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Bone Marrow Concentrate for Treatment of Knee Osteoarthritis: A Mini Review

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Abstract

Knee Osteoarthritis (KOA) is a common degenerative joint disease that affects no less than 19% of American adults aged above 45 years. The incidence of Osteoarthritis (OA) is increasing and will continue to do so as the world's population increases and continues to age. Current treatment strategies for OA include few non-invasive interventions (medications, physical therapy, activity modification and bracing) and invasive therapies (drilling, debridement, osteochondral transplantation, autologous perichondral and periosteal grafts, autologous chondrocyte implantation and arthroplasty). Many of these strategies are focused on pain reduction rather than disease modification or controlling progression. Cell based therapies are emerging as a promising approach to OA treatment and have been shown to reverse the symptoms and pathophysiology of OA. Researchers and clinicians are focusing on the beneficial effects of autologous Bone Marrow Concentrate (aBMC) for the treatment of KOA. The probable repair mechanisms and potential role of cellular and humoral components of aBMC is discussed in this review.

Keywords: Bone Marrow Concentrate; Cell Based Therapy; Knee Osteoarthritis

Abbreviations

KOA	:	Knee Osteoarthritis
OA	:	Osteoarthritis
GF	:	Growth Factor
MSC	:	Mesenchymal Stem Cell
ADSC	:	Adipose Tissue-Derived Stem Cells
SVF	:	Stromal Vascular Fraction
FDA	:	Food and Drug Administration
BM	:	Bone Marrow
BMC	:	Bone Marrow Concentrate
PRP	:	Platelet Rich Plasma
CTP	:	Connective Tissue Progenitors
RBC	:	Red Blood Cell
SDF	:	Stromal Derived Factor
CFU	:	Colony Forming Unit
IL	:	Interleukin
TNF	:	Tumor Necrosis Factor
MCP-1	:	Monocyte Chemo Attractant Protein-1
CCR2	:	C-C Motif Chemokine Receptor 2
TGF-B	:	Transforming Growth Factor
PDGF	:	Platelet Derived Growth Factor
BMP	:	Bone Morphogenic Protein

SMPCS : Synovial Mesenchymal Progenitor Cell

Introduction

Knee Osteoarthritis (KOA) is a common degenerative joint disease characterized by gradual deterioration of the articular cartilage, diseased subchondral bone, formation of osteophytes and cellular inflammation of adjacent tissues. KOA is a highly prevalent disabling disease which affects at least 19% of American adults aged above 45 years [1]. The incidence of Osteoarthritis (OA) is increasing and will continue to do so as the world's population increases and continues to age. Wallace et al recently reports that prevalence of KOA has doubled since the mid-20th century [2]. Factors that may increase the risk of developing OA are age, gender, joint trauma or overloading caused by physical labor or sports, hereditary and obesity [3]. Current treatment strategies for OA include few non-invasive interventions (medications, physical therapy, activity modification and bracing) and invasive therapies (drilling, debridement, osteochondral transplantation, autologous perichondral and periosteal grafts, autologous chondrocyte implantation and arthroplasty). Many of these strategies are focused on pain reduction rather than the disease modification or controlling progression [4].

Cell based therapies are emerging as a promising approach to OA treatment [5]. These therapies have a huge potential to contribute to novel therapeutic strategies for the repair of chondral or osteochondral defects. Cell-based therapies have been shown to reverse the symptoms and pathophysiology of OA [6]. The mechanisms of action of bone marrow derived cell therapy in tissue regeneration are related to the secretion of several cytokines, chemokines, and Growth Factors (GFs), which can improve angiogenesis, suppress inflammation, inhibit apoptosis, and stimulate endogenous repair [7]. With recent advances in regenerative medicine many clinical studies are underway to explore the capacity of stem cells to regenerate articular cartilage, suggesting a real-world potential to be translated to clinic. Cell populations used in clinical trials for KOA includes Articular Chondrocytes, Mesenchymal Stem Cells (MSCs) derived from bone marrow or adipose tissue and Bone-marrow Concentrate. Autologous cultured chondrocytes transplantation for cartilage regeneration has been used successfully over a decade [8]. Major limitations for biological cartilage repair using articular chondrocytes include lack of implanted graft mechanical stability and various side effects leading to procedure failure [9-10]. Another problem for biological cartilage repair besides the delay of treatment is the localization of the defect. These difficulties left the field open to other therapies and the most promising of these are MSCs to repair the damaged cartilage tissue. MSCs offer a potential regenerative solution given their ability to differentiate to all tissues within a joint and modulate the local inflammatory response. Although these characteristics suggest they provide ideal building blocks to restore damaged joints, a strong body of evidence supports MSC-guided regeneration through paracrine stimulation of native tissue.

