

Isolation and Characterization of Feline Wharton's Jelly-Derived Mesenchymal Stem Cells

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Abstract

Background

Wharton's jelly is a well-known mesenchymal stem cell source in many species, including human. However, there have been no reports confirming the presence of mesenchymal stem cells in wharton's jelly in cats. The purpose of this study was to isolate mesenchymal stem cells (MSCs) from wharton's jelly of cats and to characterize stem cells.

Result

In this study, Feline wharton's jelly-derived mesenchymal stem cells (fWJ-MSCs) were isolated and successfully cultured. fWJ-MSCs were maintained and the proliferative potential was measured by cumulative population doubling level (CPDL) test, scratch test and colony forming unit (CFU) test. Stem cell marker, karyotyping and immunophenotyping analysis by flow cytometry showed that fWJ-MSCs possessed characteristic mesenchymal stem cell markers. To confirm the differentiation potential, we performed osteogenic, adipogenic and chondrogenic induction under each differentiation condition. fWJ-MSC has the ability to differentiate into multiple lineages including osteogenic, adipogenic and chondrogenic differentiation.

Conclusions

This study shows that wharton's jelly of cat can be a good source of mesenchymal stem cells. In addition, fWJ-MSC may be useful for stem cell-based therapeutic applications in feline medicine.

Background

Mesenchymal stem cells (MSCs), known as multipotent stem cells or mesenchymal stromal cells, have unique characteristics such as fibroblast-like morphology, expression of specific surface markers, and multipotent differentiation capacity (1, 2). MSCs proposed as an important option in the field of regenerative medicine and immunotherapy due to its immunosuppressive properties, its ability to produce cytokines or growth factors, and its ability to differentiate multilineage such as osteogenic, chondrogenic, myogenic skeletal muscle tissue, among others. (3).

MSC has been identified in multiple tissue sources such as bone marrow, skin, skeletal muscle, placenta, umbilical cord blood and adipose tissue. (4–9). In addition, many studies in the veterinary field have reported the isolation of MSCs from various animals such as dog, cat, horse, pig, cow, mouse (10–15).

Recent studies show that feline MSC has therapeutic effects of gingivostomatitis, asthma, chronic kidney disease, and enteritis in feline medicine. (16–19). Feline MSC has been isolated from bone marrow, adipose tissue and amniotic membrane (11, 20, 21). However, there has been no report of MSC on feline wharton's jelly. Wharton's jelly is a gelatinous substance in the umbilical cord that contains fibroblast-like stromal cells (22). Wharton's jelly-derived MSC has been proposed as a promising source of stem cells for

regenerative medicine and immunomodulatory therapy (23, 24). In this study, we have established feline wharton's jelly-derived stem cells (fWJ-MSC) by characterizing mesenchymal stem cell potency and it is a good resource for stem cell therapy in feline medicine.

Results

Isolation and Culture of fWJ-MSCs

Feline wharton's jelly were obtained after cesarean section and collected samples were moved to a cell culture dish (Fig. 1A). We isolated fWJ-MSCs from the wharton's jelly and cultured cells as previously described (25). Cellular morphology was fibroblast-like and spindle-like identity (Fig. 1B). To determine self-renewal capacity of fWJ-MSCs, we calculated the proliferation via cumulative population doubling level (CPDL). A consistent increase in the rate of fWJ-MSCs proliferation via the cumulative population was observed (Fig. 1C).

Scratch test, Colony Forming Unit (CFU) test

A scratch test was performed to investigate the migration ability of fWJ-MSCs. Images taken at different time lapses after 24 hours of scratch showed the possibility of migration of fWJ-MSC (Fig. 1D). CFU test demonstrated that fWJ-MSCs can generate new colonies from a single cell and establish multiple new colonies (Fig. 1E).

Expression Pattern of Stem Cell Markers

To measure gene expression levels of stem cell markers, reverse transcriptase polymerase chain reaction (RT-PCR) was performed in passage 3. Stem cell markers (OCT4, SOX2, KLF4 and MYC) showed expression patterns as a function of stemness. (Fig. 1F).

Analysis of Karyotype

We performed karyotyping using fWJ-MSCs to identify normal chromosome numbers using our culture process. fWJ-MSCs were found to contain 38 chromosomes, which is a normal karyotype of feline (Fig. 2A).

Flow Cytometry

To confirm the immunophenotype of fWJ-MSCs, cell surface specific markers were examined by fluorescence-activated cell sorter (FACS) analysis. As a result of FACS analysis, fWJ-MSCs have expression patterns that match MSC immune-phenotypes (Fig. 2B). The results showed fWJ-MSCs were observed positive expression of CD44 (100%), CD90 (65.96%) and CD105 (60.62%), which are typical MSC markers. But fWJ-MSCs were negative for the expression of CD14 (10.16%), CD34 (11.26%) and CD45 (9.04%).

Induction of Differentiation

To determine the differentiation ability of fWJ-MSCs, 3 different types of differentiation assays (osteogenic, adipogenic and chondrogenic) were performed. Under the osteogenic differentiation conditions, fWJ-MSCs were positively stained with Alizarin Red S (Fig. 3B, C) and Von Kossa staining (Fig. 3D, E) and under the basal condition, cells were not stained with Alizarin Red S (Fig. 3G, H) and Von Kossa staining (Fig. 3I, J). Compared with the basal condition, osteogenic differentiated cells were observed 67-fold higher values of absorbance in Alizarin Red S staining (Fig. 3K). After differentiation, the gene expression levels of MSX2 were increased compared to controls (Fig. 3L). Means \pm standard deviations are plotted (** $p < 0.001$), ** $p < 0.01$).

