78] Momen-Heravi et al reported that the exosomal levels in the plasma of patients with alcoholic hepatitis were obviously higher than those of the healthy population, as were the specific miRNA profiles in their exosomes.[76]

The regenerative potential of exosomes on liver has also been investigated recently. In acute liver injury, Nojima et al found that hepatocyte-derived exosomes could promote the proliferation of hepatocytes in vitro and liver regeneration in vivo.[79] Their research suggested that the underlying mechanism might involve exosomal transfer of neutral ceramidase and sphingosine kinase 2 (SK2) to target hepatocytes. Moreover, they also found that the levels of circulating exosomes with proliferative effects also increased after liver injury. Tan et al also investigated the regenerative potential of MSCs-derived exosomes in a carbon tetrachloride (CCl_4)-induced liver injury mouse model. They reported that CCl_4-induced liver injury was notably attenuated by concurrent treatment with MSCs-exosomes, which might be achieved mainly through the activation of proliferative and regenerative responses.[80]

Recently, Yan and coworkers reported that systemic administration of hUCMSC-derived exosomes (hUCMSC-Ex) could effectively rescue mice from CCl4-induced liver failure; this protective effect was closely associated with hUCMSC-Ex-derived glutathione peroxidase1.[81] The antioxidant and antiapoptotic abilities of hUCMSC-Ex would diminish after knockdown of glutathione peroxidase1 in hUCMSCs. Nong et al also evaluated the regenerative potential of exosomes derived from human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSCs-Exo) during hepatic ischemia-reperfusion injury.[82] Their results indicated that hiPSC-MSCs-Exo administration can alleviate warm hepatic ischemia-reperfusion injury by suppressing inflammatory responses, attenuating oxidative stress responses, and inhibiting cellular apoptosis. However, the molecular mechanism by which these effects occur was not further elucidated.

Renal Regeneration

It was reported by Tomasoni et al that exosomes released by hBMSCs could promote the proliferation of cisplatin-damaged proximal tubular epithelial cells via horizontal transfer of IGF-1 receptor mRNA.[83] Zhou and colleagues also demonstrated that exosomes derived from hUCMSCs could alleviate acute kidney injuries induced by cisplatin in rats by suppressing renal oxidative stress and apoptosis, while increasing renal epithelial cell proliferation.[84] Borges et al found that tubular epithelial cells exposed to hypoxic conditions can produce exosomes enriched in transforming growth factor-β1 (TGF-β1) mRNA, which can activate fibroblasts to initiate the fibrotic repair response.[85] This study suggested that TGF-β1 mRNA delivered by exosomes constituted a rapid response to initiate tissue-regenerative responses after hypoxia injury. Their finding also enlightens the potential for exosome-targeted therapies to control tissue fibrosis.

Burger et al examined the therapeutic potential of human umbilical cord blood-derived endothelial colony-forming cells (ECFCs) and ECFC-derived exosomes in a mouse model of ischemic acute kidney injury (AKI).[86] They found that intravenous administration of ECFCs can attenuate renal injuries in mice with ischemic AKI, while direct intravenous administration of ECFC-derived exosomes had the same effect. Recently, this group demonstrated that exosomes derived from ECFCs were enriched in miR-486-5p and delivery of ECFC-derived exosomes could reduce ischemic AKI via transfer of miR-486-5p targeting PTEN.[87]

In addition, Wang et al found that MSCs that were engineered to overexpress miRNA-let7c could selectively localize to injured kidneys and upregulate miR-let7c gene expression to attenuate kidney injury.[88] The exosomes derived from these engineered MSCs were also able to selectively transfer miR-let7c to damaged kidney cells to achieve antifibrotic functions. Jiang et al also investigated the therapeutic potential of exosomes from urine-derived stem cells (USCs-Exo) on kidney injury repair in an SD rat model.[89] Their results suggested that streptozotocin-induced kidney injury could be alleviated by weekly intravenous tail injections of USCs-Exo, which could obviously inhibit podocyte apoptosis and promote vascular regeneration and cell survival. They also deduced from an USCs-Exo contents assay that the regenerative potential might be related to the enrichment of cytokines vascular endothelial growth factor (VEGF), TGF-β1, angiogenin, and bone morphogenetic protein 7 in USCs-Exo.

