The Veterinary Journal 216 (2016) 183–188

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Isolation and characterisation of peripheral blood-derived feline mesenchymal stem cells

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ARTICLE INFO

Article history:
Accepted 17 August 2016

Keywords:
Feline
Mesenchymal stem cell
Peripheral blood
Autologous plasma

ABSTRACT

The aim of this study was to isolate mesenchymal stem cells (MSCs) from feline peripheral blood (fPB-MSCs) and to characterise the cells' in vitro properties. The mononuclear cell fractions were isolated from venous blood of cats by density gradient centrifugation and cultured on plastic dishes under various culture conditions to isolate MSCs. When these cells were cultured with 5% autologous plasma (AP) and 10% foetal bovine serum (FBS), adherent spindle shaped fibroblast-like cells (fPB-MSCs) were obtained from 15/22 (68%) cats. These cells were isolated only from medium containing both AP and FBS. The morphology of these MSCs was similar to those isolated from other species and from other feline tissues. fPB-MSCs expanded steadily up to 5–6 passages, but had increased population doubling time during passaging and almost all cells stopped proliferation at passages 7–9. These cells expressed CD44 and CD90, and were mostly negative for major histocompatibility class II and CD4. The cells could be induced to differentiate into adipogenic, osteogenic and chondrogenic cell lineages. These findings indicate that fPB-MSCs can be generated but appear to require specific culture conditions.

Introduction

Mesenchymal stem cells (MSCs) are plastic-adherent cells that have high proliferation capacity, express several stem cell-associated surface markers and differentiate into a range of tissue types. MSCs have been proposed as an important option in the fields of regenerative medicine and immunotherapy due to their immunosuppressive properties, their ability to produce cytokines or growth factors and their capacity to undergo multi-lineage differentiation. Cell-based therapies using MSCs have been applied in human (Salem and Thiemermann, 2010; Farini et al., 2014) and veterinary (Fortier and Travis, 2011; Quimby et al., 2011, 2013; Spencer et al., 2011; Trzil et al., 2014; Webb and Webb, 2015) medicine.

MSCs have been isolated a range of tissues, including bone marrow, adipose tissue, foetal tissue, placenta, umbilical cord and peripheral blood (PB). Although bone marrow and adipose tissue represent excellent sources of MSCs, the tissue harvesting process is invasive and requires anaesthesia in some species. Foetal tissues, placenta and umbilical cord are potentially attractive sources of MSCs, since they contain abundant MSCs and can be collected without the requirement for invasive methods, but are not always available when needed. MSCs have been isolated from PB (PB-MSCs) of human beings, mice, sheep, horses and dogs (Roufosse et al., 2004; Lyahyai et al., 2012; Spaas et al., 2013). Since collection of blood samples is minimally invasive, PB may be a source of progenitor cells in clinical situations.

Feline MSCs have been isolated from bone marrow (Martin et al., 2002; Munoz et al., 2012), adipose tissue (Webb et al., 2012; Kono et al., 2014; Zhang et al., 2014) and foetal tissues (Jin et al., 2008; Iacono et al., 2012; Vidane et al., 2014). MSCs derived from feline bone marrow (BM-MSCs) or adipose tissue (AT-MSCs) have been applied in clinical settings, including chronic kidney disease (Quimby et al., 2011, 2013), chronic allergic asthma (Trzil et al., 2014) and chronic enteropathy (Webb and Webb, 2015). Despite this trend, basic information regarding feline MSCs is still limited, and there are no reports on feline PB-MSCs (fPB-MSCs). The aim of the present study was to establish a procedure for isolating MSCs from feline PB, to characterise the markers of fPB-MSCs and to demonstrate the capacity of these cells to differentiate.

Materials and methods

Animals

All experimental procedures complied with the Fundamental Guideline for Proper Conduct of Animal Experiments stipulated by the Science Council of Japan. The blood and bone marrow donor program has an established Animal Use Protocol approved by the Institutional Laboratory Animal Care and Use Committee at Chayagasaka Animal Hospital (protocol number 14–201, date of approval 1 September 2014; protocol numbers 15–202 and 15–203, date of approval 16 September 2015). Samples of PB were collected with informed consent from client-owned cats in private veterinary hospitals. All procedures concerned with collection of blood and bone marrow

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http://dx.doi.org/10.1016/j.tvjl.2016.08.009
1090-0233/

were performed by licensed veterinarians. Pre-experiment evaluation included physical examination, complete blood count, biochemistry profile and feline leukaemia/feline immunodeficiency virus serology.

