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Review

Stem cell therapy for osteonecrosis of femoral head: Opportunities and challenges

Yingxing Xu [a, c, d], Yaping Jiang [b, c], ChangSuo Xia [a], Yingzhen Wang [a], Zhiping Zhao [a, c, d], Tao Li [a, *]

[a] Department of Joint Surgery, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China
[b] Department of Stomatology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, 266003, China
[c] Qingdao University, Qingdao, Shandong, 266071, China
[d] Medical Department of Qingdao University, Qingdao, Shandong, 266071, China

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ABSTRACT

Osteonecrosis of the femoral head (ONFH) is a progressive disease with a complex etiology and unclear pathogenesis, resulting in severe hip pain and dysfunction mainly observed in young patients. Although total hip arthroplasty (THA) is the most effective treatment for patients with ONFH in the terminal stage, the results of THA in young patients or active populations are often not favorable, with some complications related to the prosthesis. With the development of biotechnology, an increasing number of studies pay attention to use of stem cells for the treatment of ONFH. Stem cells are characterized by the ability to self-renew and differentiate into multiple cell types, including differentiation into osteoblasts and endothelial cells to mediate bone repair and angiogenesis. Furthermore, stem cells can offer growth factors to promote blood supply in the necrotic regions by paracrine effects. Therefore, stem cell therapy has become one of the hip-preserving alternatives for ONFH. This review summarized the current trends in stem cell therapy for ONFH, from clinical applications to related basic research, and showed that an increasing number of studies have confirmed the effectiveness of stem cell therapy in ONFH. However, many unsolved problems and challenges in practical applications of stem cell therapy still exist, such as patient selection, standardized procedures, safety assessment, and the fate of transplanted cells in the body. Additional studies are required to find ideal cell sources, appropriate transplantation methods, and the optimal number of cells for transplantation.

Contents

Abbreviations: ALP, alkaline phosphatase; AMSCs, adipose-derived MSCs; BCP, biphasic calcium phosphate; bFGF, basic fibroblast growth factor; BMC, bone marrow concentrate; BMMNCs, bone marrow mononuclear cells; BMP-2, bone morphogenetic protein-2; BMSCs, bone marrow-derived mesenchymal stem cells; CD, Core decompression; CPC, calcium phosphate; CSS, cap-shaped separation; DBM, demineralized bone matrix; HHS, Harris hip score; IP-CHA, interconnected porous calcium hydroxyapatite; MRI, magnetic resonance imaging; MSCs, Mesenchymal stem cells; MVD, microvessel density; ONFH, Osteonecrosis of the femoral head; PBMSCs, peripheral blood-derived MSCs; PLGA, poly lactide-co-glycolide; RCT, randomized controlled trial; SCPP, strontium-doped calcium polyphosphate; SVF, stromal vascular fractions; THA, total hip arthroplasty; TMCs, transformed mesenchymal cells; TNF, tumor necrosis factor; UCMSCs, umbilical cord-derived mesenchymal stem cells; VAS, visual analogue scale; VEGF, vascular endothelial growth factor; WOMAC, Western Ontario and McMaster Universities Arthritis Index; XACB, xenogeneic antigen-extracted cancellous bone; β-TCP, beta-tricalcium phosphate.

* Corresponding author. Department of Joint Surgery, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China.
E-mail address: litaodoctor@sina.com (T. Li).
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1. Introduction

Osteonecrosis of the femoral head (ONFH) is a progressive disease that is characterized by interruption of blood supply, necrosis of subchondral bone, and the eventual bone collapse of the femoral head, resulting in severe hip pain and dysfunction mainly in young patients [1,2]. Although total hip arthroplasty (THA) is the most effective treatment for patients with ONFH in the terminal stages, the outcomes of THA in young adults or active populations are often not excellent, with a high failure rate caused by loosening of the prosthetic, excessive wear of polyethylene inserts, and periprosthetic infection [3–6]. Therefore, hip-preserving treatments for ONFH has become a challenge in young patients.

Stem cells have been shown to have the ability to self-renew and to differentiate into multiple cell types. Mesenchymal stem cells (MSCs) are one of the most frequently used stem cell subsets, which are widely distributed in various tissues, such as bone marrow, peripheral blood, fat, umbilical cord, etc. [7]. MSCs can differentiate into osteoblasts and endothelial cells to affect bone repair and angiogenesis [8], and can produce growth factors to promote the blood supply to necrotic regions by paracrine effects [9,10]. Furthermore, MSCs not only play a very important role in the development of disease, but also are the main source of seed cells for regenerative therapy. At present, the pathogenesis of ONFH is considered to be related to bone marrow-derived mesenchymal stem cells (BMSCs), including cell apoptosis, decrease in cell numbers, and decline in osteogenic differentiation potential [11]. Thus, stem cell therapy has become one of the hip-preserving alternatives for ONFH. It has been nearly 20 years since the first report of stem cell therapy for ONFH [12]. With the rapid development of cell biotechnology and tissue engineering, stem cell therapy has shown promising results in the treatment of ONFH. However, there still are many unresolved problems and challenges in stem cell therapy for ONFH stemming from the lack of standardized procedures, optimal accumulation of cells, and systematic security assessment.

