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Review Article

Analysis of Cell Therapies Used in Clinical Trials for the Treatment of Osteonecrosis of the Femoral Head: A Systematic Review of the Literature

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ABSTRACT

Background: Osteonecrosis of the femoral head (ONFH) is associated with regional loss of cells within bone, often resulting in pain and mechanical collapse. Our purpose was to analyze the cell-therapies used in clinical trials for the treatment of ONFH with regard to (1) cell-sources, (2) collection techniques, (3) cell-processing, (4) qualitative and quantitative characterizations, and (5) delivery methods.
Methods: A systematic review of the current literature on the use of cell therapies for the treatment of ONFH was performed. Studies with a level-of-evidence III or higher were evaluated. A total of 1483 articles were screened. Eleven studies met the criteria to be included in this review.
Results: Ten studies used bone-marrow, and 1 study used blood as the cell-source. Nine studies used freshly isolated tissue-derived nucleated cells from bone-marrow, mixed bone marrow-derived nucleated cells, 1 study used mixed blood-derived nucleated cells, and 1 study used culture-expanded cells derived from bone marrow aspirate. Cell dose varied from 2-million to 3-billion cells. Qualitative cell characterization of injected cells using surface markers was done by 5 studies using CD34. Two studies assayed the cell-population using a colony-forming-unit assay.
Conclusion: There is a lack of standardization with respect to the quantitative and qualitative characterization of methods for cell-harvest, cell-processing, and cell-transplantation/delivery. Cell-therapy holds promise as a means of restoring local cell populations that are made deficient because of injury or disease. However, the orthopedic community and patients will benefit greatly by a greater investment in blinded, randomized, controlled trials and clinical effectiveness trials that embrace rigorous standards.

Osteonecrosis of the femoral head (ONFH) is a disabling condition with a poorly understood etiology and pathogenesis [1]. Regardless of etiology or variations in ONFH pathophysiology, common abnormalities in the number or in the function of bone stem and progenitor cells have been described [2,3]. Based on the premise that ONFH progression is due to an underlying deficiency of cells capable of contributing to bone regeneration, it is rational to consider the use of cell-based therapies to potentially regenerate lost bone [4].

Hip preservation techniques are strongly preferred at earlier stages of ONFH, although there is no consensus regarding the ideal approach [5]. To date, core decompression (CD) is commonly performed for symptomatic, precollapse cases [6]. Best outcomes have been reported when CD is used in the earliest, precollapse stages of the disease with small lesions; nonetheless, the efficacy of this

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treatment remains an area of controversy [7]. With the purpose of improving the outcomes, adjunctive therapies have been proposed in an attempt to decrease the failure rate and progression to collapse stage [8].

Regions of osteonecrosis can only be restored by bone regeneration and remodeling. This requires the action of bone-forming osteogenic progenitors. Rational treatment modalities, therefore, focus on one or more of the following strategies: (1) opening pathways through necrotic tissue regions that enable the migration or conduction of regional cells into the defect site (eg, CD or drilling), (2) local or systemic targeting regional tissues using bioactive factors to promote activation, migration, proliferation, and/or differentiation of local osteogenic progenitor cells, (3) by homing of cells from systemic circulation to enhance local bone formation and remodeling (ie, chemotaxis), and (4) by transplantation of osteogenic and vascular endothelial progenitors from a remote site or tissue into the necrotic defect [9].

In Part I of this systematic review of the literature, we focused on the clinical efficacy, structural modifying effect, and safety of cell therapies in patients with early stages of ONFH [10]. This study suggested that these therapies are safe and provide improved clinical outcomes with lower disease progression rate when compared with controls [10]. Although cell therapy may hold a promising future as a stand-alone procedure or as an adjuvant therapy, its development will demand a better understanding and standardization of these therapies. The purpose of this Part II systematic review was to provide a detailed analysis of the different cell therapies used for the treatment of ONFH with regard to (1) cell sources, (2) collection techniques, (3) cell processing, (4) qualitative and quantitative characterizations, and (5) delivery methods.

Methods

Reports were identified by using an electronic search of keyword terms and combinations. A systematic review of the literature regarding the cell therapy treatment of ONFH in human patients was performed using the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, PubMed (1990-2016), and the Medline (1990-2016) [10]. The queries were performed in October 2016. Four different searching criteria were used, using the search terms: cell therapy, stem cells, hip, osteonecrosis, and avascular necrosis. Studies were included in this systematic review if the reports contained clinical and/or radiologic outcomes for cell therapy in the treatment of ONFH with a minimum follow-up of 12 months and had a level of evidence of I, II, or III. All included articles were presented in the English language and were performed on human subjects. Exclusion criteria were as follows: cadaveric studies, animal studies, basic science articles, editorials, surveys, special topics, letters to the editor, and personal correspondence.