Under adipogenic differentiation conditions, fatty droplets were observed via both unstained (Fig. 4A) and positive Oil Red O staining (Fig. 4B, C). And basal culture medium was used as undifferentiated cells, which observed negative staining (Fig. 4E, F). The differentiated cells showed approximately 3-fold higher absorbance than that of the undifferentiated cells (Fig. 4G). After adipogenic differentiation, gene expression level of adipogenic markers such as LPL, Leptin and FABP4 were increased in treated cells compared to undifferentiated cells (Fig. 4H, I, J). Means \pm standard deviations are plotted (** $p < 0.001$), ** $p < 0.01$).

Under the chondrogenic differentiation conditions in 6 well plates, the cells were positively stained with toluidine blue and undifferentiated cells were not stained (Fig. 5A ~ F). Chondrogenic differentiated cells showed 3-fold higher values of absorbance in toluidine blue staining, compared with the undifferentiated cells (Fig. 5G). After chondrogenic differentiation in tubes, we observed the pellet in the bottom of the polypropylene tube and it appeared to be ovoid and opaque (Fig. 5H). The pellet was stained with toluidine blue (Fig. 5I) which exhibited positive staining patterns. COL2A1 gene expression level were increased compared to undifferentiated conditions (Fig. 5J). Means \pm standard deviations are plotted (* $p < 0.05$).

Discussion

Feline MSCs were first isolated and characterized in the bone marrow (11) and subsequently feline MSCs have been derived from adipose tissue (20, 26–28), amniotic membrane and amniotic fluid (21, 29). In these studies, feline MSCs were characterized by morphological features, specific cell surface markers and their capacity for differentiation. Wharton's jelly is readily available and rich source of stem cell in various species including human (24, 30). But there is no study on stem cells from wharton's jelly in cats. Even though the existence of MSCs in wharton's jelly has been demonstrated in many species including human, canine, swine, bovine, equine, chicken, mouse (25, 31–35), this study was the first to show isolation of fWJ-MSCs exhibiting morphology, proliferation, karyotyping, surface markers and the capacity to differentiate into multi-lineage.

In previous study showed that WJ-MSCs have higher proliferative potential than MSCs from other sources such as bone marrow, adipose tissue, placenta and amniotic membrane (36–40). And also WJ-MSCs have primitive features, as they exhibit several characteristics of ESCs, such as ESC-like antigen

Tra-1-60, Tra-1-81, SSEA-1 and SSEA-4 (41), and numerous pluripotency genes including OCT4 and SOX2 (42). WJ-MSCs are good candidates for therapeutic applications due to ease of accessibility, lack of painful procedures, greater proliferative potential, less risk of contamination and hypoimmunogenicity (24, 43, 44). Another advantage of WJ-MSCs is that it can be cryopreserved after the birth of a baby and used for future purposes using biobanks (45).

In our study, we isolated MSCs from feline wharton's jelly tissues. Cellular morphology of fWJ-MSCs was fibroblast-like and spindle-like identity similar to other MSCs such as adipose-derived MSCs, bone marrow-derived MSCs, peripheral blood-derived MSCs and umbilical cord blood-derived MSCs (20, 25, 33, 46). Also fWJ-MSCs had similar proliferation and characteristics as MSCs derived from other feline tissues (29, 47). MSCs show positive expression of CD44, CD90 and CD105, but negative expression of CD14, CD34 and CD45, according to the International Society of Cellular Therapy (48). For FACS analysis of fWJ-MSCs, we used 6 markers (CD14, CD34, CD44, CD45, CD90 and CD105) to distinguish the MSC phenotype. fWJ-MSCs were positive by flow cytometry for CD44, CD90 and CD105 and negative for CD14, CD34 and CD105, similar to MSCs from other feline tissues and WJ-MSCs from other species (20, 49). fWJ-MSCs also had the potential for multiple lineage differentiation, including osteogenic, adipogenic and chondrogenic, similar to other species WJ-MSCs (25, 49). Based on this evidence, we suggest that cells derived from feline wharton's jelly in this study were MSCs.

There are several studies using feline MSCs in therapeutic purpose in cats. The stem cells used in most cases have been shown to originate from feline adipose tissue (16–19, 50, 51). With regard to potential therapeutic uses, there is a need for a source of stem cells of established diversity.

Conclusions

The isolated fWJ-MSCs were proved the possibility of culturing through dozens of passages. Stem cell properties were confirmed using FACS and differentiation analysis. In addition, fWJ-MSC did not show a change in the number of chromosomes through karyotype analysis. These results suggest that fWJ-MSC could potentially be used to cell therapy in feline medicine. In conclusion, we suggest that fWJ-MSCs may be a potential source of stem cells and thus may be useful for veterinary medicine.

Methods

Animals

The umbilical cord was obtained using pregnant cats ($n = 2, 4 \sim 5$ years, mixed breed) who visited Kyungpook National University Animal Hospital. In the cesarean-section delivery, the cats were pre-medicated with 0.1 mg/kg acepromazine maleate (Samwoo medical, Korea) and 5 mg/kg propofol (Myeongmun pharmaceutical, Korea) was injected intravenously. Isoflurane (Hana pharmaceutical, Korea) was used to maintain anesthesia. Under sterile conditions, umbilical cords were collected from cesarean section deliveries. Cats were hospitalized and discharged for two weeks after surgery.

