Original Article

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Characteristics of Buccal Fat Adipose-Derived Stem Cells Cultured in Autologous Human Serum- and Fetal Bovine Serum-Supplemented Media: A Comparative Study

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Abstract:

Objective: The characteristics of adipose-derived stem cells (ADSC), isolated from buccal fat pads when cultured in media supplemented, with either autologous human serum (AHS group) or fetal bovine serum (FBS group) were compared.

Material and Methods: Buccal fat tissue was harvested from six patients, who had undergone surgical removal of their maxillary third molars or underwent orthognathic surgeries. ADSC were isolated from the tissue, via an enzymatic digestion method and cultured in the media of the AHS and FBS groups (n=6/group). Colony forming units-fibroblast (CFU-F), immune-phenotyping markers, growth and multi-differentiation of the cells from both groups were compared.

Results: The number of CFU-F and the cell growth of the AHS group were significantly greater than those of the FBS group (p-value<0.05). The expressions of the mesenchymal and hematopoietic stem cell markers of both groups were not statistically different. The cells of both groups had the potential for adipogenesis, chondrogenesis and osteogenesis when cultured in inductive conditions.

Conclusion: The immunophenotype and multi-differentiation of ADSC, which were cultured in AHS- and FBS- media, were not different. However, the AHS medium could support the capacity for forming colonies and growth of the cells better than the FBS medium.

Keywords: adipose-derived stem cells, fetal bovine serum, human serum

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Introduction

Over the last decade, stem cell therapy has become an alternative method for bone reconstructive surgeries. In the field of oral surgery stem cells can be harvested from various intra-oral sources; including dental pulp tissue, periodontal ligament and buccal fat pads. The major disadvantages of dental pulp and periodontal ligament tissue are tooth extraction required and obliterated pulp chambers that are commonly found in elderly donors. Buccal fat tissue is one of the most suitable sources of adipose-derived stem cells (ADSC)[1-4]. The buccal fat pad is easier to harvest and has less donor site morbidity when compared with body fat. The tissue can be harvested during orthognathic surgeries and maxillary third molar removal. Volumes of the tissue available for isolating the stem cells are greater, when compared with other intra-oral sources. ADSC can express their immunophenotyping markers similarly to bone marrow mesenchymal stem cells (MSC)[1-4]; however, higher progenitor cell yields of ADSC have been reported[5,6]. Some studies demonstrated similar morphologies, immunophenotypes and the multi-differentiation potential of ADSC isolated from buccal fat and subcutaneous fat[7,8]. However, cell yield of the buccal fat ADSC was higher than that of the subcutaneous fat ADSC[2]. Arpornmaeklong, et al compared characteristics of buccal fat ADSC and dental pulp derived stem cells (DPSC)[9]. The authors found that cell yield at the primary passage of the buccal fat ADSC was higher than that of DPSC. The buccal fat ADSC had steady cell growth, with high osteogenic differentiation potential; whereas, DPSC had higher growth rate in early passages but their growth remarkably decreased with subsequent passages.

In general, cell cultures are performed in the laboratory using fetal bovine serum (FBS)-containing culture media. Nevertheless, FBS may pose risks of immunological reactions from the anti-FBS antibodies, and the transmission of cross-species infections to patients[10]. Moreover, avoidance of animal-derived raw materials for manufacturing cell therapeutics has been encouraged by the European Medicine Agency (EMEA/CHMP/410869/2006)[11,12]. Therefore, the xenogeneic serum-free media (XSFM) or the media containing human components; including human platelet lysate (HPL) and human serum (HS), are become alternatives to FBS. XSFM are composed of synthetic and human-derived purified substances without xenogeneic serum supplements[10,13]. Several studies demonstrated that MSC from different sources, which were expanded in XSFM, potentially retained their phenotypic gene expression, proliferation and multi-differentiation similar to those in the FBS- media[14-17]. However, the components of XSFM are highly specific to individual cell types and the optimal formulas are still being developed. In addition, XSFM is more expensive and has less volume than FBS medium; therefore, it is not practical for large batches of cell culture.