Adipose Tissue-Derived Stem Cells (ADSCs) in the form of Stromal Vascular Fraction (SVF) may offer an alternative option for MSCs and have demonstrated an ability to regenerate cartilage [5]. Jo, et al. conducted a study in which they administered different doses of ADSCs- low, middle and high in 18 patients suffering from KOA. The low- and middle-dose groups showed significant improvement in joint function and pain reduction, whereas the size of the cartilage defect increased in the low-dose group and decreased in the middle- and high-dose groups [11]. The promising outcomes demonstrated that intra-articular ADSC injection may serve as a potent and safe therapy for OA. However, the major limitation of these ADSCs is regulatory restrictions. The common practices of enzymatic and mechanical disruption of adipose tissue for isolating SVF [12] are explicitly mentioned in the Food and Drug Administration (FDA) document as "more than minimal manipulation" and is category 351 product, that is a "drug/biologic" and in need of complete FDA regulation [13].

BM derived MSCs provide an excellent therapeutic alternative for the treatment of KOA [14]. Recently, Yubo, et al. evaluated the therapeutic efficacy and safety of mesenchymal stem cells (MSCs) for the treatment of patients with knee Osteoarthritis (OA). Meta-analysis conducted by Yubo, et al. of relevant published clinical studies demonstrated that MSC-based stem cell therapy for patients with KOA was associated with significantly decreased pain scores, increased knee functions scales and low rates of adverse events [15]. However, there are limitations with the use of MSCs. Somozoa, et al. reported that MSCs have an intrinsic differentiation program reminiscent of endochondral bone formation, which they follow after exposure to specific reagents as a part of current differentiation protocols. Efforts have been made to avoid the resulting hypertrophic fate of MSCs; however, so far, none of these has recreated a fully functional articular hyaline cartilage without chondrocytes exhibiting a hypertrophic phenotype. Sequence of events and the morphology of the resulting cartilage are more comparable to that of the early phases of endochondral ossification as seen in the skeletal development or fracture repair, where it is a prelude to programmed cell death and mineralization [16]. Also, *In-vitro* culture and expansion of MSCs is associated with significant costs and regulatory requirements, which in the current financial restrictions in healthcare have made this option unfeasible for clinical application. Thus, researchers and clinicians are focusing on the beneficial effects of autologous BMC for the treatment of KOA. BMC can be safely and easily obtained from the patient while in compliance with the US Food and Drug Administration policy guidelines including minimal manipulation.

Bone Marrow Concentrate (BMC): Treatment for KOA

Autologous BMC has emerged as a novel treatment of KOA. The preparation and application of autologous BMC is a cost-effective method in delivering progenitor cells & cytokines to aid in the repair and regeneration of cartilage defects. BMC contains a range of growth factors and cytokines to support cell growth following injury [17-18]. BMC generated following