Isolation and culture of fPB-MSCs

PB (12 mL) was obtained by jugular vein puncture from 22 domestic cats (mean 5.7 years old; range 1–12 years of age; mean 5.0 kg; 10 males, 12 females). All blood sampling was achieved without sedation. This blood was collected into syringes containing 0.5 mL (500 IU) sodium heparin (Mochida) and transported at 4 °C to the laboratory within 3 h. To isolate peripheral blood-derived mononuclear cells (PBMCs), undiluted blood was layered onto 12 mL Lympholyte (Cedarlane) in a 50 mL tube and centrifuged at 300 *g* for 40 min without braking. The plasma in the top layer was collected for use as a culture medium supplement. The mononuclear cells were collected from the interphase, washed twice with phosphate buffered saline (PBS) by centrifuging at 300 *g* for 5 min and then counted. After an additional wash with PBS, cells were resuspended in culture medium (base medium) consisting of Dulbecco's modified Eagle's medium (DMEM; Sigma) with 5% separated autologous plasma (AP), 10% foetal bovine serum (FBS; Gibco) and 10 μL/mL antibiotic-antimycotic solution (Gibco). Base medium was prepared in 50 mL tubes. The largest possible quantity of this medium was created from each donor's separated plasma and stored at 4 °C until further use. Subsequently, cells obtained from each 12 mL of blood were seeded onto a 100 mm^2 tissue culture dish (Iwaki) and incubated in an atmosphere of humidified air and 5% CO_2 at 37 °C. Non-adherent cells were removed by washing the mononuclear cells twice with PBS after 72 h of incubation and fresh medium was then added to the dishes. Thereafter, the medium was changed twice weekly. Isolated colonies of putative MSCs were apparent after 4–14 days in culture and were maintained in growth medium until ~70% confluence. The cells were then treated with 0.05% trypsin–ethylene diamine tetra-acetic acid (EDTA; Sigma) and further cultured for subsequent passage in 100 mm^2 dishes at 7500 cells/cm^2 in base medium. This procedure was repeated as many times as possible. Cell doubling time (CDT) was calculated in four cats, using the following formula: $CDT = \ln(N_f/N_i)/\ln 2$, with N_f the final, and N_i the initial number of cells (Spaas et al., 2013). For population doubling time (PDT), the cell culture time (in days) was divided by the CDT (Spaas et al., 2013).

Influence of culture conditions on feline PBMCs

Several culture conditions were utilised to evaluate the influence of culture medium on feline PBMCs. PB (33 mL) was obtained by jugular vein puncture from three cats (mean 5.6 years old; mean 6.1 kg; two males, one female) under anaesthesia with mask administration of isoflurane. This blood was collected into syringes containing 1.5 mL (1500 IU) sodium heparin and transported at 4 °C to the laboratory within 3 h. PBMCs were prepared as described above. Eleven sub-fractions containing equal numbers of PBMCs were resuspended in culture medium containing either AP or FBS, then various supplements were added and the cells were seeded in a 6-well plate (Iwaki).

Eleven culture media were tested for the isolation of fPB-MSCs (Table 1). Some of the culture media were supplemented with recombinant feline granulocyte macrophage colony-stimulating factor (GM-CSF; R&D Systems) and feline BM-MSCs conditioned medium (fBM-MSCs-CM). Feline BM-MSCs were isolated from one healthy cat (4 years old, 5.4 kg, male) using a previously reported similar culture method (Webb et al., 2012). To collect bone marrow, the cat was sedated via mask administration of isoflurane, and lidocaine was administered subcutaneously at the site of skin puncture. After sterilisation, 1 mL of bone marrow was collected from the proximal humerus into a heparin-coated syringe using a bone marrow needle. Aside from the media used, the culture conditions were the same as above. After 3 weeks in culture, changes in the seeding of mononuclear cells were observed and evaluated under each condition with a light microscope.

Immunophenotyping using flow cytometry

Putative fPB-MSCs were characterised by immunophenotyping using previously identified MSC surface markers. Cells were removed from culture dishes by incubation in 0.05% trypsin-EDTA at 37 °C. Cells were resuspended in 2% bovine serum albumin (BSA; Nacalai Tesque) in PBS and then incubated with antibodies against anti-mouse CD44-fluorescein isothiocyanate (FITC; 1:100, clone IM7, eBioscience), anti-human CD90-phycoerythrin (PE; 1:100, clone 5E10, BD Bioscience), anti-human major histocompatibility (MHC) class II-FITC (1:100, clone TU39, BD Bioscience) or anti-feline CD4-PE (1:100, clone 3-4F4, Southern Biotech) in the dark at 4 °C for 45 min. Additional cells were labelled with appropriate isotype control antibodies. Following incubation, cells were washed twice with PBS and suspended in 500 μL PBS in fluorescence activated cell sorting (FACS) tubes (BD Bioscience). A total of 10,000 events were evaluated for each sample using a FACSCalibur flow cytometer (BD Bioscience) and the data were analysed with FlowJo software (Tree Star).