HPL is obtained from pooled blood from different donors for reducing batch variation. HPL can support growth of bone marrow MSC and ADSC similar to FBS without altering their immunophenotype, clonogenic ability, genomic stability and differentiation potential[18,19]. However, the potential risk for human transfusion-transmitted diseases may increase with the number of donors. Moreover, there is a lack of recommended lysis protocols, and no optimum concentration of HPL for stem cell culture[20].

Human serum also contains adequate serum proteins, growth factors, growth hormones and essential nutrients for metabolism and proliferation of cells[21,22]. Culturing stem cells in a medium containing autologous human serum (AHS) from individual patients for cell-based therapy would be a cost-saving and safer strategy, with greater patient acceptability when compared with XSFM and HPL. So far, the effects of AHS on stem cells isolated from various tissue sources, but ADSC from buccal fat pads have not yet been investigated. Yamamoto et al. demonstrated similar results of proliferation, colony forming units fibroblast (CFU-F), and expression of osteogenic

differentiation genes of bone marrow MSC when cultured in a media supplemented with 10% AHS or 10% FBS[23]. In vivo, Matsuo et al. cultured bone marrow MSC in 10% AHS or 10% FBS osteogenic induction media, and implanted the cells subcutaneously in nude mice[24]. The results showed no significant difference in terms of osteogenic potential between the cells cultured in both media. Takeda et al. cultured human bone marrow MSC in AHS- and FBS-supplemented media without osteogenic induction[25]. The authors found that the alkaline phosphatase (ALP) activity of the cells cultured in the AHS-medium was higher than that of the cells in the FBS medium. In the animals, the cells cultured in AHS-medium had excellent potential for inducing the formation of an osteoid matrix; whereas, those cultured in FBS-medium did not exhibit this characteristic. In this study, the characteristics of buccal fat ADSC when cultured in media supplemented with either AHS or FBS were compared prior to considering using an AHS-supplemented medium as our standard protocol in clinical practice.

Material and Methods

The experimental protocol was approved by the Human Research Ethics Committee, Faculty of Dentistry, Prince of Songkla University (certification: EC6012-37-P-LR).

Sample calculation

The sample size was calculated using the G*Power program (version 3.1.9.2., Heinrich-Heine-Universität, Germany). The effect size (d) of 2.00 was calculated from a previous study[26], with a significance level (α) of 0.05, power (1-β) of 0.80 and an allocation ratio of 1. The calculated sample size for each group was 6 subjects, per group.

Patient enrollment

Six volunteer patients, who signed informed consent forms to participate in the experiments, were enrolled in the study. The participants undergoing third molar removal were men or women, 20–40 years old, weighing more than 50 kg, who had maxillary impacted third molars. The participants undergoing orthognathic surgeries were men or women, 20–40 years old, weighing more than 50 kg, and having at least 35.0% hematocrit. Excluded were those patients with systemic diseases (ASA>class I); including hereditary blood diseases, disorders of the blood and blood components, blood-transmitted diseases and diabetes.

Harvesting fat tissue

The patients with impacted maxillary third molars were operated on under local anesthesia. Triangular flap incisions were created to gain access for the third molar removal. After the teeth were removed, a blunt dissection was made through the buccinator muscle, and the buccal fat tissue within the surgical field was excised. Patients who underwent orthognathic surgeries were operated on under general anesthesia. After the flap incision and retraction of the Lefort I osteotomy of the maxilla and bilateral sagittal split ramus osteotomy (BSSRO) of the mandible buccal fat tissue often leaked, and from this some parts of the tissue were harvested (Figure 1). The harvested fat tissue was immediately stored in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) at 4°C. Stem cell isolation procedures were performed within 2 hrs.