Two authors performed the initial search (N. S. P. and J. C.), and 3 investigators (N. S. P., C. P. G., and J. C.) independently reviewed the abstracts from all identified articles, and inclusion and exclusion criteria were applied based on the information presented, therein. If one or more authors selected an article, it progressed to the following phase. Full-text articles were obtained to allow further assessment of inclusion and exclusion criteria, as needed. In addition, all references from the included studies were reviewed and reconciled to verify that no relevant articles were missing from the systematic review. Data were recorded into a custom information extraction table [11].

Our initial systematic literature review yielded 1483 individual studies, of which 12 met the inclusion criteria and were identified and included for analysis. One study was excluded after communication with the authors to avoid patient duplication [12]. Based on the 11 articles included in the systematic review of the current literature on the use of cell-based therapies for the treatment of ONFH (Part I), we focused in the analysis of the different cell therapies uses. For each study, we extracted the following data: (1) CD surgery: a description of the baseline surgical procedure, (2) cell therapy (nomenclature used in the study): we included the nomenclature used in each study, (3) source site: source used to collect the cell therapy, (4) collection technique: description of the collection technique used in each study, (5) initial volume obtained (mL), (6) cell processing time, (7) processing method: method used to isolate or concentrate the cells previously to their use, (8) culture expansion (if it was done), (9) cell type: description of the cells used in each study according to the characterization shown, (10) number of cells/volume (mL) delivered, (11) number of connective tissue progenitors (CTPs): defined as the heterogeneous group of stem cells and progenitor cells that are present in native tissues that can be activated to proliferate and generate progeny and that differentiate into one or more connective tissue phenotypes (eg, bone, cartilage, fibrous tissue, fat, and muscle). The concentration, prevalence, and biological potential of CTPs in a given cell population can only be estimated using in vitro colony-forming unit (CFU) assays, (12) delivery technique, (13) delivery solution or scaffold, (14) qualitative cell characterization (surface markers), and (15) quantitative cell assessment (CFU assay; Tables 1 and 2).

Results

The cell sources, collection techniques, cell processing, qualitative and quantitative characterizations, and delivery methods varied widely between studies, as shown in Tables 1 and 2. All the studies used an autologous source of cells. Ten of 11 studies used bone marrow aspirate (BMA) as the collection technique [13–22]; however, the aspiration technique was inconsistently reported. One study used as a source peripheral blood [23]. Nine of the 10 studies that used BMA chose the iliac crest to perform the aspiration while the remaining used BMA from the subtrochanteric area [21]. The study that isolated cells from blood harvested cells from the patients by apheresis technique according to a mononuclear cell collection method after subcutaneous injections of granulocyte colony-stimulating factor at a dosage of 10 mg/kg for 4 days to mobilize the peripheral blood stem cell population [23].

The initial volume of sample collected was reported in 7 of 11 studies. The later volume varied significantly among the studies that provided the data, ranging from 10 mL of BMA [21] to 220 mL of BMA [18].

When analyzing the cell types according to the characterization done in each study, 9 studies [13–20,22] used freshly isolated tissue-derived nucleated cells from BMA: MBMNCs, 1 study [23] used mixed blood-derived nucleated cells by isolating cells from peripheral blood, and 1 study [21] used culture-expanded cells derived from BMA: culture-expanded adherent cells (CEACs). Cell dose varied from 2 million to 3 billion cells.

Qualitative cell characterization of injected cells using surface markers was done by 5 studies [13–17,23]. All 5 studies reported only on 1 surface marker: CD34. Two studies assayed the CTP prevalence cell population using a CFU assay [17,18].

Discussion

The most important findings of this study were that the utilization of cell therapies in patients with early stages of ONFH demonstrated significant heterogeneity in the choice of cells,

Table 1
Cell Therapy Description.

