J Appl Biotechnol Rep. 2024 June;11(2):1301-1312

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2024.449555.1708

Original Article

A New Xeno-free Medium for Human Mesenchymal Stem Cell Expansion *ex vivo*

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Received March 26, 2024; **Accepted** June 22, 2024; **Online Published** June 20, 2024

Abstract

Introduction: An optimal culture medium that can rapidly and efficiently proliferate cells *ex vivo* is very crucial for developing mesenchymal stem cells (MSCs)-based tissue engineering and regenerative medicine. We developed a set of MSCs *ex vivo* proliferation medium, mscGO™ XF, which consists of a basal medium and a screened human platelet lysate.

Materials and Methods: In this study, the developed mscGO™ XF medium was prepared, and then testified by human MSCs isolated from bone marrow, umbilical cord, and fat tissue. The proliferation, surface markers, differentiation, and chromosomal stability of MSCs cultured in mscGO™ XF medium were investigated.

Results: The mscGO™ XF medium could sustain MSCs at a high proliferation rate, with the population doubling time of 16 to 39 hours (depending on the type and passage number of MSCs). The proliferated MSCs could express CD105, CD90, and CD73, lack expression of CD34 and CD45; and maintain the capacity to differentiate into adipocytes, osteoblasts, and chondrocytes. Additionally, G-Band karyotyping data confirmed chromosome stability in the duration of cell culture at passage 5 and passage 7.

Conclusions: The mscGO™ XF medium could sustain MSCs proliferation *ex vivo* and exhibit the potential to be developed into a clinical-grade cell culture medium kit.

Keywords: Xeno-free Medium, Mesenchymal Stem Cells, Cell Proliferation, Cell Differentiation

Citation: Lee DC, Wang MC, Yu WL, Wu JY, Shiu HC, Cheung CS, et al. A New Xeno-free Medium for Human Mesenchymal Stem Cell Expansion *ex vivo*. J Appl Biotechnol Rep. 2024;11(2):1301-1312. doi:10.30491/JABR.2024.449555.1708

Introduction

Mesenchymal stem cells or marrow stromal cells (MSCs) are multipotent stem cells that exist primarily in bone marrow but have also been reported to be isolated from other tissues and hold great therapeutic potential for cell-based tissue engineering and regenerative medicine due to their easy isolation, multipotency, paracrine activity, and immunomodulatory properties.[1-5] In general, MSCs are characterized by three criteria: adherence to plastic; expression of specific surface markers: CD105, CD90, and CD73, and the lack of expression of CD34 and CD45; and maintaining differentiation capacity to adipocytes, osteoblasts, and chondroblasts *ex vivo*.[6] With the increasing number of clinical applications for MSCs, it is essential to ensure an adequate supply of MSCs for therapeutic purposes. The number of MSCs required for clinical applications is far exceed than those that can be isolated from the tissue itself. Therefore, identifying the optimal cell culture condition for expanding MSCs *ex vivo* is crucial.[2,7,8]

Culture conditions vary widely for each cell type including complete medium compositions, substrates, cell seeding density, and physicochemical environments, such as dissolved O_2 and CO_2 concentrations, pH, and temperature.[9] The culture medium is crucial because it provides the necessary nutrients, growth factors, and hormones for cell growth, and regulates the pH and the osmotic pressure of the culture. In general, the complete culture medium consists of a basal medium and nutrient supplements such as serum, especially, fetal bovine serum (FBS) remains the most commonly used.[9-12] However, clinical use of MSCs proliferated with FBS-containing medium raises safety and regulatory concerns. Therefore, the current trend is to use the derivatives of autologous or allogeneic human blood, such as human platelet lysate (hPL) to meet clinical applications.[13-15]

Recent studies have revealed that hPL could be used as a

nutrient supplement to sustain MSC proliferation in vitro, and the concentrations of growth factors in hPL are significantly higher than those in FBS. This may indicate that hPL supports higher cell expansion than FBS. Although hPL also has similar concerns as FBS, such as undefined ingredients and batch-to-batch variation; allogeneic immune-logical reactions, and danger of transmission of human diseases by viruses, such as human immunodeficiency virus. Under eligibility criteria and GMP production, hPL is considered a good xenogenic-free supplement for cell culture medium.[12-15]

To efficiently expand qualified MSCs *ex vivo*, we developed the mscGO™ XF medium kit. The mscGO™ XF medium is a xeno-free, user-friendly, and ready-to-use medium, composed of a basal medium and screened hPL. In this study, we evaluated and demonstrated the performance of the mscGO™ XF medium kit on three major MSCs, including adipose-derived mesenchymal stem cells (ADSCs), bone marrow mesenchymal stem cells (BM-MSCs), and umbilical cord mesenchymal stem cells (UC-MSCs). The mscGO™ XF medium could sustain MSCs at a high proliferation rate, with a doubling time 16 to 39 hours. The proliferated MSCs could express CD105, CD90, and CD73, and lack expression of CD34 and CD45; and maintain the capacity to differentiate into adipocytes, osteoblasts, and chondrocytes. In the meantime, G-banded karyotyping data confirmed the chromosome stability in the duration of cell culture at passage 5 and passage 7.

Materials and Methods

Development of mscGO™ XF Medium

The complete culture medium consists of a basal medium and nutrient supplements. In developing the basal medium, we researched commonly used basal media from published literatures and compared their ability to promote MSC proliferation in vitro.[16-18] The mscGO™ XF basal medium is derived from a combination of IMDM, MEM alpha, and DMEMHG in a specific ratio, and it is enhanced by insulin, trace elements, and proteins. The concentration of glucose is 4.0 g/L. After confirming the best ingredients, a professional media manufacturer formulated and produced the final product. The manufacturing plant we rely on has ISO9001: 2015 certification (certification number: 117 19 Q0 0039-05 R0M) and ISO13485:2016 certification (certification number: 117 19 Q0M 0046 R0M), and produces products following cGMP guidelines. Each batch of mscGO™ XF basal medium would receive a certificate of analysis (C.O.A.), and an inspection report. The nutrient supplement in mscGO™ XF medium is human platelet lysate (hPL) which is purchased commercially. The manufacturer is a cGMP compliant facility with integrated quality management systems and complies with FDA regulations 21 CFR Part 820. We add hPL to the basal medium in biosafety cabinet class II type A2 (AC2-4S9-NS, ESCO) in a positive pressure (15 pa) room to complete mscGO™ XF medium. We take out some complete medium and place it in a cell incubator for 3 days. After confirming that there is no microbial growth through microscopic examination, we continue to culture for 7 days to ensure no microbial growth.