In this review, we summarized the current trends in stem cell therapy for ONFH, ranging from clinical applications to related basic research, and briefly analyzed the existing problems and challenges.

2. Problems of current ONFH treatment

2.1. Complicated and unclear pathogenesis for ONFH

The etiology of ONFH is complicated, including impairment due to trauma, corticosteroids treatment, alcohol abuse, and connective tissue diseases [13], which ultimately leads to a disturbance in the microcirculation (Fig. 1). At present, the pathology of ONFH is considered to be related to damaged blood supply, abnormal lipid metabolism, weakened osteogenic ability of BMSCs, cell apoptosis, and gene polymorphism [11]. However, the specific pathological processes of ONFH are yet to be fully elucidated. Moreover, the repair process in different types of ONFH is inconsistent, leading to challenges in developing targeted treatments for ONFH.

2.2. Insufficient early diagnosis of ONFH

In the early stages of ONFH, no obvious symptoms are observed except slight pain in the groin, and no obvious findings are observed in X-ray images, which often leads to difficulties in the early diagnosis of ONFH. In most of the patients with a definitive diagnosis, the disease has significantly progressed, presenting as obvious collapse of the femoral head, damage in the articular cartilage, narrowing of the joint space, and deterioration of the hip into serious osteoarthritis, thus presenting a great challenge for the treatment of ONFH.

2.3. Lack of more effective treatments for ONFH

Currently, THA is the most effective treatment for patients with ONFH in the terminal stages. However, most of the patients with

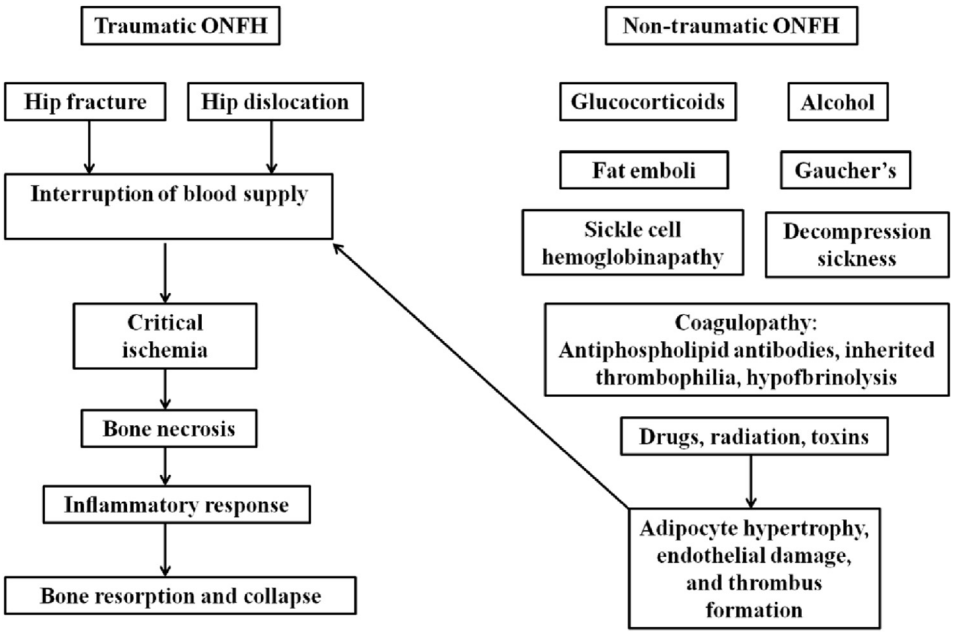

Fig. 1. Etiology and mechanisms of ONFH. Figure is from Ref. [13]. ONFH: osteonecrosis of the femoral head.

ONFH range in age from 30 to 40 years, and the bilateral hip joints are involved in 75% of patients [3]. In these patients, THA is usually associated with a high failure rate due to loosening of the prosthetic and excessive wear of polyethylene inserts [4–6], along with surgical complications such as operative stress, peri-prosthetic infection, peri-prosthetic fracture, and prosthesis dislocation [14–16]. Therefore, it is important to seek more effective and safe treatments of ONFH.

3. The theoretical basis behind stem cell therapy for ONFH

ONFH is a process that is accompanied by osteonecrosis and bone reconstruction. However, the rate of bone reconstruction is not enough to compensate for osteonecrosis due to insufficient numbers of osteoblasts in the femoral head, inevitably leading to ONFH. Currently, the pathogenesis of ONFH is considered to be related to BMSCs, including cell apoptosis, decrease in cell numbers, weakened cellular growth ability, abnormal metabolism of stem cells, and decline in osteogenic differentiation potential [11,17]. MSCs are characterized by their ability to self-renew and their potential to differentiate in to multiple cell types, and can differentiate into osteoblasts and endothelial cells for bone repair and angiogenesis [8]. Furthermore, stem cells can offer growth factors to promote blood supply to necrotic regions by paracrine effects [9,10]. Therefore, osteogenesis and angiogenesis are some of the most important mechanisms of MSCs as a treatment for ONFH.