Immunocytochemical analysis

Since feline-specific antibodies are not available for some MSC surface markers, in this study we used anti-human and anti-mouse antibodies that exhibit interspecies cross-reactivity and have been used in previous studies for identification of feline MSCs (Quimby et al., 2011, 2013; Kono et al., 2014). The putative fPB-MSCs were retrieved at passage 2 by trypsinisation and counted, then 20,000 cells were cultured in 4-well chamber slides (BD Bioscience) to 90% confluency. The culture medium was discarded and cells were gently washed three times with PBS. The cultured cells were fixed with fresh 4% paraformaldehyde (Wako) for 30 min at room temperature. They were then washed three times with PBS and subsequently rinsed with 2% BSA in PBS for 30 min at room temperature to block non-specific binding. The cells were then incubated with FITC-conjugated anti-mouse CD44 (1:500, clone IM7, eBioscience) or PE-conjugated anti-human CD90 (1:500, clone 5E10, BD Bioscience) primary antibodies in the dark at room temperature for 2 h. For negative controls, the primary antibodies were replaced with PBS alone or isotype controls. Subsequently, in order to identify the nuclei, the cells were carefully washed three times with PBS and slides were mounted in Vectashield Hard Mounting Medium with DAPI (4′,6-diamidino-2-phenylindole; Vector Laboratories). The specimens were observed at 100× magnification with a fluorescence microscope (FSX100 Bio Imaging Navigator, Olympus).

Table 1
Influence of various culture mediums on feline peripheral blood-derived mononuclear cells (PBMCs) from three different cats ($n = 3$).*

	Medium and supplement	Influence on feline PBMCs
1	DMEM with 10% FBS + 5% AP	Isolating fPB-MSCs
2	DMEM with 10% FBS	Diminish over time
3	DMEM with 20% FBS	Diminish over time
4	DMEM with 10% FBS + 10% fBM-MSCs CM	Diminish over time
5	DMEM with 10% FBS + 50 ng/mL rfGM-CSF	Diminish over time
6	DMEM with 10% FBS + 10% fBM-MSCs CM + 50 ng/mL rfGM-CSF	Diminish over time
7	DMEM with 5% AP	Grew in size and became multinucleated
8	DMEM with 10% AP	Grew in size and became multinucleated
9	DMEM with 5% AP + 10% fBM-MSCs-CM	Grew in size and became multinucleated
10	DMEM with 5% AP + 50 ng/mL rfGM-CSF	Grew in size and became multinucleated
11	DMEM with 5% AP + 10% fBM-MSCs-CM + 50 ng/mL rfGM-CSF	Grew in size and became multinucleated

All media were supplemented with antibiotics and antimycotic solution.
FBS, foetal bovine serum; AP, autologous plasma; fBM-MSCs-CM, feline bone marrow-derived mesenchymal stem cells conditioned medium; rfGM-CSF, recombinant feline granulocyte macrophage colony-stimulating factor.
* fBM-MSCs-CM was prepared from confluent MSC (passage 2) in 10 mL medium in a 100 mm^2 culture dish for 72 h. These cells were isolated from one healthy cat (4 years of age, weight 5.4 kg, male) using a culture method similar to Webb et al. (2012). Following culture, the medium was filtered through a 0.22 μm membrane and stored in aliquots of 1.2 mL –80 °C.

Trilineage cell differentiation

In order to demonstrate in vitro multi-lineage differentiation capacity, fPB-MSCs at the end of passage 3 or 4 from each of three cats were induced to differentiate into adipocytes, osteoblasts and chondrocytes. Differentiation assays were performed as described previously by Quimby et al. (2013) with minor modifications.

To assess osteogenic differentiation, fPB-MSCs were seeded at a density of 5000 cells/cm^2 in a 12-well plate and cultured under osteogenic conditions. Osteogenic differentiation was induced by approximately 21 days of incubation using DMEM supplemented with 10 nM dexamethasone, 20 mM β-glycerophosphate (Sigma) and 50 μM ascorbic acid (Sigma). The medium was changed twice weekly. After fixation in a 4% solution of paraformaldehyde for 30 min, osteogenic differentiation was confirmed by positive alizarin red staining of the extracellular calcium matrix.