Cell Culture

Human bone marrow mesenchymal stem cells (BM-MSCs), human adipose-derived mesenchymal stem cells (ADSCs), and human umbilical cord mesenchymal stem cells (UC-MSCs) were obtained from the project: Integration and Value-adding Program for Advanced and Niche Market Medical Devices, directed by the Industrial Technology Research Institute, Taiwan. The isolation and collection of primary cells were approved by the Institutional Review Board of the Industrial Technology Research Institute (ITRI), with IRB No. REC No.: 103-018-F and REC No.: 106-022-F. This study was funded by the Industrial Development Bureau, Ministry of Economic Affairs, Taiwan, under Grant No. 111121329. The protocol for cell isolation and culture was described briefly. For BM-MSCs isolation, 20-40 ml bone marrow collected from donors was subjected to Ficoll (Cat. GE17-1440-02, Sigma-Aldrich, St. Louis, MO, USA) gradient centrifugation in 4 °C. Cells in the buffy coat layer rich in BMSCs were further collected, counted, and plated in the culture dish at a density of $1x10^5$ cells/cm^2 for subsequent colony formation using the complete medium. The medium was replaced with fresh medium every three days until cells reached 80% confluency, labeled as P0.[19] For UC-MSCs isolation, the protocol was modified from Hassan G et al, published paper.[20] A human umbilical cord (UC) was collected in phosphate-buffered saline (PBS) supplemented with 1x antibiotic-antimycotic (CC501-0100, GeneDireX, Inc.) and transported to the laboratory. UC was washed with PBS to remove blood as possible as could, and cut into 5 cm^2 segment. Each segment was cut longitudinally and two arteries and one vein were carefully removed. After that, the residual tissue of the segment was transferred to a 100 mm tissue culture dish with the complete medium. The dishes were left undisturbed for 7 days, after the medium was changed for the first time and then changed every 3 to 4 days. After 2 weeks, the UC explants were removed, and the adherent cells were allowed to expand until they reached about 80% confluence. ADSCs were gift from ITRI, and ITRI purchased ADSC from the American Type Culture Collection (ATCC). In this study, we tested media including mscGO™ XF medium (CC816-0500, GeneDireX, Inc.), conventional medium: MEM alpha (12561056, Thermo Fisher) supplemented with 10% Fetal Bovine Serum, mesenchymal stem cell-qualified (12662002, ThermoFisher). The MSCs were seeded at a density of $1x10^5$ cells in 60 mm

non-coated, tissue culture dishes (PC203-0600, GeneDireX, Inc.) and incubated in a 5% CO_2, 37 °C cell culture incubator for 3 or 4 days, detached using diluted 1X Trypsin-EDTA (CC507-0100, GeneDireX, Inc.). To calculate the cell doubling time, the detached cells were stained with trypan blue and counted using a hemocytometer. The doubling level is calculated: log2 (the number of cells at the end of the proliferation duration divided by the number of cell seeded). The doubling time is calculated: duration of cell proliferation (in hours) divided by the doubling level.

Surface Markers Analysis of MSCs by Flow Cytometry

MSCs were collected for surface markers analysis by flow cytometry.[21,22] The cells were stained with fluorescein isothiocyanate (FITC) conjugated anti-marker monoclonal antibodies: CD34 (11-0349-42, 5 µl/5x10^5 cells), and CD73 (11-0739-42, 5 µl/5x10^5 cells); or Alexa Fluor™ 488 conjugated anti-human CD105 monoclonal antibody (MHCD10520, 5 µl/5x10^5 cells); or R-phycoerythrin (PE) conjugated anti-human CD45 monoclonal antibody (MHCD4504, 5 µl/5x10^5 cells); or APC conjugated anti-human CD90 monoclonal antibody (17-0909-42, 5 µl/5x10^5 cells). The antibodies mentioned were purchased from ThermoFisher. Cells were stained with different antibodies in 100 µl 1X PBS buffer for 30 minutes at room temperature in the dark, washed, and resuspended in 400 µl 1X PBS buffer. Data collection and analysis were performed using Attune NxT (ThermoFisher).

Tri-lineage Differentiation of MSCs

Ex vivo differentiation of proliferated MSCs into adipocytes, osteoblasts, and chondrocytes was achieved using commercial kits: StemPro™ Adipogenesis Differentiation Kit (A1007001), StemPro™ Osteogenesis Differentiation Kit (A1007201), and StemPro™ Chondrogenesis Differentiation Kit (A1007101), respectively. All of these kits were purchased from ThermoFisher. The differentiation protocols were carried out according to the manufacturer's instructions and briefly described below. To prepare a complete differentiation medium, the adipocyte supplement, osteogenesis supplement, and chondrogenesis supplement were thawed in a 37 °C water bath, and 10 ml of each supplement was added to the adipocyte differentiation basal medium, osteocyte differentiation basal medium, and chondrocyte differentiation basal medium, respectively. In adipogenesis and osteogenesis differentiation, MSCs were seeded into a 12 well plate at the seeding density of 1x10^4 cells/cm^2 and 5x10^3 cells/cm^2, respectively. The medium was changed every 3-4 days. After 14 days, oil droplets accumulated in the cytoplasm could be observed in the adipogenesis differentiation. To detect calcium deposition, the culture duration of osteogenesis differentiation is 21 days or longer. For chondrosphere formation, MSCs were trypsinized and prepared into a cell solution of 1x10^7 cells/ml, seeding 10 µl droplet to generate micromass culture in the center of well in a 12 well plate. The plate was left undisturbed for 30 minutes in a 37 °C incubator with 5% CO_2, then complete chondrogenesis medium was added, and the medium was changed every 3-4 days. The differentiated cells were confirmed by cytochemical staining.[21,22] Briefly, in adipocyte differentiation, the cells were stained with Oil Red O reagent (Sigma Aldrich) to examine oil-droplet generation in the cytoplasm. In osteogenic differentiation, calcium accumulation was assessed with an alizarin red stain (Sigma Aldrich). For chondrogenic differentiation, sulfated proteoglycans were assessed by alcian blue stain (Sigma Aldrich).

