Review article
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Mesenchymal stem cells and exosomes in bone defects treatment

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Abstract

Introduction Bone defect management is a critical stage of treatment and rehabilitation that still remains a challenging problem for traumatologists and orthopaedists. The need for tissue engineering techniques is due to limited abilities of the human body to correct bone tissue autoregeneration, especially in comorbid and elderly patients with osteoporosis. Bone autografts is a gold standard in those cases but is associated with certain restrictions. Regenerative medicine and stem cell biology development opened up capabilities to employ new methods for enhancement of bone tissue repair. A special interest of researchers is focused on mesenchymal stem cells and extracellular vesicles for bone tissue regeneration optimization.

Purpose of this review was to show mesenchymal stem cells and exosomes effeciency in bone defect treatment.

Materials and methods Open electronic databases of scientific literature, PubMed and e-Library, were used. The literature data search was carried out using the keywords: regenerative medicine, bone defects, exosomes, mesenchymal stem cells.

Results and discussion The review presents current ideas about mesenchymal stem cells, their microenvironment and exosomes influence on bone tissue repair. Clinical need in effective bone regeneration is still high. Mesenchymal stem cells and acellular regenerative treatments have shown good results in bone defects repair and are perspective directions. Productive use of mesenchymal stem cells and exosomes in bone defects treatment requires further study of their mechanisms of action, the regenerative techniques efficacy and safety evaluation in preclinical and clinical studies.

Conclusion The use of mesenchymal stem cells and cell-free regenerative approaches has demonstrated good results in the restoration of bone tissue defects and is a promising direction.

Keywords: regenerative medicine, bone defects, cytotherapy, exosomes, mesenchymal stem cells, bioengineering, tissue engineering

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INTRODUCTION

Despite the improvement of surgical techniques, the treatment of large bone defects caused by trauma, metastatic damage or infectious process still remains a major challenge for orthopaedic surgeons [1, 2]. Such injuries lead to delayed fracture consolidation or nonunion and ultimately impair patient's musculoskeletal function [3, 4]. Currently, bone grafting is the most commonly used treatment in those conditions [5, 6]. However, limited sources of donor tissue, complications and difficulty of graft collection, the risk of transmission of infectious diseases, short-term viability and unpredictable graft resorption restrict the widespread use of this technique and require the development of new approaches to the treatment of this pathology [7, 8]. The use of modern regenerative techniques, in particular tissue engineering, is a promising approach to the treatment of bone defects and has attracted the attention of a large number of researchers in recent years [4, 9]. Thus, the use of cell technologies could overcome osteogenic "insufficiency", which is often found in elderly patients, in whom the body's own resources are not able to restore lost bone tissue [10-12]. The angiogenic effect of exosomes would lead to improved blood supply to the developing bone tissue, thereby optimizing the process of osteogenesis [13-15].

The purpose of the study was to discuss the effectiveness of mesenchymal stem cells and exosomes to stimulate bone tissue regeneration.

MATERIALS AND METHODS

Open electronic databases of scientific literature Pubmed and e-Library were used for preparing the review. The literature search was carried out using the following keywords: regenerative medicine, bone defects, exosomes, mesenchymal stem cells. The inclusion criteria were review articles, systematic reviews, meta-analyses, multicentre studies, controlled cohort studies, uncontrolled cohort studies. Exclusion criteria were articles without a full-text version and duplicate articles. Preference was given to works published within the last five years.

RESULTS

Way to stimulate bone regeneration

Tissue engineering is an interdisciplinary field aimed at developing new biological approaches to treat a wide range of diseases [12]. The need for tissue engineering techniques in bone regeneration is due to the limited abilities of the human body for correct autoregeneration, especially in comorbid and elderly patients with osteoporosis [10].

Mesenchymal stem cells (MSCs) play a significant role in the process of bone tissue remodeling, since they are the precursors of the key regulators of this process, osteoblasts and osteoclasts, and are also able to migrate to the defect area [16]. Significant advances in the study of stem cell biology have provided new therapeutic regenerative strategies that avoid the use of autologous bone tissue [17].