Cutaneous Regeneration

Angiogenesis is of crucial importance in various physiological processes including cutaneous wound healing and tissue regeneration. It has already been established that exosomes released from cancer cells can modify the tumor environment to enable the metastasis of cancer cells and promote angiogenesis, which was reviewed in other articles.[90, 91, 92] This type of beneficial effect might also be found in exosomes derived from other sources, beyond cancer cells.

Liang et al found that exosomes released by human adipose-derived MSCs (adMSCs) can significantly promote endothelial cell angiogenesis in vitro and in vivo.[93] Further investigation indicated that exosomes derived from adipose-derived MSCs could transfer miR-125a to endothelial cells, resulting in the downregulation of angiogenic inhibitor delta-like 4. Yuan et al also demonstrated that exosomes extracted from human urine derived stem cells could enhance skin wound healing by promoting angiogenesis in vitro and in vivo.[94] However, they did not determine the underlying molecular mechanisms of this beneficial effect.

Burn injury, one of the most common causes of cutaneous damage, could significantly intensify the inflammatory reaction, including increased tumor necrosis factor α and interleukin-1β (IL-1β) levels, and decreased IL-10 levels.[95, 96, 97] Li et al found that administration of hUCMSC-exosomes could successfully reverse the burn-induced inflammatory reaction.[95] Further research suggested that miR-181c in hUCMSC-

exosomes weakened inflammation by downregulating the TLR4 signaling pathway following burn injury, which could attenuate excessive inflammation and boost tissue repair.

Zhao et al investigated the regenerative potential of exosomes derived from human amniotic epithelial stem cells on the healing of full-thickness skin defects in rats.[98, 99] Exosomes were isolated and then different concentrations were subcutaneously injected around the wound site. Eventually, they found that exosomes released by human amniotic epithelial stem cells could promote the migration and proliferation of fibroblasts, accelerating healing of full-thickness skin defect in a dose-dependent manner. Zhang et al also demonstrated that exosomes from human umbilical cord blood-derived endothelial progenitor cells possessed robust proangiogenic and wound healing effects in a diabetic rat model.[100] Microarray analyses indicated that exosomes markedly altered the expression of a series of genes involved in the Erk1/2 signaling pathway, and functional studies further confirmed that this signaling pathway was of vital importance during the exosome-induced angiogenic responses of endothelial cells. Recently, Guo and colleagues demonstrated that exosomes derived from platelet-rich plasma can effectively induce the proliferation and migration of endothelial cells and fibroblasts to promote angiogenesis and re-epithelialization in chronic cutaneous wound healing processes, highlighting the healing of chronic ulcers.[101]

Pu and colleagues reported that the survival and capillary density of flaps subjected to ischemia-reperfusion injury were significantly enhanced via the injection of exosomes released by adipose-derived stem cells.[102] Han et al had also found that exosomes derived from corneal epithelial cells could fuse to keratocytes and induce myofibroblast transformation after a corneal wound occurred.[103] Furthermore, corneal epithelial cell-derived exosomes can induce the proliferation of endothelial cells and aortic ring sprouting in vitro. Their results indicated that epithelial cell-derived exosomes might be involved in corneal wound healing and neovascularization processes, which may be applied as therapeutic interventions in the future.

Exosomes could also orchestrate controlled cutaneous regeneration in a bipolar manner. Zhang et al showed that exosomes derived from hUCMSCs could trigger the Wnt/β-catenin signaling pathway to repair damaged skin tissue during the early stages of deep second-degree burn healing, and they could also inhibit Wnt/β-catenin signaling through the induction of YAP phosphorylation to circumvent excessive skin cell expansion and collagen deposition after the remodeling phase.[104]

Skeletal Regeneration

Bone regeneration using MSCs and tissue engineering strategies is one of the most widely researched fields in regenerative medicine. Furuta and colleagues evaluated the therapeutic effects of exosomes isolated from MSC-conditioned medium (CM) in the fracture healing process in a $CD9^{-/-}$ mice model, which produces low levels of exosomes. Identical femur fractures were created in both test and control groups. Then mice in the test group were injected with exosomes, while controls were injected with exosome-free CM. The bone union rates were

measured, and the results suggested that the exosomes in MSC-CM could accelerate the fracture healing process.[12]