Chromosomal Stability Analysis by G-banding

To assess the chromosome stability of MSCs proliferated in mscGO™ XF medium, we harvested MSCs that had been proliferated with mscGO™ XF medium at passage 5 and passage 7. We then entrusted Ko's Obstetrics and Gynecology Clinic in Taipei City, Taiwan to conduct G-banded karyotyping.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Student's t-test was used for comparing two groups. Statistical significance was accepted when $p < 0.05$.

Results

Morphology and Proliferation of MSCs in mscGO™ XF Medium and Conventional Medium

We used phase contrast microscopy to examine the morphology of MSCs at different passages. The morphology of ADSCs (Figure 1A and 1B, P5, and P9, respectively) and BM-MSCs (Figure 1C and 1D, P5, and P9, respectively) cultured with mscGO™ XF medium showed an adherent, spindle-shaped, fibroblast-like cell morphology, similar to those cultured in conventional medium: MEM alpha supplemented with 10% MSC qualified fetal bovine serum (ADSCs, Figure 2A and 2B, P5, and P9; BM-MSCs, Figure 2C and 2D, P5, and P9, respectively). In addition to adult MSCs, we also used MSCs isolated from the umbilical cord, UC-MSCs. The morphology of UC-MSCs cultured with mscGO™ XF medium (Figure 1E and 1F, P5, and, P9, respectively) or conventional medium (Figure 2E and 2F, P5, and P9, respectively), showed an adherent, spindle-shaped, fibroblast-like cell morphology. Cell proliferation was determined by recording the cell seeding and proliferating numbers in each passage and calculating the cell doubling time. The doubling time of MSCs from different tissues was calculated and recorded when they proliferated in the mscGO™ XF medium and conventional medium. The result revealed that ADSCs, BM-MSCs, and UC-MSCs exhibited higher cell proliferation activity in mscGO™ XF medium than in conventional medium (Figure

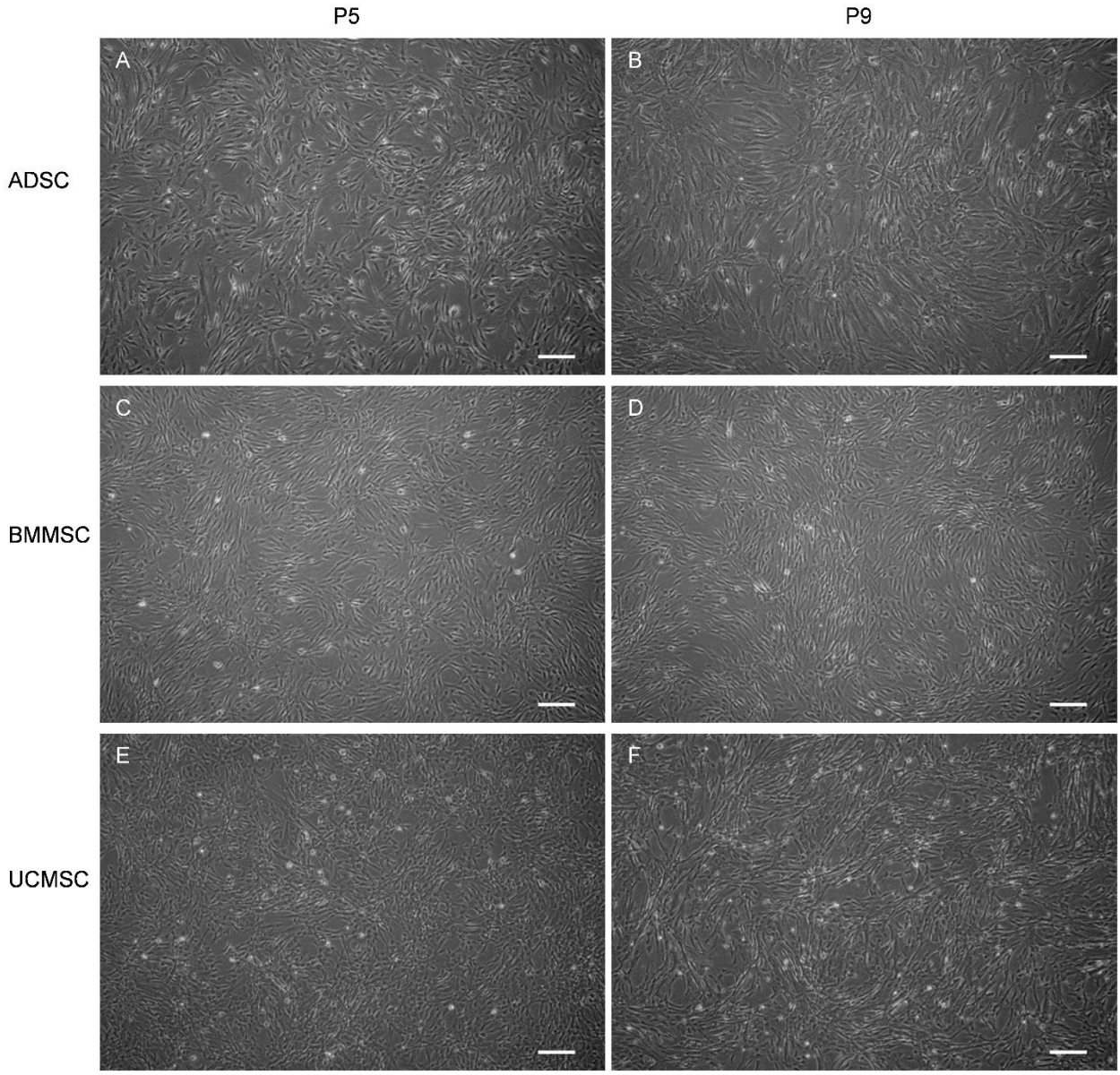


Figure 1. Morphology of Different MSCs in mscGO™ XF Medium at P5 and P9. ADSCs cultured in mscGO™ XF medium at passage 5 (A), and passage 9 (B); BM-MSCs cultured in mscGO™ XF medium at passage 5 (C), and passage 9 (D); UC-MSCs cultured in mscGO™ XF medium at passage 5 (E), and passage 9 (F). Scale bar: 100 µm.

