Research Article

MESENCHYMAL STEM CELL CHARACTERIZATION FROM MEMBRANE SYNOVIAL OSTEOARTHRITIS GRADE IV PATIENT

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ABSTRACT

The synovial membrane is the membrane that lines the synovial jointing. Stem cells can be obtained from the synovial membrane of osteoarthritis grade IV patients who perform joint replacement surgery. The aim of this study was to determine the characterization, differentiation and synovial membranes - Mesenchymal stem cells (SM-MSCs). Characterization was determined using Flowcytometry and differentiation capabilities into three cells according to the boundary of the bond. Stem cells isolated from the synovial membrane of osteoarthritis class IV verse 4 (P4) patients show potential differentiation into adipogenic, chondrogenic, and osteogenic lineage cells. The results also showed that SM-MSC isolated from OA patients was successfully characterized and had three basic criteria to be defined as mesenchymal stem cells (MSC).

Keyword: Characterization, differentiation, Synovial Membrane Mesenchymal Stem Cells

INTRODUCTION

Osteoarthritis (OA) is a joint condition indicates mild inflammation obtained from the tip of the bone. This condition consists primary osteoarthritis or degenerative arthritis and secondary osteoarthritis caused by tropism trauma or injury. OA is commonly suffered by patients published in the supporting parts of the body ¹.

OA therapy generally aims to regenerate articular cartilage. Clinical action is usually based on the main symptoms that focus on reducing pain from inflammation and the use of no steroidal anti-inflammatory drugs (NSAIDs) or total knee replacement ². The use of NSAIDs for long time has negative effect such as kidney disorders, digestive and cardiovascular disease ^{3,4}.

Synovial membranes are believed to have high regeneration abilities and play an important role in the process of tissue repair around the joints. thus, the researchers made the synovial membrane as a cell source for this study, in addition to utilizing medical waste, as well as being a source of stem cells ⁵.

Stem cells can be used in tissue regeneration, they have the ability to self-renew and differentiate as during embryonic stages, where cells can multiply into certain tissues or organs during the development process⁶. Mesenchymal stem cell (MSC) can be differentiated into adipocytes, chondrocytes and osteocytes ^{7,8}. MSC can be isolated from various tissues such as bone marrow (BM-MSC), adipose tissue (ASCs), umbilical cord, Wharton jelly, synovial tissue, and other tissues^{9,10}.

MSCs isolated from synovial membranes have the ability to differentiate into chondrocytes which are very suitable for cartilage regeneration therapy. MSCs can coalesce with cartilage defects, multiply, regenerate articular cartilage, reduce synovial fluid concentrations from prostaglandins¹¹ and reduce the progressive nature of OA¹². Therefore, research on membrane synovial potential as a mesenchymal source of OA patients can be used as an autologous therapy for OA patients. The purpose of this study was to determine the characterization, differentiation of passage 4 (P4) membrane synovial mesenchymal stem cell isolated from patients with osteoarthritis.

MATERIALS AND METHOD

This research has been out ethical feasibility test by the Research Ethics Commission of the Faculty of Medicine of Andalas University with an ethical certificate number 227/KEP/FK/2019.

MSCs Isolation from Synovial Membrane Osteo Arthritis grade IV patients

Synovial membrane was obtained from the knee in patients with osteoarthritis in Dr. M. Djamil Hospital, Padang, West Sumatera, Indonesia. Synovial membranes then washed using phosphate buffer saline (PBS) (Gibco, 14200075), 1% antibiotic and antimycotic (ABAM) (Gibco, 15240062) and 1% Amphotericin B (Gibco, 15290026). Synovial membrane cut into small pieces and cleansed from the fat and skin tissue. The synovial membrane placed into a 50 ml centrifuge tube (SPL 50015) containing