To assess adipogenic differentiation, fPB-MSCs were seeded at a density of 2500 cells/cm^2 in a 12-well plate (Iwaki) with adipogenic induction medium consisting of DMEM supplemented with 10% FBS, 0.5 μM dexamethasone (Wako), 0.5 μM 3-isobutyl-1-methylxanthine (Sigma) and 50 μM indomethacin (Wako), replaced twice weekly. After 3 weeks, cells were fixed in 10% neutral buffered formalin for 20 min, washed with 60% isopropanol and stained with oil red O for 20 min, followed by repeated washing with distilled water.

Chondrogenic differentiation was induced by micromass pellet culture. fPB-MSCs (5×10^5) were centrifuged at 300 *g* for 5 min at room temperature and resuspended in chondrogenic induction medium consisting of DMEM supplemented with 1% FBS, 10 ng/mL transforming growth factor (TGF)-β3 (Pepro Tech), 100 nmol/L dexamethasone, 50 μg/mL ascorbic acid and 40 μg/mL proline (Sigma). The cell suspensions were seeded in the centres of 12-well plates and incubated at 37 °C for 15 min. Medium was added gently and replaced twice weekly. After 2 weeks in culture, spheroid cell masses were fixed and embedded, then cut by microtome

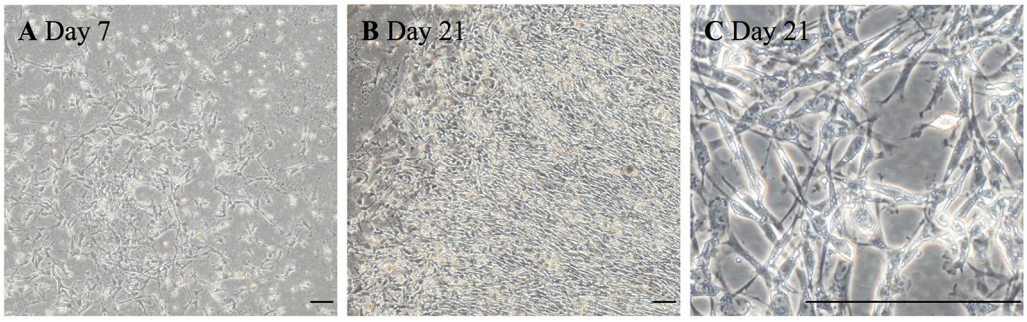

Fig. 1. Morphological characteristics of adherent putative feline peripheral blood-derived mesenchymal stem cells (fPB-MSCs) on different days. (A) Adherent, spindle-shaped fibroblastic cells formed colonies that were observed in culture dishes 7 days after seeding. Further expansion of cell colonies (B) and spindle and satellite cell morphology (C) at 21 days after seeding. Scale bar = 200 μm.

before alcian blue and periodic acid-Schiff (PAS) staining. Remaining cells in the well were also stained with alcian blue.

Results

Isolation and expansion of fPB-MSCs

Blood samples from 22 different healthy donor cats were processed. Using standard density gradient centrifugation, 2.29×10^7 and 8.47×10^7 PBMCs (mean number of cells 3.76×10^7; mean concentration of cells 3.13×10^6 cells/mL; $n = 22$ cats) were obtained. Proliferating putative fPB-MSCs were isolated from 15 samples (68% efficiency) in culture medium including 5% AP and 10% FBS. Adherent, spindle-shaped fibroblastic cells formed colonies in culture dishes 4–14 days after seeding (Fig. 1A). As time advanced, the cell colonies further expanded (Fig. 1B) and cells consistently maintained a spindle-shaped morphology (Fig. 1C). At 21–35 days after seeding, the cells became confluent. The number of colonies and the rate of cell proliferation differed between donors. Some donors formed many colonies soon after seeding and most of the colonies demonstrated fast propagation. There was no apparent association between growth properties and age, sex or breed. The number of fPB-MSCs generated from the initial culture (passage 0 to 1) was 1.10×10^6 to 8.75×10^6 (mean 3.30×10^6). PDT tended to increase with increasing passage number, but not in all cultures (Table 2). Although all fPB-MSCs steadily expanded up to 5–6 passages, almost all cells stopped proliferation at passages 7–9. Only 1/15 cultures was still proliferating at passage 10.