3A, 3B, and 3C, respectively). From passage 3 to passage 5, the average cell doubling time of ADSCs was 25 to 39 hours; while that of BMMSCs and UCMSCs was 19 to 34 hours and 16 to 25 hours, respectively.

Surface Marker Profile of MSCs in mscGO™ XF Medium and Conventional Medium

The surface marker profile had been described to identify MSCs: expression of cluster of differentiation (CD) markers: CD73, CD90, and CD105, and lack expression of CD14, CD34, and CD45. ADSCs, BM-MSCs, and UC-MSCs were propagated in conventional medium and mscGO™ XF medium, and collected for characterization of specific surface markers expression by flow cytometry analysis. The data revealed that all of the MSCs proliferated either conventional medium or mscGO™ XF medium, expressed CD73, CD90, and CD105, but did not express CD34 and CD45 (Figure 4A, ADSC, aMEM, and mscGO™ XF; Figure 4B, BM-MSCs, aMEM, and mscGO™ XF; Figure 4C, UC-MSCs, aMEM, and mscGO™ XF), the quantitative results of the ratio of surface markers of ADSCs, BM-MSCs, and UC-MSCs expanded in conventional or mscGO™ XF medium were shown in Figures 4D, 4E, and 4F, respectively.

Differentiation Potential of MSCs Propagated in mscGO™ XF Medium

One of the biological properties of MSCs is their capability

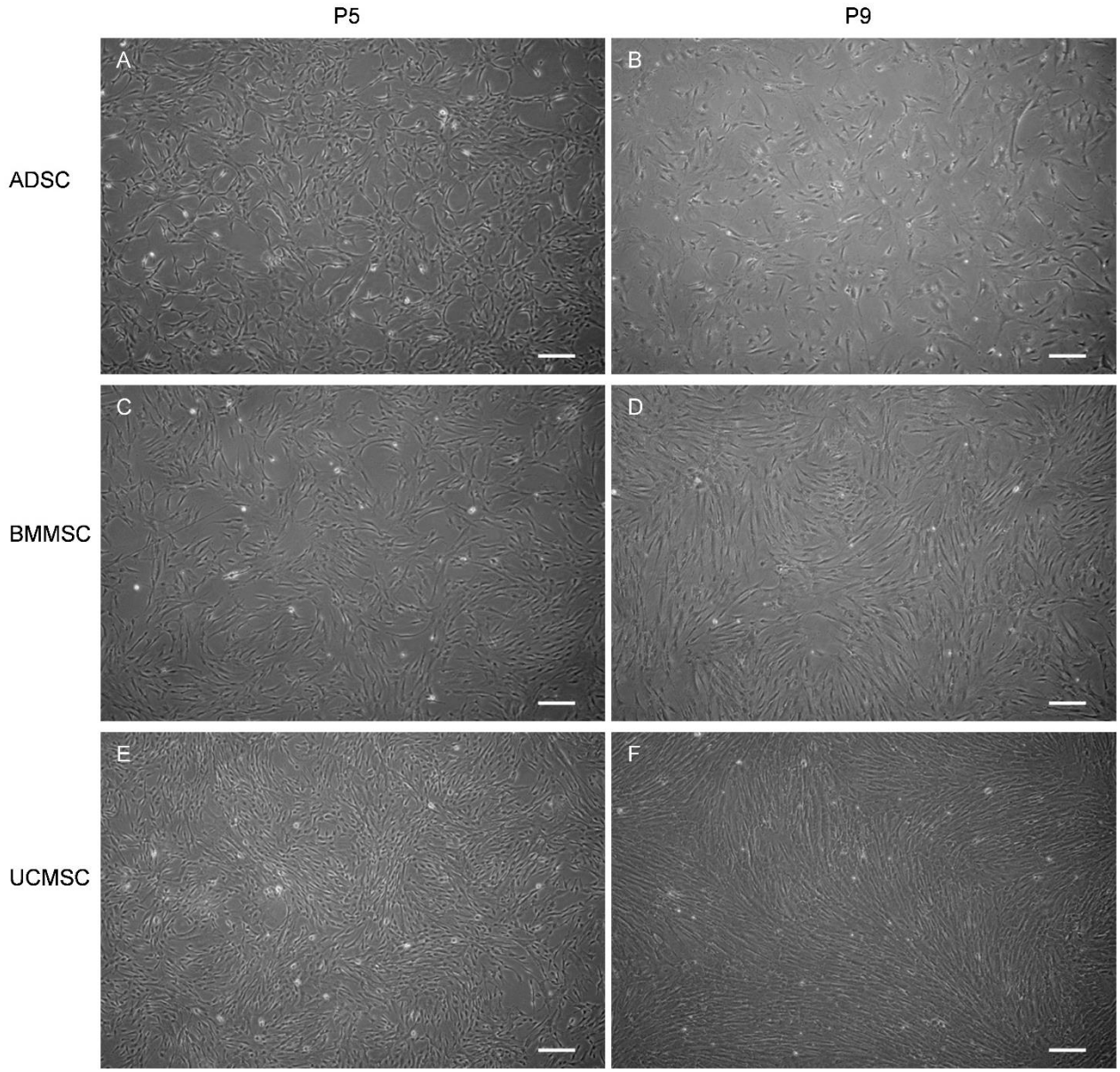


Figure 2. Morphology of Different MSCs in the Conventional Medium at P5 and P9. ADSCs cultured in conventional medium at passage 5 (A), and passage 9 (B); BM-MSCs cultured in conventional medium at passage 5 (C), and passage 9 (D); UC-MSCs cultured in conventional medium at passage 5 (E), and passage 9 (F). The conventional medium is described in material and method as MEM alpha supplemented with 10% MSC-qualified FBS. Scale bar: 100 µm.

for three lineages of mesenchymal differentiation. It means that using lineage differentiation medium, proliferated MSCs must be triggered to differentiate into osteoblasts, adipocytes, and chondroblasts. To verify the differentiation, osteoblasts could be demonstrated by calcium deposits staining with Alizarin Red S or von Kossa staining. Adipocyte differentiation is most readily demonstrated by oil droplet accumulation staining with Oil Red O. Chondroblast differentiation is demonstrated by spheroid formation and acidic glycosaminoglycans staining with Alcian blue or Safranin O. Accordance with differentiation results, ADSCs, BM-MSCs, and UC-MSCs propagated in mscGO™ XF medium in the passage 5 and passage 8, could be induced to differentiate into adipocytes, osteoblasts, and chondrocytes (Figure 5A, 5B, and, 5C; respectively). Based on these data, we verified that *ex vivo* expansion of MSCs using mscGO™ XF medium could maintain tri-lineage differentiation characteristics in both early and late passages. In this study, passage 5 and passage 8 represent earlier and later passages, respectively.