Recently, Zhang et al investigated the pro-osteogenic potential of hiPSC-MSC-Exos.[105, 106] They showed that the isolated exosomes could effectively stimulate the proliferation and osteogenic differentiation of bone marrow MSCs derived from ovariectomized rats in vitro and in vivo. These results also suggested that the therapeutic effects of hiPSC-MSC-Exos could be intensified by increasing their exosomal concentration. Bioinformatics analyses further confirmed that the PI3K/Akt signaling pathway was the principal regulator during the hiPSC-MSC-Exos-induced osteogenic differentiation of BMSCs.

Chondral Regeneration

Zhang et al showed the therapeutic effects of exosomes derived from human embryonic mesenchymal stem cells on cartilage repair. Osteochondral defects were created on bilateral trochlear grooves in a rat model. One defect was weekly intra-articularly injected with human embryonic MSC-derived exosomes for 12 weeks, and the contralateral defect was injected with phosphate-buffered saline. Eventually, complete restorations of cartilage were observed in defects treated with exosomes, whereas only fibrous repair tissues were found in the control group.[107] However, this research did not exclude the possibility that exosome injected into the trochlear groove might be transferred to the contralateral defect through blood circulation, thereby interfering with the outcome of control group.

Recently, Zhu and coworkers evaluated the regenerative potential of exosomes secreted by human synovial membrane MSCs (SMMSC-Exos) and induced pluripotent stem cell-derived MSCs (iMSC-Exos) on osteoarthritis, which is induced by failure of articular cartilage regeneration in a rat model.[108] They found that both iMSC-Exos and SMMSC-Exos could attenuate osteoarthritis by stimulating chondrocyte migration and proliferation, while the regenerative power of iMSC-Exos was stronger than that of SMMSC-Exos. This inspiring work also provided new perspectives for cell-free therapies for cartilage injury and osteoarthritis.

Muscular Regeneration

Nakamura et al showed that exosomes derived from MSCs could promote myogenesis and angiogenesis in vitro.[109] They verified that MSC-derived exosomes promoted muscle regeneration in a mouse model of cardiotoxin-induced muscle injury, which might be mediated by miR-494. Recently, Choi and colleagues also found that exosomes derived from human skeletal myoblasts during myotube differentiation could effectively induce a myogenesis response in hASCs.[110] Moreover, a laceration mouse model verified that exosomes derived from differentiating human skeletal myoblasts could accelerate skeletal muscle regeneration by reducing collagen deposition and increasing the number of regenerated myofibers in injured muscles.

Regeneration of Other Tissues and Organs

The regenerative potential of exosomes has also been investigated in other tissues and organs such as in dental pulp tissue and pancreas. Huang and colleagues have evaluated the potential of exosomes derived from dental pulp cells cultured under odontogenic differentiation conditions to induce odontogenic differentiation in naive human dental pulp stem cells and human bone marrow-derived stromal cells in vitro and in vivo.[111] After being taken up by human dental pulp stem cells and human bone marrow-derived stromal cells, exosomes could trigger the p38 mitogen-activated protein kinase pathway and increase the expression of genes required for odontogenic differentiation in a dose-dependent and saturable manner via the caveolar endocytic mechanism, boosting the regeneration of dental pulp-like tissue collectively. In addition, they found that exosomes could bind to matrix proteins such as type I collagen and fibronectin, which enabled exosomes to be tethered to biomaterials.

Diabetes mellitus is a complex metabolic disease characterized by glucose overproduction and underutilization, constituting one of the most important global health problems. Insulin, which functions to decrease blood glucose, is synthesized and secreted by pancreatic β-cells. Reductions in the amount and function of pancreatic β-cells result in relative or absolute insufficient insulin secretion, contributing to the pathophysiology underlying diabetes.[112] Oh et al investigated the regenerative potential of extracellular vesicles, including exosomes and microvesicles, derived from a murine pancreatic β-cell line.[113] They found that bone marrow cells in diabetic immunodeficient mice can effectively differentiate into pancreatic β-cells when cultured with exosomes. This inspiring finding might provide an ideal solution to the dilemma that sources of functional islets for transplantation are particularly limited.

