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A Comprehensive Review on Perfusion Method Development for Bone Marrow Collection and Stem Cell Transplantation

Narasimhulu Korrapati* and Harikrishna Y. Nanganuru

Department of Biotechnology, National Institute of Technology Warangal, Warangal-506004, India

Abstract: Bone marrow transplant (BMT) is done by the replacement of damaged bone marrow with healthy one. These healthy bone marrow cells (BMCs) are usually collected from the crest of the Ilium in humans hence these cells are used to replace damaged ones in the treatment of bone marrow related diseases such as leukemia, aplastic anemia, congenital immunodeficiency and autoimmune diseases. Even though there are different methods, perfusion method is one of the simple, safe and less contaminated methods used to harvest BMCs and it can reduce the risk in allogenic BMT. Intra bone marrow – bone marrow transfer (IBM-BMT) is one of the best procedures for allogenic BMT. Due to enlisting of hematopoietic stem cells and mesenchymal stem cells, which are derived from donor, this method has distinguishable advantages in allogenic BMT. In this paper the perfusion method (for harvesting BMCs) and IBM-BMT (for their transplantation) have been critically reviewed and showed that both methods are together will become an effective combination in allogeneic BMT.

Keywords: Allogeneic bone marrow transplantation, bone marrow cells, human stem cells, mesenchymal stem cells, intra-bone marrow bone marrow transplantation, perfusion method.

INTRODUCTION

New blood cells are produced at the tissues surrounding centre of large bones is called bone marrow. It has hemopoietic and stromal stem cells. Hemopoietic cells can produce blood cells whereas stromal cells can produce fat, cartilage and bone. Hematopoietic cells are the immature cells of the bone marrow [1]. These BMCs have effectively been using in gene therapy and organ transplantation [2-4]. BMT is a powerful strategy for the treatment of some hematologic disorders like leukemia and autoimmune diseases [1]. A perfusion method is simple, safe and better method for harvesting minimal contaminated BMCs. It decreases the risk in allogenic BMT [6, 7]. It has been used for collecting BMCs using the long bones of cynomolgus monkeys [5]. IBM-BMT is a powerful procedure to recruit donor derived hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) in animal experiments [6, 7]. It has been observed from recent animal studies that hematopoietic stem cells seem to produce other kinds of cells, such as blood vessels, bone and muscle. But they are unable to proliferate and differentiate invitro. There is no proper method for distinguishing of stem cells from other cells that recovered from the blood or bone marrow. After kidney transplantation, mesenchymal stoma cells have been suggested have potential to prevent tubular atrophy and allograft rejection in a report [8].

PERFUSION AND ASPIRATION METHODS FOR HARVESTING BONE MARROW CELLS

Human body has different types of cells. Stem cells are different among those types. Although stem cells are originated from different places of the human body, they have similar properties. They can proliferate into different types of cells that become specialized cells for specific purpose. There are three types of BMTs such as autologous, syngeneic and allogeneic. These have same procedure for harvesting of BMCs. For the processing, blood and other bone particles are removed from the harvested bone marrow then preservatives are added and are kept frozen. According to the Thomas *et al.* method, BMCs can be harvested using multiple bone marrow from the crest of ileum in humans [9]. Only very few harvested cells will be able to migrate to the bone marrow, if the harvested cells are injected intravenously (IV-BMT) and rest will be trapped in the lung [10]. Humans and some non human primates have similar stem and progenitor cell dynamics. For instinct a report has been shown that the features of mesenchymal stem cells of monkey resembles human mesenchymal stem cells. Like that these non human primate cells are useful in extracting information for the development of new transplant methods and in target gene therapy for the treatment of human diseases [11-16].

Using cynomolgus monkeys a new BMC harvesting method has been established, which is the perfusion method (Fig. **1A**).

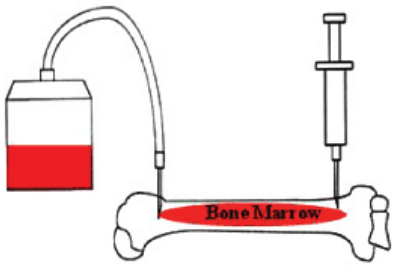
Less contaminated BMCs can be collected by Perfusion method (PM). In this method, two needles are inserted into the either ends of a long bone. One needle is connected to the 0.5ml heparin syringe and the other needle is connected to the 30ml saline syringe. Then saline is injected slowly into the medullary cavity of a long bone. Thus the saline containing less contaminated BM is collected. This collected BM using PM contains less than 10% T – cells and confirmed by the RBC: WBC and lymphocyte: granulocyte ratios whereas more than 20% in conventional method (CM). To achieve

*Address correspondence to this author at the Department of Biotechnology, National Institute of Technology Warangal, Warangal-506004, India; Tel: +919985470286; E-mail: simha7762006@gmail.com

2212-3946/14 $58.00+.00

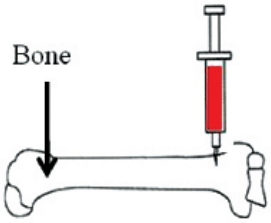
desirable results the T – cells must be removed in conventional method so there is possibility to loss some important cells during removal of T – cells but important cells will not be lost more in PM due to the less contamination. The collected BMCs using PM contains more number of cells particularly immature cells (myeloblasts and promyelocytes) than CM. According to the CFU-C assays, due to the minimal contamination, by using PM more hematopoietic progenitor cells can be collected than CM.

(A)



Perfusion Method for Harvesting BMCs

(B)



IBM-BMT

Fig. (1). (**A**) Perfusion method for harvesting BMCs. (**B**) IBM-BMT.