Study	Core Decompression Surgery	Cell Therapy: Nomenclature Used in Article	Source Site	Collection Technique	Initial Volume	Cell Processing Time	Processing Method	Culture Expanded	Cell Type
Rastogi et al, 2013 [14]	4.5-mm cannulated reamer over the guide wires subchondrally within 5 mm of the articular surface. Similarly, another parallel tract was made directed into the necrotic zone.	Isolated mononuclear cell	BMA: iliac crest	BMA with 10-mL heparinized syringe (aspiration technique not reported).	60-70 mL	1 h	FSG	No	MBMNCs
Sen et al, 2012 [15]	3 cores of 4 mm diameter were drilled from the lateral cortex to the site of lesion.	Isolated mononuclear cell	BMA: posterior iliac crest	BMA with 10-mL syringe, each time changing the site or direction of aspiration	120-180 mL	2 h	FSG	No	MBMNCs
Mao et al, 2015 [23]	Biomechanical support (porous tantalum rod implant)	G-CSF–based PBSC transplantation	Peripheral blood	PBSCs were harvested from the patients by apheresis technique using a COBE Spectra Apheresis System, after subcutaneous injections of G-CSF at a dosage of 10 mg/kg for 4 d to mobilize the PBSCs.	N/A	N/A	Apheresis	No	MBDNCs
Ma et al, 2014 [19]	10-mm diameter trephine was placed through the k-wire placed subchondraly 2-3 mm from the articular cartilage and driven toward the necrotic site.	Autologous bone graft + bone-marrow buffy coat	BMA: posterior iliac crest	BMA needle. The needle was rotated back and forth to achieve penetration through the cortical bone. A 50-mL heparinized syringe was used.	N/A	N/A	DS	No	MBMNCs
Zhao et al, 2012 [21]	Used Stryker's Navigation System, a decompression tunnel was made using a trephine through into the necrotic region 2-3 mm away from the cartilage.	Bone marrow–derived culture expanded MSCs	BMA: femur subtrochanteric area	BMA through the decompression tunnel (aspiration technique not reported).	10 mL	2 wk	FSG	Culture expanded for 2 wk	CEACs
Tabatabaee et al, 2015 [16]	Core decompression through lateral hip cortex drilled using a 2.7-mm drill, and the drill advanced into the necrotic aspect of the femoral head under fluoroscopy.	Concentrated bone marrow containing MNCs	BMA: iliac crest	Small incision was made over the iliac crest, and a needle was advanced between the cortical tables of the crest (aspiration technique not reported).	200 mL	1 h	DS	No	MBMNCs
Gangji et al, 2011 [17]	3-mm trephine under fluoroscopic control through greater trochanter into necrotic lesion, placed at a distance of 2-3 mm from articular cartilage.	Bone marrow cells	BMA: iliac crest	Aspiration technique not reported	N/A	N/A	Apheresis	No	MBMNCs
Lim et al, 2013 [13]	Decompression was performed using a percutaneous approach with a 3-mm diameter trephine; 2 or 3 drilling holes	BMMC	BMA: posterior iliac crest	Deep insertion of a beveled needle (6-8 cm long and 1.5 mm in internal diameter), aspiration with 50-mL plastic syringe. Successive small-fraction aspirations were performed, turning the needle 45° after each aspiration with several perforations.	N/A	N/A	DS	No	MBMNCs
Liu et al, 2013 [20]	10-mm diameter trephine was placed through 2.5 mm k-wire	BMMC	BMA: posterior iliac crest	Needle used to aspirate slowly and continuously. Direction	150-200 mL	1.5 h	DS	No	MBMNCs

(*continued on next page*)

Table 1 (*continued*)

Study	Core Decompression Surgery	Cell Therapy: Nomenclature Used in Article	Source Site	Collection Technique	Initial Volume	Cell Processing Time	Processing Method	Culture Expanded	Cell Type
	placed subchondraly 5 mm from the articular cartilage and driven toward the necrotic site. Expanding reamers of different diameter.			was changed after aspiration of 5 mL of bone marrow, and aspiration depth was changed after each 20 mL (1 mL of heparin saline every 5 mL of bone marrow). BMA stored in a collection bag containing acid citrate dextrose.					
Yamasaki et al, 2010 [22]	Guide wire inserted into subchondral bone. Holes drilled with 6 and 10 mm burr.	BMMC	BMA: iliac crest	2 mm needle with a 10-mL syringe containing 1 mL of heparinized saline.	700 mL	N/A	DS	No	MBMNCs
Pepke et al, 2016 [18]	Three 2.0-mm k-wires drilled subchondraly into necrotic area (2-3 mm from articular cartilage). Centrally placed k-wire was over drilled using 5-mm trephine.	BMMC	BMA: iliac crest	Aspiration technique not reported	200-220 mL	N/A	DS	No	MBMNCs

BMA, bone marrow aspirate; BMMC, bone marrow mononuclear cells; CEACs, culture-expanded adherent cells; DS, density separation; FSG, ficol separation gradient; MBDNCs, mixed blood-derived nucleated cells; MBMNC, mixed bone marrow–derived nucleated cells; MNC, mononucleated cells; N/A, not available; PBSC, peripheral blood stem cells.

method of cell processing, cell characterization, quantitative and qualitative assessment of the cells used, and surgical methods of cell delivery. Furthermore, this broad variation and deficit of standards makes generalizable inferences and reproducibility challenging. Despite these limitations noted, these studies showed overall improved clinical outcomes and represent the prologue and the foundations to begin building the standards required for optimizing biological regenerative approaches to hip preservation [10].

