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Original Article

Optimization of human mesenchymal stem cell isolation from synovial membrane: Implications for subsequent tissue engineering effectiveness

Norihiko Sugita [a], Yu Moriguchi [a], Morito Sakaue [a], David A. Hart [b], Yukihiko Yasui [a], Kota Koizumi [a], Ryota Chijimatsu [a], Syoichi Shimomura [a], Yasutoshi Ikeda [a], Hideki Yoshikawa [a], Norimasa Nakamura [a, *]

[a] Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita 565-0871, Japan
[b] McCaig Institute for Bone & Joint Health, University of Calgary, 3330 Hospital Drive Northwest, Calgary, Alberta T2N 4N1, Canada

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ABSTRACT

Synovium-derived mesenchymal stem cells (SDMSCs) are one of the most suitable sources for cartilage repair because of their chondrogenic and proliferative capacity. However, the isolation methods for SDMSCs have not been extensively characterized. Thus, our aim in this study was to optimize the processes of enzymatic isolation followed by culture expansion in order to increase the number of SDMSCs obtained from the original tissue. Human synovium obtained from 18 donors (1.5 g/donor) was divided into three aliquots. The samples were minced and subjected to collagenase digestion, followed by different procedures: Group 1, Tissue fragments were removed by filtering followed by removing floating tissue; Group 2, No filtering. Only floating fragments were removed; Group 3, No fragments were removed. Subsequently, each aliquot was sub-divided into two density subgroups with half. In Group 1, the cell-containing media was plated either at high (5000 cells/cm^2) or low density (1000 cells/cm^2). In Groups 2 and 3, the media containing cells and tissue was plated onto the same number of culture dishes as used in Group 1, either at high or low density. At every passage, the cells plated at high density were consistently re-plated at high and those plated at low density were likewise. The expanded cell yields at day 21 following cell isolation were calculated. These cell populations were then evaluated for their osteogenic, adipogenic, and chondrogenic differentiation capabilities. The final cell yields per 0.25 g tissue in Group 1 were similar at high and low density, while those in Groups 2 and 3 exhibited higher when cultured at low density. The cell yields at low density were $0.7 \pm 1.2 \times 10^7$ in Group 1, $5.7 \pm 1.1 \times 10^7$ in Group 2, $4.3 \pm 1.2 \times 10^7$ in Group 3 (Group 1 vs Groups 2 and 3, $p < 0.05$). In addition, the cells obtained in each low density subgroup exhibited equivalent osteogenic, adipogenic, and chondrogenic differentiation. Thus, it was evident that filtering leads to a loss of cells and does not affect the differentiation capacities. In conclusion, exclusion of a filtering procedure could contribute to obtain higher number of SDMSCs from synovial membrane without losing differentiation capacities.

1. Introduction

Mesenchymal stem cells (MSCs) can be isolated from various tissues and have the potential to self-renew and differentiate into multiple lineages such as osteogenic [1], chondrogenic [2], adipogenic [3,4], myogenic [5] and neurogenic [6] specificities. Among the MSC sources, synovium-derived mesenchymal stem cells (SDMSCs) have been demonstrated to exhibit superior chondrogenic and proliferation potentials compared to MSCs derived from other tissue [7–9]. A considerable number of studies of cartilage repair have been conducted using SDMSCs with promising results [10–13]. For successful cell-based therapy, securing a sufficient number of cells is critical. It depends on the delivery method, specific to our "scaffold-

* Corresponding author.
E-mail address: norimasa.nakamura@ohsu.ac.jp (N. Nakamura).
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free tissue engineered construct" procedure [14], we need in average 1.3×10^8 cells for treatment cartilage defect and quality inspection in our clinical trial (UMIN000008266), [15]. However, optimized methods for culturing SDMSCs, including the isolation from synovial membranes have not been fully characterized. Most of the previous studies on the culture of SDMSCs reported the isolation of cells using a standard collagenase digestion followed by filtering to remove debris [7–13,16–23] before starting the primary culture. However, there may be a potential loss of additional MSCs in the filter-trapped undigested tissue fragments. We hypothesized that the use of undigested tissue fragments would lead to increases in the number of SDMSCs available from the original synovial tissue. Moreover, it was previously reported that plating density can influence the proliferation of MSCs [8,24]. Taken together, it was important to quantify how the filtering process, as well as the plating density of cells thereafter could affect the number of SDMSCs obtained within a clinically relevant duration of cell culture.

