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Review article

The potential of exosomes in the therapy of the cartilage and bone complications; emphasis on osteoarthritis

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ABSTRACT

Osteoarthritis is a prevalent worldwide joint disease, which demonstrates a remarkable adverse effect on the patients' life modality. Medicinal agents, exclusively nonsteroidal anti-inflammatory drugs (NSAIDs), have been routinely applied in the clinic. But, their effects are restricted to pain control with insignificant effects on cartilage renovation, which would finally lead to cartilage destruction. In the field of regenerative medicine, many researchers have tried to use stem cells to repair tissues and other human organs. However, in recent years, with the discovery of extracellular microvesicles, especially exosomes, researchers have been able to offer more exciting alternatives on the subject. Exosomes and microvesicles are derived from different types of bone cells such as mesenchymal stem cells, osteoblasts, and osteoclasts. They are also recognized to play substantial roles in bone remodeling processes including osteogenesis, osteoclastogenesis, and angiogenesis. Specifically, exosomes derived from a mesenchymal stem cell have shown a great potential for the desired purpose. Exosomal products include miRNA, DNA, proteins, and other factors. At present, if it is possible to extract exosomes from various stem cells effectively and load certain products or drugs into them, they can be used in diseases, such as rheumatoid arthritis, osteoarthritis, bone fractures, and other diseases. Of course, to achieve proper clinical use, advances have to be made to establish a promising regenerative ability for microvesicles for treatment purposes in the orthopedic disorders. In this review, we describe the exosomes biogenesis and bone cell derived exosomes in the regenerate process of bone and cartilage remodeling.

1. Introduction

Osteoarthritis (OA) affects about 10% of men and 18% of women over the age of 60. The symptoms of OA chiefly occur in the knee and hip bone as well as the soft-tissue framework in around the joint. These structures include synovium and ligaments might be a manifest inflammatory condition, consequently, which become impotent [1]. Prevalent pharmacologic remedy for OA include some monoclonal antibody like as Tanezumab (against nerve growth factor) and acetaminophen, sprifermin/recombinant human fibroblast growth factor-18, and Nonsteroidal anti-inflammatory drugs (NSAID) [2]. These drugs are not efficient on the restoration of cartilage homeostasis and mortality rate, and there are not suitable alternatives treatment for joint surgery; however, these surgeries may have side effects, such as limited prostheses life, infection, and high costs [3].

Various signaling pathways like paracrine and endocrine play a key role in retaining cellular and molecular homeostasis, and can lead to the onset and spread of many diseases [4,5]. Some soluble factors like growth factors, chemokine and cytokines, are the main form of paracrine communication approaches between cells [6]. Extracellular vesicles are classified according to their origin or biological function (Table 1) [9]. In recent studies, extracellular vehicles (EVs), especially exosomes, have been identified as another important intermediate cell mediator ([10,11]). Some studies declare that, size of exosomes as being 30–100 nM, and micro-vesicles are often larger than exosomes as being 100–300 nM; but, typically the size of the exosomes is considered 40 to 150 nm with a density ranging from 1.09 to 1.18 g/ml. Exosomes were detected for the first time in the cultured sheep erythrocytes

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Table 1
Classification of extracellular vesicles.

Type of vesicles	Exosomes	Microvesicles	Apoptotic bodies
Origin	Endosomes from many cell types	Plasma membrane of many cell types	Plasma membrane from endoplasmic reticulum
Size	40–100 nm	50–1000 nm	50–4000 nm
Density	1.12–1.22 g/cm^3	None	1.17–1.29 g/cm^3
Markers	CD9, CD63, CD81, CD82, Alix, TSG101, HSP 70, flotilin-1	Integrin, CD40 metalloproteinase, Selectin, flotilin-2	Phosphatidylserine and histones
Lipids	Ceramide, cholesterol, sphingomyelin and lysophosphatidic acid	Cholesterol	Phosphatidylserine
Molecular cargo	mRNA, miRNA, nc RNAs, mtDNAs	mRNA, miRNA, nc RNAs, mtDNAs	Nuclear fractions and cellular organelles
Reference	[7]	[8]	[9]

supernatant liquids [12]. Currently, exosomes are found in almost all body fluids, including milk, urine, serum, amniotic fluid, and saliva [13,14].