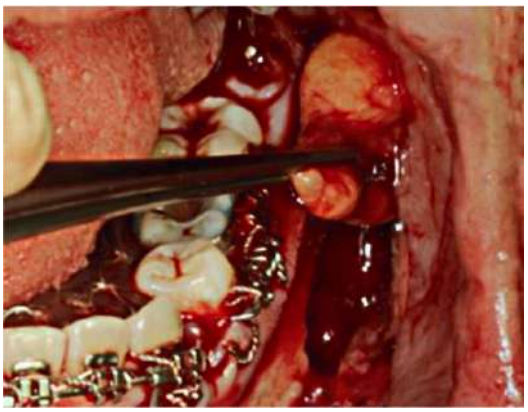


Figure 1 Harvesting fat tissue during BSSRO of the mandible is demonstrated

Blood collection and AHS preparation

For each patient undergoing third molar removal, 25 ml of venous blood was drawn from the median cubital vein or the cephalic vein prior to the operation, and another 25 ml was drawn at the follow-up visit for stitch removal. For each patient, undergoing orthognathic surgery, 50 ml of venous blood was drawn from the side opposite to the intravenous infusion arm. The blood was collected in centrifuge tubes with a clot activator (VACUETTE® Serum Clot Activator Tubes, Greiner bio-one, USA) and left for blood clotting. Afterward, the tubes were centrifuged at 2,000×g for 15 min, and then the supernatant of the serum from each tube was collected and sterilized through a 0.2 µm pore syringe filter (Minisart High Flow Syringe Filter, Sartorius, Germany). The total volume of serum from each patient was recorded, and a portion of the serum was used as a medium supplement for isolating and culturing ADSCs. For later usage, aliquots of the sterile AHS were kept at -20°C.

Isolation of ADSC

The fat tissue from each patient was washed with phosphate-buffered saline (PBS, Gibco, USA) to remove debris and red blood cells. The volume of fat tissue was measured using a 5-cc sterile disposable syringe (Nipro, Belgium). Afterwards, the fat tissue was incubated in 3 mg/ml type I collagenase (Gibco, USA) in PBS, at 37°C, with gentle agitation for 60 min. The stromal vascular fraction (SVF) was obtained as cell pellets after centrifugation at 1,200×g for 10 min. The cell pellets were resuspended by pipetting and filtered through a 70 µm cell strainer (Corning, Merck KGaA, Germany). The cells in the solution that passed the filter were equally seeded into 6-well plates (Corning, Merck KGaA, Germany), which were then divided into 2 groups.

AHS group: The cells were cultured in 200 µl of DMEM supplemented with 10% AHS and 50 µg/ml penicillin–streptomycin (Gibco, USA).

FBS group: The cells were cultured in 200 µl of DMEM supplemented with 10% FBS (Gibco, USA) and 50 µg/ml penicillin–streptomycin.

The cells of both groups were cultured in a humidified atmosphere with 5% CO_2, at 37°C, until reaching 70.0-80.0% confluence; sub-culturing was then performed. The morphologies of the cells were observed via light microscope (Zeiss, Germany). The cells of passages 1–5 were used for the following experiments (n=6/group/testing).

Capacity for forming a colony

The cells of passages 1–2 of each group were seeded into 6-well plates, at a density of 100 cells/well, and cultured to measure their CFU-F. After 10 days, the cells were fixed in 4% formaldehyde (Sigma-Aldrich Inc., USA) and stained using 0.1% toluidine blue (Sigma-Aldrich Inc., USA). The morphologies of the cells in CFU-F were examined via light microscope. The number of CFU-F was counted as the number of colonies consisting of at least 50 cells or >2 mm in diameter.

Cell growth

The cells of each group, at passages 2–3, were seeded into 24-well culture plates (Corning, Merck KGaA, Germany); at a density of $5x10^3$ cells/well, and cultured in 300 µl of the medium of each group (n=6/group/time point). On days 1, 3, 7, 14, and 21 after seeding, the number of viable cells in each well was measured using resazurin-based solution (PrestoBlue Reagent, Thermo Fisher Scientific Inc., USA); according to the manufacturer's instructions. The absorbance of each well was read at 600 nm using a microplate reader (Thermo Fisher Scientific, Finland). The levels of optical density (OD) were compared with a standard curve of OD of 3,000-50,000 cells to infer the number of cells.

Flow cytometry analysis

Fluorochrome-conjugated monoclonal antibody

cocktail (MSC Phenotyping Kit human, Miltenyi Biotec, Germany) was used for detecting the MSC immunophenotypes of the cells, following the criteria of the International Society for Cellular Therapy (ISCT)[27]. For the positive markers, $5x10^5$ cells at passages 2-3 of both groups were incubated in the antibodies of the cluster of differentiation (CD)73, 90, and 105 surface antigens; whereas, those against CD14, 20, 34, and 45 were the negative markers. The antibodies of CD271 and 146, which are specific to neural crest and pericyte cells, respectively, were also the markers in the sequences[28,29]. Each sample was measured for at least 10,000 events using a fluorescence-activated cell sorting machine (CytoFLEX S, Beckman Coulter, Germany). The data were analyzed using analysis software (CytExpert, Beckman Coulter, Germany).