The shortage of tissue grafts has also stimulated the development of regenerative medicine technologies that apply natural biomaterials that have positive properties such as biocompatibility, bioactivity, controlled degradation and structural similarity to native tissue extracellular matrix [18]. One of the philosophies of regenerative medicine is creation of such scaffold biomaterials that simultaneously mimic the extracellular matrix and positively modulate the activity of proper/exogenous stem cells to achieve maximum regenerative potential [19].

Currently, the following materials have been used to improve bone regeneration [10]:

1. Tissue grafts of:
   – autologous origin (bone grafts, cancellous grafts);
   – allogeneic origin (bone grafts, bone graft product);

2. Components of the extracellular matrix:
   – collagen (types 1, 2 and 4);
   – fibronectin;
   – laminin;
3. Decellularized extracellular matrix:
   – demineralized bone matrix;
   – decellularized bone extracellular matrix;
   – calcined bovine bone;
4. Ex vivo cultured therapeutic cells:
   – MSC transplantation;
   – extracellular matrix formed by cells;
   – exosomes.

The use of mesenchymal stem cells and extracellular vesicles as strategies to optimize bone tissue regeneration is of particular interest to researchers [4].

Mesenchymal stem cells

Currently, there are two main strategies for using MSCs to improve bone tissue regeneration: the release-mobilization of endogenous MSCs and the use of exogenous stem cells [20]. The use of exogenous stem cells is possible in several ways: systemic administration by intravenous infusion [21] and local application of cell suspensions, sheets and spheroids [22]. In systemic use of MSCs, in addition to the osteogenic effect, modulation of the immune status and, accordingly, restoration of the microenvironment have been demonstrated; however, the percentage of cells that reach the bone defect remains low thus decreasing the therapeutic efficacy [21, 23]. The use of cell sheets [24] and spheroids [25] with an extracellular matrix formed by stem cells promotes the release of a large number of growth factors and cytokines that improve bone tissue regeneration, which is successfully used in the treatment of bone defects [26] and to improve the bone-to-graft bonds [27]. Thus, the study of Vishnevsky et al. [28] showed that the addition of MSCs to bone grafts in a rabbit rib cage defect model promoted an increase in the proportion of mature bone matrix and more intense osteogenic differentiation.

In recent years, much attention has been paid to the microenvironment of cells, since its significant influence on the functional activity of stem cells and their reproducible therapeutic effects has been discovered [6]. In a pathological microenvironment, the viability and differentiation of MSCs decreases; thus, during cytotherapy, both the donor microenvironment and the recipient microenvironment play a significant role in determining the therapeutic effectiveness of transplanted stem cells [29]. Important systemic factors affecting bone regeneration and cell microenvironment include the level of steroid hormones [30] and blood glucose [31], as well as the activity of the inflammatory process [32]. Decreased estrogen levels in postmenopausal women have been shown to result in decreased proliferation and osteogenic differentiation of bone marrow (BM) MSCs, decreased bone mineralization, accumulation of reactive oxygen species, adipogenic cell differentiation, imbalance between osteoblastogenesis and osteoclastogenesis, and ultimately, in bone loss [33]. Elevated levels of corticosteroids also suppress the proliferation and osteogenic potential of BM MSCs [34]. At the cell level, the energy metabolic profile has a significant impact on the functions of stem cells, so increased glucose concentration causes dysfunction of BM MSCs [35]. Oxidative stress and accumulation of advanced glycation end products lead to decreased viability and osteogenic differentiation of stem cells [36]. Despite the essential role of inflammation

in bone healing, the proinflammatory microenvironment is a key pathogenetic mechanism underlying various osteopenic disorders, as inflammatory cytokines lead to impaired proliferation and osteogenic differentiation, excess production of reactive oxygen species and apoptosis of stem cells [31]. Thus, to improve the regenerative potential of stem cells, it is necessary to analyze and adjust the level of steroid hormones and glucose in both the donor and the recipient, as well as modulate the inflammatory microenvironment [36]. For this purpose, gene expression regulators, such as rapamycin, a signaling inhibitor of mTOR, can be used in the clinic [37]; DAPT, Notch signaling inhibitor [38]; PDTC, nuclear transcription factor-kappaB (NF-κB) signaling inhibitor [39]; GSK2606414, PERK inhibitor [40]; antioxidant NAC [41]; licochalcone A [42], etc. The effectiveness they demonstrated indicates the potential use of the technique of normalizing the microenvironment of stem cells in order to increase the efficiency of MSC-mediated bone healing [43].

