

Review Article

Application of bone marrow mesenchymal stem cells to the treatment of osteonecrosis of the femoral head

Cheng Wang, Yu Wang, Hao-Ye Meng, Xue-Ling Yuan, Xiao-Long Xu, Ai-Yuan Wang, Quan-Yi Guo, Jiang Peng, Shi-Bi Lu

Institute of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China

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Abstract: Osteonecrosis of the femoral head (ONFH) is a type of common and refractory disease in the orthopedic clinic that is primarily caused by a partial obstruction of the blood supply to the femoral head, resulting in a series of pathological processes. Mesenchymal stem cells (MSCs) comprise a mixture of various stem cells in myeloid tissue with multipotential differentiation capacity. They can differentiate into bone cells under specific conditions and can be used to treat ONFH through cell transplantation. This review summarizes research on MSCs in the field of ONFH in recent years, reveals the inner characteristics of MSCs, describes their potential to treat osteonecrosis disease, and analyzes the existing challenges of using MSCs in clinical applications.

Keywords: Osteonecrosis, mesenchymal stem cells, femoral head, gene-transfected, tissue engineering, transplantation

Introduction

Osteonecrosis of the femoral head (ONFH), which is a type of progressive and refractory disease, can cause great pain in patients. It is caused by a lack of appropriate blood supply to trabecular bone in the femoral head and bone cell death due to various reasons, leading to articular cartilage collapse and subsequent osteoarthritis [1]. ONFH primarily influences patients aged from 30 to 40 years, and the morbidity rate for men is three times that for women; in addition, the bilateral hip joints are involved in 75% of patients [2]. Bone marrow mesenchymal stem cells (BMMSCs) have powerful self-proliferation ability and multi-potential differentiation capacity and can undergo osteogenesis through induction. Elucidation of the characteristics of BMMSCs of a controlled culture *in vitro* and of osteogenic differentiation after implantation could lead to new breakthroughs for the treatment of ONFH [3].

Mechanism of ONFH

The pathogenesis of ONFH is complicated, mainly due to factors such as trauma, application of hormones, intemperance, and connective tissue diseases [4, 5]. Microcirculation dis-

turbance is the common pathogenesis of idiopathic ONFH, and the factors that determine its pathologic evolution include its pathogenesis, the equilibrium relationship between osteoclast-mediated bone absorption and the effective bony remodeling rate, and biomechanical effects. The pathogenesis ultimately causes damage to the microcirculation, necrocytosis of bone cells and myeloid tissue, reduction of osteogenic differentiation capacity of BMMSCs, and absorption enhancement of osteoclasts toward unprotected trabecular bone. Such bone will become sparse and small due to the absorption, and it also loses mineral metabolism function due to the necrocytosis, decreasing its mechanical strength. When stress is transmitted, trabecular bone is easily fractured. The bone absorption caused by osteonecrosis is accompanied by repair, and the strength of repair depends on the blood supply status and functional status of the bone marrow.

The process of rebuilding necrotic trabecular bone requires BMMSC proliferation, connective tissue rich in fibroblast-like cells in the osteonecrotic area, the regeneration of blood capillaries, and the synthesis of bone matrix by osteoclasts and osteoblasts during BMMSC differen-

tiation; the absorption rate and effective remodeling rate of new bone maintain a certain type of equilibrium that is ultimately determined by the activities of osteoclasts, the osteoblast differentiation capacity of BMMSCs, and the functional status of the osteoblasts [6, 7]. Overall, the features of ONFH include blood insufficiency, repair and further collapse of the femoral head, and degenerative arthritis of the hip joint.

The pathogenesis of traumatic osteonecrosis is well understood and primarily includes fracture and dislocation. However, the pathogenesis of atraumatic osteonecrosis varies and includes hereditary susceptibility, with factors related to anatomy and the absence or dysplasia of blood vessels in the joint capsule being the most common abnormalities. Abnormal distribution of vasculature may be related to ONFH, indicating that osteonecrosis is more likely to occur when patients have abnormal microcirculation of the femoral head in addition to other risk factors such as long-term application of hormones, intemperance, and diseases of connective tissue [8].

In the early stage of ONFH, no obvious symptoms are noted except slight pain in the groin, and no obvious findings are observed in X-ray images. With gradual progression of the disease, pain becomes apparent, and signs of typical changes begin to appear in X-ray images. Subsequently, the femoral head displays obvious collapse, articular cartilage becomes damaged, joint space narrows, and the joint as a whole deteriorates into serious osteoarthritis [9].