Multi-differentiation potential

Adipogenic differentiation

Differentiation of the cells into an adipogenic lineage was induced using an adipogenic differentiation kit (MesenCult™, STEMCELL Technologies Inc, Canada). In brief, the cells at passages 3-5 of both groups were seeded at $3x10^4$ cells/well, in 6-well culture plates, and cultured in 2 ml of adipogenic differentiation medium in 5% CO_2, at 37°C, for 10–14 days. When lipid vacuoles of the cells were detected, via low magnification under a light microscope, they were fixed in 10% formaldehyde for 1 hr and then stained with 20mg/ml of Oil red O in isopropanol (Sigma-Aldrich Inc., USA) for 15 min. The stained cells in the well plates were scanned using a slide scanner (Aperio, Leica Biosystems, USA) to create image files for the descriptive assessment.

Chondrogenic differentiation

Differentiation of the cells into a chondrogenic lineage was induced using a chondrogenic differentiation kit (MesenCult™-ACF, STEMCELL Technologies Inc, Canada). The cells at passages 3-5 of $5x10^5$ from each group were suspended in 0.5 ml of complete MesenCult™-ACF medium in a 15 ml polypropylene tube (Corning, Merck KGaA, Germany). The suspension was centrifuged at 300×g for 10 min at room temperature to form cell pellets, and then 0.5 ml of the complete medium was added into each tube to reach a final volume of 1 ml. The tubes were incubated in 5% CO_2, at 37°C, with their caps left loosened to allow gas exchange. The medium was changed every 3 days, without disturbing the cell pellets; until day 21. On day 21, the chondrogenic cell pellets were fixed in 10% formalin for 30 min, and then embedded in paraffin for standard histological processing. Six-micron-thick histological sections were stained with Alcian Blue (Sigma-Aldrich Inc., USA).

Osteogenic differentiation and mineralization

Differentiation of the cells into an osteogenic lineage was induced using an osteogenic differentiation kit (MesenCult™, STEMCELL Technologies Inc, Canada). The cells at passages 3-5 of both groups were seeded at $2x10^4$ cells/well, into 24-well culture plates and cultured in 300 µl of osteogenic differentiation medium in 5% CO_2; at 37°C, for 21 days. The medium was changed every 3 days. The osteogenic differentiation and mineralization matrix of the cells were assessed using ALP staining and Alizarin Red S (AR) staining, respectively.

ALP staining

On day 14, ALP staining was performed using an alkaline phosphatase staining kit (BioVision, USA). The medium of each well was removed, and the cells were rinsed twice with the wash buffer. Afterwards, 250 µl of the ALP staining reagent was added into each well, and then the well plates were incubated for 30 min in 5% CO_2, at 37°C. The reagent in each well was removed and replaced with 250 µl of wash buffer prior to observing the areas of staining via light microscopy.

AR staining

The mineralization matrix produced by the cells on days 7, 14, and 21 was determined via AR staining (Sigma-Aldrich Inc., USA). On the day of the experiment, the cells were washed with PBS and fixed in 4% formaldehyde for 10 min. After that, the cells were washed with distilled water and covered with 2% AR solution for 5 min, then the unincorporated dye was washed many times with distilled water. To quantify the mineralization, the AR stain was solubilized by incubation in 160 µl of 10% acetic acid for 30 min. Afterwards, the supernatant was collected in 1.5 ml microcentrifuge tubes (Eppendorf SE, Germany), and then 100 µl of mineral oil was added. The tubes were heated at 85°C for 10 min and centrifuged at 20,000×g for 15min. The solutions were transferred into a 96-well plate, and the absorbance of each well was measured at 405 nm using a microplate reader.

Statistical analysis

The measured parameters were analyzed using statistical analysis software (SPSS version 14, SPSS Inc., IL, USA). The independent T-test was applied to compare the differences between the two experiment groups; including the number of CFU-F and immunophenotyping CD markers. One-way ANOVA, followed by Tukey's HSD were applied to compare the differences of the cell growth and the mineralization matrix of the experimental groups among the time points. A p-value<0.05 was the level of statistical significance.

