Ream content a stem cell source for bone defects

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Bone grafts are normally used for the treatment of bone defects and non-union fractures, and the most common donor site is the iliac crest. The reamer-irrigator-aspirator (RIA) is an innovative technology to obtain osseous particles during intramedullary reaming in femur fractures. Discarded RIA can provide abundant native bone marrow mesenchymal stem cells (BM-MSCs) compared to the iliac crest. Autograft obtained from the use of RIA shows osteogenic potential at least equal to the iliac crest autograft with less donor site morbidity. The disadvantages of using the iliac crest including small volume, invasive harvesting, and pain for the patient make RIA-harvested autograft bone preferable because it has good properties and is amenable for use as a sole alternative to the iliac crest graft or as a graft expander in conjunction with autologous iliac crest bone marrow aspirate.

**Keywords:** Bone marrow; Reamer-irrigator-aspirator; Iliac crest; Mesenchymal stem cells; Bone repair

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**Introduction**

Extensive segmental bone loss, infection, or tumor, and delayed healing or non-union of long bone fractures remain challenging surgical problems in orthopedic patients. The basic treatment principle for such cases is to provide stable mechanical fixation and biological support such as bone grafting [1, 2]. Autograft is the most commonly used type of bone graft [3] and the donor site with the most potential is the iliac crest. Since the volume is limited, complications associated with harvesting bone from the iliac crest, potential donor site morbidity, and pain may occur. Hence, it is reasonable to search for alternative techniques to support bone formation in multi-fragmentary fractures with bone defects or pseudarthrosis formation, periprosthetic fractures, or bone defects after tumor diseases or infection. Recently, a reamer-irrigator-aspirator (RIA) device has become an alternative way of collecting bone material [4-7]. The RIA system was used for fracture fixation to prepare long bones with intramedullary nails. The RIA device allows removal of biological debris of the medullary canal by simultaneous irrigation and suction during the reaming procedure. With the advent of this new bone graft harvesting device, an alternative source of bone is now available to treat fracture non-unions. The biologic content of the RIA graft is shown to be superior to the iliac crest and the volume of available graft is on average 50 ml or more [6]. Further studies on the biomechanical effect of graft harvest have shown no critical weakening of the donor femur after graft harvesting [8, 9]. Furthermore, the system allows the aspiration of intramedullary debris containing various growth factors such as fibroblast growth factor (FGF-a), platelet-derived growth
factor (PDGF), insulin-like growth factor (IGF), transforming growth factor (TGF-b), and bone morphogenetic protein-2 (BMP-2) [10] and of bone marrow progenitor cells exhibiting characteristics typical of mesenchymal stem cells (MSCs) [11-14]. The cells obtained from RIA have a fusiform shape, fibroblast-like growth pattern and the expression of characteristic surface molecules such as CD90, CD105, CD73-positive, and CD34- and CD45-negative [13]. In addition, the cells can be differentiated along the osteogenic, neuronal [13], adipogenic, and chondrogenic lineage [12, 15].

This review will focus on the fact that the concentration of bone marrow mesenchymal stem cells (BM-MSCs) differs depending on the harvest site.

**Bone marrow mesenchymal stem cells (BM-MSCs)**

Bone marrow is a complex tissue of the body, including two main branches of stem cells containing MSCs and hematopoietic stem cells (HSCs) [16]. They can be distinguished from each other by their cell surface markers [17]. BM-MSCs, also called mesenchymal stromal cells, were discovered by Friedenstein et al. who observed a certain population of plastic-adherent, highly proliferative cells that were capable to form a colony of fibroblasts (CFU-F) [18]. Evidence suggests that MSCs exists not only in bone marrow, but also in almost all organs [19] like: adipose tissue [20], umbilical cord [21], gingival connective tissue [22], amniotic membrane [23], permanent teeth [24], lungs [25], chorionic villi of the placenta [26], peripheral blood [27], fetal liver [28], and even in exfoliated deciduous teeth [29]. The number of MSCs isolated from these tissues may be different in terms of the yield, donor age, cell passage, and the quality even when the cells are obtained from the same donor [30-32]. Other sources of MSC can be used for therapeutic purposes [15] like: homing efficiency, differentiation potential, tissue engineering, production of trophic factors, and immunomodulation [34]. One of the distinguishing characteristics of stem cells are their ability to self-renew to regenerate after injury [35]. Also, multi lineage differentiation ability into a variety of cell types including osteoblasts, chondrocytes, adipocytes, myoblasts, and neuroblasts has been described extensively. Because it is not necessary to match BM-MSCs in transplantation and they do not cause immunological rejection, BM-MSCs transplantation has been suggested as a therapeutic method [36]. BM-MSCs are able to differentiate into these cell types in vitro under specific induction medium for each of them, as well as to form new bone and cartilage tissues [37]. Differentiation ability into particular lineages is done in relation to the source tissue and induction medium. Expression of genes and proteins in specific cell types are different and detectable in BM-MSCs [38]. BM-MSCs are capable of stably expressing CD29, CD44, CD73, CD105, and HLA. However, a number of researchers have indicated MSCs do not express major histocompatibility complex (MHC) class II and do not express or have low expression levels of MHC class I. Moreover, BM-MSCs do not express T cells co-stimulatory molecules such as B7-1 and B7-2, and do not express or stimulate apoptosis molecules such as CD40, CD80, CD86, and FasL [36].