Influence of culture conditions on feline PBMCs

A summary of different types of culture media effects is shown in Table 1. fPB-MSCs were only isolated using medium containing both FBS and AP. In contrast, culture medium containing only FBS resulted in a diminished numbers of seeded PBMCs over time, and putative fPB-MSC colonies could not be obtained. In culture medium containing only AP, the seeded cells gradually increased in size and became multinucleated; these cells were suspected to be giant macrophages, although definitive cell phenotyping was not performed. Although these cells became confluent after 3 weeks, no spindle-shaped putative fPB-MSCs were obtained. Culture medium supplemented with recombinant feline GM-CSF and fBM-MSCs-CM did not result in the isolation of putative fPB-MSCs.

Table 2
Population doubling time (PDT) in days of putative peripheral blood-derived feline mesenchymal stem cells of four cats.

Passage	Donor 1	Donor 2	Donor 3	Donor 4
P0 to P1	–3.14	–4.04	–8.53	–9.31
P1 to P2	1.74	2.69	1.58	3.62
P2 to P3	3.34	4.61	2.86	3.13
P3 to P4	3.61	3.94	2.73	3.7
P4 to P5	4.68	4.13	2.64	3.09
P5 to P6	5.06	4.18	3.21	4.57
P6 to P7	4.39	3.06	2.76	5.6
P7 to P8	7.46	4.27	3.19	9.30
P8 to P9	8.65	5.83	3.02	*
P9 to P10	*	9.9	5.36	

* fPB-MSCs did not reach a confluent state.

Immunophenotyping assays

Immunophenotyping using flow cytometry was performed in five different cats. These donors were randomly selected from 15 cats from which putative fPB-MSCs were isolated in previous experiments. The putative fPB-MSCs were strongly positive for CD44 and CD90. In contrast, MHC class II and CD4 were uniformly negative (Fig. 2A) or were expressed only in a small number of cells. Summaries of the flow cytometry analysis results for each marker are shown in Fig. 2B. On the basis of immunocytochemistry, CD44 and CD90 were detected strongly on almost every putative fPB-MSC (Fig. 3).

In vitro differentiation

After 3 weeks of culture using osteogenic differentiation medium, fPB-MSCs were stellate or irregular. Differentiated cells showed multiple alizarin red-stained calcium deposits (Fig. 4A). The cell morphology changed from a spindle-shaped to a stellate configuration after 3 weeks of culture in adipogenic medium and many cells contained numerous oil red O-positive lipid droplets (Fig. 4B). fPB-MSCs formed macroscopically spherical colonies after 3 days of culture with chondrogenic induction medium. These colonies increased in size and formed opaque cartilaginous nodules over time. The nodules were fixed and the remaining cells in each well were evaluated after 14 days. Unsulphated proteoglycans in the extracellular matrices were stained with PAS and alcian blue, confirming the formation of cartilage lacunae (Fig. 5A and C). Conversely, spherical colonies that had initially formed in the base medium (negative control) gradually decreased in size, did not have lacunae and did not exhibit positive staining with alcian blue (Fig. 5B and D).

Discussion

Feline MSCs were first isolated by Martin et al. (2002) from bone marrow and subsequently feline MSCs have been derived from adipose (Webb et al., 2012; Kono et al., 2014; Zhang et al., 2014) and foetal (Iacono et al., 2012; Vidane et al., 2014) tissue. In these studies, feline MSCs were characterised by morphological