Collagenase enzyme type I (Gibco, 17100017), Hyaluronidase (Sigma, H-3506) and Trypsin-EDTA 0.1% (Gibco, 25200072). The tube stored in CO₂ resistant shaker (Thermo, SHKE2000CO2) at 300 rpm for 16 hours in the incubator (Thermo, 8000DH) of 5% CO₂ and 37°C. The remaining synovial membrane was removed, and the supernatant was centrifuged (MPW-260R) at 1600 rpm for 5 min. The supernatant was removed, and the pellet was mixed with complete medium: Mem- α (Gibco, 10950080) which supplemented by 10% Fetal Bovine Serum (FBS) (Gibco, 10270106), and 1% antibiotic-antimycotic (ABAM) (Gibco, 15240062), 1% Amphotericin B (Gibco, 15290026) addition. SM-MSCs was cultured at density of 2 x 10⁶ cells in T-flask 25cm³ (TPP, 90026). The cells were incubated on T25 Flask (TPP, 90026) at 5% CO₂ and 37°C incubator. The experiment was approved by the Local Ethic Committee (number of ethical clearance 227/KEP/FK/2019)

SM-MSCs CD Markers Characterization

SM-MSCs that reached 80% confluency were harvested to analyze the positive and negative CD markers using flowcytometry (Macsquant, Analyzer 10). The cells were stained with hMSCs analysis kit components (CD90 FIT C, CD105 PerCP-Cy5, CD73 APC, CD34 PE, CD116 PE, CD19 PE, CD45 PE, HLA-DR PE and CD44 PE) (BD stem flowTMkit, 562245) and the procedure was according to the manufacture protocol. The experiments and measurement were performed in triplicate^{13,14}.

Differentiation of SM-MSCs

In osteogenic differentiation, SM-MSCs were seeded at density 5 x 10³ cells in 24 well plate (Nunc, 72296-18) using StemPro

Osteogenesis Differentiation Kit (Gibco A10072-01) for 3 weeks. Calcium deposits as the osteogenic differentiation marker were visualized using Alizarin Red S (Sigma, A5533). For chondrogenic differentiation, SM-MSCs were seeded at density 5 x 10³ cells in 4 well plate (Nunc, 176740) using StemPro Chondrogenesis Differentiation Kit (Gibco A10071-01) for 3 weeks. Glycosaminoglycan as the chondrogenic differentiation marker were visualized using Alcian Blue (Sigma, A5268). Adipogenic differentiation of SM-MSCs was performed by StemPro Adipogenesis Differentiation Kit (Gibco A10070-01) for 3 weeks. Oil Red O staining was used (Sigma, 00625) to confirm lipid droplets in the culture as the marker of adipogenic differentiation.

RESULTS AND DISCUSSION

Isolation mesenchymal stem cell from membrane synovial Osteoarthritis grade IV

There are 3 basic criteria of the cells to be define as MSCs based on the International Society for Cellular Therapy, such as: 1) have the adherent type of culture, 2) have positive expression for several CD markers such as CD73, CD90, CD44 and CD105 and showed negative expression for CD14, CD34, CD45 and Human Leucocyte Antigen (HLA-DR), 3) have differentiation capability into 3 different lineage cells, osteocyte, chondrocyte, and adipocyte. The culture of SM-MSCs at 4th passage as the result of cells isolation from knee-joint of OA patients showed in Figure 1. SM-MSCs exhibited adherent type of culture, elongated and resulted in spindle-shaped cells. This result indicates that the cells isolated from the synovial membrane of OA patients have conformed with the first basic criteria of MSCs.