Chromosomal Stability of MSCs Propagated in mscGO™ XF Medium

MSCs are considered a promising source for tissue engineering and regenerative medicine. Therefore, cytogenetic chromosome analysis is crucial for verifying the safety and

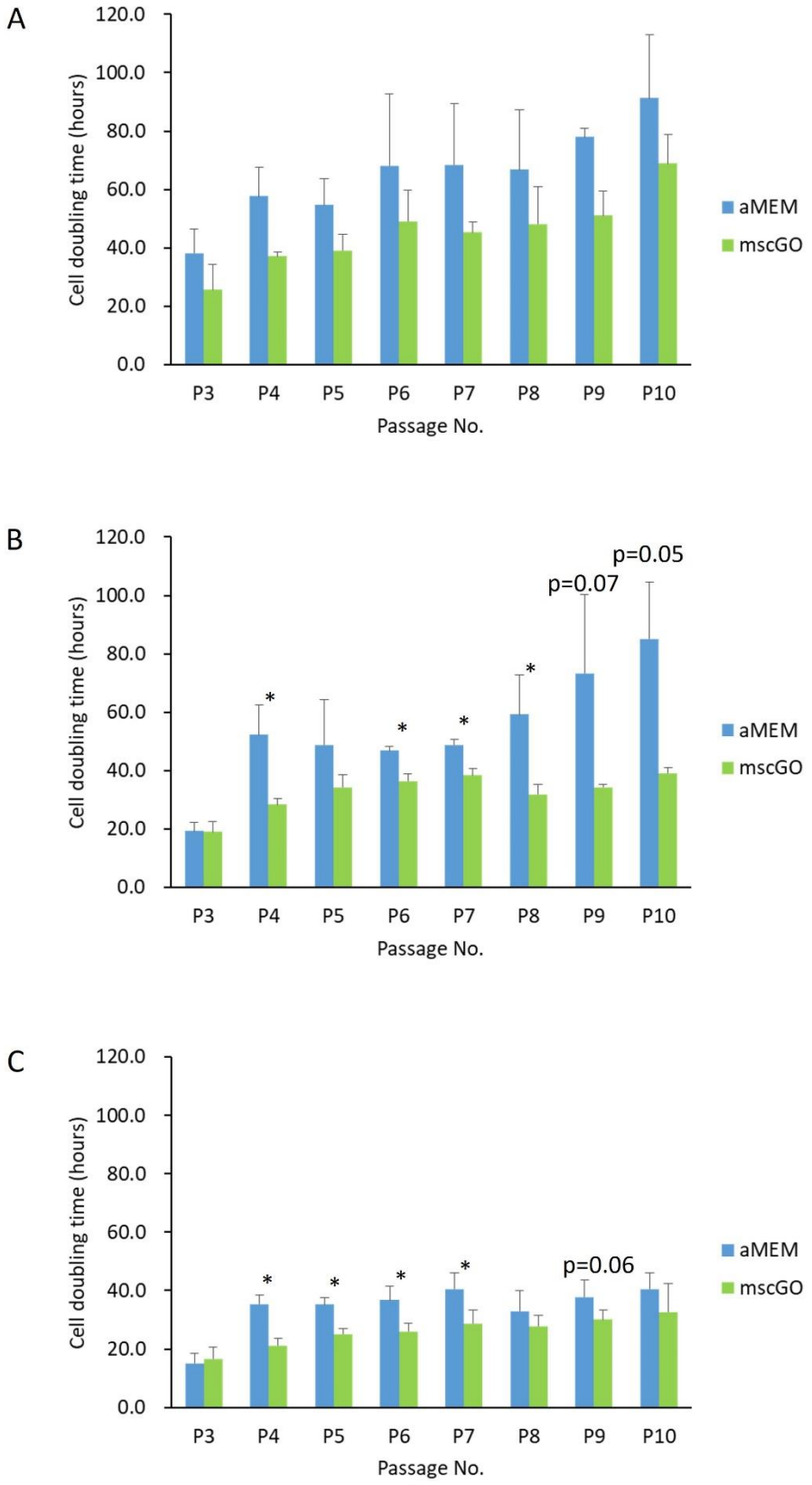


Figure 3. The Population Doubling Time of Different MSCs. The population doubling time of ADSCs cultured in mscGO™ XF medium (A, mscGO) and conventional medium described in material and method (A, aMEM). The population doubling time of BM-MSCs cultured in mscGO™ XF medium (B, mscGO) and conventional medium (B, aMEM). The population doubling time of UC-MSCs cultured in mscGO™ XF medium (C, mscGO™ XF) and conventional medium (C, aMEM). ADSCs (n = 2), BM-MSCs (n = 3), and UC-MSCs (n = 3). *Statistical significance between aMEM and mscGO™ XF was accepted when p <0.05.