Results

Demographic data

The demographic data on the six subjects are presented in Table 1.

Cell morphologies

After seeding the cell suspension, adherent cells could be detected around days 3–7, which then gradually proliferated with time. By observation, the cells of both groups had spindle-shaped or fibroblast-like morphology.

Capacity for forming colony

Within 10 days, the cells at low seeding density in both groups were able to form colonies that characterized their self-renewal. By observation, the CFU-F of the AHS group occurred earlier and resulted in larger and denser colony formation than did those of the FBS group (Figure 2). The numbers of CFU-F in the AHS group were significantly greater than those in the FBS group (p-value=0.012) (Figure 3).

Cell growth

Profiles of the cell growth in both groups are demonstrated in Figure 4. The cells in the AHS group

Table 1 Demographic data of the subjects

Case	Age (years)	Gender	Fat volume (ml)	Hct (%)	Operation	Blood volume (ml)	AHS volume(ml)	AHS/blood volume (%)
1	24	Male	5	44.3	BSSRO setback	50	23	46
2	21	Female	3	-	Surgical removal of 18	50	20	40
3	23	Female	3	-	Surgical removal of 28	50	21.5	43
4	36	Female	5	38.8	BSSRO advancement	50	22	44
5	21	Male	4	49.6	BSSRO setback	50	19	38
6	26	Female	5	43.4	BSSRO setback	50	20	40

AHS=autologous human serum, BSSRO=bilateral sagittal split ramus osteotomy

rapidly grew after day 3, and reached their maximum growth on day 14, whilst those in the FBS group gradually grew and reached their maximum growth on day 21. The mean amounts of cells in the AHS group were significantly greater than those in the FBS group at days 14 and 21 (p-value=0.001 and p-value<0.001, respectively).

Flow cytometry analysis

The expression of the MSC immunophenotypes of both groups is demonstrated in Table 2. The profiles of all markers of both groups were not statistically different (p-value>0.05). The cells in both groups expressed negative markers at less than 2.0%.

Multi-differentiation potential

Adipogenic differentiation

After 14 days of culture in the adipogenic induction medium, Oil red O-stained lipid droplets in the cytoplasm of the cells in both groups were seen, implying their adipogenic differentiation potential (Figure 5 A and B).

Chondrogenic differentiation

After 21 days of culture in the chondrogenic induction medium, the cell pellets of both groups exhibited Alcian blue staining of glycosaminoglycans (GAGs) in the extracellular matrix (ECM) (Figure 5 C and D).

Osteogenic differentiation and mineralization

After 14 days of culture in the osteogenic induction medium, positive staining was detected in the cells of both groups, indicating ALP production by the differentiated cells (Figure 5 E and F). The levels of AR extracted from the cells of both groups steadily grew with time over 21 days (Figure 6). The levels in the AHS group were greater than those in the FBS group at all observation timepoints (p-value>0.05).