Early treatment of ONFH primarily includes reducing the mechanical load pressure of the femoral head, rotary osteotomy, tantalum rod transplantation [10-13], and techniques such as marrow core decompression surgery and shockwave therapy to increase repair ability in the area of necrosis [14, 15]. Subsequent treatment involves total hip arthroplasty THA. However, due to the uncertain curative effect of artificial hip joints and because the age of onset of ONFH is becoming younger, better methods are required to prevent femoral head collapse.

MSCs

MSCs are widespread in tissues such as the bone marrow, adipose tissue, periosteum, mus-

cle, synovial membrane, muscle tendon, and blood vessels of the umbilicus, dental pulp, and blood vessel periphery [16-19]. MSCs that have been separated and extracted from those tissues have high capacity for multipotential differentiation, self-renewal, and proliferation [20]. They can differentiate into bone, cartilage, adipose tissue, muscle, muscle tendon, vascular endothelial cells, and nerve cells under different induction and differentiation conditions [21].

BMMSCs have many advantages such as easy acquisition, quick expansion *in vitro*, minor immunological rejection, long-term coexistence in the host, maintenance of differentiation ability after repeated passages, and ease of transfection [22, 23]. Friedenstein et al. [24] transplanted BMMSCs *in vivo* after culturing them *in vitro*. After several weeks, fibroblastic colony-forming cells were confirmed to have differentiated into bone tissues and they were called osteogenic precursor cells.

Bianco et al. [25] confirmed that BMMSCs are easy to grow adhering to the wall in *in vitro* culture, and that they form colonies, differentiate into committed progenitor cells, and expand. BMMSCs have the basic characteristics of stem cells described above but they also differentiate into various types of connective tissue cells such as osteoblasts, chondroblasts, myoblasts, lipoblasts, and matrix cells supporting hematopoiesis. They can also differentiate into cardiac muscle cells, nerve cells that traditionally serve as terminal cells, and neural glia cells. In addition, their differentiation shows tissue specificity, indicating that the tissue micro-environment reached by BMMSCs can induce directional differentiation.

Stem cells used to treat osteonecrosis

For many refractory diseases, cellular transplantation is undoubtedly an effective method. Transplanting cells with specific functions according to damaged body part not only can repair the partial functions of the part but can also avoid toxic and other side effects due to traditional drug therapy [26, 27]. MSCs have been widely applied to repair bone and cartilage defects, tissue damage, ONFH, rarefaction of bone and osteoarthritis, and other clinical areas [28, 29]. Presently, research is ongoing concerning transplantation of MSCs into osteo-

Treatments of osteonecrosis of the femoral head

Table 1. Examples of therapeutic application of stem cells in patients with osteonecrotic femoral head

Cell	Case	Age	Hip	Stage	Follow-up duration	Method	Outcome	Reference
bone marrow stromal stem cells	62	22-54	78	I (16) II (52) III (10)	9-13 years	MSCs* were infused into the femoral head artery	The Harris scores were increased. 18 hips got artificial joint replacement.	[31]
bone marrow stromal stem cells	10	20-48	10	3A (6) 3B (4)	24 months	MSCs mixed with β -tricalcium phosphate (β -TCP) granules in combination with vascularized iliac bone grafts	The average clinical score improved from 65.6 \pm 25.5 points to 87.9 \pm 19.0 points.	[32]
Cord mesenchymal stem cells	30	19-63	49	II (24) III (25)	12 months	UC-MSCs** were infused into the femoral head artery	The Harris scores were increased significantly at 3, 6, and 12 months posttransplant	[33]
bone marrow stromal stem cells	38	21-73	40	I (7) II (25) III (8)	36 months	core decompression combined with MSCs	At 36 months, 33 patients achieved clinical and radiographic healing.	[34]
bone marrow mesenchymal stem cells	8	19-43	16	II a (4) II b (2) II c (3) III (1)	12-42 months	core decompression and MSCs transplantation	The Harris scores and VAS scores were increased. Femoral head collapsed 12 months after operation in 1 case of stage III	[35]
bone marrow mesenchymal stem cells	3	25-30	3	4A (1) 4C (2)	27-48 months	MSCs cultured with beta-tricalcium phosphate (β -TCP) ceramics	Osteonecrosis did not progress any further and early bone regeneration was observed	[36]

*MSCs: Mesenchymal stem cells. **UC-MSCs: umbilical cord mesenchymal stem cells.

Treatments of osteonecrosis of the femoral head

necrosis patients in an attempt to promote the repair process [30] (Table 1).