density-gradient centrifugation has increased numbers of stem/progenitor cells and growth factors. The concentrate possesses, anti-inflammatory, angiogenic and potent immunomodulatory properties that can potentially enhance cartilage repair [19]. Pre-clinical studies conducted for treatment of cartilage pathology have reported promising results with the use of BMC [20,21]. The studies generated enthusiasm and have led to early clinical trials. Review conducted by Filardo and colleagues found 5 clinical trials and 2 pre-clinical studies focusing on the use of BMC for the treatment cartilage regeneration [22]. The studies show the potential of BMC as a promising treatment for cartilage regeneration and can potentially be translated for future therapies. Several clinical studies have reported the safety of BMC and its potential effectiveness in treatment of early KOA and moderate focal chondral defects [14,18,23-26]. Hendrich and colleagues conducted a 101 patients study with an average follow-up time of 14 months. The group reported no adverse effect or morbidity from the harvest site [27]. Recently, Shapiro et al published results of a prospective, single-blind, placebo-controlled trial on 25 patients with bilateral KOA, and reported that the use of BMC is safe. The authors did not find any significant difference between BMC and the placebo group, although both groups showed improvement in pain at 1 week, 3 months, and 6 months [26]. Two other studies reported by Centeno et al and Kim et al showed the beneficial effect of BMC injection for KOA in terms of improved functional activity scores and pain scores. Centeno et al combined the injection of BMC with PRP and platelet lysate and compared for the treatment of OA with and without adipose tissue. The data indicates that there is no significant difference between the 2 groups. Addition of an adipose graft to the BMC did not provide a detectible benefit over BMC alone treated group [14].

Similarly, Kim, et al. reported BMC injection along with adipose tissue improved functional activity scores and pain scores in the patients with degenerative arthritis of knee [24]. The studies lacked a control group and BMC injections were performed concomitant to other treatments thus making the interpretation of the results challenging. Sampson et al carried out intra-articular administration of autologous, nonculture expanded BMC with subsequent follow-up injection of Platelet Rich Plasma (PRP) at 8 weeks post BMC injection in 125 patients suffering from moderate to severe osteoarthritis. Among 125 patients, no patient reported a significant side effect from the treatment, and the median pain reduction among the 87 patients with complete data at a median follow-up of 148 days was 71.4%. Furthermore, median patient satisfaction for the 84 patients that completed the post procedure satisfaction survey was 9.0 out of 10 [25]. In general, there were no significant adverse events in the above-mentioned studies highlighting the safety of BMC injection for the treatment of KOA. Another systematic review conducted by Chahla, et al. concluded that intra articular BMC injections for KOA and focal cartilage defects are safe and showed clinical benefit. The studies included in this systematic review reported good results, but they used different outcome measures and this heterogeneity does not allow for direct comparison [18]. Although basic science and animal models have shown that stem and progenitor cell therapies may potentially perform as disease-modifying treatments for KOA through proposed mechanisms of tissue regeneration or immunomodulation, this effect still needs to be further proven. It is still not yet clear how BMC can be best utilized for the treatment and which of the components of BMC are predominantly responsible for the desired effect. Furthermore, optimized delivery of BMC may better address the pathophysiology of subchondral bone with intraosseous infiltration [28].

Role of cellular and humoral components of BMC

Initial experiments using BMC in treating cartilage pathology have reported clear benefits [20,21]. BMC is a cost-effective method in delivering MSCs to aid in the repair and regeneration of cartilage defects. MSCs have been reported to enhance the quality of cartilage repair by increasing aggrecan content and tissue firmness [19]. Alongside MSCs, BMC contains a range of growth factors and cytokines to support which are assigned to have anabolic and anti-inflammatory effects thus supporting cell growth following injury [18,29-30]. However, it is still not clear how BMC can be best utilized for the treatment of different conditions and which of the components of BMC are predominantly responsible for supporting the growth and regeneration of chondrocytes.

Cellular composition of BMC

Previous studies have reported the clinical value of bone marrow concentrates, showing a positive correlation between the number of applied bone marrow progenitors and favorable clinical outcomes in OA and other orthopedic indications [11]. Despite the advantages of using bone marrow aspirates or concentrates, the quality of these samples remains difficult to assess and is poorly controlled. There is an overall lack of consensus on which stem cell markers to characterize BMC. Recently, Jawhari, et al. proposed an optimized method of counting $CD45^{low}CD271^{high}$ cells and tested it as an indicator of bone marrow sample quality [31]. This assay for counting $CD45^{low}CD271^{high}$ cells may provide a useful measurement of bone marrow quality. While the specificity of this measurement of $CD45^{low}CD271^{high}$ cells remained low in the defined experimental conditions, $CD45^{low}CD271^{high}$ cell counts were positively and modestly correlated with the prevalence of Connective Tissue Progenitor (CTPs). Thus, assessing the CD271 fraction in BMC might help us to identify the responders and non-responders of the cellular therapy and might help in planning future therapies.