**Cell sources of bone marrow mesenchymal stem cells:**

**I-Iliac crest bone marrow mesenchymal stem cells (ICBM-MSCs)**

MSCs are commonly isolated from an aspirate of bone marrow harvested from the superior iliac crest of the pelvis in humans [39, 40]. Iliac crest bone marrow (ICBM) was collected with a Jamshidi needle [41]. Human bone marrow contains multipotent cells that have the potency for differentiation into a number of mesodermal lineages; adipocytes, osteoblasts, and other mesodermal pathways [42, 43]. MSCs are present at a concentration of less than 1 in 100000-500000 mononuclear cells in bone marrow aspirates from adults, and MSCs must be culture expanded to obtain sufficient numbers for clinical use [44]. BM-MSCs are the most appropriate cells for inducing bone repair, as they have a strong osteogenic potential and are easily obtained by culturing iliac crest aspirates [45, 46]. Thua et al. have shown that the implantation of bone marrow is efficient in the repair of bone non-union in the initial outcome and may provide an available alternative to autologous cancellous bone graft [47]. Bone marrow contains osteogenic progenitors and the injection of bone marrow is considered the potential way for effective bone regeneration [48, 49]. Ohgushi et al. have shown that the implantation of bone marrow affects the local biology and optimizes the potential of osteoprogenitors [50]. In another study it has been shown that autologous bone marrow injection increases cellular numbers and stimulates the cells to fabricate a structural osteoconductive substrate with extremely low donor site morbidity [51]. Some studies have reported that bone marrow injection is a mini-invasive technique and contributes to bone union in cases of stable delayed or non-union [52-54]. Autologous bone grafting has some serious drawbacks, such as a prolonged operation time [55] and donor site morbidity in about 10-30 % of the cases [56-58]. The isolation of MSCs from bone marrow is highly invasive and causes pain and discomfort to the patient. In addition to that, the frequency of BM-MSCs also depends on the volume of aspiration. The numbers of MSCs per milliliter will decrease with the increased volume of aspirated marrow for each puncture because of the dilution of the bone marrow sample with peripheral blood [29, 36]. These major drawbacks
have led many researchers to explore alternative methods for more abundant and accessible sources for MSCs using the least invasive collection procedures.

1-Ream-irrigator-aspirator mesenchymal stem cells (RIA-MSCs)

Intramedullary nailing was popularized by Kuntscher in 1940 for the treatment of femoral and tibia non-union fractures [61, 62]. By the late 1990’s the RIA system was designed and patented, and after several refinements, it was made accessible for clinical use in 2003 [63]. The RIA system was originally designed as a simultaneous aspiration and reaming system to reduce intramedullary pressure, heat generation, operating time, and systemic effects of reaming like fat embolism [61, 64-66]. The RIA system has several theoretical advantages that may make it a more suitable technique than others. First, continuous irrigation and suction assists with the whole removal of infected bone debris and the collected debris can then be sent for microbiological analysis. Second, irrigation and sharp cutting flutes decrease heat generation, and therefore, endosteal thermal necrosis [67]. Additionally, patients who are not appropriate candidates for iliac crest harvesting because of obesity or other premorbid conditions are potential candidates for medullary bone graft.

Animal data confirm that the RIA decreases intramedullary temperature, pressure, and fat embolization, and attenuates many of the pathologic processes seen in the setting of pre-existing lung contusion or hypovolemic shock. Using a porcine model Husebye et al. have observed that the application of RIA actually led to a significant decrease of intramedullary pressure in the femur to almost baseline levels [68]. Beck et al. have shown that in bone healing RIA bone grafts cause an increase in bone volume to the 3-month graft in the preclinical sheep model compared to an iliac crest wing graft [7].

In 1986, a researcher indicated that reaming products contain viable bone, osteocytes, and mesenchymal stem cells. The researcher showed that RIA bone graft has growth factors and stem cell concentrations that either equal or surpass those found in the iliac crest bone [13, 14, 69, 70] and have viable osteocytes and osteoprogenitor cells [71, 72] [10, 73]. Porter et al. have shown that the cells extracted from RIA are adherent to culture plastic and fibroblast cells displayed on a surface marker profile indicative of MSCs. They have also shown that these cells differentiate into osteogenic, adipogenic, and chondrogenic cells. Their finding approved the potential of RIA aspirate for bone healing [75]. RIA is becoming more common place in treating segmental defects. Several authors have found that RIA reaming contain multipotent stem cells that are capable of osteosynthesis [13, 72, 74].