Exosomes can be used as potential biomarkers for early diagnosis of cancer, as well as drug carriers (for gene therapy) in the treatment of malignancies [15]. In recent years, other applications has been introduced for exosome, including promoting the regeneration of tissues, bone and cartilage healing, and reducing the risks of direct stem cell transplantation (immune rejection and cell renewal capacity) [16,17].

In recent years, researchers have been looking for approaches to rebuild human tissues and organs; hence one of these methods is the use of human stem cells. Indeed, the use of mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), Wharton's jelly stem cells, and embryonic stem cells (ESCs) showed promising results. Most of their therapeutic effects are mediated via EVs, such as exosomes and growth factor. Today, researchers have isolated exosomes from different sources and used solely or in combination with other factors like cytokines [18,19].

If EVs could produce a paracrine signal for renewal procedure, they might be considered as therapeutic alternative with some advantages over stem cells therapy [20]. Regenerative effects of EVs derived from MSC have been widely reported in pre-clinical models of the kidney and lung injury, myocardial infarction, and liver injury [21,22]. EVs have some special effects like as augmenting angiogenesis, prohibiting apoptosis, and reducing the oxidative stress, and also production of adenosine triphosphate (ATP) by surface kinases, which is assumed to enhance endogenous cell survival in the injured area [23]. In this review article we are going to talk about studies that have been carried out in the field of cartilage and bone regeneration in recent years with the help of exosomes.

2. Methodology

2.1. Criteria for considering studies for this review

2.1.1. Types of studies

Observational studies including (prospective and retrospective), Experimental studies and quasi-experimental studies, cross-sectional studies were evaluated.

The inclusion criteria were studies (i) exosomes in the therapy of the cartilage and bone complications were included, (ii) exosomes in the therapy of osteoarthritis (iii) exosomes in the therapy of bone fracture healing. Abstracts and studies without a control group were excluded.

2.1.2. Search strategy

A preliminary search of MEDLINE (PubMed) was conducted according to our search strategy and main keywords including “exosomes; osteoarthritis; cartilage and bone complications, bone fracture healing”. The secondary search of MEDLINE (PubMed), Cochrane Library, Embase, scopus databases for published articles and search of Google Scholar, and ProQuest (thesis and dissertation) was conducted for gray literature and unpublished studies. Studies that met our inclusion criteria were included in the study.

3. Characteristics of exosomes

The production of exosomes initially begins with the penetration of micro domains with a clathrin coating on the cell membrane [24]. For releasing of exosomes, vacuoles should become the primary endosome, which is then carried with the assistance of endosomal sorting complex required for transport (ESCRT). Exosomes stem from the endosomes, which originate from endocytosis of the cytoplasmic membrane. Then a number of substances, such as the coating of two-layer lipid-enriched with cholesterol, sphingomyelin, and ceramide, are added to these vesicles and, eventually exosomes are released [25]. ESCRT regulates vesicular trafficking processes, and might have an assistant role in a number of chaperones such as heat-shock protein (HSP) 70 and HSP90. The ESCRT complex consists of a number of cytosolic proteins, called as ESCRT-I, ESCRT-II, and ESCRT-III. ESCRT confers the membrane of exosomes a flexibility state that leads to their transportation through the cytoplasm. Exosomes also contain the protein TSG101, which binds to ubiquitinated cargo proteins and is required for the sorting of endocytic [26].

The exosomal surface membrane also has some other markers, such as CD9, CD63, CD82, CD81, heat shock proteins (HSP), major histocompatibility complex (MHC), and lipid raft like as Flotillin-1, which are markers that could be utilized for the exosome sorting and detecting [7,27]. The internal contents of the exosomes include nucleic acids, proteins, various microRNAs (miRNAs) and DNA [28]; besides, various lipid compounds are found in exosomes, such as ceramide, sphingomyelin, phosphatidyl choline, phosphatidylserine, phosphatidyl ethanolamine, and cholesterol (Fig. 1) [29]. At first, exosomes were considered as useless cellular metabolic waste, and then, with subsequent studies, more functions were discovered. For example, the exosomes derived from cancer cells through different mechanisms lead to remodeling of an extracellular matrix and intervention against immune cells, subsequently, increasing in metastasis and angiogenesis of malignant cells [30] (Fig. 2).