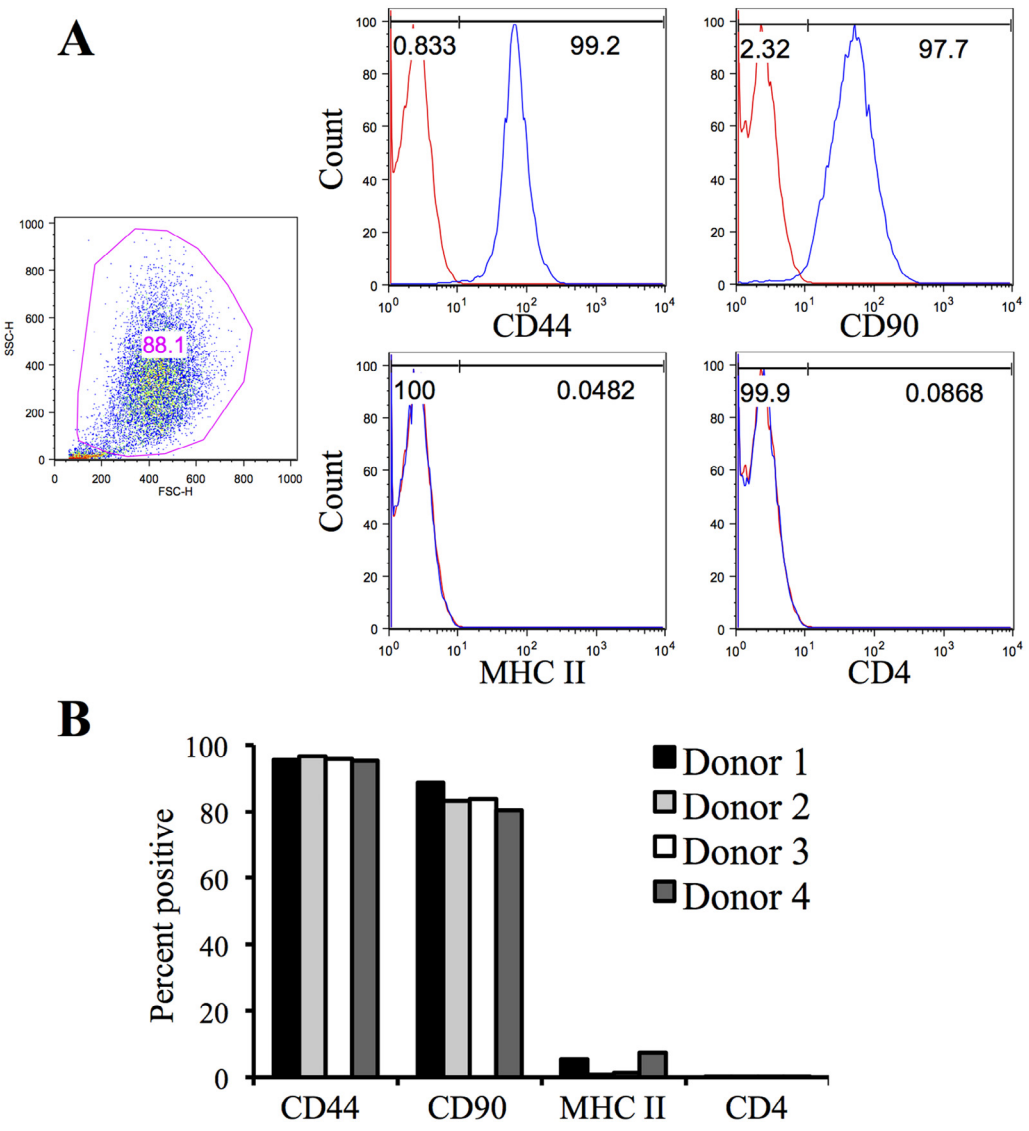

Fig. 2. (A) Representative results from one cat showing the immunophenotype of feline peripheral blood-derived mesenchymal stem cells (PB-MSCs) (passage 2) by flow cytometry. Most feline PB-MSCs expressed CD44 and CD90, but did not express major histocompatibility complex (MHC) class II or CD4. The blue lines represent specific staining, red lines are identical cells stained with isotype controls. (B) Immunophenotypes of PB-MSCs (passage 2) from four cats. The specific surface marker examined is listed on the *x* axis, and the percentage of expressing cells is shown on the *y* axis.

features, cell-surface antigen profiles and their capacity for differentiation. Although the existence of MSCs in PB has been demonstrated in many species, our study is the first to demonstrate the feasibility of isolating fPB-MSCs exhibiting appropriate morphology, mesenchymal surface markers and the capacity to differentiate into osteoblasts, chondroblasts and adipocytes.

Canine PB-derived fibroblast-like cells have been isolated in the presence of interleukin 6 (Huss et al., 2000). Tondreau et al. (2005)

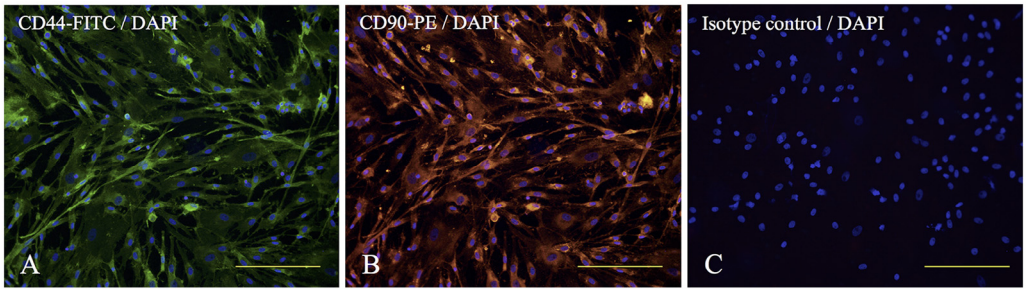

Fig. 3. Representative fluorescent images for mesenchymal stem cell markers. Feline peripheral blood-derived mesenchymal stem cells were labelled at passage 2. There is strong staining for CD44-fluorescein isothiocyanate (FITC) (A) and CD90-phycoerythrin (PE) (B) compared to the isotype control (C). Cells were co-labelled with 4′,6-diamidino-2-phenylindole (DAPI; blue). Scale bar = 100 μm.

