The Biological Characteristics of Placenta Derived Mesenchymal Stem Cells Cultured in Human Serum

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Objective: *Evidence exists indicating that mesenchymal stem cells (MSCs) are promising candidate for therapeutic applications. One major obstacle for their clinical use is the biosafety of fetal bovine serum (FBS), which is a crucial part of all media currently used for culture of MSCs. Although some recent studies recommended substituting FBS with human serum (HS) for the expansion of MSCs for clinical use, the characteristics and functional capacity of the expanded cells has only been partially explored. In addition, limited experience indicates that HS may replace FBS in some but not all culture systems. Currently, relatively little is known about using HS instead of FBS for isolation and expansion of placenta derived MSCs. Therefore, this study aimed to compare the exploit of HS and FBS as a supplement in terms of their impact on biological characteristics of MSCs.*

Material and Method: *MSCs derived from placenta were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum or 10% human serum. The morphology, the expression of MSC markers, the differentiation ability and the proliferation characteristics were examined.*

Results: *The results demonstrated that MSCs cultured in DMEM supplemented with 10% HS had similar characteristics to MSCs cultured in DMEM supplemented with 10% FBS. Interestingly, MSCs cultured in DMEM supplemented with 10% HS had greater expansion potential than that of MSCs cultured in DMEM supplemented with 10% FBS.*

Conclusion: *The results obtained from this study imply some application in the use of HS instead of FBS for expansion of placenta derived MSCs. The HS-expanded MSCs might be useful and safe for use as a therapeutic tool in regenerative medicine.*

Keywords: *Mesenchymal stem cell, Human serum, Placenta*

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Mesenchymal stem cells (MSCs) have become an innovative tool for cell based therapy of degenerative disorders over the past 2 decades[1]. Besides their capacity for differentiation into cell lineages of mesenchymal origin (adipocytes, osteoblasts, and chondrocytes), a body of evidence suggests their potential to differentiate into non-mesenchymal cell lineages as well, such as neural-like cells, hepatocytes, and pancreatic-like cells[2,3]. The characteristics that qualify MSCs as a favorable tool for cellular therapies of autoimmune and chronic diseases manifested both as inflammation and tissue injury are their ability to suppress alloreactive T, B, and NK cell responds[4]. In addition, they have immunomodulatory properties for which they are used in the prophylaxis and treatment of graft versus host disease (GvHD)[5]. MSCs have been shown to prevent graft failure and to promote engraftment in hematopoietic stem cell transplantation (HSCT)[6]. Currently, the expansion of MSCs for their clinical use has been performed under different culture conditions, most of which were based on the addition of fetal bovine serum (FBS) as a supplement[2]. Under these culture conditions, anti-FBS antibodies have been detected in most patients infused with MSCs cultured in the presence of FBS[7]. The identification of suitable culture conditions for optimal expansion and appropriate functional capability of MSCs still remains a crucial

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matter. The recent interest in obtaining high numbers of MSCs for clinical use has led to the development of therapeutic protocols based on the use of hemo-components such as autologous and allogeneic human serum (HS) or plasma, cord blood serum or human platelet lysate (HPL)[7]. HS-containing media were recently described as possible substitutes for FBS-containing media for the expansion of MSCs for clinical use[7]. Indeed, HS-containing media markedly increase proliferation of MSCs that displayed neither immunogenicity nor oncogenic potential[7]. Little is known about the effect of HS-containing media on the functional properties of MSCs and hence on their efficacy in clinical application. Notably, HS contains numerous bioactive molecules as well as growth factors that may influence MSCs expansion and function[8]. In this context, some recent reports have addressed the influence of HS on the generation and expansion of MSCs[7,9]. These studies mainly focused on morphology, proliferation, differentiation, immunophenotype, karyotype and tumorogenicity. Telomerase activity, transformation and senescence have also been investigated due to the rapid proliferation of MSCs[10]. Various studies, mostly in bone marrow derived MSCs, reported similar phenotypic and functional effects of MSCs expanded in HS and in FBS-containing media[11]. Conflicting results have been obtained on the effect of HS on mitogen-stimulated or alloantigen-induced T-cell proliferation[12]. There are no data addressing the effect of HS on the biological characteristics of placenta derived MSCs. This information is important in view of an alternative sources of MSCs expanded in animal free system for human therapeutic application.