Figure 1. light microscopy of a heterogenous population of MSCs isolated from membrane synovial a) stem cell after isolation (100x), b) synovial membrane (SM) (100x), seven days of cultivation

The second criteria for MSCs is the positive and negative expression of specific surface markers owned by the cells. MSCs naturally have positive surface marker of CD90, CD73 and CD105 and negative surface marker of CD45, CD34, CD11b, CD19, and HLA-DR. Several studies also confirmed that MSCs have positive surface marker of CD44. As in the Table 1. and Fig. 2, the cells isolated from synovial membrane of OA patients at 4th passage was showed positive for CD90, CD44, CD105, and CD73, and negative expression for negative lineage such as CD34, CD45, CD11b, CD19 and HLA-DR. Hence, the cells isolated from synovial membrane of OA patients also conformed with the second basic criteria of MSCs. The two different passages, 4th passage of the cells showed no difference CD markers expression. Both passage-4 were detected to have the same level expression of certain positive and negative surface markers.

Table 1. Antigen surface markers of synovial membrane mesenchymal stem cells

Passage	CD90	CD44	CD105	CD73	Negative Lineage
P4	92,52 $\pm$ 0,15	87,02 $\pm$ 0,58	98,34 $\pm$ 0,02	95,15 $\pm$ 0,59	0,42 $\pm$ 0,18

Data are presented as mean $\pm$ standard deviation of surface markers of synovial membrane mesenchymal stem cell. Negative lineage marker containing CD45, CD34, CD11b, CD19, HLA-DR.

Figure 2. Analysis CD markers expression on SM-MSCs P4 by flow cytometer. SM-MSCs was confirmed as MSCs because exhibited high expression for positive marker, CD73=95.15%; CD90=92.52%; CD105=98.34%, and low expression of negative lineage (0.72%)

This result has the same finding with the previous research that the stem cells isolated from different sources was also exhibited high expression of CD90, CD44, CD105, CD73 and low expression of CD34, CD45, CD14, CD19, and HLAII both early (P4)^{24,25,27}. It was also conformable with previous research that MSCs from various sources, including AT-MSCs, had positive markers for CD73, CD90, and CD105 and negative markers of

CD11b or CD14, CD19, CD34, CD45, and HLA-DR in their cell-surface immunophenotyped⁷.

Differentiation of MSCs

The result of SM-MSCs differentiation into 3 different types of cells such as adipocyte, chondrocyte and osteocyte have shown in Table 2.

Table 2. Qualitative analysis of SM-MSCs differentiation at passage 4 by Alcian Blue, Alizarin Red S, and Oil Red O staining for chondrogenic differentiation, osteogenic differentiation, and adipogenic differentiation, respectively.

From the differentiation analysis result after 21 days of incubation, the cells that isolated from synovial membrane showed the ability to differentiate into 3 different lineages (adipocytes, osteocytes and chondrocytes) of cells as the third basic criteria to define MSCs. There was no difference result of differentiation between passage 4 as indicates from the qualitative staining result. Osteogenesis was confirmed by the accumulation of calcium deposit which detected by alizarin red S, and the positive result showed by the red color. Alcian blue staining is specific polyvalent dye that is used to identify acidic polysaccharides such as glycosaminoglycans as well as the marker of chondrogenesis. As seen at the table, the cells were associated in the process of differentiation and stained as blackish blue. Oil droplet formation is one of adipogenic differentiation marker and was stained by Oil Red O. Oil Red O is a diazo dye that is used to stain the triglycerides and lipid formation^{7,13,14}. According to these results, the cells isolated from the synovial membrane was considered as MSCs because exhibited 3 basic criteria to be defined as MSCs, adherent to the plastic substrates, positive and negative expression to the certain CD markers, and be able to differentiate into 3 types of cells. Therefore, the method used in the cells isolation has successfully used to produce SM-MSCs and considered as the first step of application in stem cell mediated therapy for OA patients.

CONCLUSION

This study showed the characterization mesenchymal stem cell. The cells isolated from synovial membrane of OA patients are considered as MSCs because exhibited the 3 basic criteria for MSCs. SM-MSCs in this study are adherent to the plastic substrates, have positive surface markers (CD90, CD44, CD105 and CD73) and negative surface markers (CD45, CD34, CD11b, CD19, HLA-DR) expression, and capable to differentiate into adipocyte, chondrocytes and osteocyte.

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