Advantages of RIA-MSC

Cox et al. have shown that RIA contains a large number of MSCs that could be used for bone repair without enzyme digestion and prior cell expansion. They have compared MSCs from the iliac crest with RIA in terms of their capacity for differentiation and have shown that RIA has more potential than the iliac crest. They have suggested that 1 L of the iliac crest would be required to achieve a similar number of MSCs that are in the RIA fraction [75]. In 1958, it was recognized that intramedullary reaming products have osteoinductive potential [76]. Researchers have shown that RIA reaming products such as bone graft have both osteoinductive and osteoconductive properties and the ability to form mechanically viable callous [69]. Osteogenic cells from RIA with osteoconductive scaffold and osteoinductive growth factors such BMPs have shown high healing rates in the treatment of non-union [77]. We have shown that RIA-MSCs were attached to collagen scaffold and had great potential to differentiate to osteoblast [78]. Kuehlflick et al. in a study compared the MSCs from three different sources: the RIA fraction, iliac crest, and adipose. They showed that MSCs from RIA have osteogenic potency in vitro and in vivo, and they suggest that MSCs from RIA are a suitable source for bone regeneration and an alternative source for iliac crest bone graft [79]. Yoshikawa et al. in their study have shown that osteocalcin concentration correlates with the amount of newly formed bone [80] and RIA-MSCs show a higher expression of osteocalcin compared to the iliac crest [79]. Drik Henrich et al. have shown that RIA content is a rich source for various types of autologous progenitor cells, which can be used to accelerate the healing of bone and other musculoskeletal tissue [14]. Churchman et al. in a study enumerated and characterized MSCs extract from RIA and compared them to MSCs from iliac crest bone marrow. The results suggest that long bones contain very large numbers of MSCs, transcriptionally-similar to ICBM MSCs; they can be procured by reaming using the RIA device and used, following concentration, as autologous and potentially allogeneic bone repair therapy [81]. Kay Sinclair et al. have recommended that the lipid-rich fat layer of the aspirate may be a source of mesenchymal stem cells that, each alone or in aggregation with currently available synthetic bone graft material, could be used to stimulate new bone growth [82]. Widemann et al. have also found that RIA reamings have increased osteogenic elements compared with the iliac crest [83]. The biologic potential of RIA thus has led to the investigation of the RIA graft in non-union surgery. Some studies on the use of RIA bone graft approve the osteogenic potential reported in basic science investigations. Suk et al.
have reported the usage of RIA graft in post-traumatic foot defects [84]. He used the RIA graft to treat a post-traumatic medial-column defect in the foot after a gunshot wound. Several studies have reported on the technical aspects of obtaining graft and on the potential morbidities related to RIA graft harvesting [4, 5, 85]. Several researchers have shown that the aspirate from the reaming system was laden with bioactive substrates like osteoblasts and growth factors like BMP-2 after performing cell cultures and flow cytometry [15, 71, 72, 86, 87]. Schmidmaier et al. have shown that RIA filtrate have significant levels of growth factors known to be involved in bone healing, like PDGF, vascular endothelial growth factor (VEGF), FGF-2, IGF-I and TGF-b1. They also found BMP-2 within the filtrate, but below the limit of detection (100 pg/ml) for the ELISA assay used in the present study and they have shown that levels of VEGF, PDGF, and BMP2 compared between other growth factors were higher in the RIA content compared to the iliac crest [10]. Otherwise, Porter et al. support the findings and show measurable levels of FGF-2, IGF-1, and latent TGF-b1 [15]. In another study, it has been shown that growth factors increased and angiogenic factors decreased in the RIA fractions [69]. They found higher levels of the five out of seven growth factors obtained from intramedullary reaming compared with iliac crest graft. These included PDGF, fibroblast growth factor (FGFA), IGF-1, BMP-2, and TGF-B1.

Another study has reported that RIA compared to the iliac crest bone graft had a complication rate in the iliac crest bone graft (19.4%), which was approximately three times that seen with RIA (6%) [88]. These results are important when selecting a stem cell treatment method and patients should know the complications with iliac crest bone graft.

Conflicting interests

The authors have declared that no conflicts of interests exist.

Abbreviations

RIA: Reamer-irrigator-aspirator; BM: Bone marrow; MSCs: Mesenchymal stem cells; RIA-MSCs: Ream-irrigator-aspirator mesenchymal stem cells; BM-MSCs: Bone marrow mesenchymal stem cells; ICBM-MSCs: Iliac crest bone marrow mesenchymal stem cells; BMP-2: Bone morphogenetic protein-2; PDGF: Platelet-derived growth factor; VEGF: Vascular endothelial growth factor; FGF-2: Fibroblast growth factor; IGF-1: Insulin-like growth factor; TGF-b1: Transforming growth factor.


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