Material and Method

Ethical consideration

This study was approved by the Human Ethics Committee of Thammasat University No. I (MTU-EC-DS-3-139/57). All subjects participated in the study after giving written informed consents. The placenta was obtained from pregnant women after normal delivery at Thammasat Chalermprakiat Hospital. Peripheral blood samples were collected from healthy volunteers. Subjects who had any clinical history of malignancy, metabolic disorder, or infectious disease, were excluded.

Preparation of human serum

Human serum was donated from 20 infection-free, healthy volunteers. From each donor, 20 ml of whole blood was drained into 50-ml sterile tubes without anticoagulant and allowed to clot overnight at 4°C. The serum was separated by centrifugation at 2,000xg for 30 min at room temperature. Subsequently, the supernatants were carefully aspirated and pooled into new sterile tube. The pooled serum was then sterilely filtered through 0.22-μm pore filters (Costa, corning, USA), aliquoted into 15-ml sterile tubes and frozen at -20°C until use. Once thawed, sterile pooled human serum was centrifuged to remove the aggregated material and then maintained at 4°C to avoid refreezing.

Isolation and culturation of MSCs derived from placenta

Placental tissue (size 3x3x1 cm) obtained from pregnant women after normal delivery were dissected under aseptic condition. The tissues were extensively washed with phosphate buffer saline (PBS), pH 7.4 and minced into small pieces (approximately 1-2 mm^3). Subsequently, the tissues were digested with 1.6 mg/ml collagenase (Sigma-Aldrich, USA) and 200 mg/ml deoxyribonuclease I (Sigma-Aldrich, USA) for 4 hour at 37°C with shaking. After washing twice with PBS, the cells and all pellets were resuspended in MSC growth medium [Dulbecco's Modified Eagle Medium (DMEM; Gibco®, USA) supplemented with either 10% human serum or 10% fetal bovine serum (FBS; Gibco®, USA) and plated into in 25 cm^2 tissue culture flasks (Costa, corning, USA). The cultures were maintained at 37°C in a humidified tissue culture incubator with 5% carbon dioxide. The culture medium was changed every 3-4 days. The cells were observed continuously until developing colonies of fibroblast-like cells were formed. The plastic-adherent cells (about 80-90% confluence) were sub-cultured using 0.25% trypsin-EDTA (Gibco®, USA) and re-plated at density of $1x10^4$ cells/cm^2 for further expansion. Some batches of continuously sub-culture cells were cryopreserved in freezing medium (90% FBS and 10% DMSO) and stored in liquid nitrogen for future use.

Immunophenotypical characterization of MSCs using flow cytometer

The cells cultured in DMEM supplemented with either FBS or HS at passage 3-5 were detached with 0.25% trypsin-EDTA and washed twice with PBS. In each sample, $4x10^5$ cells were resuspended in 50 μl of PBS and incubated with 10 μl of fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated antibodies against CD34 (Bio Legend, USA), CD45 (Bio Legend, USA), CD73 (Bio Legend, USA), CD90 (Bio Legend, USA) or CD105 (BD

Bioscience, USA) for 30 min at 4°C in the dark. After washing with PBS, the cells were fixed with 1% paraformaldehyde in PBS. The positive cells were identified by comparison with isotype-match controls [FITC-conjugated mouse immunoglobulin G1 (IgG1) and PE-conjugated mouse immunoglobulin G2a (IgG2a)]. At least twenty thousand labeled cells were acquired and analyzed using flow cytometry (FACS calibur™, Becton Dickinson, USA) and CellQuest® software (Becton Dickinson, USA).

Proliferation assay

For the assessment of growth characteristics of MSCs expanded in DMEM supplemented with HS in comparison to MSCs expanded in DMEM supplemented with FBS, MSCs at passage 2-4 were cultured in DMEM supplemented with either 10% HS or 10% FBS at a density of $5x10^2$ cells/cm^2. The cells were maintained at 37°C in a humidified tissue culture incubator with 5% carbon dioxide. The cells were harvested to determine cell number by hemocytometer every day. The mean of the cell counts was calculated and plotted against culture time to generate a growth curve.

