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Original Article

Comparative study of different centrifugation protocols for a density gradient separation media in isolation of osteoprogenitors from bone marrow aspirate

Noel Ye Naung [a,*], *Srisurang Suttapreyasri* [b], *Suttatip Kamolmatyakul* [c], *Thongchai Nuntanaranont* [b]

[a] *Department of Oral Sciences, School of Dentistry, University of Otago, Dunedin, New Zealand*
[b] *Department of Surgery, Faculty of Dentistry, Prince of Songkla University, Hatyai, Thailand*
[c] *Department of Preventive Dentistry, Prince of Songkla University, Hatyai, Thailand*

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ABSTRACT

Introduction: Human bone marrow contains osteoprogenitors capable of differentiating into osteoblasts. Density gradient centrifugation (DGC) is a commonly used method to isolate osteoprogenitors from bone marrow. Numerous studies used different dilution and centrifugation protocols, which might affect cell yields and quality. Moreover, the relative isolation efficiencies of the different separation protocols have not been investigated. This study compares the enrichment efficacy of the two different centrifugation protocols for a commonly used DGC media in isolation of osteoprogenitors.

Material and method: Bone marrow was aspirated from human anterior iliac crests. Osteoprogenitors are isolated with Ficoll DGC media. A centrifugal force of 400 g and 1:1 dilution was compared with the centrifugal force of 1000 g after three dilution times with a buffer.

Results: The average numbers of isolated cells were significantly higher when using lower centrifugal force with 1:1 dilution, however, there was no detectable difference between Colony-forming unit-fibroblast (CFU–F) forming capacity, STRO-1 positivity, osteogenic differentiation or mineralization abilities between protocols.

Conclusion: Both protocols could isolate competent and functional osteoprogenitors, while a lower centrifugal force (400 g) with 1:1 dilution produced recovery of more osteoprogenitors.

* *Corresponding author.*
E-mail address: noel.naung@otago.ac.nz (N.Y. Naung).
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1. Introduction

Autogenous bone is the gold standard grafting material and its osteogenic capacity resides in the bone marrow.[1] The bone marrow stromal system contains multipotent mesenchymal stem cells and osteoprogenitor cells.[2] Bone marrow aspirate was considered the richest and most readily available source of osteoprogenitors, which can be a valuable alternative bone graft substitute to prevent grafting related morbidity and complications.[1]

The osteogenic potential of bone marrow aspirate was first reported in 1869 by Goujon.[3] In 1970, Friedenstein and colleagues cultured adult bone marrow cells and demonstrated the ability to form fibroblastic colonies known as colony-forming units – fibroblasts (CFU–F).[4] These cells are capable of differentiating into bone, cartilage, fat and other mesenchymal lineage cells.[5,6] Each CFU–F is derived from a single cell, therefore the number of CFU–F colonies reflects the number of stem or progenitor cells.[4] The early preosteogenic stem cell surface marker, monoclonal antibody STRO-1, can also be used to characterize the osteogenic precursor cells from aspirates of human bone marrow.[7]

The osteoprogenitor cells are limited in number, containing only approximately 0.005% of the nucleated cells in the fresh bone marrow aspirates.[1] Hernigou treated 60 non-union or osteonecrosis patients with bone marrow aspirates and seven atrophic non-unions failed to heal as a result of significantly lower numbers and concentration of osteoprogenitors injected.[8] Therefore, the ability to isolate the maximum number of marrow stromal mesenchymal stem and osteoprogenitor cells with the highest replication and differentiation potential is crucial for the success of bone tissue engineering applications. In addition, contamination of the osteoprogenitors with red blood cells impairs the efficacy of the cell therapy.[9]

Bone marrow stromal cells can be isolated and purified by removing unwanted cells using different techniques including density gradient centrifugation. Density gradient centrifugation is a technique that allows the separation of cells depending on their density. Ficoll, a high molecular weight sucrose polymer, has been widely used for isolation of mesenchymal stem and osteoprogenitor cells from bone marrow.[10] Ficoll–Paque PREMIUM 1.073 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was chosen in this study because, when compared to standard Ficoll (1.077 g/ml), a lower density (1.073 g/ml) has proven benefits in isolating the lower density mononuclear cells and mesenchymal stromal cells with higher proliferation potential, which can ultimately benefit all clinical applications.[11]

A number of studies using Ficoll to remove the mononuclear cells from the bone marrow used different centrifugation forces (from 2600 g to 2500 g) as well as different ratios of dilution (1:1 to 1:3).[12–14] A centrifugal force of 400 g and 1:1 dilution is the general protocol recommended by the manufacturer, which has been used with success to isolate the mononuclear cells from the blood, however, the optimum result may not be obtained due to the difference in nature of the bone marrow and the blood such as cellularity, viscosity and weight. According to the basic principles of centrifugation, differences in the centrifugal force could affect the sedimentation rate and the viability of cells.[15] Accordingly, the ratio of dilution, in other words, the viscosity of the bone marrow, could also affect the efficacy of separation.[15] Therefore, we assumed that different centrifugation forces and the ratio of dilution might affect the quantity and quality of the isolated bone marrow stromal cells and osteoprogenitor cells.

Currently, to our extent of knowledge, the relative isolation efficiencies of the different separation protocols have not yet been investigated. The aim of the study was to compare the enrichment efficacy of the two different centrifugation protocols for the Ficoll density gradient separation media on bone marrow osteoprogenitor cell separation.

2. Materials and methods

2.1. Bone marrow harvesting

Bone marrow samples were obtained by aspiration from the anterior iliac crests of ten patients undergoing alveolar bone grafting surgery at the dental hospital, Prince of Songkla University after informed consent. Ethical approval was obtained from the Ethical Board of the Faculty of Dentistry.

2.2. Bone marrow aspiration technique

Bone marrow was aspirated from the anterior iliac crests under general anesthesia. A 2 mm stab incision was made through skin and subcutaneous tissue on the anterior iliac crest about 2 cm posterior from the anterior superior iliac spine. A Klima-Rosegger bone marrow aspiration needle (diameter 14 G, 1.5 inches long) was inserted into the cancellous bone of the iliac crest between the inner and outer tables of the iliac crest. After removing the obturator, the marrow was aspirated in small fractions (<4 ml) and continuous aspiration (more than 6 s) was avoided to reduce the degree of dilution by peripheral blood[16] (Fig. 1). Perforations were made 1 cm apart from each site to avoid dilution by bleeding from the previous area.

Twenty milliliters of aspirated marrow was collected into the 50 ml sterile Falcon tube containing 1 ml of anticoagulant solution (1000 units of heparin in sterile normal saline solution).

2.3. Grouping

The experiment was performed to compare between 2 different centrifugation forces and dilution protocols as shown in Table 1. A total of 20 ml aspirated bone marrow was equally divided and put into two 50 ml plastic tubes.

2.4. Bone marrow processing

The bone marrow was first filtered through a 100 μm cell strainer to remove bone fragments, cell clumps and fat. The bone marrow in each tube was diluted with a buffer in accordance with each protocol.