Cell Isolation

Cell isolation was performed through some modifications as previously described (1, 2, 25). The collected wharton's jelly was placed in a sterile culture dish and washed with 0.9% physiological saline and vessel was removed. Tissue was then finely chopped and resuspended in 2 mg/ml collagenase type I solution (US Sigma-Aldrich) at 37 °C for about 20 minutes. After enzymatic digestion, it was centrifuged at 3,000 rpm for 5 minutes and washed in phosphate buffered saline (PBS) (Gibco, USA). Cell pellets were resuspended in culture medium in hypoglycemic Dulbecco's Modified Eagle's Medium (LG-DMEM; Gibco, USA) containing 10% FBS (fetal bovine serum; Gibco, USA) and 1% Penicillin-Streptomycin solutions (Gibco, USA). fWJ-MSCs were seeded in T-75 flasks (Corning, USA) and incubated in a humidified atmosphere with 5% CO₂. The culture medium was changed three times a week and passed when the cells reached 80–90% confluence.

Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from the cultured cells with the RNeasy minikit (Qiagen, Germany) according to the manufacturer's protocol. RNA concentrations were measured by Nanodrop 2000 (ThermoScientific, USA). cDNA was prepared by 1 mg of total RNA for reverse transcription using Superscript II reverse transcriptase (Invitrogen, USA) and oligo dT primers (Invitrogen, USA). The cDNA was amplified using T100™ Thermal Cycler (Biorad, USA). Primers are shown in the Table 1.

Table 1
List of RT-PCR Primer

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Product size
OCT4	GCCCGAAAGAGAAAGCGAAC	CGACGATTGCAGAACCACAC	161 bp
SOX2	GCCCTGCAGTACAACCTCCAT	TGGAGTGGGAGGAAGAGGTA	175 bp
KLF4	ACCAAGAGCTCATGCCACCT	AAGGCTTCTCACCTGTGTGG	183 bp
MYC	AGGAGAAACGAGCTGAAACG	GTTCTCGTCGCTTCCTCAAC	181 bp
GAPDH	GTGGAGGGACTCATGACCAC	GTGAGCTTCCCATTTCAGCTC	176 bp

Cumulative Population Doubling Level Analysis

Proliferation and growth efficiency of fWJ-MSC was determined by CPDL using the formula $CPDL = \ln(N_f/N_i) / \ln 2$, where N_i is the initial seeding cell number, N_f is the final harvest cell number and \ln is the natural logarithm. fWJ-MSCs (5×10^4 cells) were plated three times in 6-well culture plates (Corning, USA) and passaged 4 days later. Final cell count was counted and 5×10^4 cells were plated again. To calculate the cumulative doubling level, we calculate the doubling population for each pass and then multiply by the doubling of the previous passages.

Scratch test, Colony Forming Unit test

In scratch test, fWJ-MSCs were incubated in 6-well plates until 90% confluence was reached. Cell scratches were made with a 100 μ l pipette tip. DMEM medium containing 5% FBS was used for 24 hours. After 24 hours, inverted microscope was used to observe the healing state of the cells.

In CFU test, Passage 5 fWJ-MSCs were seeded at 1×10^3 cells in 6-well plates and incubated DMEM culture medium containing 10% FBS. After 2 weeks, the plastic adherent colonies were stained with 1% toluidine blue (Scytek, USA).

Karyotype Analysis

In order to detect any chromosomal abnormalities in the fWJ-MSC, karyotyping was performed by the conventional methods at passage 5. fWJ-MSCs were arrested with 500 μ l colcemid (Gibco, USA) in the incubator (37 $^{\circ}$ C, 5% CO₂) for 1 hours. The cells were suspended in a hypotonic solution (0.075 M KCl; Sigma-Aldrich, USA) and incubated for 20 min at 37 $^{\circ}$ C. And the cells were fixed by washing in Canoy's fixative (methanol : glacial acetic acid = 3 : 1; Sigma-Aldrich, USA). Chromosome diffusion was obtained by pipetting the suspension into a clean glass and air drying. fWJ-MSCs undergoing metaphase were pictured with a CCD camera (Olympus, Tokyo, Japan), chromosomes were counted, and pattern was analyzed by software (ChIPS-Karyo; GenDix Inc, Korea).

Flow Cytometry

To establish the immunophenotype of fWJ-MSCs, cells were stained with 6 antibodies for FACS analysis, following the protocol provided by the manufacturer (Beckman Coulter, USA). fWJ-MSCs were trypsinized and washed several times with PBS at passage 5. The suspended cells were aliquoted (approximately 1×10^6 cells) for specific antibody staining. The cells were immunostained with the following antibodies shown in the Table 2. The antibodies were conjugated with Fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Analysis was determined by the use of FACS (Gallios Flow Cytometer; Beckman Coulter, USA) and software (Kaluza for Gallios; Beckman Coulter, USA).

Table 2
List of FACS antibodies

Marker	Antibody	Company / Catalog#
CD105	MOUSE ANTI HUMAN CD105	BIO-RAD / MCA1557
CD90	PE Mouse Anti-Human CD90	BD Pharmingen / 555596
CD45	FITC Mouse Anti-Human CD45	BD Pharmingen / 555482
CD44	PE anti-mouse/human CD44 Antibody	BioLegend / 103024
CD34	FITC Mouse Anti-Human CD34	BD Pharmingen / 555821
CD14	MOUSE ANTI HUMAN CD14	BIO-RAD / MCA1568

Osteogenic Differentiation

Osteogenic differentiation medium (StemPro Osteogenesis Differentiation Kit; Gibco, USA) was used to prove osteogenic differentiation capability. At passage 5 when cells reached confluency of 80 to 90%, medium was changed to the osteogenic induction medium, and incubated for 3 weeks, changing once every 3 days. After 3 weeks, calcium deposition was found by staining with Alizarin Red S and Von Kossa. For Alizarin Red S staining, cells were washed with PBS and fixed with 70% ethanol for 1 hour at 4° C. The cells were then washed several times with distilled water. Cells were stained with Alizarin Red S (IHCworld, USA) for 10 minutes at room temperature. Cells were washed with nonspecific dye with 5 distilled water. For quantitative measurements, Alizarin Red S stain was solubilized for 1 hour using 100 mM cetyl pyridinium chloride (Sigma-Aldrich, USA). Solubilized Alizarin Red S absorbance was measured at 570 nm using a spectrophotometer. For Von Kossa staining, stain cells with 5% silver nitrate (Scytek, USA) for 30 to 60 minutes while exposing the cells to UV light, then stain with 5% sodium thiosulfate (Scytek, USA) for 2–3 minutes, then counterstaining for 5 minutes with nuclear red stain (Scytek, USA).