In vitro differentiation assay

At the third passage, placenta derived MSCs cultured in DMEM supplemented with 10% HS were induced to differentiate into adipocytes and osteoblasts. The differentiation of placenta derived MSCs cultured in DMEM supplemented with 10% FBS were performed in parallel with the MSCs those were cultured in DMEM supplemented with 10% HS.

Adipogenic differentiation

The adipogenic differentiation potential of placenta derived MSCs cultured in DMEM supplemented with either 10% FBS or 10% HS were examined using passage 4 of cultured cells. Briefly, the cells were seeded at a density of $2x10^4$ cells/cm^2 and allowed to adhere to the dishes overnight. Subsequently, the cells were washed with PBS, then 2 ml of adipogenic differentiation medium [DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.5 mM isobutyl-methylxanthine (Sigma-Aldrich, USA), 1 µM dexamethasone, 10 µM insulin (Sigma-Aldrich, USA), 100 µM indomethacin (Sigma-Aldrich, USA)] was added. The cells were cultured at 37°C in a humidified tissue culture incubator with 5% carbon dioxide. The medium were changed every 3 days. After 4 weeks of culture, cytoplasmic inclusions of neutral lipids were then stained with oil red-o (Sigma-Aldrich, USA). Control cultures without the differentiation stimuli were carried out in parallel to those of the experiments and stained in the same manner.

Osteogenic differentiation

The osteogenic differentiation potential of placenta derived MSCs cultured in DMEM supplemented with either 10% FBS or 10% HS were examined using passage 4 of the cultured cells. Briefly, the cells were seeded at a density of $1.2x10^4$ cells/cm^2 and allowed to adhere to the dishes overnight. Subsequently, the cells were washed with PBS and 2 ml of osteogenic differentiation medium [DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.5 mM isobutyl-methylxanthine (Sigma-Aldrich, USA), 1 µM dexamethasone, 10 µM insulin (Sigma-Aldrich, USA), 100 µM indomethacin (Sigma-Aldrich, USA)] was added. The cells were cultured at 37°C under humidified atmosphere of 5% CO_2 in air. The medium was replaced every 3 days. After day 7, 10 µM β-glycerophosphate (Sigma-Aldrich, USA) was added into the cultured. After 3 weeks of culture, the cells were washed with PBS and fixed with 4% paraformaldehyde at 4°C for 20 min. Then, the cells were washed with PBS and stained with 0.5% alizarin red s (Sigma-Aldrich, USA) for 30 min at room temperature. Subsequently, the cells were washed twice with distilled water and observed under inverted microscope (Nikon TS100, Japan). Control cultures without the differentiation stimuli were carried out in parallel to those of the experiments and stained in the same manner.

Statistical analysis

Data were presented as mean ± standard error of mean. Mann-Whitney U test was used to assess the significance of differences between observed data. The *p*-value <0.05 was considered to be statistically significant.

Results

The characteristic of PL-MSCs cultured in DMEM supplemented with human serum and FBS

Placenta derived mesenchymal stem cells (PL-MSCs) were isolated from placental tissue according to their adherence to culture plates. The cells were maintained in DMEM supplemented with 10% HS or 10% FBS after isolated from the tissue. The media were changed every 3-4 days and the number of non-adherent cells was gradually diminished. Approximately

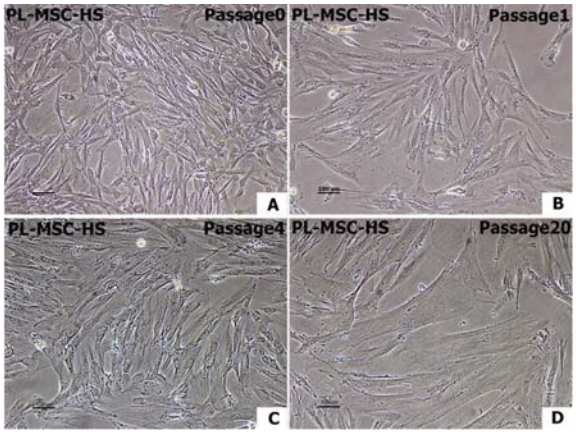

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Fig. 1 The spindle-shaped morphology of PL-MSCs cultured in DMEM supplemented with 10% human serum.