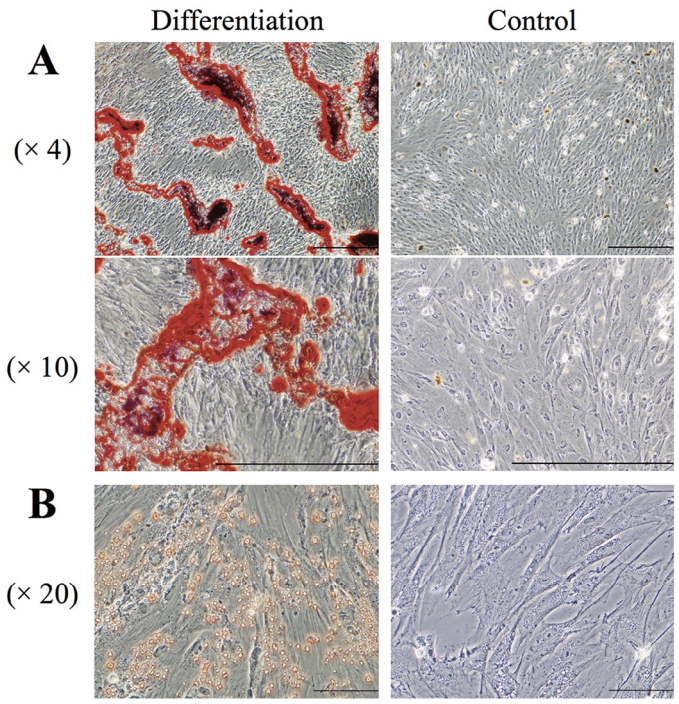

Fig. 4. Representative images of feline peripheral blood-derived mesenchymal stem cells (fPB-MSCs) cultured in adipogenic and osteogenic induction medium. (A) Osteogenic differentiation was demonstrated by calcium deposition shown by alizarin red S staining. Scale bar = 500 μm. (B) Adipogenic differentiation of fPB-MSCs was confirmed based on production of lipid droplets using oil Red O staining. Scale bars = 100 μm. Respective negative control cells are shown on the right.

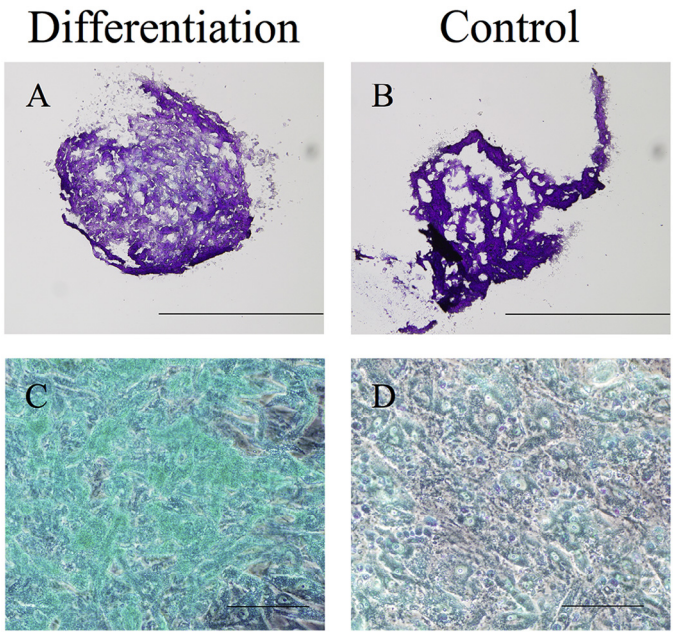

Fig. 5. Chondrogenic differentiation of feline peripheral blood-derived mesenchymal stem cells. (A) Sectioned nodules treated with chondrogenic medium were stained by PAS and alcian blue methods. The stained tissue displayed typical cartilaginous tissue phenotype. (C) The presence of acidic proteoglycan has been demonstrated at monolayer cells treated with chondrogenic medium by alcian blue staining. (B and D) Respective negative controls are shown on the right. Scale bar = 200 μm.