4. Current stem cell therapy for ONFH

4.1. Cell source

MSCs are one of the most frequently used stem cell subsets, and are widely distributed in the various tissues, such as bone marrow, peripheral blood, fat, umbilical cord, etc. [7]. Therefore, depending on the source of the tissue, MSCs are named bone marrow-derived MSCs (BMSCs), adipose-derived MSCs (AMSCs), peripheral blood-derived MSCs (PBMSCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs) (Fig. 2).

4.1.1. BMSCs

Bone marrow is the most commonly used stem cell source in the treatment for ONFH. In a suitable environment, BMSCs can differentiate into musculoskeletal system cells, such as trabecular bone, articular cartilage, tendon, etc. [18]. At present, it would be of clinical interest to improve the osteogenic differentiation of BMSCs, which is influenced by growth factors, glucocorticoids, small-molecule drugs, and gene polymorphism. For example, the combination of bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) could promote the osteogenic differentiation of BMSCs [19]. Low-dose dexamethasone enhances the osteogenic differentiation potential of BMSCs by upregulating the expression of alkaline phosphatase (ALP), which is an early osteogenic differentiation marker [20]. Icariin can also promote osteogenesis in BMSCs by increasing the expression of P-glycoprotein [21]. In addition, the study by Li et al. has showed that miRNA-291a-3p promoted osteogenic differentiation of BMSCs by directly inhibiting the expression of DKK1 and activating the Wnt/β-catenin signaling pathway [22].

Notably, the clinical applications of BMSCs mainly include three forms: bone marrow concentrate (BMC), cultured cells, and bone marrow aspirates, with the mostly used form being BMC [23]. The study by Rastogi et al. [24] demonstrated that the injection of isolated mononuclear cells was more effective than direct injection of bone marrow in the treatment of ONFH, because isolated mononuclear cells had a higher density of cells at the implanted site.

4.1.2. AMSCs

Adipose tissue is another reliable stem cell source for the treatment for ONFH. AMSCs have some advantages such as easy accessibility, higher productivity, and similar differentiation potential as BMSCs [25,26]. In the induction of osteogenic differentiation, transplantation of autologous AMSCs can improve osteogenesis, and increase bone mass and bone density in rabbit model of ONFH [27]. The over-expression of VEGF could promote osteogenesis and angiogenesis by AMSCs [28]. According to the report by Abudusaimi et al. [29], core decompression combined

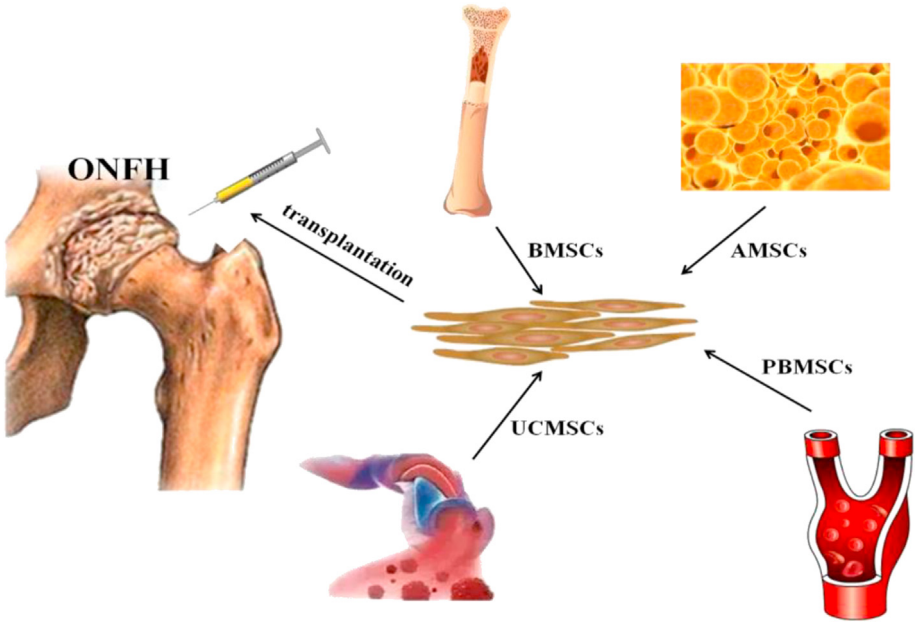

Fig. 2. The main source of stem cells for transplantation. ONFH: osteonecrosis of the femoral head. BMSCs: bone marrow-derived mesenchymal stem cells; AMSCs: adipose-derived mesenchymal stem cells; PBMSCs: peripheral blood-derived mesenchymal stem cells; UCMSCs: umbilical cord-derived mesenchymal stem cells.

with AMSCs transplantation improved the microstructure and osteogenesis of the necrotic area by increasing the expression of osteocalcin in rabbit models of ONFH. Furthermore, Pak et al. [30] injected autologous adipose stromal vascular fractions (SVF) percutaneously into hip joints, which improved patients' symptoms and promoted bone regeneration in the area with osteonecrosis. However, most of these promising results are based on the basic research studies, and the evidence offered by clinical trials still requires further elucidation.