Core decompression and MSC transplantation

Core decompression surgery is an important method for treating ONFH in the early stage to get through the sclerosis band that will hinder osteonecrosis repair, relieve the high-pressure environment in the bone, and improve blood supply in the osteonecrosis area [37-39]. MSC transplantation based on core decompression surgery can provide seed cells to promote repair and restoration of the femoral head and accelerate reconstruction and creeping substitution of new bone [40, 41].

Hernigou et al. [42] adopted core decompression and autologous bone marrow transplantation to cure 189 hips of 116 ONFH cases. With an average 7-year follow-up after surgery, for 145 hips operated on during Ficat I-II, only 9 hips needed reoperation for joint replacement; in addition, for 44 hips treated during Ficat stages III-IV, only 25 hips needed reoperation for joint replacement. They believed that this technique could be used to improve repair in the osteonecrosis area, at least in the earlier stages before mechanical failure of the femoral head occurs. Rastoqi et al. [43] concluded similarly that core decompression combined with implantation of autologous BMMSCs was an effective and safe procedure. Cuervas-Mons et al. [44] assessed the efficacy of BMMSCs for the treatment of femoral heads infused using core decompression. The procedure improved hip function and avoided total hip replacement in 75.3% of patients with ONFH treated during the first 2 years.

However, the abovementioned results are debatable. Lim et al. [45] compared the clinical and radiographic results of two groups for the treatment of ONFH: one involved multiple drilling and stem cell implantation and the other involved core decompression, curettage, and a bone graft. No statistically significant difference was found between the groups.

The treatment of ONFH is based on patient age, periodization, the area of osteonecrosis, position and collapse risk, and individual choice. Only by correctly mastering the therapeutic principles and adopting proper methods specific to different stages can the best therapeutic effect be achieved. Regarding early-stage ONFH, core decompression and BMMSC trans-

plantation have shown good surgical effects; however, for incurably ill patients, the curative effect of this procedure is poor, and the treatment method must be selected with discretion. Once collapse has occurred in intermediate or later stages (the degree of collapse is more than 4 mm, and the duration is more than 6 months), no additional specific treatment other than an artificial joint is effective.

MSCs and bone tissue engineering technology

Tissue engineering is performed according to principles in cell biology and engineering, planting living cells cultured *in vitro* onto a natural or artificial polymer scaffold with good biocompatibility and biodegradability, and repairing and reconstructing defective areas of tissues and organs *in vivo* [46]. The three main components of engineered include seed cells, signal factors, and scaffolds [47-49]. Ideal bone seed cells should have a relatively simple structure, regardless of the presence of primitive cells with specific functions; be easy to collect, with little injury to the body; show strong proliferation capacity when cultured *in vitro*; exhibit self-renewal and differentiation toward a specific direction under certain conditions; have stable expression of the osteoblast phenotype; be able to continue to generate osteogenetic activities after implantation; and lack oncogenicity [50].

Regarding seed cell selection, MSCs have powerful differentiation potential and proliferation capacity, and their origin, separation method, and types of differentiation tissues have unique advantages in repairing bone defects of ONFH, which is the ideal choice of seed cells of bone tissue engineering [51]. Composite scaffolds made of MSCs can structurally strengthen defect repairs, hinder the entry of surrounding tissues, and serve as a biological carrier of cells, growth factors, and genes. In addition, MSCs can integrate cells and interact with receptors to adjust cell function. In a previous study, a BMMSC-loaded bone matrix scaffold effectively stimulated bone regeneration in a preclinical femoral head osteonecrosis model in sheep [52]. Some researchers have used MSCs as seed cells in clinical or experimental research with very successful results [53].

Kawate et al. [36] cultured MSCs with beta-tricalcium phosphate (beta-TCP) ceramics and a

Treatments of osteonecrosis of the femoral head

free vascularized fibula and transplanted them into patients with ONFH. The disease did not progress any further, and early bone regeneration was observed, indicating that this tissue-engineered approach has potential for the treatment of ONFH.

The increase in the use of bone tissue engineering brings new hope to the treatment of ONFH using BMMSCs. Bone tissue engineering can construct a three-dimensional structure in the necrotic femoral head that is beneficial to the in-growth of new capillaries, surrounding tissues, and osteoprogenitor parent cells [54]. In addition, this approach can provide a good carrier for signal factors, enabling BMMSC differentiation toward targeted tissue more effectively in the presence of osteogenic factors to improve the induction ability of biological materials [55]. At the same time, the use of a scaffold guarantees a higher concentration of BMMSCs in the area of osteonecrosis, further promoting osteogenesis [56, 57]. However, many scaffolds are currently under investigation and their development is limited by factors such as high cost and technical immaturity; moreover, their clinical effects have not been verified.

