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A comparison of distinct bone marrow-derived cells on cartilage tissue engineering

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ABSTRACT

Repair of articular cartilage damage has been a great clinical issue due to the difficulties of heal and regeneration once cartilage is damaged. Tissue engineering has emerged as a promising trend for cartilage repair. However, there are some limitations to cartilage tissue engineering, and one of them is the cell source. Using various cell sources for cartilage repair or tissue engineering would lead to distinct outcomes. Therefore, the effect of utilizing diverse bone marrow-derived cell source including bone marrow concentrate (BMC) and bone marrow-derived mesenchymal stem cells (BMMSCs) for cartilage tissue engineering was investigated in this study. The biological constructs containing BMC/BMMSCs and articular tissue fragments were examined *in vitro*. Scanning electron microscopic images revealed the cells in the constructs with BMC grew and attached into tissue fragments well. Histological results displayed neotissue formations with positive Alcian blue staining in the BMC-articular tissue fragment constructs. Moreover, the gene expression of type II collagen in the constructs with BMC was higher than ones with BMMSCs after 28 and 42 days of culture. Our results demonstrated the biological constructs containing BMC and articular fragments contributed better chondrogenesis. BMC would be a potential candidate of cell source for cartilage tissue engineering and cartilage repair.

Introduction

Articular cartilage is a highly compressive resistant tissue that covers the surfaces at the ends of long bones [1,2]. It plays an important role in load transmission, shock absorption, and maintenance of the functional integrity of the joint. Within the synovial joint, articular cartilage could lubricate the surface to promote frictionless movements. However, unlike other connective tissues, articular cartilage itself lacks blood supply to support tissue repair and remodeling once damaged [3,4]. This contributes to a limited capacity for self-repair and regeneration.

Cartilage injures may be caused by trauma, biomechanical imbalance, or degenerative changes of joint, and may lead to progressive damage and degeneration such as osteoarthritis (OA). Microfracture and subchondral drilling that are common treatments are invasive, resulting in damaging subchondral bone and a poor biomechanical repair [5–7]. Tissue engineering has emerged as a potential method for cartilage repair and regeneration. However, one of the major challenges is cell source. Recent studies have suggested that different cell sources or types give rise to various outcomes of cartilage repair, thus it will be critical to select appropriate cell source in order to develop a better treatment for cartilage repair.

Autologous chondrocytes were the first cells used clinically. However, mature chondrocytes do not readily proliferate and easily lose their phenotypes during *in vitro* expansion [8,9]. Mesenchymal stem cells (MSCs) have become an alternative source for cartilage repair, our previous study has shown that MSCs have ability to differentiate into chondrocytes in a single-step procedure [10] and accumulating researches have demonstrated that MSCs indeed can

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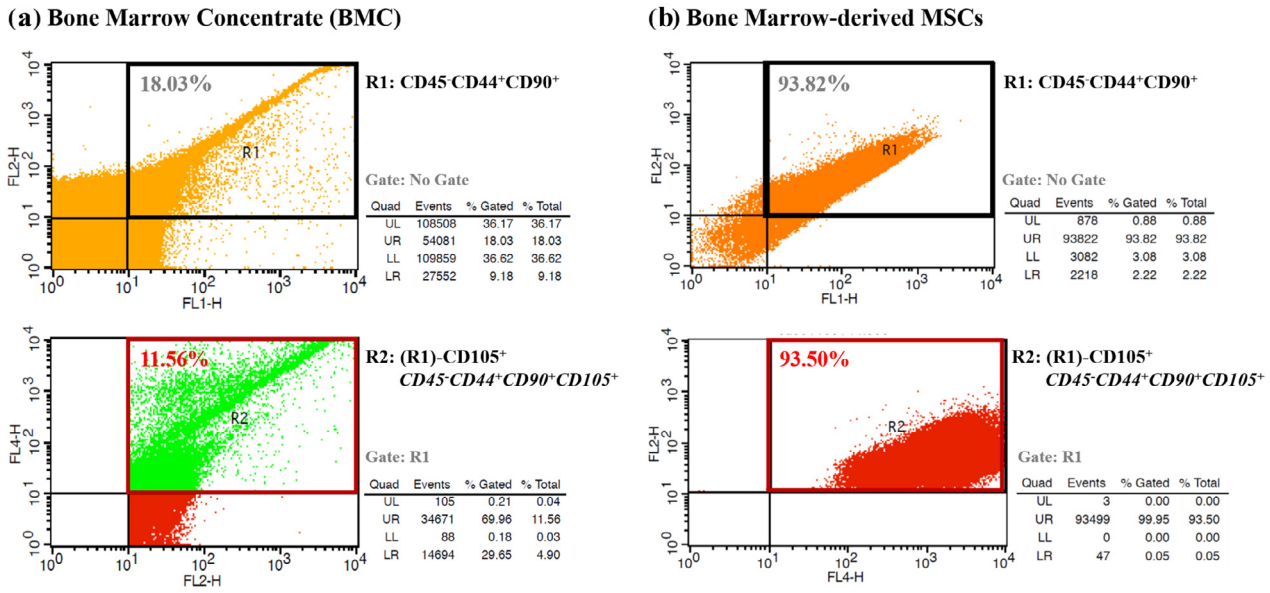