4.1.3. PBMSCs

Peripheral blood is also one of the sources of MSCs, and is considered to be superior to bone marrow. Compared with BMSCs, PBMSCs have many advantages, such as easy accessibility, higher cost-effectiveness, and lower risk of contamination from tumor cells [31–33]. The studies by Song et al. [34,35] demonstrated that transplantation of PBMSCs alleviated ischemic femoral head necrosis in the liquid-nitrogen refrigeration rabbit model by promoting new bone formation. In addition, Fu et al. [36] transplanted PBMSCs into the necrotic area in rabbit ONFH models. They found that PBMSCs transplantation could increase the bone density and improve the microstructure of the femoral head by upregulating the expression of BMP-2 and downregulating the expression of PPAR-γ. In the clinical trials, Mao et al. [37] combined PBMSCs with porous tantalum for the treatment of ONFH in the early and medium stages. This study showed that PBMSCs transplantation improved the efficacy of biomechanical support in the treatment of ONFH, compared to biomechanical support alone. However, this treatment combination is not suitable for patients with painful cap-shaped separation (CSS) ONFH [38].

4.1.4. UCMSCs

UCMSCs have apparent stem cell attributes and lower immunogenicity. UCMSCs are also easier to isolate and expand in vitro, and have a remarkable potential to promote tissue regeneration [39]. Kuang et al. [40] reported that exosomes derived from human UCMSCs could prevent the progress of glucocorticoid-induced ONFH in rats by reducing osteocyte apoptosis. Furthermore, this effect is achieved by regulating the miR-21-PTEN-AKT signaling pathway. In clinical trials, Chen et al. [41] grafted UC-MSCs to treat ONFH by intra-arterial infusion. On magnetic resonance imaging (MRI), they found that this method could reduce the necrotic volume of the femoral head, and increase the oxygen transport based on the oxygen delivery index analysis. The authors speculated that this result was due to the migration of UC-MSCs into the necrotic area and the osteogenic differentiation of UC-MSCs. Furthermore, Cai et al. [42] investigated the efficacy of the combination of allogeneic UCMSCs and autologous bone marrow mononuclear cells (BMMNCs) transplantation in the treatment of ONFH. The study demonstrated that this therapeutics approach gradually improved the clinical symptoms of patients, including pain and claudication.

4.2. Implantation methods

4.2.1. Combination of core decompression and MSC transplantation

Core decompression (CD) is the most classical procedure for treatment of ONFH in the early stages, and reduces intraosseous pressure, removes the necrotic tissue, and stimulates the formation of new bone [43]. However, the efficacy of core decompression is variable, and favorable outcomes are observed only in patients with small necrotic lesions [44]. Therefore, a combination of core decompression and MSC transplantation has become a common approach in the treatment of ONFH. Briefly, stem cells are isolated from the relevant tissue, and then injected into the necrotic area of femoral head following core decompression. Tabatabaee et al. [45] performed the combination of core decompression and autologous bone marrow transplantation on patients with ONFH. They found that the combination treatment improved the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and visual analogue scale (VAS) significantly, and promoted the repair of necrotic areas, as observed by MRI. In addition, the study by Rastogi et al. [24] also demonstrated that core decompression combined with autologous BMMNCs implantation could improve Harris hip score (HHS) and reduce the size of lesions in patients with ONFH. Sen et al. [46] performed a randomized control study, which showed that the clinical score and mean hip survival were significantly better in

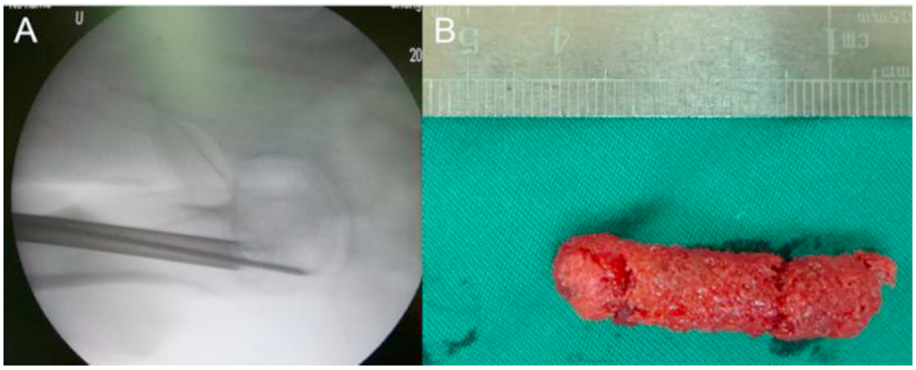

Fig. 3. Combination of core decompression and bone marrow buffy coat for the treatment of ONFH. (A) The X-ray showed that core decompression was performed by the Kirschner wire and 10-mm diameter trephine in the operation; (B) The long cylindrical bone obtained by core decompression was implanted into the bone tunnel after mixing with bone marrow. Figure is from Ref. [47]. ONFH: osteonecrosis of the femoral head.

patients treated with the combination of core decompression and autologous bone marrow mononuclear cell transplantation, compared with patients treated with core decompression alone. Ma et al. [47] conducted a prospective, double-blinded, randomized controlled trial (RCT) to examine the efficacy of core decompression combined with bone marrow buffy coat grafting for the treatment of ONFH (Fig. 3). They found that the combination treatment improved the clinical symptoms and slowed the disease progression of ONFH.