In the present study we aimed to maximize the yield of cultured human SDMSCs starting from equivalent weights of synovial membrane material. The results presented indicate that both the MSC isolation method, as well as the propagation density, significantly influences the assessed outcomes.

2. Materials and methods

2.1. Harvest of synovial membrane and isolation of cells

Our study protocol was approved by the institutional committee for medical ethics. Written informed consent was obtained from all patients. Human synovial membranes were obtained (1.5 g per patient) from 18 patients (10 male and 8 female donors; mean age, 25.5; range 16–48 years: Table 1) during arthroscopic surgery. Synovial tissues from each donor were divided into three aliquots (0.5 g each) and meticulously minced using surgical scissors. The minced tissues were then digested in a collagenase solution [440 u/ml collagenase A, Type AFA (Worthington Biochemical Corporation, Lakewood, NJ, USA)] in growth medium containing high-glucose Dulbecco's Modified Eagle Medium (DMEM, Wako Chemical Corp., Osaka, Japan), supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich, St. Louis, MO, USA) and 1% antibiotic–antimycotic solution (Sigma–Aldrich) according to the previously established protocol [12,16,25]. Specifically, we used the same, animal origin free, collagenase at the same concentration as we used in our clinical trial (UMIN000008266) according to the manufacturer's instruction. Following 3 or 16 h of incubation, undigested tissues were removed from the cell-containing liquid with a 70-μm nylon filter (BD Falcon, Franklin Lakes, NJ, USA) followed by centrifugation (1500 rpm for 5 min). Subsequently, the floating undigested tissue fragments were removed (Group 1). In Group 2, No filtering was performed but floating undigested tissue fragments were removed after centrifugation. In Group 3, No filtering and the floating undigested tissue fragments were not removed, and all components used for the subsequent cell culture. Therefore, Group 1 contained only cells; Group 2 contained cells and the precipitated undigested tissue fragments; while Group 3 contained cells plus both the precipitated and floating undigested tissue fragments (Fig. 1). In each group, all the contents were re-suspended in 10 ml of complete media.

For microscopic observation, cell-containing media (10 μl) from Groups 1–3 were applied onto cell counter plates for analysis.

DNA was extracted from the cell-containing liquid (100 μl) after collagenase digestion in all groups with a DNeasy Blood & Tissue Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Briefly, cells and small tissues were digested with lysis buffer and proteinase K, DNA was purified, and the DNA content was quantified in a spectrophotometer. Total DNA content per 0.5 g tissue was calculated according to the ratio of sampling volume (100 μl) to the total volume (10 ml) of the cell-containing suspension.

2.2. Plating and subsequent primary cell culture

Each aliquot (Groups 1–3) from individual donors was further divided into two subgroups (5 ml each) for subsequent plating at two different cell densities.

For Group 1, the cells were plated at high (5000 cells/cm^2) or low density (1000 cells/cm^2). For Groups 2 and 3, media containing cells and undigested tissues was plated onto the same number of culture dishes used in Group 1, either at high or low density. Cells were cultured in the growth medium containing 10% FBS at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The medium was replaced every 4 days.

2.3. In vitro expansion of cell populations

The cultured cells were subjected to passaging when reaching 80% confluency. Cells were harvested by treatment with trypsin–EDTA (0.25% trypsin and 1 mM EDTA; Gibco BRL, Life

Table 1
Synovial samples used in this study.

Sample number	Age	Sex	Diagnosis
1	16	Male	Anterior cruciate ligament injury
2	30	Male	Anterior cruciate ligament injury
3	23	Male	Synovitis (After anterior cruciate ligament reconstruction)
4	35	Female	Synovitis (After anterior cruciate ligament reconstruction)
5	30	Male	Anterior cruciate ligament injury
6	23	Male	Anterior cruciate ligament injury
7	44	Female	Anterior cruciate ligament injury
8	16	Male	Osteochondromatosis
9	20	Female	Anterior cruciate ligament injury
10	18	Female	Anterior cruciate ligament injury
11	44	Male	Anterior cruciate ligament injury
12	19	Male	Anterior cruciate ligament injury
13	23	Female	Anterior cruciate ligament injury
14	16	Male	Anterior cruciate ligament injury
15	17	Male	Meniscal injury
16	18	Female	Anterior cruciate ligament injury
17	19	Female	Meniscal injury
18	48	Female	Anterior cruciate ligament injury