VISUAL CONFIRMATION OF ISOLATED BONE MARROW MESENCHYMA
STEM CELLS AND DIFFERENTIATED CARDIOMYOCYTE-LIKE CELLS

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ABSTRACT
This project attempted in vitro protocols to isolate, culture, expand and induce bone marrow mesenchymal stem cells (BM-MSCs) into cardiomyocyte-like cells. Mixed hematopoietic and mesenchymal bone marrow cells were isolated from Sprague Dawley rats. They were then cultured for 15 days in complete Dulbecco’s Modified Eagle Medium (DMEM), during which the media was changed every 3 days to remove unattached floating hematopoietic cells. Inverted phase contrast microscope was used for morphological detection of BM-MSCs. Immunocytochemistry was also done to confirm BM-MSCs characteristics based on specific protein markers (positive markers: CD44 and CD117; negative marker: CD34) and secondary antibody IgG Alexa Fluor, visualized by fluorescence microscope. Subsequently, 10-15 days separate treatments of synthetic demethylating agents, 5-azacytidine and zebularine, were conducted to induce differentiation of BM-MSCs into cardiomyocyte-like cells. Acquired mixed bone marrow cells of hematopoietic and mesenchymal cells were seen morphologically roundish: spherical in shape at the initial culturing. Progressively, more BM-MSCs displaying spindle-shaped fibroblast morphology were observed. Immunostained cells successfully confirmed these BM-MSCs. The separate treatments by selected inducers resulted in morphologically cardiomyocyte-like cells: each having extended cytoplasmic processes and a myotube-like structure. It can be concluded that the protocols used could successfully develop BM-MSCs to cardiomyocyte-like cells.

Keywords: Bone Marrow, Stem Cell, Cardiomyocyte-like Cell

INTRODUCTION
Stem cells are the undifferentiated and unspecialized cells, which not only have the abilities for self-renewal, but also to differentiate into specific cells of various lineages [1, 2]. There are two types of stem cells: embryonic stem cells (ESC) and adult stem cells. If the differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies with lesser ethical issues than using ESCs [3, 4]. Bone marrow provides a renewable source of adult mesenchymal stem cells (MSCs) that can be expanded readily in culture. The culture-expanded and characterized MSCs have been actively studied for their potential to differentiate into several lineages in vitro and enhance tissue repair in vivo [5, 6]. Since the possibility of MSCs to differentiate into myogenic cells was first reported by Wakitani and associates [7], the transplantation of cultured cardiomyocytes into damaged myocardium has been proposed as a potential future therapy for heart treatment [8, 9]. Myocardial cell transplantation research has generated significant interest since myocardial infarction is a leading cause of morbidity and mortality in civilized countries. Adult cardiac muscle unfortunately lacks the ability to regenerate damaged region and post-infarction function is often seriously compromised [10]. The current study attempted in vitro protocols to isolate, culture, expand and induce bone marrow mesenchymal stem cells (BM-MSCs) using two demethylating agents into cardiomyocyte-like cells.

MATERIALS & METHODS
Mixed hematopoietic and mesenchymal bone marrow cells were isolated from tibia and femur of Sprague Dawley rats. They were then cultured for 10-15 days in complete Dulbecco’s Modified Eagle Medium (DMEM), during which the media was changed every 3 days to remove unattached floating hematopoietic cells. Cultures were maintained in a humidified environment with 5% CO2 at 37°C using CO2 incubator. This culture is termed as the primary culture (Passage 0 or P0 cells). This primary culture contained both hematopoietic and BM-MSCs, with the later having the ability to adhere on to the plastic surface of the flask. Non-adherent hematopoietic cells were removed by changing the culture medium. Inverted

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