Probable detrimental effect of RBCs in progenitor stem cell functionality

Previous experimental studies and clinical pilot trials showed that a reduction of functional activity of the infused cells was associated with reduced therapeutic effects [32,33]. However, the impact of the composition of the cell product and the potential effect of contaminating cells such as Red Blood Cells (RBCs) was unclear. Assmus, et al. (2010) carried out experimental studies to demonstrate that the addition of Red Blood Cells (RBCs) impairs

BMC cell function *in-vitro* and *in-vivo*. The data published in Journal of the American College of Cardiology (JACC) suggest that the number of contaminating RBCs in the purified BMC population might have influenced the functionality of the cells used for cell therapy. Co-incubation experiments were carried out to study the effect of RBCs on the functionality of the cells. The RBCs dose-dependently reduced the viability of BMCs as measured by trypan blue exclusion assay. Furthermore, the invasion capacity of BMCs at baseline and after stimulation with Stromal Derived Factor-1 (SDF-1) was significantly reduced by RBC addition. In fact, the highest dose of RBC contamination completely abolished the migratory capacity of isolated BMCs toward SDF-1. Incubation of BMCs with RBCs further reduced the Colony Forming Unit (CFU) capacity, thus indicating that RBCs directly affect cell viability and cell functionality [34].

Based on previous studies it is known that stem cell competence, migration and survival is dependent on the integrity and function of mitochondria [35-37]. Therefore, Assmus, et al. tested the mitochondrial function of BMCs by measuring the mitochondrial membrane potential with JC-1 staining after addition of RBCs. They found that contamination with RBCs dose-dependently reduced the quantitative mitochondrial membrane potential of BMCs, indicating that RBCs impair the mitochondrial function of BMCs. Taken together, this data confirms that RBC contamination dose dependently impairs the *in vitro* and *in vivo* functions of isolated bone marrow-derived mononuclear cells. Bleeding disorder like haemophilia leads to recurrent joint hemorrhages which leads to inflammation, damage of articular cartilage, and eventually to destruction of the whole joint. *In vitro* studies have shown that the combination of monocytes/macrophages and red blood cells, as present in whole blood, leads to long-lasting disturbance of cartilage matrix turnover [38]. Mechanism proposed for this irreversible damage is the conversion of hydrogen peroxide and catalytic iron, supplied by damaged red blood cells, into hydroxyl radicals [39-40]. Hydrogen peroxide is produced by chondrocytes under the influence of interleukin-1 (IL-1) formed by activated monocytes/macrophages. Hydroxyl radicals cause chondrocyte apoptosis resulting in permanent cartilage damage, [41] since the chondrocyte is the only cell type of cartilage and responsible for maintenance of the cartilage matrix. Moreover, there are also direct harmful effects exerted by intra-articular blood on cartilage, as demonstrated by *in vitro* studies. Indeed, it has been reported that a short four-day exposure of human cartilage to whole blood at concentrations up to 50% may induce long lasting inhibition of cartilage matrix proteoglycan synthesis and a prolonged decrease in proteoglycan content [42-45]. Thus, reduced hematocrit in the BMC would help in better functionality of the stem cells used in the cell therapy. However there have been technological challenges and limitations to commercially separate out the RBC while not depleting the native monocyte fraction.