Adipogenic Differentiation

To determine if fWJ-MSCs can be differentiated into adipocytes, cells were treated with adipose production differentiation medium (StemPro Adipogenesis Differentiation Kit; Gibco, USA). At passage 5, when the cells reached 80–90% confluence, the medium was changed to adipose production differentiation medium and incubated for three weeks, changing once every three days. After 3 weeks, oil red O staining (Scytek, USA) was performed to detect lipid fibrosis. Cells were fixed with 10% neutral buffered formalin to fix for at least 1 hour and rinsed with 60% isopropanol before incubating for 10 minutes in freshly diluted Oil Red O. Oil red O stain was solubilized using isopropanol and absorbance was measured using a spectrophotometer at 500 nm.

Chondrogenic Differentiation

To confirm that fWJ-MSCs are differentiated into chondrocytes, cartilage formation differentiation medium (StemPro Chondrogenesis Differentiation Kit; Gibco, USA) was used. First, cells (5×10^5 cells) were seeded in 15 ml polypropylene tubes and centrifuged with pellets at passage 5. The pellet was incubated in 1 ml of cartilage forming differentiation medium and incubated for 4 weeks. Differentiation medium was replaced 3 times a week. After differentiation, pellets were embedded in paraffin and 4 μ m sections were cut. For histological evaluation, sections were stained with toluidine blue (Scytek, USA). For quantitative measurements, toluidine blue staining was solubilized with 100 mM cetylpyridinium chloride (Sigma-Aldrich, USA) for 1 hour. Solubilized toluidine blue absorbance was measured at 600 nm using a spectrophotometer.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative Real-time PCR (qRT-PCR) was performed by mixing cDNA with primers and LightCycler® 480 SYBR Green I Master (Roche Diagnostics, Germany). qRT-PCR was performed using an LightCycler 480 II with supplied software (Roche applied science, Germany) according to the manufacturer's instructions. RNA expression levels were compared after normalization to endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences used in this study are listed in Table 3.

Table 3
List of Real-time PCR Primer

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Product size
MSX2	GCCTCCAAGACACATGAGC	CCTGGGTCTCTGTGAGGTTTC	185 bp
LPL	TGGCGGAGGAATTTCACTAT	AGGAGAAAGGCGACTTGGAG	176 bp
LEPTIN	AGCAGCTTGGCTGACAATTT	CCAGCAATCACTCCTGGTCT	178 bp
FABP4	CATCAGTGTGAATGGGGATG	CCACTTCTGCACCTGTACCA	169 bp
COL2A1	CCCTAGAGGTCCTCCTGGTC	CAAAGGCAGACATGTCGATG	188 bp
GAPDH	GTGGAGGGACTCATGACCAC	GTGAGCTTCCCATTTCAGCTC	176 bp

Statistical Analysis

The data were analyzed by Student's t-test using Excel software (Microsoft, USA) and expressed as the mean \pm standard error. Statistical significant data are indicated by asterisks ($***p < 0.001$, $**p < 0.01$, $*p < 0.05$).

Abbreviations

MSCs: mesenchymal stem cells; fWJ-MSCs: feline wharton's jelly-derived mesenchymal stem cells; CPDL: cumulative population doubling level; CFU: colony forming unit; RT-PCR: reverse transcriptase polymerase

chain reaction; FACS: fluorescence-activated cell sorter; qRT-PCR: quantitative real-time polymerase chain reaction.

Declarations

Ethics approval and consent to participate

Applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research. This study was performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and with approval from the Institutional Animal Care AND Use Committee of Kyungpook National University (KNU 2019-0128). Written informed consent was obtained from all owners.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this article. These data are available on request from the corresponding author.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of the article

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Authors' contributions

All authors have been involved in the following aspects of this manuscript; MSS and KKK were involved in drafting the manuscript and interpretation of data. SHY and YSK have been responsible for the study conception and design. SKO, SES and KSK were involved in the study design and interpretation of data.