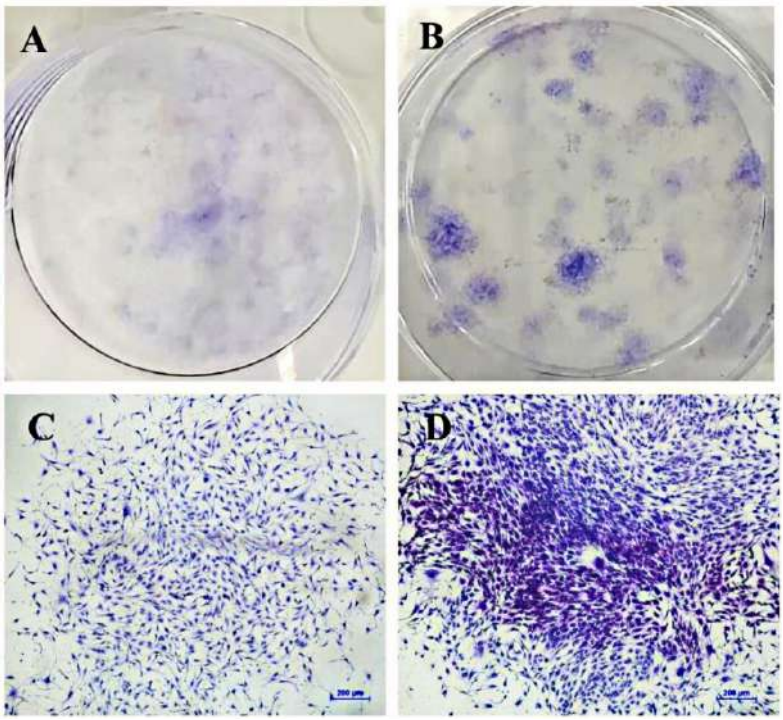
Discussion

This study demonstrated methods of harvesting and isolating ADSC from buccal fat pads, cultured in AHS for expansion. Additionally, expressions of CD271 and 146 of the cells were assessed that would imply their embryonic origins. Those protocols were practical and economical. Additionaly, combining osteogenic differentiated stem cells with some types of bone grafts and osteoconductive scaffolds can be applied clinically to promote new bone formation. The major advantages of AHS over allogeneic human serum (Allo-HS) are their biocompatibility of individual growth factors, proteins, hormones and having no risk of Rh sensitization nor blood-transmitted diseases from other donors[10,21,22]. Although commercial allo-HS prepared from AB-blood group donors is available, it is still up to 4-5 times more expensive than FBS. In this study, the volumes

Table 2 The percentages of immunophenotype markers among the cells

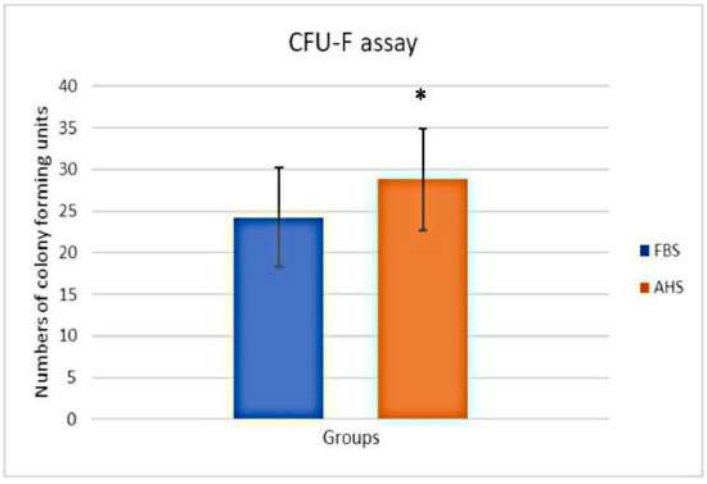
CD Markers		FBS Group (%)	AHS Group (%)
MSC markers (Positive markers)	CD73	80.1±8.6	81.0±7.4
	CD90	66.3±8.2	66.0±8.4
	CD105	41.6±8.1	41.2±13.1
Hematopoietic markers (Negative markers)	CD14, 19, 34, 45	0.2±0.2	0.2±0.1
	HLA-DR	0.4±0.1	0.4±0.2
Additional markers	CD271	7.5±7.1	7.1±5.1
	CD146	4.1±2.3	5.5±2.2

CD=cluster of differentiation, MSC=mesenchymal stem cell, HLA-DR=human leukocyte antigen-DR isotype, FBS=fetal bovine serum, AHS=autologous human serum



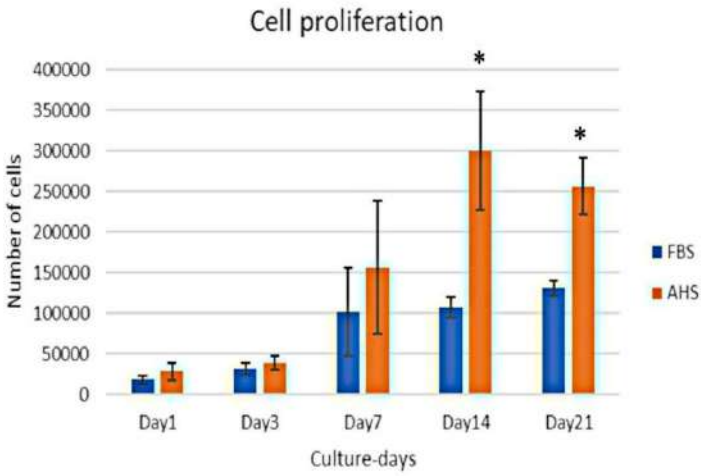
CFU-F=colony forming units-fibroblast, FBS=fetal bovine serum, AHS=autologous human serum