Role of BMC Cytokines on the functionality of progenitor stem cells

In the osteoarthritic knee there exists an imbalance of the chondrocyte's cellular catabolic and anabolic functions. This inequality leads to degradation of the extracellular matrix of hyaline cartilage and is mediated by cytokines such as interleukin-1 (IL-1), chemokines like the C-C class of the beta chemokine family and Tumor Necrosis Factor (TNF). The chronic inflammatory process that ensues causes further cartilage damage, and eventually leads to mechanical and biological dysfunction within the joint. Though the complete role of inflammation in OA is unknown, elements of inflammation have been directly implicated in the progression of the disease and the degeneration of the cartilage surfaces of the joint. Interleukin 1b (IL-1b), a pro-inflammatory cytokine has been demonstrated to play a central role in the pathophysiology of cartilage damage and degradation in arthritis. In OA patients, increased IL-1b has been observed within the synovium, synovial fluid, and cartilage itself. IL-1b has been directly implicated in the inhibition of chondrogenic differentiation and inhibition of specific extra cellular matrix proteins required for cartilage function [46]. In addition to IL-1b, Monocyte Chemoattractant Protein-1 (MCP-1) is also present in the inflamed joints. MCP-1 is a member of the C-C class of the beta chemokine family and one of the key factors involved in the initiation of inflammation. It triggers chemotaxis and trans-endothelial migration of monocytes to inflammatory lesions by interacting with the membrane C-C Motif Chemokine Receptor 2 (CCR2) in monocytes [47]. Harris et al demonstrated that MCP-1 inhibited the chondrogenesis of Synovial Mesenchymal Progenitor Cell (sMPCs) at the gene, protein, and primitive tissue levels. These anabolic sMPCs are present in the adjacent synovial lining and synovial fluid and are capable of differentiating into cartilage both *in vitro* and *in vivo*. They have also demonstrated that MCP-1 increases the proliferative potential of these cells. Exposure to physiological (OA knee joint synovial fluid) levels of MCP-1 triggers changes in the transcriptome of sMPCs and prolonged exposure to the chemokine induces the expression of MCP-1 in sMPCs, resulting in a positive feedback loop from which sMPCs cannot apparently escape. After prolonged exposure to MCP-1, sMPCs begin to express MCP-1 which "locks" the joint in a viscous cycle of ineffective repair. Thus, arising a need of the adjunctive which breaks this viscous cycle by inhibiting the secretion of MCP-1 [48]. Therefore, a potential biologic inhibiting both IL-1b and MCP-1 is required for effective treatment of OA.

One of the known antagonists to IL-1b cytokine and MCP-1 is IL-1 receptor antagonist protein (IL-1ra). Brown et al reported IL-1 receptor antagonist inhibits MCP-1 generation by human mesangial cells [49]. While marrow concentrate after density gradient centrifugation contains a relatively small population of MSC (0.001%-0.01%) It is an important biological tool with a rich source of growth factors, including Platelet-Derived Growth Factor (PDGF), transforming growth factor-beta (TGF-β), and Bone Morphogenetic Protein (BMP)-2 and BMP-7, which are reported to have anabolic and anti-inflammatory effects [18]. Cassano, Fortier and colleagues reported that BMC has a significantly greater concentration of interleukin-1 receptor antagonist (IL-1RA) [50]. Although there does not seem to be a consensus on the minimum level of IL-1ra necessary to achieve therapeutic benefit, it has been generally reported in the literature that a ratio of IL-RA to IL1β on the order of 10-1000 to 1 is sufficient to effect blockade of

the IL-1 receptors or the IL-1 triggered effects, thereby alleviating the degenerative effects of IL-1 [51]. Thus, injecting BMC might inhibit IL-1 catabolism and MCP-1 secretion and therefore may be responsible for the beneficial symptomatic pain relief.

Conclusion and Future Directions

KOA is a chronic disease characterized by the slow degradation of cartilage which results in pain and disability in patients impacting on the quality of a patient's life. The development of new therapeutic approaches involving cell-based therapies, may become a viable alternative for the treatment of KOA. However, this will require overcoming multiple challenges by basic scientists such as cell source, harvesting techniques, effective cell dose and composition and clinical studies with good sample size and long term follow up are requisite. Use of autologous BMC is growing exponentially as it is proven to be a safe and easy to be performed. With regards to the cellular and cytokine content of BMC it remains unclear which makeup with or without RBC and which applications lead to the best outcome in KOA. Further well designed clinical trials are needed to establish the long-term effects of BMC in treatment of KOA.

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