Exosomes impress the target cells by several approaches. First, exosomes interact with the target cells receptors, and therefore, activate the signaling cascade in the cells. Second, exosomes can integrate own cargo with target cells, either directly or by an endocytosis pathway, and then release mRNAs, miRNAs and functional proteins to cytosol, leading to numerous biological processes. Exosome secretion can be accelerated by various chemical substances, environmental conditions (low pH and oxygen), and mechanical excitation [8,31].

Isolation of exosomes is a challenge and it is necessary to achieve an optimal purity before any therapeutic application [32]. The EVs isolation is carried out by several methods including, density gradient, commercially kits, centrifugation, and then are recognized through particular biomarkers (Table 2) [33]. Isolated exosomes can be characterized based on quantity, surface biomarkers, size, zeta potential, and many supplementary approaches such as, western blot, droplet digital PCR (ddPCR), transmission electron microscopy (TEM), and

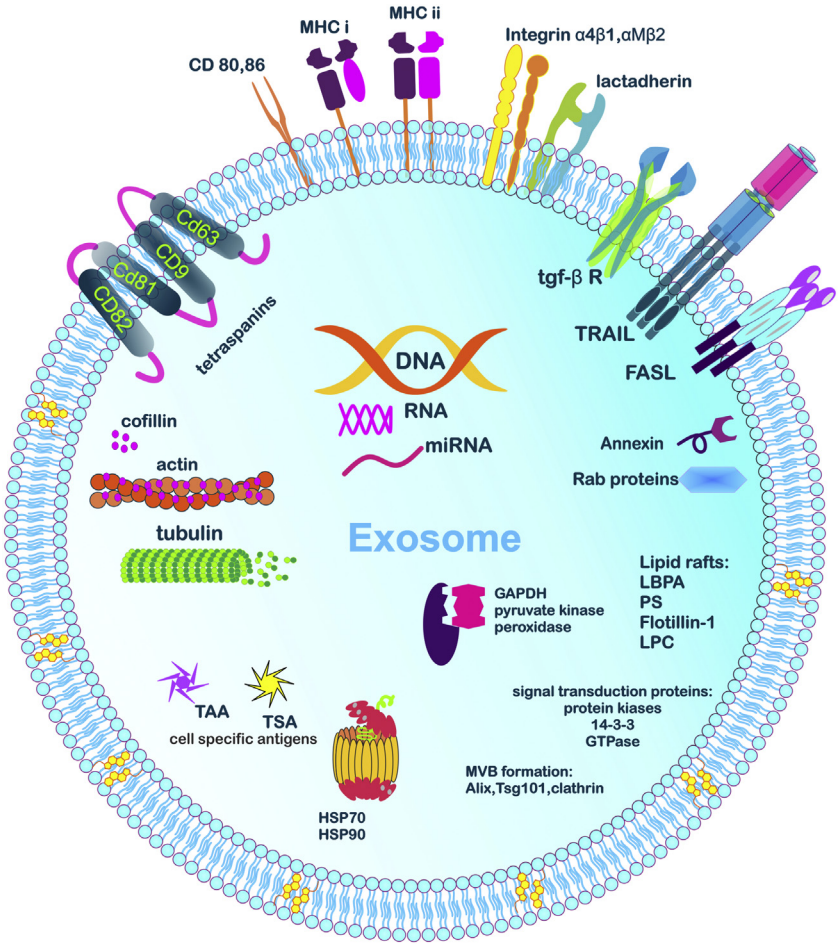

Fig. 1. Exosome molecular composition.