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Figures

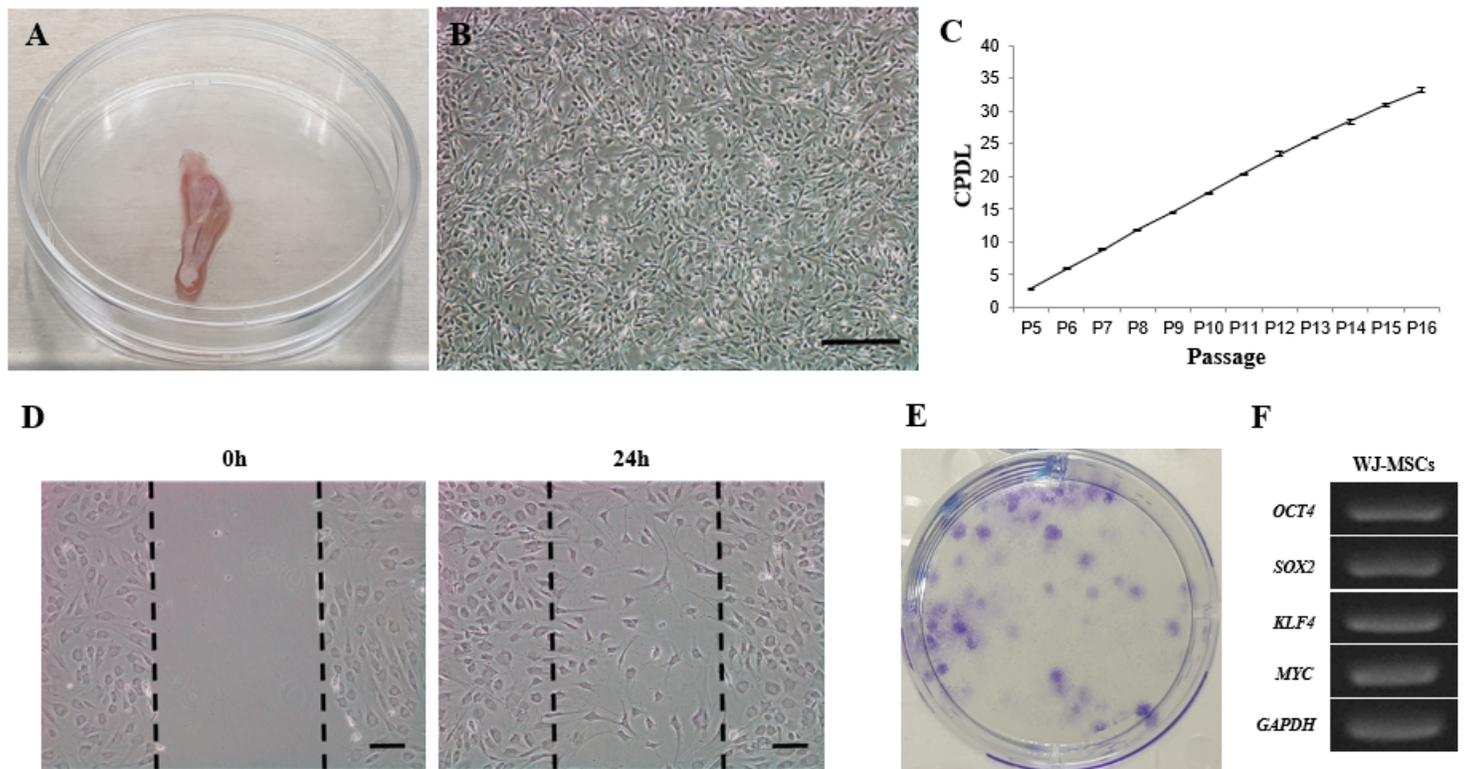


Figure 1

Primary culture of fWJ-MSCs and identification of CPDL. (A) Extracted feline umbilical cord tissue. (B) Image of isolated fWJ-MSCs. Cells exhibited spindle shape similar to other mesenchymal stem cells. Scale bar : 100 μ m. (C) Cumulative growth curve of fWJ-MSCs. CPDL was measured from passage 5 to 16. (D) Scratch test. Figure shows representative images of fWJ-MSCs migration post-scratch assay obtained at after 24h. Scale bar : 10 μ m. (E) Colony forming unit test. At 2 weeks of culture, the CFU were stained with toluidine blue to visualize the colonies generated. (F) RT-PCR. OCT4, SOX2, KLF4, MYC expression determined stemness. Vinculin expression showed adhesive ability.