In all, this combination treatment not only has some advantages of core decompression, but also provides seed cells to improve the repair and remodeling of the necrotic area. Therefore, in situ implantation of MSCs into the necrotic area can significantly improve the therapeutic efficacy of core decompression in ONFH.

4.2.2. MSCs arterial perfusion

Arterial perfusion of MSCs is another treatment method for ONFH, the purpose of which is to improve the blood supply to the femoral head. The positive effect of MSCs on angiogenesis has been reported in previous studies. According to the study by Kinnaird et al. [48], MSCs augment arteriogenesis by inducing the release of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Qian et al. [49] reported that the transplantation of autologous BMSCs could promote angiogenesis in rabbit ischemic hind limbs. Based on this theory, the efficacy of MSCs arterial perfusion in ONFH was explored in preclinical and clinical trials. Jin et al. [50] performed arterial perfusion of MSCs in the Beagle dog model of ONFH. They found that arterial perfusion of MSCs promoted the angiogenesis and vascular repair of the femoral head by increasing the expression of VEGF and by increasing microvessel density (MVD), which improved the blood supply and reconstruction of the necrotic area. However, arterial perfusion of MSCs is often combined with biomechanical support in clinical applications. Mao et al. [37] combined the targeted intra-arterial perfusion of PBMSCs with porous tantalum in the treatment of ONFH in the early and medium stages of the disease (Fig. 4). His study showed that arterial perfusion of PBMSCs improved the efficacy of biomechanical support in the treatment of ONFH, compared to biomechanical support alone. However, this method did not apply to severe lesions, but was applicable in early and medium stage ONFH. In addition, the study of Ying et al. [38] also indicated that this combination treatment was not suitable for patients with painful CSS ONFH.

4.2.3. Cytokine pretreated or gene-modified MSCs transplantation

The current studies have demonstrated that many cytokines are involved in promoting the osteogenesis and angiogenesis of MSCs,

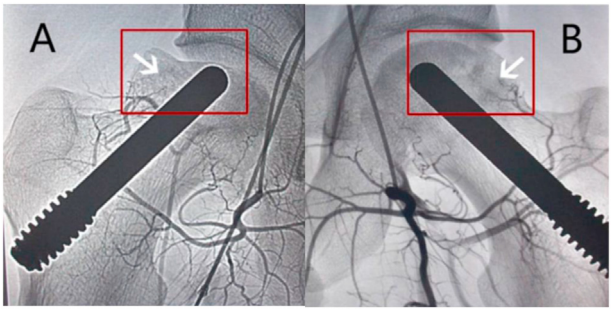

Fig. 4. Combination of arterial perfusion of PBMSCs and porous tantalum rod for the treatment of ONFH (36 months after operation). **(A)** Without vascular regeneration was observed in the control group, and the box showed the collapsed femoral head; **(B)** Vascular regeneration around the femoral head was found in the combination treatment group, and the box showed the intact and round femoral head. Figure is from Ref. [37]. PBMSCs: peripheral blood-derived mesenchymal stem cells; ONFH: osteonecrosis of the femoral head.

and improving the repair of ONFH, including BMP, VEGF, bFGF, and tumor necrosis factor (TNF) [51]. Therefore, these cytokines are used to pretreat MSCs to establish an effective treatment of ONFH. Wang et al. [52] performed the transplantation of AMSCs pretreated with BMP-2 after drilling through the growth plate in a juvenile rabbit model of femoral head epiphyseal ischemic necrosis. This study showed that the combination treatment could increase new bone formation in the necrotic area and prevent the collapse of the femoral head.

Furthermore, with the rapid development of genetic engineering techniques, gene-modified MSCs have been widely employed as seed cells for the treatment of ONFH in preclinical trials. Ma et al. [53] investigated the effect of BMSCs modified by VEGF-165 and BMP-2 gene for the treatment of ONFH in a rabbit model. The results suggested that VEGF-165/BMP-2 gene transfection augmented the osteogenic effects of BMSCs, increased the number and quality of new bones, and accelerated the repair of ONFH. In addition, Peng et al. [54] combined the BMSCs transfected with BMP-2 and bFGF genes with a demineralized bone matrix (DBM) to repair ONFH in Beagle dogs. They found that the transplantation of BMP-2 and bFGF-transfected MSCs in conjunction with DBM could successfully promote the repair of the necrotic area by increasing osteogenesis and angiogenesis. The study by Zhang et al. [55] also demonstrated that the combination of BMSCs overexpressing FGF-2 gene and xenogeneic antigen-extracted cancellous bone (XACB) could effectively increase vascular regeneration, and promote the repair of ONFH in a rabbit model. Subsequently, Peng et al. [56] investigated the effects of the transplantation of bFGF-2 gene-

transfected MSCs and XACB on TNF-α expression in a rabbit model of ONFH. They found that FGF-2/MSCs/XACB could promote the repair of ONFH via the inhibition of TNF-α expression.