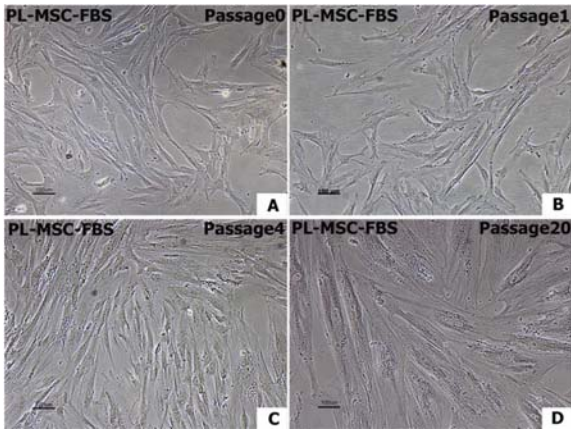

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Fig. 2 The spindle-shaped morphology of PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum.

1-2 weeks in the culture, cells exhibited a uniform spindle-shaped morphology. There was no visible difference in morphology of PL-MSCs cultured in DMEM supplemented with either 10% HS (Fig. 1) or 10% FBS (Fig. 2). PL-MSCs cultured in DMEM supplemented with 10% HS could be maintained in cultured at least 20 passages similar to those of PL-MSCs cultured in DMEM supplemented with 10% FBS. Regarding to the proliferation capacity, the proliferation capacity of PL-MSCs cultured in DMEM supplemented with 10% HS was not different from those of PL-MSCs cultured in DMEM supplemented with 10% FBS during the early period (passage 2). At passage 3-4, PL-MSCs

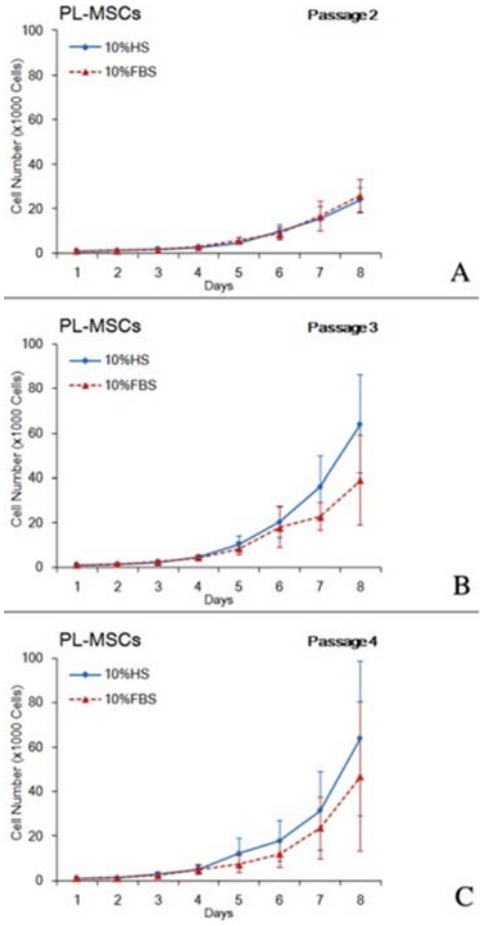

Fig. 3 Growth characteristic of PL-MSCs cultured in DMEM supplemented with 10% human serum in comparison to PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum. Data shown are the mean ± SEM.

cultured in DMEM supplemented with 10% HS had higher proliferative capacity than those of PL-MSCs cultured in DMEM supplemented with 10% FBS (Fig. 3). However, the differences in the proliferative capacity among these PL-MSCs were not statistically significant.