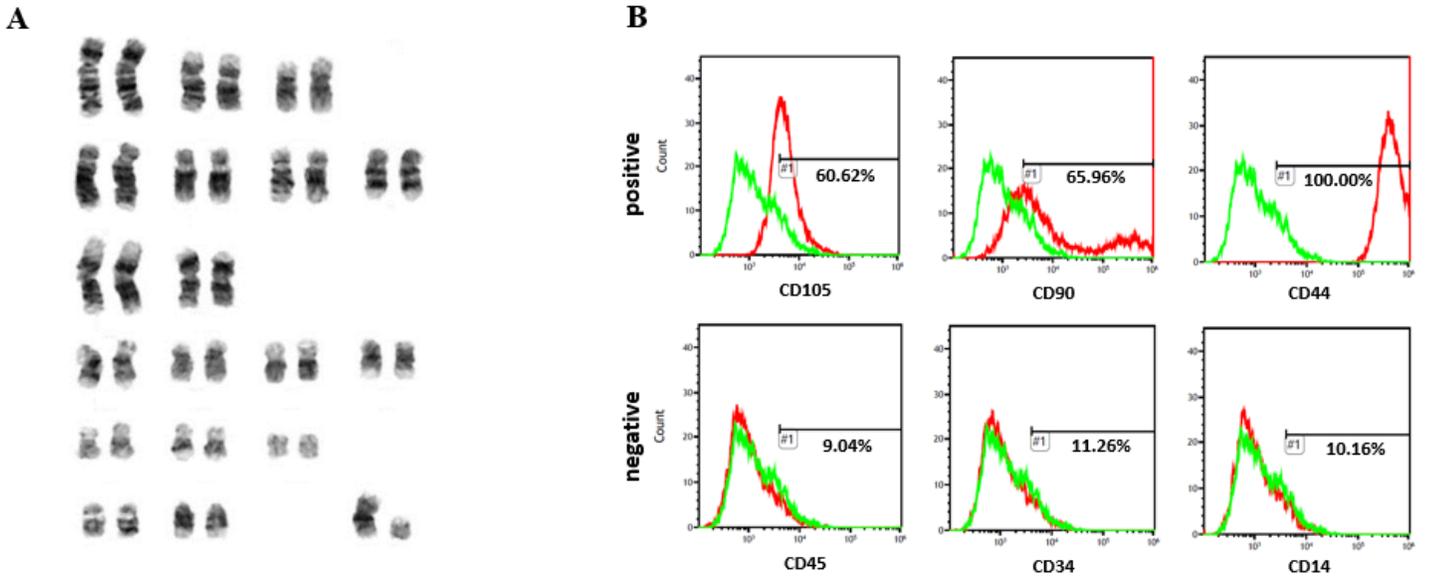


Figure 2

Karyotyping and Flow cytometry analysis. (A) Karyotype of fWJ-MSCs at passage 5 showing a euploid number of chromosomes. (B) FACS analysis was performed at passage 5. Values show the intensity of the indicated antigen.

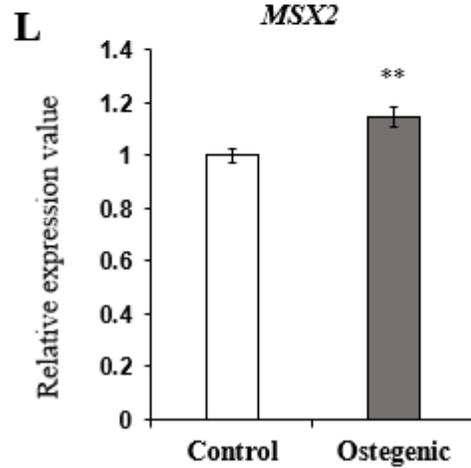
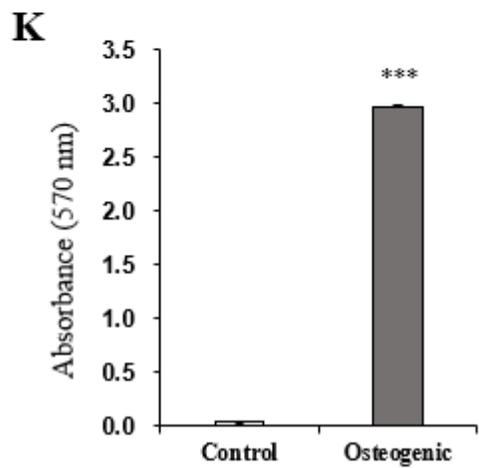
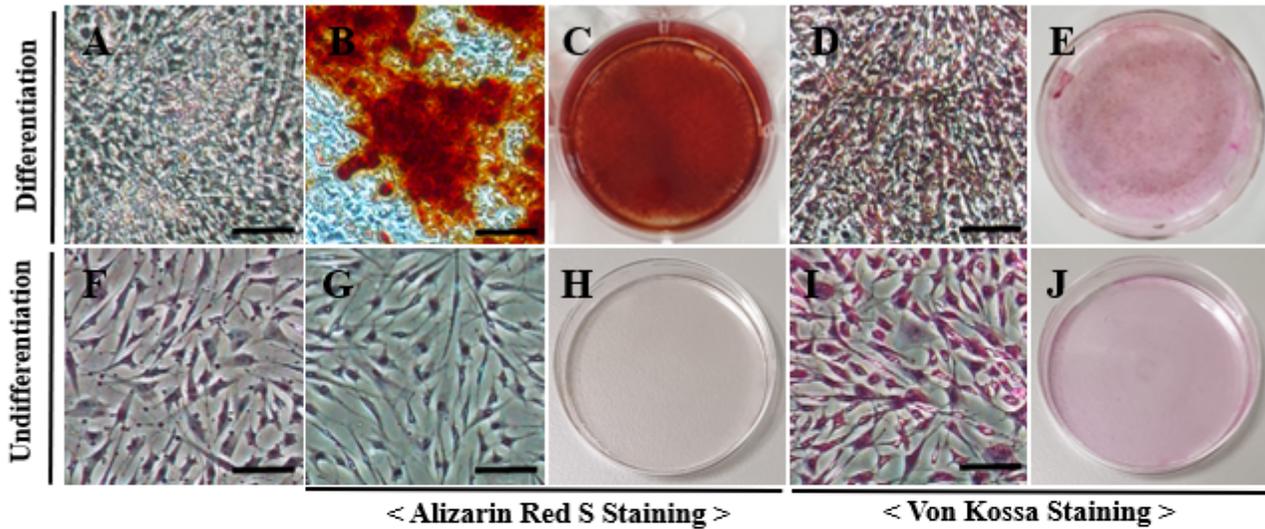


Figure 3

Osteogenic differentiation. (A-J) Osteogenic differentiation of fWJ-MSCs. (A,F) Bright field image of cells in osteogenic condition and control condition. (B-E, G-J) Alizarin Red S and Von Kossa staining after 3 weeks of osteogenic induction and control condition. Osteogenic differentiated cells (A-E) were grown in osteogenic differentiation medium. Control cells (F-J) were grown in basal medium with 10% FBS. Differentiated cells stained with Alizarin Red S (B,C) and Von Kossa (D,E). Scale bar : 25 μ m. (K) For quantification, Alizarin Red S stained cells were solubilized with 100 mM cetylpyridinium chloride, and the absorbance was measured at 570 nm by spectrophotometer for 0.5 seconds. Compared with the control, differentiated cells showed 67-fold higher absorbance values. (L) qRT-PCR for detection of mRNA expression level of osteogenic markers: *MSX2*. (Control = Undifferentiated fWJ-MSC)