Exosomal Engineering for Tissue Repair and Regeneration

Recent evidence indicates that exosomes secreted by most cell types can mediate transfer of proteins, mRNAs, and microRNAs; theoretically, endogenous exosomes could be promising candidates for natural drug delivery due to their small size, low immunogenicity, nontoxicity, permeability of physiological barriers, and stability in circulation.[114, 115, 116, 117] Emerging exosomal engineering strategies have achieved this miraculous aim.

Reported investigations proved that exosomes could be specially preloaded with therapeutic agents, such as short-interfering RNA (siRNA), miRNA, DNA, and some proteins and compounds.[115, 117] By this means, the desired agents can be protected from degradation and deactivation after administration, and effectively transferred to target cells for special medical purposes. Besides, the surface of exosomes could also be engineered with particular ligands with affinity to specific cell types, ensuring that the engineered exosomes can be efficiently and selectively captured by target cells to evade the off-target effects.[118] Generally, modifying exosomal content and surface markers will not interfere with the other natural features of exosomes. Moreover, it has been recognized that the microenvironment, which parental cells are exposed to, can influence the

functional potential of the exosomes these cells produce.[119, 120] Existing evidence regarding exosomal engineering strategies is presented in the following section, and the potential applications in regenerative medicine will also be discussed herein.

Engineering the exosomal content with compounds

Zhuang et al were the first to encapsulate an anti-inflammatory compound, curcumin, into exosomes derived from EL-4, a murine lymphoma cell line.[121] After incubation with exosomes loaded with curcumin, RAW 264.7 cells, a murine macrophage cell line, released lower levels of proinflammatory cytokines IL-6 and tumor necrosis factor α compared with cells incubated with curcumin alone. Additionally, they demonstrated that the curcumin delivered by exosomes is more stable and more highly concentrated in blood circulation. They found that the solubility, stability, and bioavailability of curcumin were significantly increased after they were encapsulated in exosomes. Moreover, intranasal administration of exosomes encapsulating curcumin leads to rapid delivery of curcumin to the brain, because the modified exosomes were selectively taken up by microglial cells, and subsequently induced apoptosis of microglial cells. In contrast, curcumin is difficult to penetrate the blood-brain barrier when it is delivered alone. Therefore, the exosomes-mediated curcumin delivery is superior to delivery of curcumin alone. This proof-of-concept investigation successfully validated that exosomes could be modified to deliver specific therapeutic agents to target cells. In the future, with assistance from exosomes engineered with anti-inflammatory compounds, clinicians might be able to beneficially modulate the inflammatory response as soon as tissue damage occurs. It is also reasonable to imagine that engineering exosomes with compounds that can boost tissue regeneration may help improve overall tissue repair.

Engineering the exosomal content with nucleic acids

Another exosomal engineering strategy would be encapsulating nucleic acids into exosomes. Alvarez-Erviti and colleagues demonstrated that systemic injection of engineered exosomes containing siRNA against GAPDH or BACE1 could be effectively delivered to mouse brain tissues and that the expression of target genes in recipient cells could be repressed; these findings indicate that efficient in vivo delivery of nucleic acids through exosomes is feasible.[122]

Recently, de Rivero Vaccari et al isolated exosomes from embryonic cortical neuronal cultures and loaded them with an siRNA against apoptosis speck-like protein containing a caspase recruitment domain (ASC) and administered the exosomes to spinal cord-injured animals.[123] Surprisingly, they found that neuronal-derived exosomes could cross the injured blood-spinal cord barrier and deliver their cargo in vivo, resulting in the knockdown of ASC protein levels. These findings also indicate that exosomal siRNA delivery might be a promising route to block the devastating inflammation activation that occurs after CNS injury. In the future, proregeneration nucleic acids, such as proinflammatory, antiproliferation, or antiangiogenic genes might also be transferred via engineered exosomes to promote tissue repair.

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