Immunophenotype of PL-MSCs cultured in DMEM supplemented with human serum and FBS

Flow cytometry was used to investigate the immunophenotype of the PL-MSCs cultured in DMEM supplemented with 10% HS from passage 3 to passage 5. The results demonstrated the high expression of MSCs surface markers including CD73, CD90, and CD105 in PL-MSCs cultured in DMEM supplemented with 10% HS (98.49±0.73%, 92.41±3.72%, 83.76±1.57%,

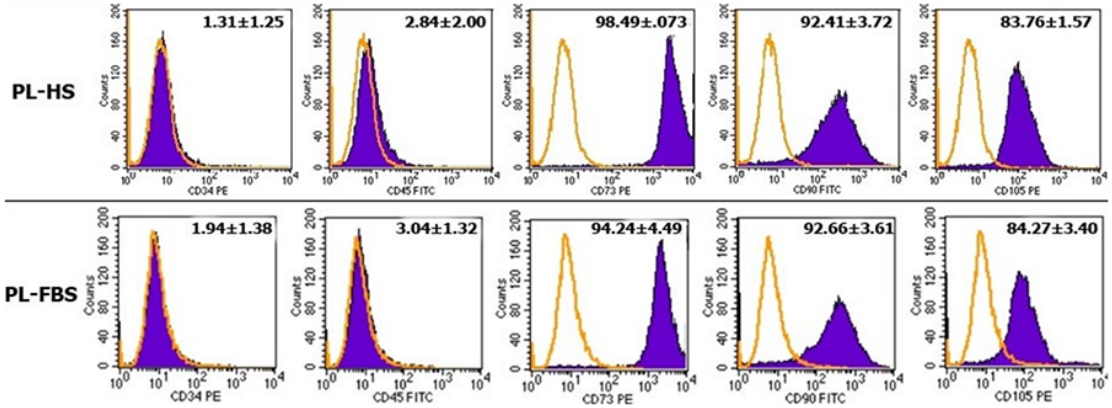

Fig. 4 The expression of cell surface markers in PL-MSCs cultured in DMEM supplemented with 10% human serum in comparison to PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum. The yellow lines show the profile of negative control.

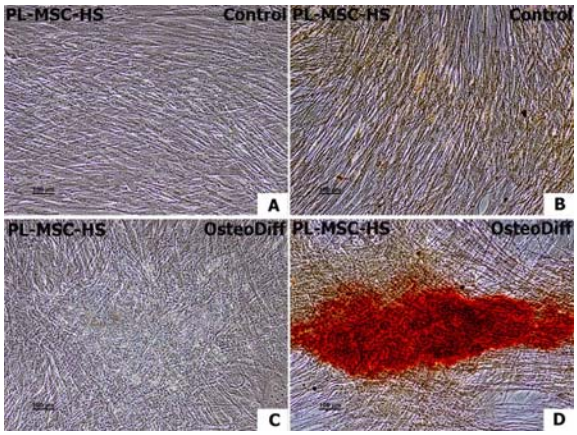

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Fig. 5 Osteogenic differentiation of PL-MSCs cultured in DMEM supplemented with 10% human serum. A) PL-MSCs cultured in DMEM supplemented with 10% human serum for 21 days. B) Alizarin red staining of PL-MSCs cultured in DMEM supplemented with 10% human serum for 21 days. C) PL-MSCs cultured in osteogenic differentiation medium for 21 days. D) Alizarin red staining of PL-MSCs cultured in osteogenic differentiation medium for 21 days.

respectively) similar to those of PL-MSCs cultured in DMEM supplemented with 10% FBS (94.24±4.49%, 92.66±3.61%, 84.27±3.40%, respectively). Very low expression of hematopoietic markers including, CD34 and CD45 was detected in PL-MSCs cultured in DMEM supplemented with 10% HS (1.31±1.25%, 2.84±2.00%, respectively) similar to those of PL-MSCs cultured in DMEM supplemented with 10% FBS (1.94±1.38%, 3.04±1.32%, respectively). There was no statistically significant difference in the expression of MSCs surface markers of PL-MSCs cultured in DMEM supplemented with either 10% HS or10% FBS (Fig. 4).