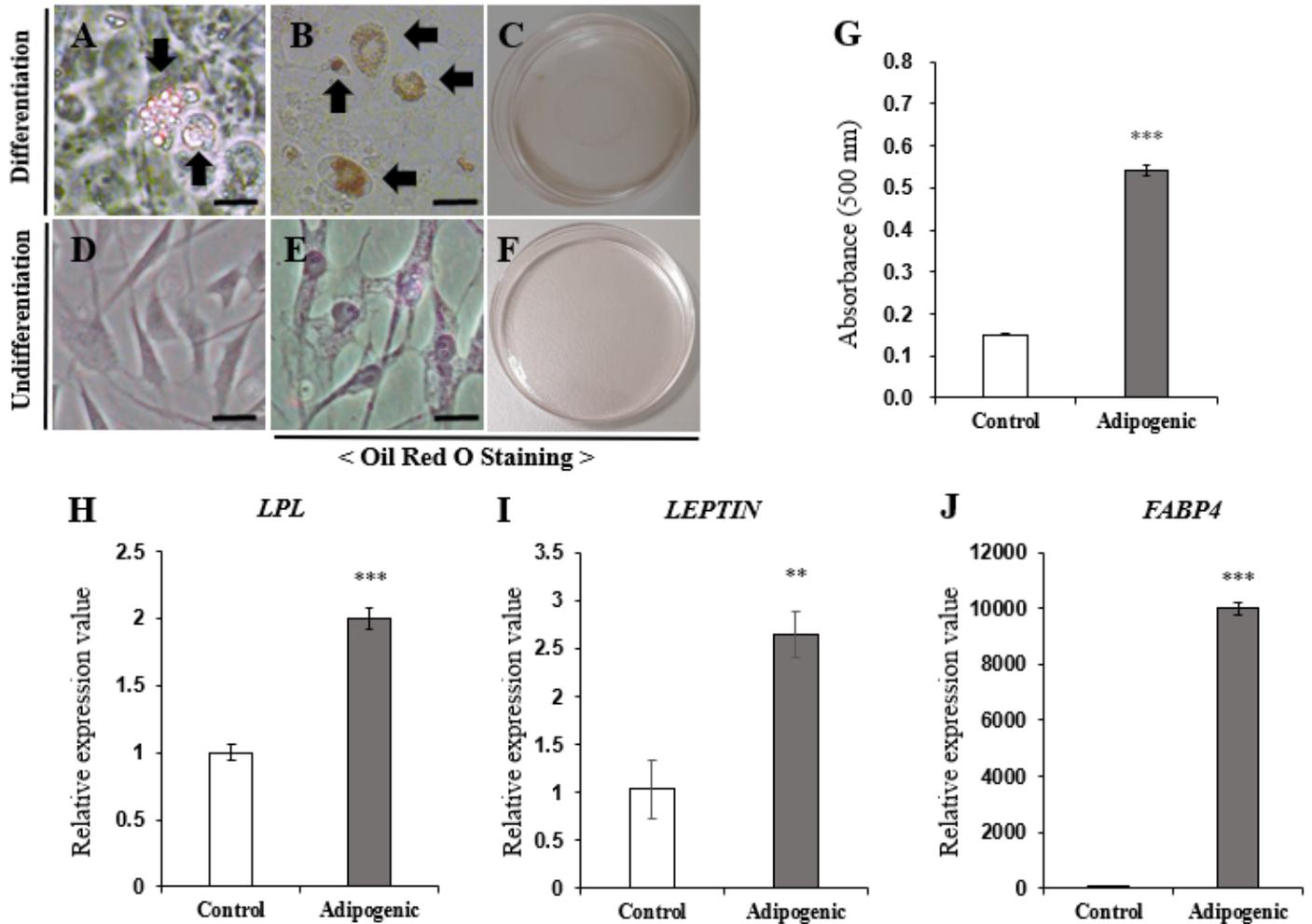


Figure 4

Adipogenic differentiation. (A-F) Adipogenic differentiation of fWJ-MSCs. Oil Red O staining was performed after 3 weeks of adipogenic differentiation. (A-C) Differentiated cells were grown in adipogenic differentiation medium. Fatty droplets were stained with Oil Red O. (Arrow : fatty droplet) (D-F) Negative controls showed no staining with Oil Red O. Scale bar: 10 μ m. (G) For quantification, stained fWJ-MSCs were solubilized with 100% isopropanol, and the absorbance was measured at 500nm for 0.5 second by spectrophotometer. Compared with the control, differentiated fWJ-MSCs showed 3 fold higher absorbance values. (H-J) qRT-PCR for detection of mRNA expression level of adipogenic markers: LPL, LEPTIN and FABP4. (Control = Undifferentiated fWJ-MSC)