At the moment, the use of adipose tissue MSCs (ADSCs) has become increasingly attractive for clinical specialists as it is the least traumatic method for the patient [44]. However, the limited potential of ADSCs for osteogenic differentiation and the natural tendency towards adipogenic differentiation hinder their widespread use in bone tissue regeneration [45]. At the same time, it is known from the literature that circular RNAs (circRNAs) play a significant role in determining the further path of development of stem cells and progenitor cells [46]. A study by D Zhang et al. [44] assessed the modulating effect of circRNA on the osteogenic differentiation of adipose tissue stem cells. CircRNA is a closed, continuous ring of RNA, which makes it more stable than linear RNA due to the absence of a free end available for enzymatic degradation [47]. Some effects of circRNA are reproduced by acting on microRNAs (miRs) that regulate the expression of target genes [48]. Thus, in the course of this review, it was found that circRNA-vgll3 directly binds to miR-326-5p in the cytoplasm of cells like a “sponge” and inhibits its activity, which leads to an increase in the expression of integrin α5 (Itga5) [44]. Itga5 is known to play a significant role in cell adhesion to the extracellular matrix, improves the functional activity and survival of osteoprogenitors, and also carries out some mechanisms of osteogenic growth factors, such as BMP2, TGFβ and PTH [49]. Increased expression of Itga5 through the circRNA-vgll3/miR-326-5p/integrin α5 signaling pathway leads to improved ADSC homing, recruitment of osteoprogenitor cells, and improved osteogenic differentiation of adipose tissue stem cells [44]. The use of a circRNA-vgll3 inhibitor led to a decrease in the mRNA expression of ADSC osteogenic differentiation genes (Runx2, OSX, Col1a1, OPN, OCN and BSP) [44]. The therapeutic effect of a combined use of circRNA-vgll3-modified calcium phosphate scaffolds and ADSCs was also assessed in a rat model of bone defect of the skull [44]. An increase in mineral density and an increase in the volume of newly formed bone tissue were revealed when compared to the control group [44]. The results of that study show the promise of using circular RNAs, namely circRNA-vgll3, to improve the osteogenic differentiation of adipose tissue stem cells and their further use for the treatment of bone defects [44].

Literature data on research into the effectiveness of stem cells in bone tissue restoration are not limited to animal trials alone. Thus, VN Bordakov et al. [50] provided data on the successful use in their clinical practice of tissue-engineered constructs based on calcium hydroxyapatite, fibrin glue and bone marrow MSCs in the treatment of defects of long bones. A histological assessment of the regenerate one month after grafting discovered developing bone tissue, and the result of treatment was subsequent consolidation of the fracture and functional recovery. However, the authors emphasize the importance of further studying this combination of biologically active components in subsequent studies [50].

Exosomes

Traditional tissue engineering is based on the use of scaffolds, cultured cells, and growth factors [51]. However, the use of cells has a number of disadvantages: limited sources, insufficient cell activity, immunological reactions and high costs for clinical use [52]. Nevertheless, the therapeutic potential

of exosomes is similar to the paracrine functions of stem cells and overcomes the limitations associated with their transplantation [1]. This is why safer acellular tissue engineering may become an alternative to cell therapy [2, 53, 54]. Thus, the studies of the regenerative potential of MSC exosomes on a model of bone defect in rats performed by IV Mayborodin et al. [55, 56] demonstrated faster healing and an increase in bone density, as well as the formation of less rough callus compared to the control group.