Fig. 1. Characteristics of the cells. (a) Surface marker expression of fresh bone marrow cells. (b) Surface marker expression of purified BMMSCs after 4 passages.

be applied in cartilage repair [11–14]. Bone marrow concentrate (BMC), which contains MSCs, is an alternative cell source for cartilage repair. Following bone marrow aspiration, BMC is easily obtained after centrifugation and is available for a minimal manipulation of cells on the same day [15]. However, the role of BMC in treating cartilage diseases should be further investigated and explored the feasibility in cartilage tissue engineering.

Our previous works have developed a biological construct containing MSCs and articular tissue fragments to promote chondrogenesis for cartilage repair [10], and provided an *in vitro* culture procedure to assess the feasibility of biological constructs prepared with different cell types for cartilage tissue engineering. In this study, we explored the effect of using distinct bone marrow-derived cell sources including BMC and purified MSCs for cartilage tissue engineering.

2. Materials and methods

2.1. Isolation of bone marrow concentrate and bone marrow-derived mesenchymal stem cells

Bone marrow aspirates were harvested from the iliac crest of miniature pigs. All procedures were approved by the Animal Care and Use Committee of Yuanpei University of Medical Technology (No. 101,001). The bone marrow aspirates were then filtered through a 70-micron nylon mesh for further isolation. The gradient was prepared by adding 15 ml of a 17.5% sucrose solution into a 50-mL polystyrene conical centrifugation tube according to previous studies [16,17]. 10 ml of bone marrow was carefully overlaid onto the sucrose solution. Rapid isolation of buffy coat was followed by centrifugation of the gradient solution at 1500 rpm for 5 min. Finally, the bone marrow concentrate (BMC) containing MSCs was collected.

The BMC was further centrifuged and the supernatant was then discarded to obtain the cell pellet. The cells were plated in 100-mm dishes in Dulbecco's modified Eagle's medium containing 1 g/l of glucose (DMEM-LG) (Hyclone Co., Logan, UT, USA), 100 U/ml penicillin, 10 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 10% fetal bovine serum (FBS). They were then grown in an incubator at 37 °C under 5% CO_2 atmosphere. The medium was changed 7 days later, and subsequently was changed twice a week. Monolayer cells were subcultured by trypsinization and subculture with a 1:3 split every week. Cells were used in the study before passaging into the 4th generation. Finally, bone marrow-derived MSCs (BMMSCs) were obtained.

2.2. Characteristics of bone marrow concentrate and bone marrow-derived mesenchymal stem cells

The phenotypic characteristics of BMC and BMMSCs were further assessed by flow cytometric analysis of specific surface antigens. Tested markers included CD44, CD45, CD90, and CD105 (Novus Biologicals). The cells were washed with DPBS and stained with markers for 1 h on ice. The samples were washed and then analyzed by FACScalibur flow cytometer (Becton Dickinson).

2.3. Harvest of cartilage fragments and synovial tissues

Cartilage and synovial tissues were harvested from porcine knee purchased at a traditional market. Briefly, the tissues were washed with DPBS solution several times and minced into small fragments.

2.4. Preparation of biological constructs and in vitro *culture*

Two constructs of distinct cell types including BMC and BMMSCs were prepared and tested in this study. In brief, the cells containing 5×10^5 tested cells, 60 mg of cartilage fragments, and 60 mg of synovial tissues were mixed together. After centrifugation, the supernatant was discarded and a Cell-Cartilage-Synovium mixture was obtained. The mixture was then wrapped into 0.2 ml fibrin gel and allowed to form into a construct in a 48-well plate. Finally, the Cell (BMC or BMMSC)-Cartilage-Synovium construct was acquired.

The formed biological constructs were divided into 2 groups according to cell type: (1) Group 1: BMC–Cartilage–Synovium constructs, and (2) Group 2: BMMSC–Cartilage–Synovium constructs. All the constructs were cultured in MEM-α containing 10% fetal bovine serum (FBS; Gibco) without growth factors on 24-well plates, with the medium changed every 3 days.

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