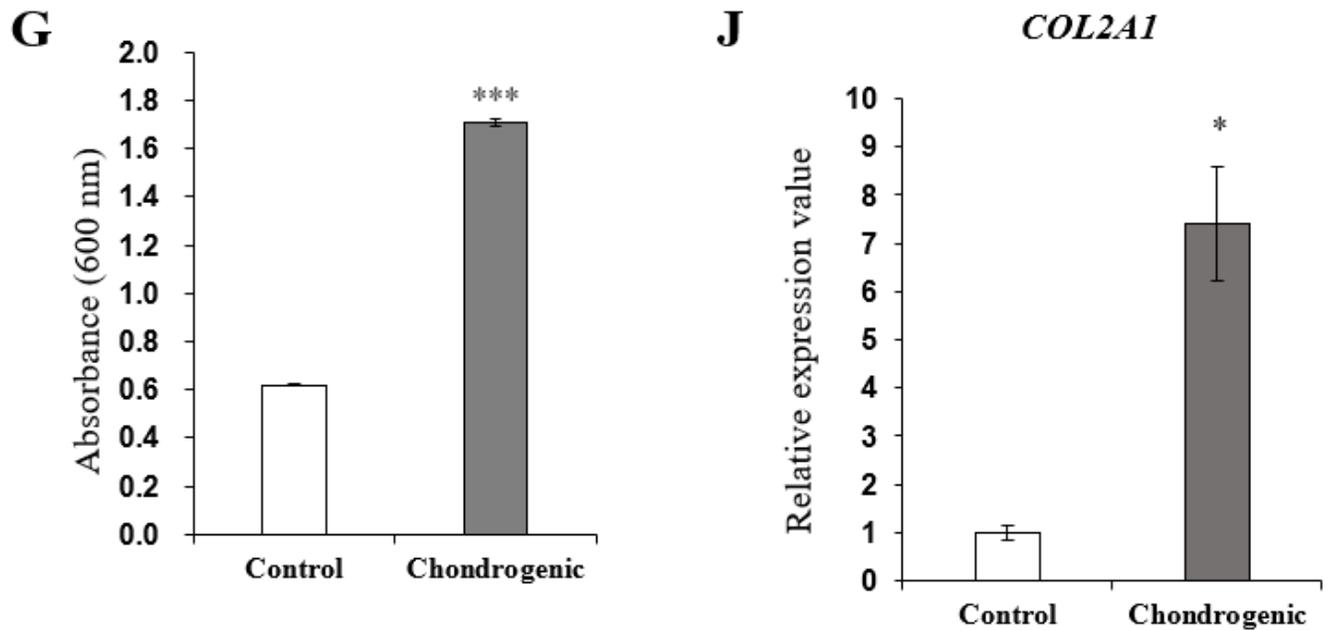
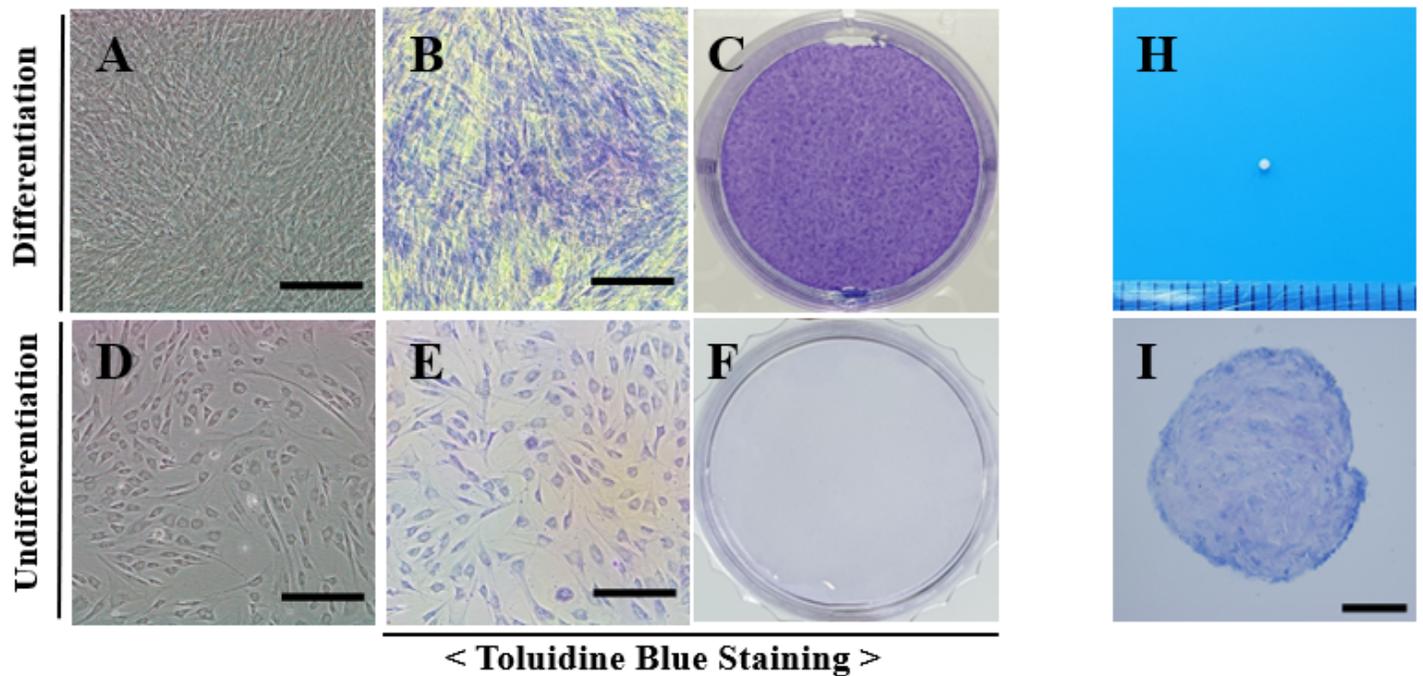


Figure 5

Chondrogenic differentiation. (A-F) Chondrogenic differentiation of fWJ-MSCs. Toluidine blue staining was conducted after 3 weeks of chondrogenic induction. Scale bar : 50 μ m. (G) qRT-PCR for detection of mRNA expression level of chondrogenic-specific markers: COL2A1. GAPDH was used as reference for evaluating the quality of mRNA. (H) The shape of chondrogenic pellet. (I) Toluidine blue staining for chondrogenic pellet. Scale bar: 100 μ m. (J) qRT-PCR for detection of mRNA expression level of chondrogenic markers: COL2A1. (Control = Undifferentiated fWJ-MSC)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ARRIVEchecklistfWJMSCsBMCVR.pdf](#)