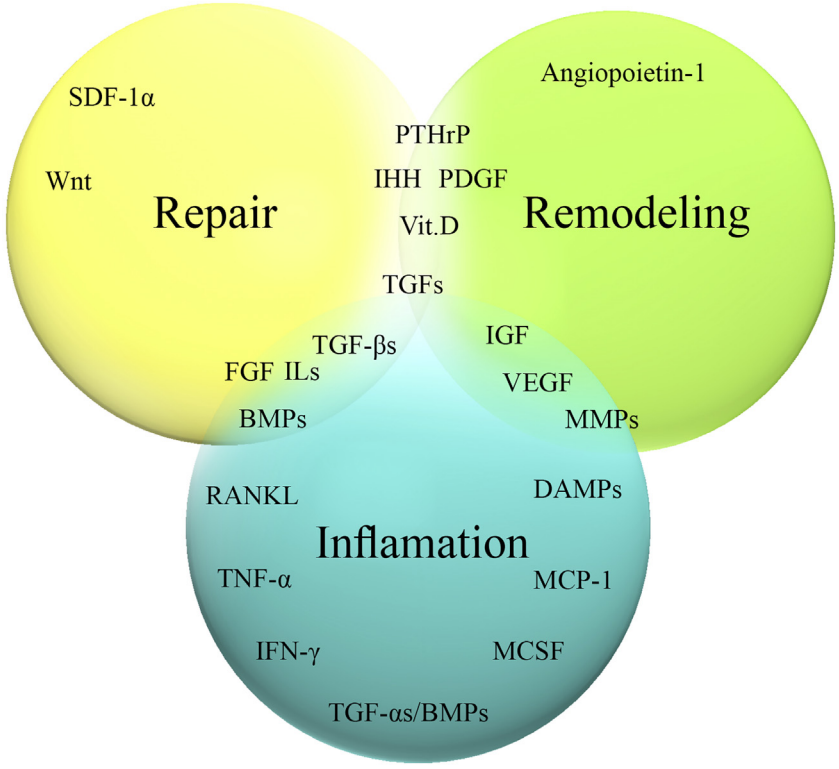

Fig. 2. Key factors involved in the bone healing phases. The positioning of the factors at the interfaces among the circles demonstrates overlapping functions.

Table 2
Comparison of exosome isolation techniques.

Isolation technique	Ultracentrifugation based techniques	Exosome precipitation	Size-based techniques	Microfluidics based technique	Immunoaffinity capture-based techniques
Isolation principle	Density, size, and shape based	Altering the solubility of exosomes	Entirely based on the size diversity between exosomes and other extra cellular particle	Based on a variety of exosomes features like immunoaffinity, size, and density	Based on specific interaction between exosomes membrane-bound antigens and immobilized antibodies
Advantage	Cost-effective and feasible for Large sample and yields have great quantities of exosomes	Easy and does not need advanced equipment, Potential for high capacity utilization	Fast, does not need advance equipment, good portability, high-purity yield	Fast, low costs, portable	Appropriate for specific exosomes isolation, extremely purified yield much better than other techniques
Disadvantage	Costly equipment, time-consuming, low portability, not available everywhere, high speed centrifugation may affect the exosomes	Co-sedimentation of other non-exosome such as proteins and polymeric materials, time-consuming and need pre-and post-cleanup	Moderate exosomes purity, Entering shear stress, exosomes loss owing to sticking to the membranes, need precise equipment, not suitable for large volumes	In general, it is not a standard method and is not suitable for large-scale experiments on clinical specimens	High reagent cost, low capacity and yields, the antigenic epitope may be blocked or masked.

nanoparticle tracking analysis (NTA) [34]. After isolation, exosomes are used in several ways in regenerative medicine, including direct injection into desired tissue or circulation method, mix with hydrogel, deposited on electrospun fibers by chemical linkers or with bio-degradable gels like as fibrin, and special tags engineered on to the exosomes [35].