Osteogenic differentiation potential of PL-MSCs cultured in DMEM supplemented with human serum and FBS

PL-MSCs cultured in DMEM supplemented with 10% HS were induced to differentiate into osteogenic lineage by culturing in osteogenic differentiation medium. After 3 weeks of induction, the cell aggregate with mineralized matrix was detected in PL-MSCs cultured in DMEM supplemented with 10% HS (Fig. 5) similar to the osteogenic differentiation of PL-MSCs cultured in DMEM supplemented with 10% FBS (Fig. 6). In contrast, MSCs cultured in medium without any osteogenic differentiation stimuli did not show any expression of mineralized matrix.

Adipogenic differentiation potential of PL-MSCs cultured in DMEM supplemented with human serum and FBS

After 4 weeks of adipogenic induction, PL-MSCs cultured in DMEM supplemented with 10% HS became a large cells which containing numerous lipid droplets in their cytoplasm (Fig. 7) similar to adipogenic differentiation of PL-MSCs cultured in DMEM supplemented with 10% FBS (Fig. 8). These lipid droplets were positive for oil red-o staining. PL-MSCs cultured in DMEM supplemented with either 10% HS or 10% FBS serve as a control cultures. They did not have any lipid droplets in their cytoplasm and negative for oil red-o staining.

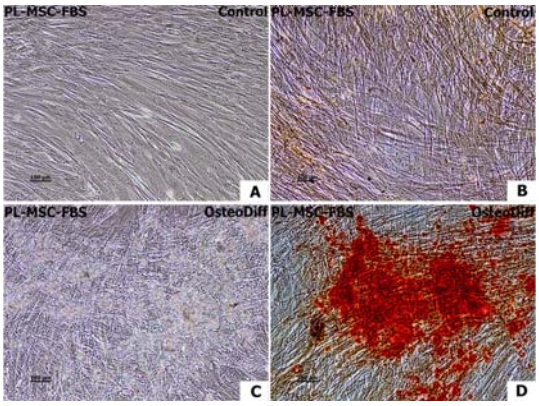

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Fig. 6 Osteogenic differentiation of PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum. A) PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum for 21 days. B) Alizarin red staining of PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum for 21 days. C) PL-MSCs cultured in osteogenic differentiation medium for 21 days. D) Alizarin red staining of PL-MSCs cultured in osteogenic differentiation medium for 21 days.

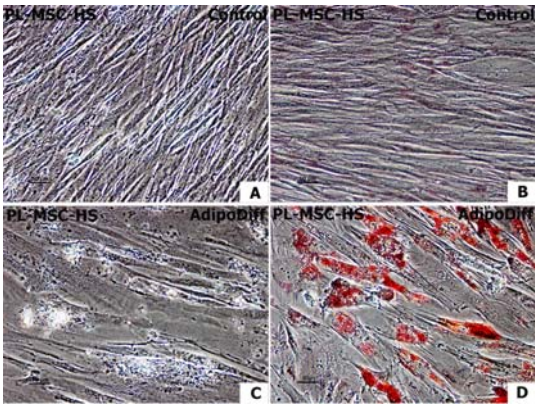

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Fig. 7 Adipogenic differentiations of PL-MSCs cultured in DMEM supplemented with 10% human serum. A) PL-MSCs cultured in DMEM supplemented with 10% human serum for 28 days. B) Oil-red-o staining of PL-MSCs cultured in DMEM supplemented with 10% human serum for 28 days. C) PL-MSCs cultured in adipogenic differentiation medium for 28 days. D) Oil-red-o staining of PL-MSCs cultured in adipogenic differentiation medium for 28 days.

Discussion

Several studies have shown that mesenchymal stem cells, mostly isolated from bone marrow (BM-MSCs), have multi-lineage differentiation potential[13]. In addition, they have been characterized as immune privileged cells, in that they have the ability to suppress alloreactive T-lymphocyte[14,15]. Therefore, MSCs may serve as a promising tool for therapeutic application in various incurable diseases. Despite these attractive properties, the implementation of MSCs into clinical setting still has a concern, especially the issue about biosafety of FBS in culture medium. Since, the expansion of MSCs has been performed under different culture conditions, most of which were based on the addition of FBS as a supplement. Under these culture conditions, anti-FBS antibodies have been detected in most patients infused with MSCs cultured in the presence of FBS[7,16,17]. Because of safety issues for clinical use, defined serum-free and xeno-free culture system has been developed to avoid animal-derived reagents[18,19]. However, the fully defined factors of xeno- and serum-free culture system are not available[20]. The cell isolation and early expansion are needed to carry out using serum-containing medium[18,19]. Previous studies reported that serum-containing medium also necessary for MSCs differentiation[20]. Therefore, the identification of suitable culture conditions for optimal expansion and appropriate functional capability of MSCs still remains a crucial matter. This study demonstrated that PL-MSCs cultured in human serum supplemented medium has similar biological characteristics to those of PL-MSCs cultured in FBS supplemented medium in term of morphology, immunophenotype, differentiation and proliferation capacities. Previous studies, mostly in BM-MSCs, reported the similar phenotypic and functional effects of MSCs expanded in HS and in FBS-containing media[11].