Figure 2 Toluidine blue staining of CFU-F of the FBS group (A) and (C), and the AHS group (B) and (D) after 10 days of culture. The 5x magnified images (C) and (D) demonstrate the morphologies of the CFU-F (scale bars=200 μm). The cells of both groups were fibroblast-like spindle shaped and the amount of the cells per colony of the AHS group was higher than that of the FBS group



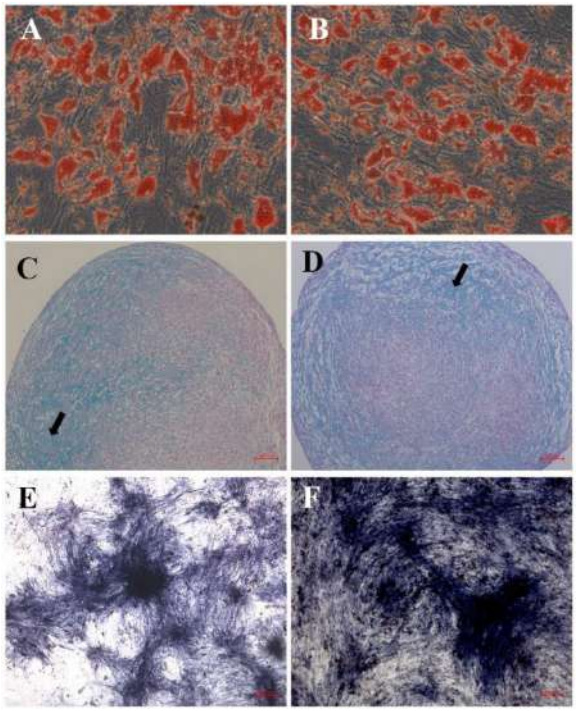
CFU-F=colony forming units-fibroblast, FBS=fetal bovine serum, AHS=autologous human serum

Figure 3 The numbers of CFU-F in the FBS and AHS groups. The numbers of CFU-F in the AHS group were significantly greater than those in the FBS group (*p-value<0.05)



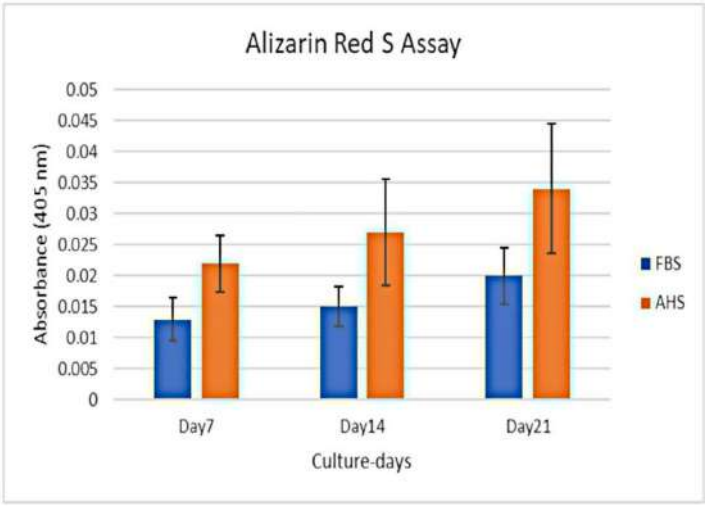
CFU-F=colony forming units-fibroblast, FBS=fetal bovine serum, AHS=autologous human serum

Figure 4 The growth profiles of the FBS and AHS groups over 21 days. The amounts of cells in the AHS group were significantly greater than those in the FBS group at days 14 and 21 (*p-value<0.05)



FBS=fetal bovine serum, AHS=autologous human serum, GAGs=glycosaminoglycans, ECM=extracellular matrix, ALP=alkaline phosphatase