4. The potential of exosomes in treatment of OA

Bone diseases associated with hereditary and environmental factors, such as rheumatoid arthritis and OA have become worldwide skeletal disorders [36]. Pathological symptoms include bone destruction (especially during aging), bone margin expansion or along joint margins (osteophytes), increased subchondral bone thickness, and inflammatory conditions. These diseases may also disbalance bone regeneration (because of defect in osteoblast bone absorption), which increases the risk of bone fractures [37]. Besides, anatomical factors related to individuals, such as knee alignment, bursa morphology, leg length inequality, and hip dysplasia have a strong association with OA [38]. The precise etiology of OA is unknown, but there is evidence that loss of cartilage matrix ingredients structural macromolecules, such as proteoglycans (PGs) and collagens due to an excessive activation of extracellular proteinases (mostly matrix metalloproteinase), and decreased synthesizing of new matrix for repair, is involved (chondrocytes in normal individual maintain a balance between synthesis and degradation of extracellular matrix ingredients) [39]. In OA patients, the metabolic activity of the chondrocytes is anomalistic. However, OA is considered as a noninflammatory ailment, but proinflammatory cytokines like as interleukin (IL)-1 have a critical role in this disease pathogenesis. IL-1 increases the production of nitric oxide (NO) and prostaglandin E2 (PGE2) by chondrocytes [40]. Studies have shown that NO can prevent the biosynthesis of proteoglycans and induce chondrocyte apoptosis in OA patients, On the one hand, PGE2 may play an anabolic role, leading to an increase in the biosynthesis of collagen and PG [41]. Moreover, IL-1 significantly intensifies the expression of matrix-degrading proteinases like matrix metalloproteinase (MMP)-1, -2, -3, -7, and 13, and prevents the production of PG and collagen [42].

Insulin-like growth factor (IGF)-I is considered as a serum factor responsible for stimulating the biosynthesis of PG, which also acts as a promoting factor for expression of collagen II in chondrocytes. IGF-I expression is significantly higher in OA patient cartilage than normal individual [43]. In addition to IGF-I, three isoform of the transforming growth factor (TGF)-β family and bone morphogenetic protein (BMP) family are the main stimulators of chondrocyte biosynthesis. Most of the BMPs also have other names, including osteogenic protein (OP) or growth and differentiation factor (GDF) [44].

Osteoclasts have a key role in bone resorption. Two cytokines such as macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL) are involved in osteoclastogenesis. Various transcription factors, signaling pathway, and co-regulators play an essential role in modulation of osteoblastogenesis. Numerous researches revealed that, some factors, such as runt-related transcription factor 2 (Runx2), Wnt pathway, TGF-β, BMPs, and some miRNA are responsible for the final differentiation of osteoblasts to the bone mass [45]. Osteoclast-derived exosomes cause the formation of osteoclasts in vitro and also prevention of osteoclastogenesis process. RANK levels were reported to be high in osteoclastic exosomes (low levels of RANK during culture conditions prevent the formation of osteoclasts). Thus, exosomes from osteoclast are paracrine controllers of osteoclastogenesis [46]. Solberg et al. revealed that exosomes from osteoblasts carried RANKL, Tartrate-resistant acid phosphatase (TRAP) enzymes, osteoprotegerin (OPG), leading to the development of osteoclastogenesis. Moreover, osteoblasts-derived exosomes improved bone regeneration via upregulating Runx2 and alkaline phosphatase [47]. Exosomal miRNAs derived from human bone marrow mesenchymal stem cells (BM-MSCs) have been shown to induce the Wnt signaling

pathway, leading to osteogenic differentiation [48].

Studies to date on exosomal miRNAs have shown that miR-30d-5p, miR-199b, and miR-133b-3p prevent the *RUNX2* gene expression, consequently, suppressing osteoblast differentiation [49]. It was found that miR-140-3p reduced osteoblast activity by inhibiting the BMP-2 expression. On the other hand, miR-885-5p negatively regulated BMP-2 expression, therefore, accelerated the osteoblast differentiation and mineralization [50]. If specific miRNAs could reduce inflammation or tissue destruction in OA, they could be packaged in exosomes or nanoparticles to treat OA patients. Cong Tao et al. revealed that human synovial mesenchymal stem cells-derived exosomes (hSMSCs) obviously stimulated chondrocyte proliferation and migration. But these exosomes had a crucial defect of preventing the synthesis of some proteins, such as aggrecan and collagen II. To confirm this, the mRNA expression levels of Wnt family members in hSMSCs was measured, and results indicated that Wnt5a was overexpressed, leading to the activation of YAP signaling pathways for chondrocyte proliferation and differentiation. The activation of YAP resulted in the suppression of extra cellular matrix (ECM) formation [51].