4.2.4. MSCs transplantation and bone tissue engineering technology

Currently, bone tissue engineering technology is the main field of orthopedic research, including the culture of living cells in vitro, composite transplantation of cells in vivo, and the repair and reconstruction of bone tissue. Bone tissue engineering technology is characterized by seed cells, carriers and scaffolds. MSCs are the most ideal seed cells for bone tissue engineering due to their excellent differentiation capacity, strong proliferation activity, and low immunogenicity. The carriers or scaffolds can be used to regulate cellular function by not only integrating cells and receptors, but also by providing biomechanical support for the necrotic area of the femoral head. Therefore, the combination of MSCs and carriers or scaffolds has become a new approach for the treatment of ONFH.

Autologous bone tissues are the common scaffolds for MSCs in the treatment of ONFH in the clinic. Kang et al. [57] combined autologous BMSCs transplantation with auto-iliac cancellous bone grafts to treat ONFH. They believed that this procedure was more suitable for patients who have medium sized lesions. Kawate et al. [58] combined autologous MSCs cultured with beta-tricalcium phosphate (β-TCP) ceramics with a free vascularized fibula to treat three patients with steroid-induced ONFH (Fig. 5). After treatment, osteonecrosis did not progress any further and early bone regeneration was observed. They observed that the tissue engineering approach had great potential for the treatment of osteonecrosis.

In addition, synthetic scaffolds characterized by spatial structure and mechanical support similar to normal bone tissue have been applied to ONFH therapies, including DBM, XACB, tantalum rods, biphasic calcium phosphate (BCP) ceramic scaffolds [59], biomimetic synthetic scaffold poly lactide-co-glycolide (PLGA) [60], PLGA calcium phosphate (CPC) composite scaffold [61], and strontium-doped calcium polyphosphate (SCPP) [62]. Kang et al. [62] combined BMSCs with SCPP to treat ONFH in a rabbit model. They found that the combination treatment improved angiogenesis and remodeling into the new trabecular bone by increasing VEGF expression and promoting osteogenesis. Yamasaki et al. [63] evaluated the clinical results of the combination therapy comprising

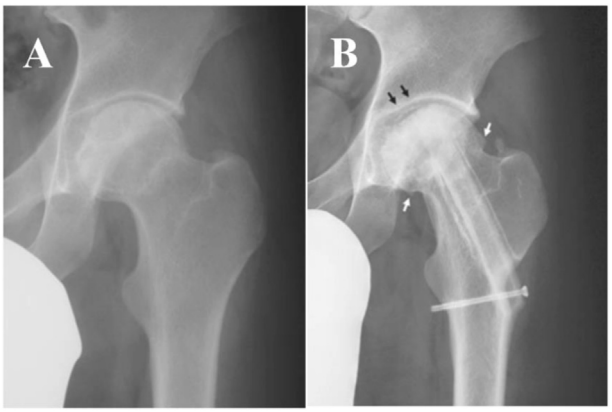

Fig. 5. Combination of transplantation of autologous MSCs cultured with β-TCP ceramics and a free vascularized fibula for the treatment of ONFH. (A) A large necrotic area of femoral head was found in the preoperative X-ray. **(B)** Although the sclerosis area and osteophytes were found in the latest X-ray, the patient did not feel uncomfortable. Figure is from Ref. [58]. MSCs: mesenchymal stem cells; β-TCP: beta-tricalcium phosphate; ONFH: osteonecrosis of the femoral head.

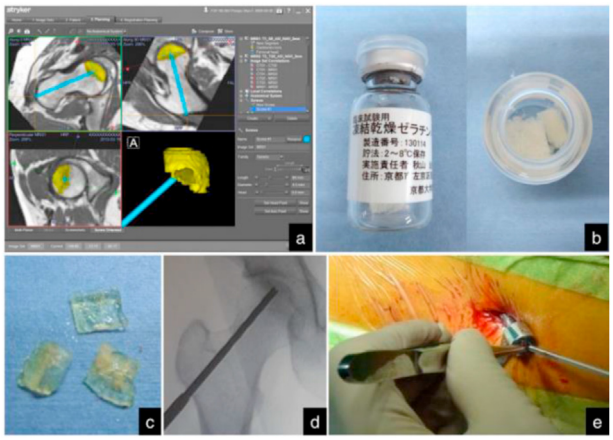

Fig. 6. Controlled release of rhFGF-2 for the treatment of ONFH. (a) Pre-operative planning. (b) Preparation of rhFGF-2-impregnated gelatin hydrogel. (c) Pieces of the rhFGF-2-impregnated gelatin hydrogel. (d) Intra-operative fluoroscopic image after drilling. (e) Percutaneous administration of the gelatin hydrogel. Figure is from Ref. [78]. rhFGF-2: recombinant human fibroblast growth factor-2; ONFH: osteonecrosis of the femoral head.

BMMNCs transplantation and interconnected porous calcium hydroxyapatite (IP-CHA) on early bone repair in ONFH. After treatment, the size of the osteonecrotic area in the BMMNC group was reduced, and hypertrophy of the bone was observed in the transition zone. Therefore, the study showed that combined BMMNCs and IP-CHA implantation could promote the repair of ONFH and prevent the collapse of the femoral head.