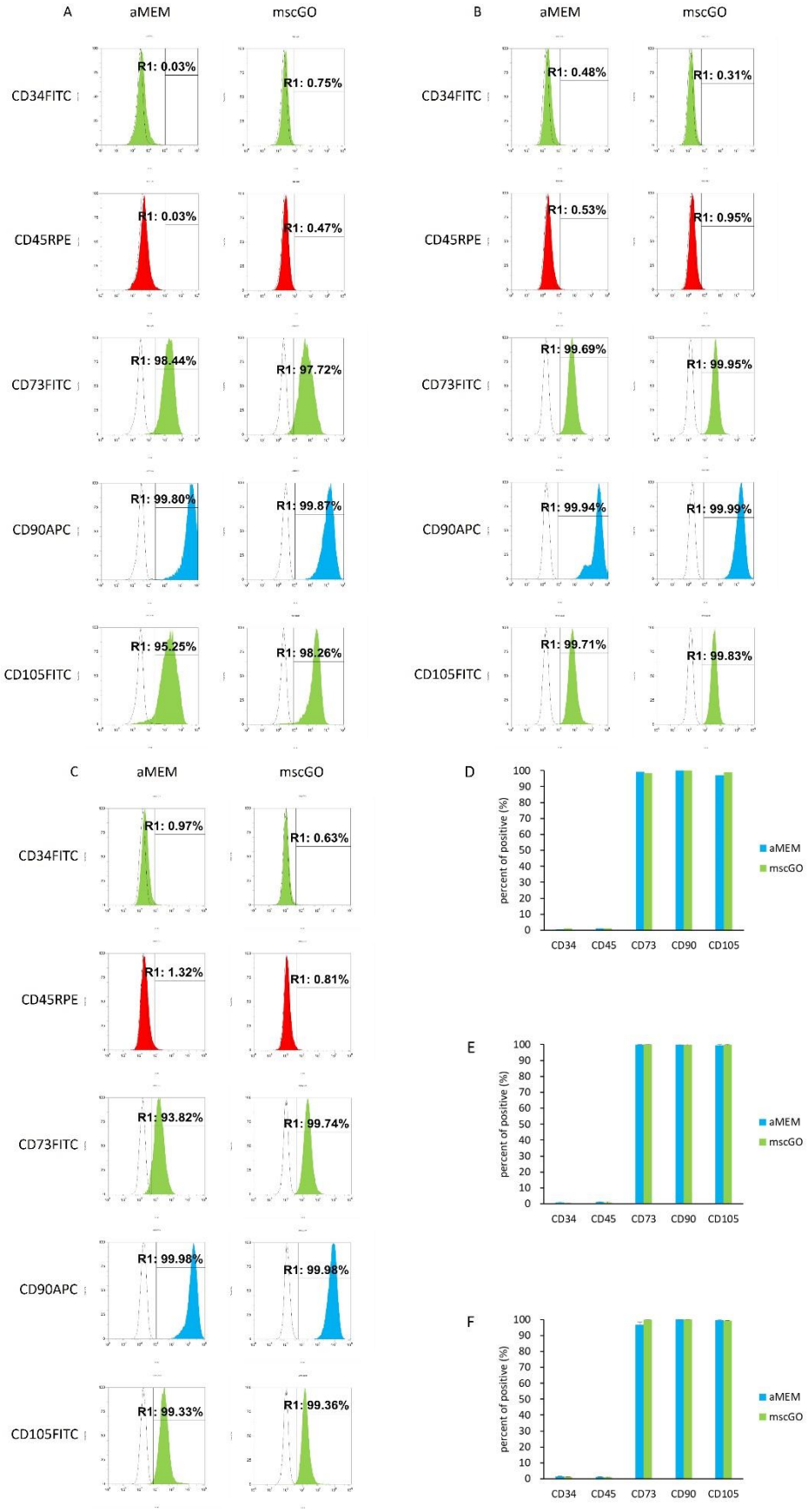
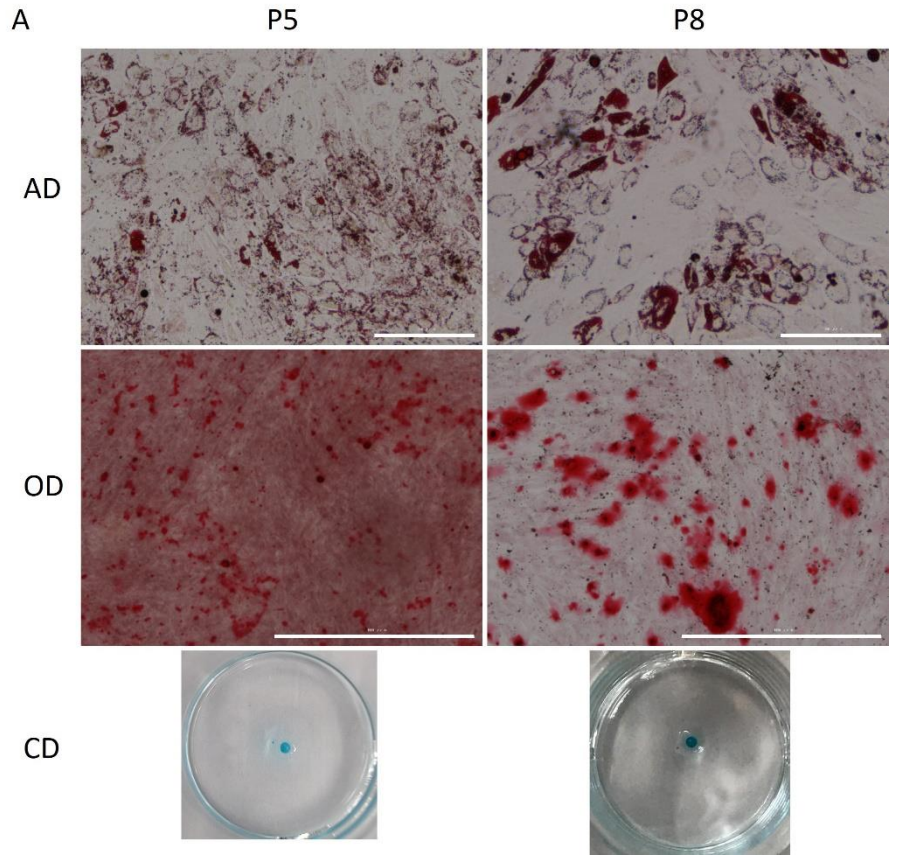