Cosenza et al. found that exosomes derived from MSCs had immunosuppressive properties, which lead to declining T and B lymphocyte proliferation, and also inducing regulatory T (Treg) cell differentiation. They found that, exosomes had minor effect in inhibiting helper T (Th) 1 cell population, subsequently, they were more stimulating Treg and Tr1 cells in vitro experiment. The reason for this observation is not well-known, but the preliminary analysis showed that most exosomes contain TGF-β1 [52]. Recently, other studies in this regard have shown that extracellular microsomal cells have little effect on T cells, and may also indirectly inhibit the population of B and natural killer (NK) cells [53]. This partial disagreement may be due to the isolation protocols, culture conditions, and EVs sources. A study conducted based on the assumption that stimulation of human MSCs before exosomes isolation could enhance the immunomodulatory exosomes feature [54], which were almost able to achieve a positive outcome. On the other hand, another article reported that MSCs stimulation did not increase the immunomodulatory effects of exosomes derived from MSCs [55].

The inducible nitric oxide synthase (iNOS) has been indicated to have immunomodulatory effects. This enzyme can suppress T cell population thorough the production of nitric oxide (NO) [56]. Mao and colleagues observed that miR-92a-3p in exosomes cargo interfered with the cartilage growth and degradation through Wnt5a signaling pathway. Their data revealed that miR-92a-3p was expressed in the late stage of chondrogenic differentiation [57]. Other studies have found that miR-92a-3p correlates with SRY-box 9 (SOX9) and collagen type II alpha 1 chain (COL2A1) overexpression, and also cause a delay in the progression of OA through ADAM metallopeptidase with thrombospondin type 1 motif (ADAMTS) 4 and ADAMTS5 inhibition [58]. The Wnt5a has two anabolic and catabolic functions. For instance, it can trigger MMPs gene expression and inhibit collagen II expression in chondrocytes [59]. Conversely, some paper offered that Wnt5a signaling is initiated through TGF-β and is essential for ECM synthesis in smooth muscle tissues [60].

5. Bone fracture healing by exosomes

Bone fractures are one of the common problems affecting 2% of the population per year, and the obesity and low physical activity levels are considered as risk factors [61]. Bones are formed via a well-known advancement schedule called as endochondral ossification, which basically involves cartilage production [62]. The bone fracture is ameliorated by a similar endochondral process; the break gap is bridged through a cartilaginous callus organized via nearby periosteum-derived precursor cells. The chondrocyte hypertrophy and calcification lead to the callus to be changed into bone form [63]. Bone regeneration can often be compromised for some reasons, such as critical-sized bone defects, growth factor levels, and biomechanical factors (bone fixation resistance). These factors could be impressing in the healing process. Large bone defects are determined by ischemic surroundings, with a severe shortage of oxygen and nutrients around the core [64,65]. This concept is a big challenge to be applied in the cell-based therapies (because of a decrease in the glucose reserves) [66].

After an injury, platelets or the complement system (in lack of bleeding) trigger the healing process. This activation results in the secretion of both vasoactive mediators and chemotactic agents that recruits the neutrophils, macrophages and fibroblasts cells to the damage site [67]. These cells can release several necessary factors for preparing of the fresh tissue formation and Set up renovation events. In the early inflammatory stage, short-lived inflammatory cells like neutrophils, migrate to the damage location. During the acute inflammatory phase, monocytes and macrophages release some factors such as chemoattractant protein 1 and IL-6 [68]. These chemokines, along with the toll-like receptors (TLRs) and NOD-like receptors (NLRs) signaling pathways, recruit tissue-resident macrophages and cause secrete various cytokines like as IL-6, IL-1β, and IL-1. Macrophages have important role in various phases of bone renewal, including intramembranous ossification and endochondral bone formation [69]. Macrophages generally have two types, including pro-inflammatory (M1-like) and anti-inflammatory (M2-like) macrophages. In the acute inflammation phase, macrophages represent mostly a M1-like phenotype. Th1 and cytotoxic T cells ($CD8^+$) by producing tumor necrosis factor (TNF)-α and interferon (IFN)-γ are involved in the development of M1-like phenotype [70]. M1 macrophages release inflammatory mediators, including TNF-α, IL-1β, and IL-6. However, it has been shown that chronic expression of these cytokines has a negative effect on bone regeneration [71]. For example, TNF-α triggers apoptosis of stem cells, thus, inhibits the regenerative potential. On the other hand, it can improve bone regeneration by activating muscle stromal cells [72]. Th2 cells produce anti-inflammatory cytokines such as IL-4 and IL-13, which trigger the development of M2 anti-inflammatory phenotypes [73]. The exact effect of T cells in tissue regeneration is still unclear. However, it seems that distinct T cells subtypes are involved in tissue renewal steps and their function varies in different tissues [74].