Our systematic review presents several limitations. This study has the inherent limitations of the available literature. For example, the stratification of the lesion, as per size and location varied, making difficult to determine the parameters or indications for which these could be utilized. Also, there are a small number of studies available for assessment, and it is difficult to determine which of the various cell preparations is the most efficacious at this point. Nevertheless, we believe it was valuable to point out the tremendous variations in cell sources, collection techniques, cell processing, qualitative and quantitative characterizations, and delivery methods.

All studies reported utilized CD as a baseline control, and then assessed various means of sourcing and transplanting populations of cells containing presumptive progenitors of cell (ie, a combination of strategies 1 and 4 from the introduction). However, the methods used for CD involved widely different techniques including from 2.7-mm drill holes to 10-mm cores and different number of holes drilled.

Cell sourcing and particularly processing strategies also varied widely. BMA was used as the cell source in 10 of 11 studies, and peripheral blood was used in one [23]. Even though aspiration techniques are known to have profound effects on the concentration of cell and osteogenic progenitors and the optimal technique stipulates that less than 2-4 mL of the aspirate must be taken per site limiting hemodilution from peripheral blood [24,25], the details of aspiration technique were not consistently documented in the majority of the studies. This variation and uncertainty in technical methods potentially makes reproducing many studies challenging. In future studies, the field will benefit from more details documenting the aspiration technique used including: aspiration site, needle, volume in each aspirate, anticoagulant, total volume and spacing, and aspiration rate.

BMA has been the most common source for harvesting stem and progenitor cells (especially from the iliac crest) because of its accessibility for surgeons [17,24,26–31]. CTP concentration (number of stem and progenitor cells in the native tissue) accounts only for a small population within the bone marrow: averages from 1000 to 2000 CTPs/mL of aspirate, with a CTP prevalence between 1×10^{-4} and 1×10^{-6}. Processing of the bone (eg, density separation by centrifugation) can increase both the concentration and prevalence of CTPs, by removing red blood cells, serum, and non-CTPs cells [24,29,32–36]. All studies used some method to increase the concentration of nucleated cells that were transplanted, but methods varied from density separation (centrifuge; 6 studies [13,16,18–20,22]), to density gradient separation (eg, Ficol; 3 studies [14,15,21]), and then to apheresis (2 studies [17,23]).

The number of osteogenic progenitors required to induce remodeling and repair of the osteonecrotic area has not been determined yet. In our analysis, studies varied widely in the number of cells transplanted (ie, cell dose), with a range from 2 million to 2 billion cells per site. No conclusions can be obtained through this observation, and future dose-ranging studies will be needed to address this issue.

Beyond cell numbers and concentration, further methods for quantitative characterization of the attributes of the cells being

Table 2
Cell Therapy Description.

Study	Cell Type	Number of Cells/ Volume	Number of CTPs	Delivery Technique	Delivery Solution or Scaffold	Qualitative Cell Characterization: CD Markers	Quantitative Cell Assessment (CFU Assay)
Rastogi et al, 2013 [14]	MBMNCs	Approx. 1.1 × 10*8 cells/5 mL	N/A	Long core biopsy needle and gel foam was used to plug the canal	No	CD34+	N/A
Sen et al, 2012 [15]	MBMNCs	Approx. 5 × 10*8 cells/2 mL	N/A	N/A; bone wax to prevent backflow	No	CD34+ (5 × 10*7 CD34 + cells)	N/A
Mao et al, 2015 [23]	MBDNCs	2.47 × 10*8 MNC	N/A	Intra-arterial delivery through the medial circumflex femoral artery.	Porous tantalum rod implant	CD34+ (1.71 × 10*6 CD34+ cells)	N/A
Ma et al, 2014 [19]	MBMNCs	Approx. 3 × 10*9 MNC/ 1 mL	N/A	Through a trephine	Cylinder of bone from the femoral neck	N/A	N/A
Zhao et al, 2012 [21]	CEACs	2 × 10*6 cells/mL/2 mL	N/A	Injected with puncture needle through plug	2 mL normal saline solution	N/A	N/A
Tabatabaee et al, 2015 [16]	MBMNCs	>2 × 10*6 cells	N/A	Spinal needle was inserted and aimed at the necrotic femoral head through the opened cannel and advanced within 2-3 mm of the joint line. Channel was closed with an allograft bone plug from the corticocancellous iliac crest.	No	CD34+	N/A
Gangji et al, 2011 [17]	MBMNCs	1.9 ± 0.2 × 10*9 MNC/ 49.7 ± 2.3 mL	92.6 ± 22.4 × 10*7 cells	N/A	No	CD34+ (2.0 ± 0.3 × 10*9 leukocytes [1% ± 0.2% CD34+])	N/A
Lim et al, 2013 [13]	MBMNCs	8.7 ± 4.6 × 10*8 MNC	N/A	Through a small trocar	No	CD34+ (1.69 × 10*7 CD34+ cells)	N/A
Liu et al, 2013 [20]	MBMNCs	31.4 ± 4.8 × 10*6 MNC/ 5 mL	N/A	Through bone tunnel using a pushing bar	Nanohydroxyapatite/ poly-amide 66 composite bone-filling material	N/A	N/A
Yamasaki et al, 2010 [22]	MBMNCs	1 × 10*9 MNC/40mL	N/A	Cell-seeded scaffold	Interconnected porous calcium hydroxyapatite	N/A	N/A
Pepke et al, 2016 [18]	MBMNCs	118.9 ± 15.1 × 10*6/10 mL	50 ± 16	Through the cannulated trephine	No	N/A	f-CFU assay manual count