Monocytes and macrophages are known to be key regulators of tissue healing processes, and different macrophage phenotypes have different effects on repair processes [57]. During the repair process, the recruitment of monocyte macrophages occurs, a transition from the M_1-phenotype, pro-inflammatory, to the M_2-phenotype, anti-inflammatory [58]. Current research is focused on studying the influence of different macrophage phenotypes on tissue regeneration [59]. Thus, F Loi et al. [60] suggested that consistent changes in macrophage phenotypes make a significant contribution to the process of osteogenesis. Macrophages control the physiological process of bone repair by secreting various factors that are osteoinductive and, conversely, inhibiting bone regeneration [61]. Intercellular interactions are mediated by the release into the local environment of exosomes (Exos) that are extracellular vesicles ranging in size from 40 to 150 nm, containing proteins, lipids and nucleic acids, including miRs [62, 63]. Exosomes captured by target cells have a biological effect on them, change their behavior pattern and activate signaling pathways [64]. The study of M Kang et al. [59] investigated the functional role of paracrine factors, Exos M_0, M_1 and M_2 macrophages, in the treatment of critical size defects in a rat calvarial defect model. It was revealed that Exos M_0 and M_2-macrophages promote bone repair while Exos M_1 affects bone regeneration [59]. Exos M_1, and namely miR-155, may affect RUNX2 and BMP signal ways, in particular BMP2 and BMP9 that result in the decrease of osteogenic differentiation of MSC, while Exos M_0 and M_2, miR 378a, promote expression of osteoinductive genes of MSC [59]. CT evaluation conducted after 3 weeks showed impairment in bone formation in Exos M_1 group and improvement in Exos M_0 and M_2-macrophages group [59]. Other studies demonstrated that osteogenesis improved as a result of common cultivation of M_2-macrophages with osteoprogenitors while M_1-macrophages decreased expression of osteogenic markers and impaired bone tissue mineralization [65, 66]. The study confirms the results of other studies and indicates the differential and opposite effects of polarized macrophages and their exosomes on bone tissue regeneration, and also provides grounds for the prospects of using exosomes of M_0 and M_2-macrophages as osteogenesis stimulators [67-69].

The pathogenetically important anti-inflammatory effect of exosomes was demonstrated in a study by X Wang et al. [70], where the effect of a polycaprolactone (PCL) scaffold in combination with MSC exosomes and S-nitrosoglutathione (GSNO) on bone tissue repair was studied. PCL is a biocompatible but bioinert polymer, what limits its stand-alone application in bone engineering [71]. Nevertheless, GSNO, which is a donor of NO, regulates the activity of the blood coagulation system, has an anti-inflammatory effect and prevents the destruction of bone tissue [72]. The work demonstrated a decrease in the expression of pro-inflammatory genes (IL-6, TNF-α, iNOS и Il-1β), an improvement in the osteogenic differentiation of BM MSCs, what was manifested by an increase in the expression of mRNA ALP, Col-I and Runx2, as well as an increase in ALP activity [70]. Researchers have suggested a synergistic effect of GSNO and exosomes on the expression of pro-inflammatory cytokines [70]. The results of that study show the promise of using bioactive agents such as GSNO and exosomes in combination with scaffolds to improve bone tissue regeneration [70].

Filling of large bone tissue defects is often accompanied by insufficient vascularization of the resulting tissue [73]. Angiogenesis plays a significant role in the process of bone tissue remodeling; therefore, for effective healing, improvement of both osteogenesis and angiogenesis is necessary [13]. A study by Y Zha et al. [2] examined an acellular tissue engineering system using encapsulated vascular endothelial growth factor (VEGF) gene in exosomes on the surface of a 3D-modulated PCL scaffold in a radius bone defect model in rats. Micro-CT analysis after 6 and 12 weeks revealed a significant

improvement in bone regeneration in the experimental group, and histological analysis showed the presence of more mature collagen fibers compared to the control group [2]. The study demonstrated the dual role of exosome-modified scaffolds: as an inducer of osteogenic differentiation of BM MSCs and as a VEGF depot that ensures remodeling of the vascular network [2]. That work demonstrated the possibility of using exosomes as a biovector for the delivery of biologically active substances to improve bone tissue regeneration [2].