Treg cells could modify the local inflammation via releasing immunosuppressive cytokines like as IL-10, IL-35 and TGF-β, which are involved in the expansion of the M2-like macrophage. Additionally, γδT cell subset have crucial role in bone repairing processes by secreting IL-17A and, therefore, improves the performance of osteoblasts [75]. The bone fracture causes a laceration of blood vessels in the damaged area and, therefore, the blood flow is significantly decreased. The primary fracture environment is a powerful inducer of the pro-angiogenic factors secretion [76]. Pro-angiogenic factors leads to the organization of new capillaries in the damaged area. PDGF, vascular endothelial growth factor (VEGF), and angiopoetin-1 are the crucial factors in promoting the vessels in new bone [77]. The major effect of VEGF is vascularization, and it has been implicated in the osteoprogenitor cells differentiation and regulation of several osteoinductive agents, such as TGF-β1, IGF, and FGF-2. New vessels supply the source of circulating factors, such as parathyroid hormone and vitamin D, which are important for bone homeostasis [78].

Stromal cell-derived factor-1α (SDF-1α) is an important factor for stimulation of systemic and local progenitor cells [79]. In the acute phase of bone repairing, SDF-1α is extremely released via the periosteum that is involved in the migration of mobilized cells to the bone formation area. Subsequently, Osteoprogenitor cells in BM increase the CD44 and CXCR4 expression for receiving osteopontin and SDF-1 factors [80]. During the development of bones, the density, expansion, and differentiation of mesenchymal pregenital cells are regulated by combining signaling pathways, such as Wnt, FGF, and BMP. FGF and Wnt signaling are involved in the controlling the limb growth and prohibiting chondrogenesis, while confer a proliferative and osteogenic phase to the progenitor cells [81]. On the one hand, BMP and TGF-β

signaling are essential for the aggregation and differentiation of the SOX9-positive chondrogenic progenitors [82].

Recently, bone renewing medical profession focuses on the application of iPSCs and induced MSC (iMSC), which has more advantages over the autologous ESCs and MSCs. IPSCs and iMSCs are considered as a valuable source of exosomes. First, iMSCs and iPSCs harvesting could be carried out as a non-invasive approach than other sources, such as Bone-marrow (BM) and synovial membrane (SM) MSCs (SMMSCs). Second, it is thought that iPSCs and iMSCs transplantation can theoretically overcome some problems, such as the ethical issues and the demand for immunosuppression drug. Third, autologous iMSCs could be a stable origin of MSCs that have the potential to be applied clinically [83]. Another EV source for bone fracture treatment is monocyte/macrophage cells. There are different opinions whether the EVs derived from which macrophage phenotype is a more appropriate source [84]. The therapeutic efficacy of EVs is related to the parent cells and also how these cells are prepared before harvesting. Studies have shown that different populations of stem cells prior to the preparation of EVs, if subjected to low oxygen conditions, increase their proangiogenic activity, vasculogenic, and chemotactic property [85]. Through the incubation of MSCs to a hypoxic condition, the indirect effect to bone healing has been improved. Moreover, by genetically manipulating the parent cells, the EVs repairing potentials can be enhanced [86]. Anderson and colleagues treated MSCs with ischemic conditions, enriched with some mediators like platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). The result of this experiment revealed that endothelial cells-derived exosomes were very effective in the formation of tubules in vitro [87]. Studies have shown that chondrogenesis is very important for osteogenesis. In addition, the hypertrophic cartilage is essential. During bone reconstruction, hypertrophic cartilage within the break callus undergoes mineralization, vascularization, and finally remodeling into the bone [88]. On the other hand there are several problems in the cartilage repair compared to other tissues. First, the cartilage tissue has a low number of cells and poor metabolic activity. Second, these cells have limited access to nutrients and can only be spread via the synovial fluid. Third, cartilage is present in a hard-biomechanical environment along with tensile and frictional forces [89].