isolated MSCs from human PB using GM-CSF and culture medium containing conditioned medium from human BM-MSCs. Standard isolation methods used for BM-MSCs can be employed to isolate PB-derived precursor cells from several mammals, such as sheep and horses (Koerner et al., 2006; Lyahyai et al., 2012). Although we tried to isolate fPB-MSCs using previously described culture methods for isolation of feline BM-MSCs (Martin et al., 2002; Munoz et al., 2012; Webb et al., 2012), the seeded PBMCs diminished over time. Additionally, cell proliferation was not observed, despite the use of several different types of culture media, for example media containing feline GM-CSF or fBM-MSCs-CM. Culture medium containing both FBS and AP resulted in the enhanced adherence and propagation of fPB-MSCs. Our results suggest that methods used to isolate PB-MSCs differ between animal species.

In several species, MSCs have been successfully cultured and grown in autologous serum or platelet-rich plasma instead of medium supplemented with FBS (Stute et al., 2004; Edamura et al., 2012; Fukuda et al., 2015). To the best of our knowledge, no studies have used a culture medium supplemented with both AP and FBS to isolate MSCs. Culture medium supplemented with only FBS or AP did not facilitate the isolation of fPB-MSCs. It is unknown which components of feline plasma are responsible for the ability to isolate and maintain proliferation of fPB-MSCs. Growth factors, such as TGF-β and fibroblast growth factors, affect proliferation, morphogenesis and survival of MSCs (Rodrigues et al., 2010). We are investigating which factors are required during the growth stage of fPB-MSCs. Our preliminary findings suggest that the propagation of passaged fPB-MSCs might not require AP (data not shown).

fPB-MSCs had similar morphology, proliferation and growth properties to MSCs derived from other feline tissues (Vidane et al., 2014; Arzi et al., 2015), although the initial culture time to reach near confluence in fPB-MSCs was longer than that of feline BM and AT-MSCs (Martin et al., 2002; Kono et al., 2014). fPB-MSCs were positive by flow cytometry for CD44 and CD90, and mostly negative for CD4 and MHC II, similar to MSCs derived from other feline tissues and PB-MSCs from other mammals (Webb et al., 2012; Spaas et al., 2013). fPB-MSCs had multi-lineage differentiation potential, similar to PB-MSCs of other species (Lyahyai et al., 2012; Spaas et al., 2013). On the basis of this evidence, we believe that the cells derived from feline PB in the current study were MSCs.

BM-MSCs or AT-MSCs have potential for use in feline regenerative medicine and treatment of inflammatory diseases (Quimby et al., 2011, 2013; Trzil et al., 2014; Webb and Webb, 2015). Since the collection of PB is minimally invasive and does not usually require anaesthesia, the harvesting procedure used in our study for fPB-MSCs may be a useful source of MSCs.

Iazbik et al. (2007) and Barfield and Adamantos (2011) concluded that a maximum of 10–12 mL/kg blood can be donated at one time by healthy cats. We selected 12 mL as the blood volume that can be safely collected without the use of sedation from most healthy cats. However, collection of blood from sick cats might have several limitations, such as volume restriction and altered isolation rates and growth properties of MSCs. The nature of MSCs may change with the health status of the donor (Jones and Schäfer, 2015). The growth properties and differentiation potential of MSCs may be adversely affected by increased donor age (Choudhery et al., 2014). We were not able to identify a similar trend in our study, but further investigation may be required using larger numbers of cats. In our study, samples were taken only from healthy, client-owned cats, but MSCs could not be generated from PB from seven cats using our study techniques. Infection with feline foamy virus may affect the ability to isolate feline MSCs (Arzi et al., 2015), but infection with this virus was not investigated in our study. A general screening test for infectious diseases that might affect PB-MSCs is recommended for feline blood donors.

Conclusions

A simple and reproducible method was established for isolation and propagation of MSCs from feline PB. Isolation of

fPB-MSCs was only achieved using culture medium containing both FBS and AP. The cells derived using this method had a spindle cell morphology and expressed the mesenchymal markers CD44 and CD90, but with low level expression of MHC II and CD4. Trilineage differentiation of the fPB-MSCs towards osteoblasts, chondroblasts and adipocytes was confirmed. Additional studies are needed to further characterise fPB-MSCs and identify the components of feline plasma that facilitate the isolation of these cells.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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