4.3. The number of transplanted MSCs

The efficacy of MSCs transplantation on ONFH is related to the number of cells in the local femoral head, which can affect the repair capacity of MSCs in the necrotic area. Nevertheless, the optimal number of transplanted cells is still undetermined. In theory, Hernigou et al. [64] suggested that the number of osteoblasts or osteocytes needed for bone reconstruction in ONFH was approximately 3×10^8. In fact, the realization of ideal cell numbers largely depends on the osteogenic differentiation ability of MSCs, and not only the number of transplanted cells. Remarkably, there is a decrease in the number of MSCs in the proximal femur of patients with corticosteroid-induced osteonecrosis [65], which means that the microenvironment of ischemia and hypoxia in the necrotic area can affect the proliferation and differentiation of MSCs. Moreover, the severity and extent of necrosis also need to be evaluated carefully before transplantation of MSCs. Although Hernigou et al. [66] believed that the number of MSCs transplanted should be as large as possible, the safety of maximum transplantation of MSCs needs to be investigated further. According to the current reports, the number of MSCs used in most of the studies ranged from 10^6 to 10^9, with the most commonly used cell number being 10^8 [24,67,68].

5. Comparison between stem cell therapy and other treatments for ONFH

5.1. Stem cell therapy and THA for ONFH - how should we select?

To date, THA, the most effective treatment for ONFH patients in the terminal stages, contributes to the excellent outcomes in pain relief and functional improvement of the hip joint. In the past, it was thought that prosthetic loosening and excessive wear of

polyethylene inserts were the primary causes for high failure rate of THA in young patients [69,70]. However, the outcomes of THA in young patients with ONFH have been further augmented due to improvements in operative techniques, prosthesis design, and bearing materials. The study by Byun et al. [71] showed that utilization of third-generation ceramic-on-ceramic bearings in THA could provide excellent clinical results in active patients younger than 30 years. Similar results were found in other studies [72]. In addition, THA was demonstrated to have good implant survival and long-term outcomes for treatment of ONFH in young patients, with a mean time to follow-up of 14 years [73]. Nevertheless, several concerns still remain, such as the potential for ceramic fragmentation, prosthesis impingement, abnormal sounds, dislocation, difficulty and risk of revision. We should note that a 5% revision rate at 8.4 years in patients younger than 30 years after THA was shown in a systematic review and meta-analysis [74]. Thus, after THA, at least one or more revisions will be required in young patients during their lifespan. Meanwhile, a previous study also reported that the complication rate is high, and the survivorship is low in young patients after THA revision [75]. As a result, longer follow-up times and more systematic evaluation for THA in young patients with ONFH need to be performed in future studies.

Compared with THA, stem cell therapy serves as a minimally invasive technique which retains more joint function and facilitates postoperative recovery, in addition to the promotion of bone regeneration in necrotic areas. Importantly, the clinical results of future THA are not affected by stem cell therapy; therefore, this technique can be used as a part of a step-by-step treatment for ONFH.

Taken together, THA is suitable for terminal-stage ONFH with collapse of the femoral head, narrowing of joint space, and osteoarthritis or in patients who have failed joint-preserving treatment; meanwhile, stem cell therapy is considered as a hip-preserving alternative for ONFH.

5.2. Stem cell therapy and basic fibroblast growth factors (bFGFs) for ONFH, which is better?

bFGF, also known as fibroblast growth factor-2 (FGF-2), is one of the peptides which exerts the beneficial effects on the modulation of bone remodeling and wound healing by promoting the proliferation and differentiation of MSCs and increasing the regeneration of capillary vasculature [76]. However, the effective half-life period of bFGF is relatively short in vivo due to protein instability, which leads to limitations in the conventional application of bFGF. As a result, a controlled release technique was used for the application of bFGF in previous studies. In an adult rabbit model of ONFH, the necrotic area was significantly repaired, and progression of the femoral head collapse was inhibited after the local injection of recombinant human FGF-2 (rhFGF-2) microspheres [77]. Furthermore, Kuroda et al. [78] used gelatin hydrogel as a slow-release carrier, and performed the direct administration of rhFGF-2-impregnated gelatin hydrogel for the treatment of collapsed ONFH. The authors found that the visual analogue scale (VAS) for pain scores, University of California, Los Angeles (UCLA) activity scores were significantly improved after treatment one year. Meanwhile, bone regeneration in the necrotic area of femoral head was investigated by computed tomography (Fig. 6). Moreover, Moncion et al. [79] used acoustically-responsive scaffolds to control the release of bFGF for angiogenesis. Nevertheless, the optimal concentration, explicit release time, the biologic stability, and long-term clinical results of bFGF need to be confirmed in future studies.

Stem cell therapy appears to be simpler and more effective in theory because it is believed that MSCs can be easily harvested and expanded in vitro and be induced to differentiate into osteoblasts and chondrocytes. Furthermore, increasing attention has been paid to the biological function of exosomes secreted from MSCs in stem cell therapy. A number of studies have confirmed that exosomes secreted from MSCs could promote angiogenesis [80,81], osteogenesis [82], cell proliferation and inhibit cell apoptosis [83]. Thus, the paracrine effect of stem cells is another important advantage in the treatment of ONFH, compared with the single application of bFGF. In view of this, MSCs pretreated by cytokines or modified by genes related with osteogenesis and angiogenesis, such as BMP, VEGF, bFGF and TNF, have been widely employed as seed cells for treatment of ONFH in some preclinical trials [51–56].