CD, core decompression; CEACs, culture-expanded adherent cells; CFU, colony-forming unit; CTPs, connective tissue progenitors; G-CSF, granulocyte colony-stimulating factor; MBDNCs, mixed blood-derived nucleated cells; MBMNC, mixed bone marrow–derived nucleated cells; MNCs, mononucleated cells; N/A, not available.

transferred was described in 6 of the 11 studies, using CD34 cell-surface markers [13–17,23]. CD34 is associated with hematopoietic stem cells and, therefore, is a rational metric of the quality of BMA tissue in the setting of traditional bone marrow transplantation. However, CD34 is not a marker that is associated with osteogenic progenitors. As a result, it does not directly correlate with osteogenic potential [37]. Future studies may consider characterization of marrow quality using cell-surface markers that are associated with osteogenic marrow-derived cells (eg, CD146, CD90 [38,39], and surface-bound hyaluronan [40,41]). Moreover, it would be ideal if the studies provided a functional assay to characterize the prevalence of colony-forming CTPs from the cells used and the capacity of the progeny of those to differentiate to express an osteogenic phenotype [30,32–34].

Using precise and consistent nomenclature and metrics to describe the cells used in therapy is of outmost importance when reporting on the use of cell therapies. The term CTP is used to describe the heterogeneous populations of stem cells and progenitor cells that are present in all native musculoskeletal tissues (eg, bone marrow, bone, and fat) [30,32,34]. The concentration and prevalence of CTP differs from one tissue source to another, and the biological potential of CTPs also varies. To date, no single surface marker or set of markers have been described to distinguish between CTPs and non-CTPs in native tissues. As a result, the only quantitative assay for CTPs is an in vitro CFU assay [9]. Automated methods of CFU assay can provide concentration, prevalence, and biological potential among the colony-founding CTPs in a given tissue [33,34,42]. Only 2 studies reported CFU assays [17,18].

When cells are freshly isolated from tissues, and CTP prevalence and function is not measured based on colony formation, it is most appropriate to define that population of cells as a “mixed tissue-derived nucleated cells”. Following this rationale, 9 studies [20,24,25,27–32] used MBMNC, and 1 [23] used mixed blood-derived nucleated cells. The remaining study used culture-expanded adherent cells [21].

The method of cell delivery also varied widely between studies. Ten studies [20,24–32] directly instilled or delivered the cell therapy through the core tract, although the precise methods were not necessarily described in a manner that would allow the technique to be reliably duplicated. One study performed intra-arterial delivery through the medial circumflex femoral artery [23], a minimally invasive strategy that demands advanced technical skills, which may not be available in every center. The distribution of the injected cells and their possible extravasation into adjacent or distant tissues was not examined.

Conclusion

There is a lack of standardization with respect to the quantitative and qualitative characterization of cellular therapies combining cell harvest, cell processing, and cell transplantation/delivery. Cellular therapies hold promise as a means of restoring local cell populations that are made deficient because of injury or disease. However, the orthopedic community and our patients will benefit greatly by a greater investment in blinded, randomized, controlled trials and clinical effectiveness trials that embrace rigorous standards. Of particular importance is increased rigor in documentation and qualitative characterization of methods for cell harvest, processing, and delivery, as well as uniform assessments of clinical outcomes.

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