Gene-transfected BMMSCs transplantation

The method of transferring target genes into BMMSCs via a carrier, using various carrier materials, and implanting them into osteonecrotic areas of the femoral head has achieved exciting results. Bone morphogenetic proteins (BMPs) can facilitate bone formation and promote ectopic osteogenesis. Xiao [58] showed that BMPs are a good choice for repairing experimental defects of ONFH using BMMSC-seeded bio-derived bone materials combined with rhBMP-2. Tang et al. [59] indicated that porous beta-TCP loaded with BMP-2-gene-transduced BMMSCs are superior for the treatment of early-stage ONFH. Wen et al. [60] concluded that the combination of core decompression and transplantation of hepatocyte growth factor transgenic autologous BMMSCs enhanced blood vessel regeneration and bone reconstruction in an ONFH model. Using biotechnology, BMMSCs with certain transfected genes can be used to treat ONFH.

MSC arterial perfusion

As described above, ONFH refers to a series of pathological processes caused by partial obs-

truction of the blood supply to the femoral head. Therefore, the main direction of treatment is to improve the blood supply to the femoral head, and the existence of autologous stem cell transplantation vascular regeneration technology provides a wider prospect for treatment [61].

MSCs can be used to partially protect blood vessel endothelium, improve the repair and regeneration of vascular endothelial cells, and enhance vascular proliferation. Kocher [62] confirmed that MSCs and endothelial progenitor cells facilitate blood flow in experimental models with insufficient blood supply. Kinnaird et al. [63] found that injecting cultured osteogenic stem cells toward the ischemic rear limb muscle of a rat promoted collateral circulation and limb functional recovery, and that the gene expression level of cell factors related to angiogenesis increased. Selective arterial perfusion of MSCs can improve venous flow, reduce introsseous pressure, restore and improve the blood supply to the femoral head, and improve or increase blood circulation around osteonecrotic areas of the femoral head to produce a good specific environment. Mao et al. [64] treated 62 ONFH patients (78 hips) with BM-MS-C perfusion via the medial circumflex femoral artery, and achieved satisfactory clinical results in 92.31% (72 of 78) of the hips; only 6 hips (7.69%) required replacement. They concluded that their method is an effective, safe, and minimally invasive treatment strategy for early-stage ONFH.

Compared to other ONFH treatments, selective arterial perfusion of MSCs creates a good environment for stem cell survival and differentiation, is minimally invasive, and has little untoward effect and good patient compliance.

Conclusion

Along with the rapid development of molecular biology and cytobiology, research on MSCs will be deepened. The study of various biological factors that support and promote the growth, differentiation, and maturity of MSCs; the use of extracellular matrix to simulate microenvironments *in vivo* will become popular research areas with the hope of making major breakthroughs and creating a new era for minimally invasive surgical treatment of ONFH [65, 66].

Presently, For the various stages in the pathological process of osteonecrosis, more research

Treatments of osteonecrosis of the femoral head

on the treatment mechanism of ONFH using MSCs can be performed on the role of stem cells [67]. However, some problems persist with the method itself; for example, sources of MSCs are limited, and the bone marrow used for treatment is currently autogenous iliac bone marrow. However, according to some research [68, 69], the viability of MSCs decreases during the course of osteonecrosis, and bone marrow disorder is systemic, not partial.

In future research, some questions related to the biological characteristics of MSCs can be addressed. For example, during the process of osteogenesis, how do MSCs interact? What are the phenotypes and effects of immune cells on BMMSCs? Is the differentiation potential of MSCs from different sources the same? Are their abilities of self-renewal and multi-directional differentiation potential after repeated culture the same? How different are bones formed by implanted MSCs from normal bones in terms of histology and biomechanics? In addition, the risk of forming a teratoma at the implanted site is a limitation in the clinical application of MSCs. Although many problems persist that need to be further researched, MSCs will certainly be applied clinically.

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Disclosure of conflict of interest

None.

Address correspondence to: Jiang Peng, Orthopedics Research Institute of Chinese PLA, General Hospital of Chinese PLA, Fuxing Road 28, Haidian District, Beijing 100853, P. R. China. Tel: +86 10 6693 9205; Fax: +86 10 6816 9305; E-mail: pengjiang301@126.com

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Treatments of osteonecrosis of the femoral head

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Treatments of osteonecrosis of the femoral head

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Treatments of osteonecrosis of the femoral head

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