Figure 4. Flow Cytometry Analysis of MSCs Surface Markers. All of the MSCs: ADSCs, BM-MSCs, and US-MSCs, cultured in mscGO™ XF medium (mscGO™ XF in A, B, and C; respectively) and conventional medium: MEM alpha+10% MSC-qualified FBS (aMEM in A, B, and C; respectively) expressed CD73, CD90, and CD105; did not express CD34 and CD45. The quantitative results of the ratio of surface markers of ADSCs, BM-MSCs, and UC-MSCs expanded in conventional or mscGO™ XF medium were shown in Figures 4D, 4E, and 4F, respectively.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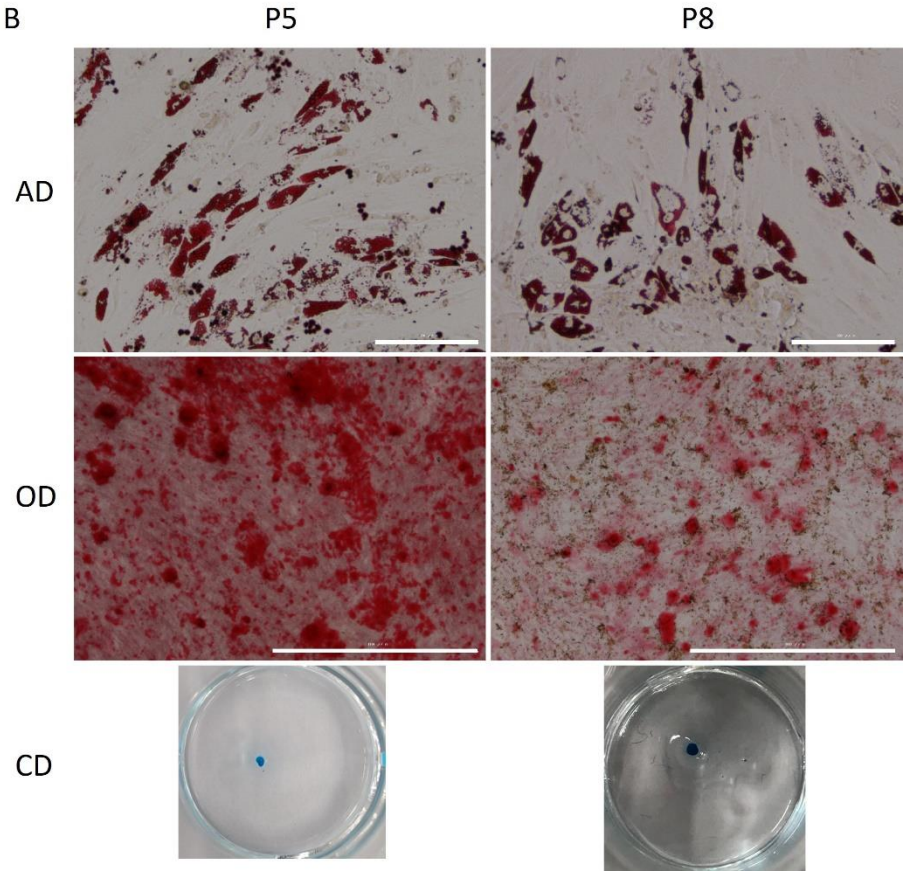
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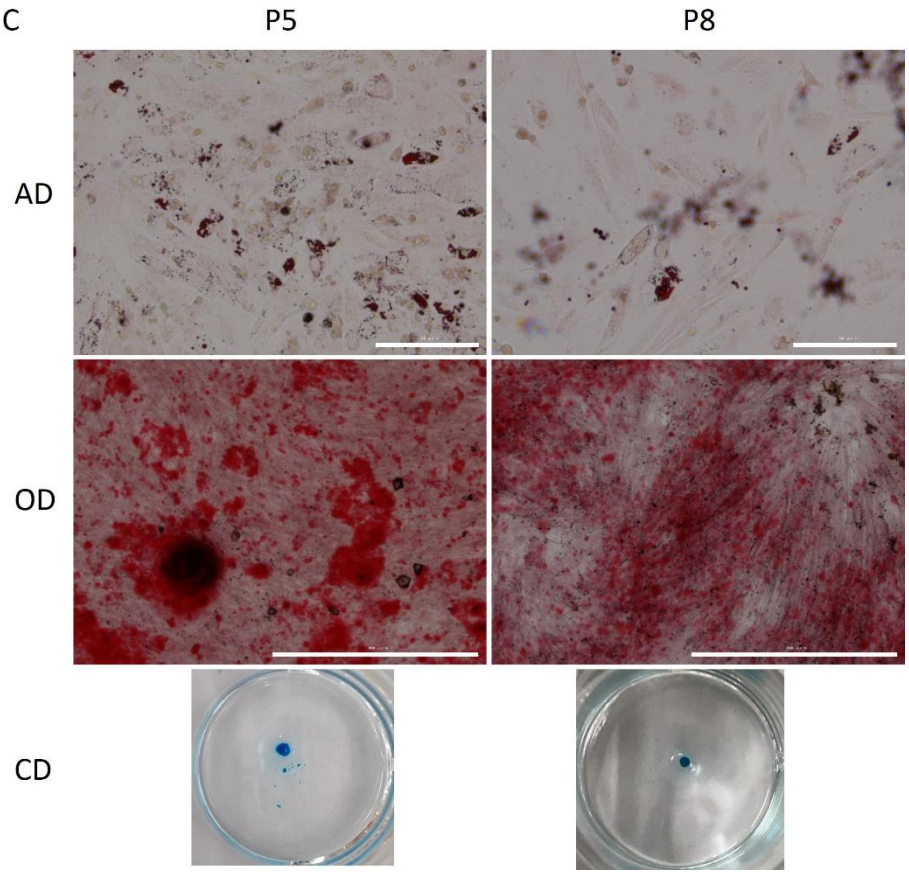

Figure 5. Tri-lineage Mesenchymal Differentiation Potential of MSCs at P5 and P8. ADSCs, BM-MSCs, and US-MSCs propagated with mscGO™ XF medium sustain tri-lineage mesenchymal differentiation potential of MSCs at P5 and P8 shown in A, B, and C, respectively: Adipocytes were confirmed by oil red stained oil droplets in the cytoplasm (AD, Scale bar: 200 µm). In osteogenic differentiation, calcium accumulation was assessed with alizarin red S stain (OD, Scale bar: 1000 µm). The chondrosphere is formed with chondrogenic medium treatment and sulfated proteoglycans were stained by alcian blue stain (CD).

stability of MSCs. G-banding (using Giemsa stain) is a karyotyping technique that can detect chromosomal abnormalities.[23,24] In this study, ADSCs, BM-MSCs, and UC-MSCs were cultured with mscGO™ XF medium at passage 5 (P5) and passage 7 (P7), were prepared for G-banded karyotyping. More than 20 cells in each sample were analyzed and the number of chromosomes in ADSCs at P5 and P7 was 46, XY, in BM-MSCs at P5 and P7 was 46, XY, and in UC-MSCs at P5 and P7 was 46, XY (data not shown). The karyotype analysis of ADSCs at P5 and P7 was presented in Figure 6A, while the karyotype analysis of BMMSCs and UCMSCs were presented in Figures 6B and 6C, respectively. The G banding patterns were consistent between each diploid chromosome in all the analyses.