6. Challenges of stem cells therapy for ONFH

6.1. Confined patient selection

Many studies showed that the efficacy of stem cell therapy on ONFH was related to patient selection, which depends on the stage of ONFH [47]. The reports with a good outcome are mainly focused on patients in stage I and stage II, while the outcomes in stage III or IV cases, especially those with collapse of the femoral head, have proven to be poor [84]. In addition, etiology of ONFH is another important factor that affects clinical outcomes. It was reported that there were better outcomes of stem cell therapy in the patients with post-traumatic ONFH than in the non-traumatic cases [46]. Moreover, the modified Kerboul angle, which is relevant to the collapse of the femoral head, is a risk factor for the failure of stem cell therapy in ONFH [85,86]. In view of this, the decisions on how to improve the accuracy of early diagnosis in ONFH, select appropriate cases, and identify optimal indications remain the primary challenge for stem cell therapy in ONFH.

6.2. Lack of standardized procedures

A successful surgical technique depends on the presence of standardized procedures, and stem cell therapy in ONFH is no exception. Primarily, there is a lack of standardized cell management before operation, including the isolation, expansion, preservation, and the delivery of stem cells. Actually, most stem cells are prepared in research laboratories before clinical application, leading to a lack of sufficient manufacturing quality control. Therefore, the reproducibility of clinical results is not guaranteed. In addition, the surgical operation is not uniform and specific, and many controversies exist in the use of stem cell therapy for ONFH, such as how to choose the source of cells, how to adopt a suitable transplantation method, and how to determine the optimal number of cells for transplantation. Furthermore, the standard operation protocol also needs to be clarified when stem cell therapy is combined with other methods, including core decompression, carriers, and scaffolds.

6.3. Unclear safety

Although mild complications of stem cell therapy in ONFH have been reported [87], including hematoma, wound infection and pain at the site of bone marrow aspiration, the systematic assessment of safety in this technology in long-term follow-up should not be ignored.

Tumorigenicity is the most concerning safety issue. To some extent, some characteristics of stem cells are similar to cancer cells, such as the excellent viability, the relative ability to evade apoptosis, and the extensive proliferation capabilities [88]. As is known, the extensive expansion of MSCs in vitro is required usually to obtain sufficient cell numbers for clinical applications. However, the long-term expansion in vitro could alter the morphology and

phenotype of MSCs, and could make MSCs highly tumorigenic, which have led to multiple fast-growing lung deposits after injection into immunodeficient mice [89]. The same result was confirmed by Ren et al. [90]. They found that the spontaneous transformation was observed in MSCs derived from adult cynomolgus monkey following culture in vitro. Importantly, these spontaneously transformed mesenchymal cells (TMCs) had a significant difference in growth patterns and morphology compared to normal MSCs, and had a high level of tumorigenicity, leading to the generation of subcutaneous tumors after injection into NOD/SCID mice. Nevertheless, there is still a lack of enough evidence for malignant transformation in vivo after MSCs transplantation in clinical studies, and the tumorigenicity of stem cells still needs to be elucidated. All in all, a systematic risk assessment in the body should be established to evaluate the safety of stem cell therapy in ONFH.

6.4. Unknown fate of transplanted cells in vivo

Although the efficacy of stem cell therapy in ONFH has been reported in preclinical and clinical trials, the fate of transplanted stem cells in vivo is still unknown. How are transplanted stem cells distributed in vivo? What is the number of transplanted cells in the necrotic area of the femoral head? In terms of arterial perfusion of stem cells, it is unknown whether the transplanted cells will spread with blood flow after transplantation. In addition, it is also not clear whether stem cells undergo mutation, apoptosis, or even necrosis, and how many stem cells differentiate into osteoblasts. In view of this, despite the insufficient research on stem cells tracing due to the limitation of tracer safety, effectiveness and activity, monitoring of transplanted stem cells via cell tracing technology must be pursued [91].

7. Conclusion

ONFH is a progressive disease with complex etiology and unclear pathogenesis and lacks optimal treatment, especially for young patients. With the development of biotechnology, stem cell therapy in ONFH has become the focus of current research. This review summarized the current trends in stem cell therapy in ONFH - from clinical application to related basic research - and showed that an increasing number of studies have confirmed the effectiveness of stem cell therapy in ONFH. However, there still are many unsolved problems and challenges in practical application, such as patient selection, standardized procedures, safety assessment, and the fate of the transplanted cells in the body. Therefore, more studies are required to find the ideal cell sources, appropriate transplantation methods, and optimal number of cells for transplantation. Moreover, systematic safety assessment and stem cell tracing in vivo are also necessary. With the resolution of the above problems, it is certain that stem cell therapy may become the most effective hip-preserving alternative to THA for the treatment of ONFH.

Declaration of competing interest

The authors declare that they have no conflict of interest to this study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.reth.2020.11.003.

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