Figure 5 Images of Oil red O-stained cells from the FBS group (A) and the AHS group (B) demonstrate the red areas of the accumulation of lipid-rich vacuoles within the adipocytic differentiated cells (scale bars=50 μm). Images of Alcian-blue-stained pellets from the FBS group (C) and the AHS group (D). The arrows indicate the blue staining of GAGs deposition in the ECM (scale bars=100 μm). ALP staining images of the FBS group (E) and the AHS group (F) demonstrate bluish-purple precipitation, indicating an increase in ALP (scale bars=200 μm)



AR=alizarin red, FBS=fetal bovine serum, AHS=autologous human serum

Figure 6 The levels of AR extracted from the cells of both groups. The levels in the AHS group were greater than those in the FBS group at all observation timepoints

of 10% AHS supplemented medium were adequate for the entire culture period; however, a small volume of AHS from each donor is still the major limitation for long-term culture. Therefore, further studies should be performed to assess whether lesser volumes of AHS can support longer periods of cell culture, without compromising characteristics of the cells. Regarding the results, ADSC cultured in the AHS medium had greater capacity for forming colonies, when compared with those cultured in the FBS medium. By observation, the colonies of the AHS group were larger and denser than of those of the FBS group. This implies that the type of serum might relate to the number of cells forming per colony[23]. The growth of the cells in the AHS group was significantly better than that of the cells in the FBS group. The highest number of cells growing in the AHS group was reached on day 14; whereas, the FBS group was reached on day 21. This meant that AHS could accelerate cell growth and could thus reduce the time spent on culturing the cells as well as their exposure to culture reagents. Regarding cell differentiation, the ADSCs cultured in the media of both groups were able to differentiate into three lineages of adipogenesis, chondrogenesis and osteogenesis. Therefore, it is presumed that AHS is superior to FBS in terms of promoting the proliferation of ADSC while retaining their adipogenic, chondrogenic and osteogenic differentiation potential.

Regarding the essential characteristics of MSCs defined by ISCT, at least 95.0% of the cell population must express MSC surface antigens of: CD73, CD90 and CD105, and express hematopoietic surface markers of: CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR in less than 2.0%. It is known that buccal fat is highly vascularized tissue, and various types of progenitor cells are present in the perivascular niche around the adipose tissue. These cells include tissue-resident mesoderm-derived cells, circulating bone-marrow-derived cells, pericytes and neural-crest-derived cells. Although, adipose tissue derives from the mesoderm, the developmental origin of ADSCs during embryogenesis remains unclear. Some studies in the literature support the assertion that some subsets of bone marrow, dental pulp and adipose MSCs originate from the neural crest[30]. Cuevas-Diaz Duran et al.[31] and Quirici

et al.[32] reported amounts of CD271-positive cells, isolated from human subcutaneous adipose tissue, using magnetic labeling beads of 2.9% and 4.4%, respectively. Another theory hypothesized that the perivascular zone is the niche of ADSCs, which arise from a pericytic origin[29, 33]. CD146 is an early surface marker of MSCs derived from perivascular cells[34]. In this study, the CD271- and 146-positive cells of the FBS group accounted for 7.5±7.1% and 4.1±2.3%, respectively; whereas, those of the AHS group accounted for 7.1±5.1% and 5.5±2.2%, respectively. This could imply that CD 271 and 146 define the subpopulation of isolated buccal fat ADSCs. Regarding the ISCT criteria, the results did not meet the criteria. The possible reason for this is that the method utilized for isolating ADSCs from buccal fat tissue might not provide a homogeneous mesenchymal population[35]. However, the amount of stem and progenitor cells obtained through this method did not retard their self-renewal capacity, proliferation, or multi-differentiation potential. For the application of stem-cell-based bone tissue engineering, the higher matrix mineralization of the cells cultured in the AHS medium would also indicate the benefit of using AHS for culturing ADSCs for clinical use.

Conclusion

The immunophenotype and multipotentiality for differentiation of ADSCs, which were cultured in AHS- and FBS- media were not different. However, the AHS-supplemeted medium was able to support the capacity for forming colonies and growth of the cells better than the FBS-supplemeted medium. Therefore, it is possible to use AHS-supplemented medium as a standard protocol for culturing stem cells.

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Conflict of interest

There are no conflicts of interest.

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