It was previously shown that BMSC-Exos and magnetic nanoparticles (Fe_3O_4, γ-Fe_2O_3) in combination with a static magnetic field (SMF) have a positive effect on both osteo- and angiogenesis [74, 75]. Wu et al. [1] assessed the effect of magnetic nanoparticle-modified exosomes (BMSC- Fe_3O_4-Exos) in combination with SMF on the functional characteristics of human umbilical vein endothelial cells (HUVEC) and BMSC, as well as the restoration of calvar defect on rat models. Researchers have demonstrated the osteogenic effect of this modification of exosomes in combination with a static magnetic field, which was manifested by improved mineralization of bone tissue, increased ALP secretion and mRNA expression of osteogenic markers (OPN, RUNX2, COL-1) [1]. When co-cultured with HUVEC, effects such as accelerated cell migration, a greater number of tube-like structures, and an increase in the expression of mRNA of pro-angiogenic factors (VEGF, ANG-1, HIF-1α) were noted, which confirms the angiogenic effect of BMSC-Fe_3O_4-Exos-SMF [1]. Micro-CT analysis demonstrated an increase in the amount of newly formed bone and blood vessels in the experimental group, which is confirmed by the results of other studies [76-81]. The positive effect on osteo- and angiogenesis was explained by an increase in the concentration of miR-1260a and its effect on the transcription of certain genes [1]. Thus, in BMSC, miR-1260a inhibits the expression of HDAC7 and thereby reduces its suppressive effect on the expression of OPN, RUNX2, OCN, ALP and COL-1, a similar mechanism of action is observed in HUVEC, where miR-1260a inhibits COL4A2, thus increasing secretion of VEGF, ANG-1 and HIF-1α [1, 75, 82]. This kind of angiogenic effect was demonstrated in the work of Lu et al. [14]. The study showed that miR-29a-3p, through post-transcriptional inhibition of VASH-1 which negatively affects angiogenesis, contributed to improved proliferation and migration of endothelial cells, and an increase in the number of osteocalcin-positive osteoblasts, bone mineral density and trabecular bone volume was noted, compared with the control group [14]. The results of this study demonstrate the therapeutic potential of exosomes in the repair of bone defects [1].

DISCUSSION

Management of large bone defects remains a challenging problem for orthopaedic surgeons, and therefore there is an active search for methods to increase the efficiency of bone regeneration [1, 4]. A large number of studies have been focused on the use of mesenchymal stem cells and exosomes as stimulators of osteogenesis. Analysis of current medical literature allows us to draw definite conclusions:

– All the studies we reviewed show a positive effect of mesenchymal stem cells and exosomes on the process of bone tissue remodeling.

– Local use of mesenchymal stem cells is characterized by a more pronounced regenerative effect compared to systemic administration [21, 23, 26, 28].

– Developing methods for increasing the differentiation of mesenchymal stem cells of adipose tissue in the osteogenic direction would enable to use alternative sources of multipotent cells to bone marrow in the future, and, accordingly, reduce the traumatic nature of the procedure, which is especially important in the case of a severe patient's condition [44].

– To improve the regenerative potential of mesenchymal stem cells, it is necessary to consider the endocrine status of both the donor and the recipient, as well as carry out anti-inflammatory therapy to create an optimal microenvironment of transplanted cells [31-36].

– Exosomes of anti-inflammatory phenotypes of macrophages have a positive effect on the process of bone repair [60, 65-69].

– Exosomes of mesenchymal stem cells have an anti-inflammatory, angiogenic and osteogenic effect that ensure a more efficient bone tissue regeneration process [1, 2, 14, 55, 56, 70, 74, 75].

CONCLUSION

Despite the active development of medicine, the clinical need for effective bone regeneration still remains at a high level. The use of mesenchymal stem cells and acellular regenerative approaches has demonstrated good results in bone defect management and is a promising direction. Further study of the mechanisms of action is necessary for the productive use of mesenchymal stem cells and exosomes in the treatment of bone defects along with assessment of the effectiveness and safety of these regenerative techniques in preclinical and clinical studies.

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