Table 1. Comparing CM and PM for Harvesting BMCs.

	CM	PM
Insert times	more	less
T cells contamination	high	low
Number of hematopoietic progenitors	low	high
Ratio of RBC: WBC	high	low
Ratio of lymphocyte: granulocyte	high	low

Progenitor activity of the collected BMCs in PM is more than CM. being more progenitor activity; because of short-term reconstitution of these progenitors by donor cells this is an advantage to the donor cells. The harvested BMCs using PM from the aged donor's ileum are useful for the BMT across major histo compatibility complex (MHC) barriers and if the donors are brain dead it can be used for organ transplantation. These are treated with irradiation and transplanted directly into the bone marrow cavities of the long bones of recipient cynomolgus monkeys. A report has been shown in cynomolgus monkeys that the performance of IBM – BMT more on the both sides of humeri than tibiae [17].

INTRA BONE MARROW-BONE MARROW TRANSPLANTATION (IBM-BMT)

The replacement of damaged bone marrow with healthy one is called the bone marrow or cord blood transplantation. There are some diseases such as metabolic diseases, hematopoietic diseases and genetics diseases that must need BMT. BMT is the best option to treat leukemia, aplastic anemia and autoimmune disorders [18-20]. The graft rejection risk can be forbidden by replacing the HSCs and MSCs to be employed through the IBM-BMT. And also there are some more features to pose IBM-BMT as best are rapid hemopoietic recoveries, complete restoration of T-cell functions, avoids graft failure at reduced radiation doses and avoids the development of GvHD even after the injection of whole BMCs. Thus this is more effective than IV – BMT [21, 22]. Some factors secreted form MSCs have been shown to raise tissue repair [23] by migrating to the site of injury of particular tissues like kidney [30], liver [31], lung [32] and heart [33], activate the endogenous tissue progenitors proliferation and differentiation [24], reduce the inflammatory action and immune reactions [25], regulate the immune responses through the suppression of T – cell [26] by soluble factors like IL-6, M-CSF, IL-10, TGFβ, HGF and PGE2 [34, 35]. According to the recent report, the therapeutic benefits of MSCs have been extended to T cell-mediated diseases such as GvHD [27], Crohn's disease [28] and the prevention of organ transplantation rejection [29]. And also MSCs are capable of regulating the cells of the innate and adaptive immune systems and their proliferation, differentiation, maturation and function, and persuading an anti-inflammatory phenotype [34, 35]. Some more activities of MSCs have been shown that modulating DC function [36], different aspects of rejection process, including the inhibition of DC differentiation [38], suppressing DC function during allogeneic islet transplantation [37], regulating the activity of the T and B cell activity and preventing the GvHD development [36].

INTRA BONE MARROW-BONE MARROW TRANSPLANTATION IN ANIMALS

Phosphate-buffered saline (PBS) was injected into the medullary cavities of the femurs and tibias of donor mice and collected BMCs with PBS. Then BMC suspension is filtered using a 70-μm nylon mesh and centrifuged. Supernatant is collected after the centrifugation. Then 3×10^9 BMCs are adjusted per 1ml. These are then sent to irradiation. These prepared BMCs are used in the IBM – BMT 24 Hrs after irradiation [39]. After anesthetization, the area from the inguinal region to the knee joint of the recipient mice is shaved. 26-G needle is then injected into the BM cavity by drilling the tibia. One micro syringe is then connected to 26-G needle and prepared BMCs are injected into the BM cavity. Organ transplantation, including skin [40], pancreas [41], leg [42] and hear can also be successful with IBM – BMT. Even if the radiation doses were reduced and without using any immunosuppressant, the achievement of donor cell engraftments can be done. According to a report, the serum insulin and lipid levels in KK-Ay were improved from normal BALB/c mice after 4 months of BMT. Due to the MSC-stromal cell disorder, the abnormality is occurred in endothelial progenitor cells. By the oxidative stress and decreases in

adiponectin MSC-stromal cell disorder is exacerbated. This has been suggested by the HO^{-1} induction's beneficial effect [43]. HO^{-1} expression has been up regulated, while reducing the iNOS levels in the kidney of db/db mice and the adiponectin secretion has been induced immediately after enhancing the signaling pathway of pLKB1-AKT-AMPK during the IBM-BMT^{+} thymus transplantation (TT) [44, 45]. Thus IBM-BMT has been established as an efficient method to human disorders and also shown superior to IV-BMT in case of severe combined immunodeficient mice, where the human cells are reconstituted [46-50]. IBM-BMT can efficiently transfer donor whole BMCs into recipients and this method can be used to quickly replace not only donor-derived-HSCs but also MSCs.

CONCLUSION

Perfusion method can efficiently be applied to the long bones and crest of Ilium in monkeys. During the collection of BMCs from either the long bones or crest of Ilium using PM, no accidents have been occurred. Moreover, it has better features than CM that are lower insertion time, lower T – cell contamination, lower ratio of RBC:WBC and lymphocyte: granulocyte and higher progenitor activity. So PM is an efficient and safe method to humans as well. It is not only efficient for BMCs and also for the collection of HSCs and MSCs with minimal contamination. IBM-BMT is best method, due to rapid hemopoietic recoveries, complete restoration of T-cell functions, avoids graft failure at reduced radiation doses and prevents the development of GvHD even after the injection of whole BMCs. The combination of the Perfusion method and IBM-BMT will become a powerful new strategy for allogenic BMT and regeneration therapy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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Received: March 11, 2014 **Revised: March 19, 2014** **Accepted: March 19, 2014**