To treat bone problems, exosomes are ideally transmitted directly to the defective site compared with systemic delivery; but there may be a need for higher doses to obtain the same drug concentration, which may increase side effects in non-target tissues. Large defects generally need insemination of a gap-filling scaffold, which helps the growth of inner renovation cells. Therefore, cell scaffolds can be used to propagate the local EVs. This method has been applied for the delivery of osteogenic agents, like as BMP-2, to bone repair [90]. And for this idea, collagen-based scaffolds were synthesized that could control BMP-2 secretion. However, side effects such as swelling and postoperative pain and resorption of close intact bone were observed [91]. These side effects may also be seen when EVs are used with synthesized scaffolds. Hence, further studies on the biomaterial used in the construction of these scaffolds and the mechanism of materials release from inside them can contribute to the optimization of this therapeutic approach. On the other hand, it is possible to target the desired area by attaching a specific receptor on the EVs surface, which leads to an enhanced per-cell delivery of therapeutic cargo, allowing the total administered EVs dose to be decreased. Further experiments are still needed to identify the suitable target cells and surface receptors for better bone renewal [89].

Overall, bone regeneration processes are performed by a wide range of molecules that can be potentially used to increase recovery during an abnormality. Jia and colleagues reported the effects of endothelial progenitor cells-derived exosome on osteogenesis in a rat Distraction osteogenesis (DO) model. Endothelial progenitor cells derived exosomes (EPC-exosomes) was positionally syringed into the injury site at the beginning of the consolidation stage. Consequently, injection of EPC-exosomes led to remarkably intensified callus formation and mineralization, and bone tissue quality improved significantly in the reconstructed gap at 2 to 4 weeks. EPC-exosomes had some advantages than EPCs, including avoiding the likely problem of EPCs implantation such as emboli, immunologic rejection, and malignant mutation [92]. Xu and colleges found that exosomes derived from BM-derived stem cells contained miR-302b, miR-203, miR-218, miR-148a, miR-135b, miR-199b, miR-219, miR-299-5p, and let-7a that are known to trigger osteogenesis [50]. Wei et al. found that merging the BMP-2-activated macrophages-derived exosomes with titanium nanotubes could be regulatory molecules for improving osteogenesis. Another study, applying BMP-2 titanium nanotubes, demonstrated useful effects of this combination on the MSCs duplication and differentiation (F. [93]). Effective bone repair is a major challenge in orthopedic surgery. To date, autologous and allogeneic bone grafting has been used to treat these defects. But this method has problems, including restricted source of graft substance and damage to harvest sites. Allogeneic bone grafts often have poor mechanical consistency and immunological rejection [94].

6. Conclusion

Our review paper concentrated on the recent methods towards the exosome's application in the cartilage and bone repairing procedure. In recent years, the studies that have been done in this regard are very promising, while some problems are still existing. The use of natural carriers like as exosomes has some advantages and disadvantage than synthetic carrier agents (liposomes or any kind of nanoparticles), including less toxic or immunogenic features, more stability, maintainability over a long period of time, little probability of aneuploidy occurrence, and low risk of immunological rejection following in vivo allogeneic injection. These reasons have caused exosomes to have a high potential for clinical administration. But, on the other hand, suitable cell sources for obtaining exosomes and being usable in the clinic should have several conditions. It should be efficient and suitable for regeneration, available in large quantities, isolation and purification should be possible on a large scale. Moreover, because exosome contain different molecules like proteins, mRNAs, and miRNA that affect the physiological function, special storage conditions are required. If exosomes are packaged with optimal biological dosages and certain specific products, they can be used in clinical applications and, hopefully, be able to provide a suitable alternative to common treatments.

Declaration of competing interest

Authors declare no conflict of interest.

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