Discussion

At present, there is no consensus on the appropriate nutrient supplement for the isolation and proliferation of human mesenchymal stem cells (hMSCs) *ex vivo* for clinical applications. The most commonly used nutritional supplements are fetal bovine serum (FBS) and human platelet lysate (hPL). However, the exact composition of FBS and hPL in terms of proteins, cytokines/growth factors, and elementary components is not fully defined. And both exhibit in batch-to-batch variations, besides, there is no standardized process for hPL manufacturing. Most studies indicates that hPL-supplemented medium promotes a higher proliferation rate of hMSCs compared to other serum-free medium, or FBS-supplemented medium. Furthermore, it is important to note that utilizing hMSCs propagated in FBS-containing medium for clinical applications is seen as a potential risk due to the possibility of exposure to infectious agents and animal antigens. In recent years, human blood derivatives, particularly, platelet lysates have been recognized as beneficial nutrient supplements for isolating and expanding hMSCs from various tissues.[12-14]

When culturing cells *in vitro*, the proliferation rate is a commonly usable parameter to evaluate the health of the cells. Research reports have indicated that using hPL to culture hMSCs can shorten the cell doubling time and increase the number of cell population doubling.[12,25-27] Meanwhile, the size of hMSCs cultured in hPL supplemented medium is smaller compared to those in FBS supplemented medium.[25,26] Interestingly, Liao G et al. described that utilizing

Figure 6. Chromosomal Stability of MSCs at P5 and P7 Using G-banding. Karyotype analysis of ADSCs, BM-MSCs, and US-MSCs, at P5 and P7, cultured in mscGO™ XF medium with G-banding was shown in A, B, and C, respectively.

hPL in UC-MSCs expansion *ex vivo* could reduce the level of senescence.[26] Additionally, hPL sustained increasing BM-MSCs proliferation without alterations in telomere length.[25] In the study of MSCs surface marker expression, there was no significant difference between MSCs expanded with hPL or FBS.[25,26] Although the hPL is from a different donor than MSCs, it does not seem to induce HLA-DR expression.[12,27] In terms of their differentiation capability, hMSCs can achieve adipogenic, osteogenic, and chondrogenic differentiation, whether utilizing hPL or FBS. However, hPL-expanded

hMSCs show better potential in osteogenic and chondrogenic differentiation.[12,25-27] In the study of the immunomodulatory properties of hMSCs expanded with hPL or FBS, the data shows contradictions.[12] The precise way in which hPL-expanded hMSCs exert their immunomodulatory effects is not well understood, and further studies are suggested. Production of hPL following Good Manufacturing Practice (GMP) standards is crucial and essential. Furthermore, standardizing of the composition of hPL is beneficial for reducing the batch-to-batch variation and clarifying the effects of the using hPL in cell isolation and proliferation.[12,13]

The mscGO™ XF medium is a xeno-free medium composed of a basal medium supplemented with 5% hPL. When compared to the conventional culture medium which consists of a basal medium supplemented with 10% to 20% FBS with or without specific growth factors, mscGO™ XF is an FBS-free, xeno-free, and ready-to-use medium. According to our experimental data, mscGO™ XF medium could be used to propagate adult MSCs: ADSCs and BM-MSCs, and fetal MSCs: UC-MSCs *ex vivo*. These proliferated cells meet the criteria of MSCs: by adhering to the plastic tissue culture dish, expressing specific surface antigens (CD73, CD90, and CD105), and exhibiting tri-lineage differentiating capability including adipocyte differentiation, osteoblast differentiation, and chondrocyte differentiation. Therefore, mscGO™ XF medium could be used to proliferate human mesenchymal stem cells *ex vivo*.

Genetic stability during cultivation is crucial for cell proliferation *ex vivo*. Therefore, we have confirmed the chromosome stability of MSCs proliferated with mscGO™ XF medium through G-banding karyotyping, a method regularly used to diagnose various chromosomal abnormalities in individuals. While the resolution of G-band karyotyping to detect chromosomal variation is typically on a “megabases” scale, it is sufficient to diagnose certain categories of abnormalities. For example, aneuploidy, caused by the absence or addition of chromosomes is easily detected by karyotyping. Besides, cytogeneticists can also frequently detect much more subtle deletions or insertions as deviations from normal banding patterns, such as translocations which are readily apparent on karyotypes. MSCs propagated using mscGO™ XF medium at passages 5 and 7, exhibited a normal chromosome karyotype, 46 XY, and the same G-banding patterns. These results are beneficial to illustrate that the chromosomal stability of MSCs cultured with mscGO™ XF medium is reliable. Summarizing the experimental results above, we believe that the mscGO™ XF medium is a kit suitable for culturing and expanding MSCs *ex vivo*. It shows promise to develop into a clinical application medium in the future.

Conclusion

To summarize, mscGO XF medium could be used to proliferate adult and fetal MSCs including ADSCs, BM-MSCs, and UC-MSCs *ex vivo*. These cells exhibited spindle shape, fibroblast-like morphology, and adherence to the plastic tissue culture dish; expressing specific surface antigens (CD73, CD90, and CD105); exhibiting tri-lineage differentiating capability including adipocyte differentiation, osteoblast differentiation, and chondrocyte differentiation. According to these experimental data, these proliferating cells meet the criteria of MSCs. Therefore, we believe that the mscGO XF medium can be used to culture and proliferate MSC *ex vivo*.

Authors’ Contributions

All authors contributed equally to this study.

Conflict of Interest Disclosures

Don-Ching Lee, Chi-Shiu Cheung, and Wen-Che Tsai are employees of GeneDireX, Inc. I confirm that Don-Ching Lee, Chi-Shiu Cheung, and Wen-Che Tsai have a financial or other interest in the subject/matter of the work in which I will be involved, which may be considered as constituting a real, potential, or apparent conflict of interest.

Acknowledgment

All the authors gratefully acknowledge support of this work by the Industrial Development Bureau, Ministry of Economic Affairs, Taiwan, under Grant No. 111121329, and also GeneDireX, Inc. Research team.

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