HS-containing media were recently described as possible substitutes for FBS-containing media for the expansion of MSCs for clinical use[7]. Notably, HS contains numerous bioactive molecules as well as growth factors that may influence MSCs expansion and function[21]. In this context, some recent reports have addressed the influence of HS on the generation and expansion of MSCs[7,9]. Telomerase activity, transformation and senescence have also been investigated due to the rapid proliferation of MSCs cultured in human supplemented serum[10]. Of the most important, the knowledge attained from this study will be the step that will lead to the advanced understanding of defined factors in human serum those are involved in the in vitro expansion of MSCs that could be applied

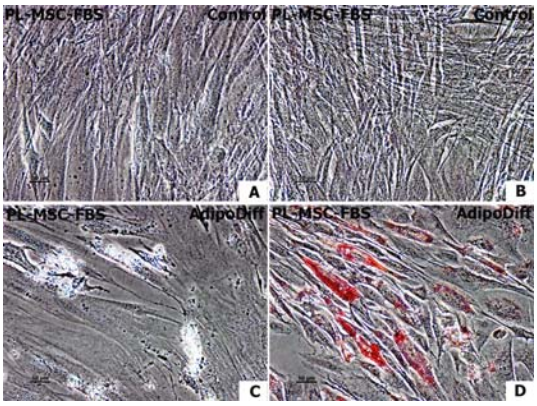

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Fig. 8 Adipogenic differentiations of PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum. A) PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum for 28 days. B) Oil-red-o staining of PL-MSCs cultured in DMEM supplemented with 10% fetal bovine serum for 28 days. C) PL-MSCs cultured in adipogenic differentiation medium for 28 days. D) Oil-red-o staining of PL-MSCs cultured in adipogenic differentiation medium for 28 days.

for clinical study in the future.

Conclusion

The present study demonstrated that PL-MSCs cultured in human serum supplemented medium has similar biological characteristics to those of PL-MSCs cultured in fetal bovine serum supplemented medium in term of morphology, immunophenotype, differentiation and proliferation capacities. The results obtained will ultimately yield a readily available source of MSCs cultured in animal free medium for therapeutic application.

What is already known on this topic?

Mesenchymal stem cells (MSCs) have become an innovative tool for cell based therapy of degenerative disorders. MSCs isolation and expansion need to carry out using serum-containing medium, most of which are based on the addition of FBS as a supplement. Under this culture condition, anti-FBS antibodies have been detected in most patients infused with MSCs cultured in the presence of FBS. Because of safety issues for clinical use, defined serum-free and xeno-free culture system has been developed to avoid animal-derived reagents. Until now, the fully defined factors of xeno- and serum-free culture system are not available.

What this study adds?

Human serum can use as a supplement for MSCs culture as the evidence that showed in this study. PL-MSCs cultured in human serum supplemented medium has similar biological characteristics to those of PL-MSCs cultured in fetal bovine serum supplemented medium in term of morphology, immunophenotype, differentiation and proliferation capacities. This study will ultimately yield a readily available source of MSCs cultured in animal free medium for therapeutic application.

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Potential conflicts of interest

None.

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คุณลักษณะของเซลล์ต้นกำเนิดมีเซนไคม์จากรกที่เพาะเลี้ยงในน้ำยาที่มีส่วนผสมของซีรัมจากมนุษย์

เสริมพร ทวีทรัพย์พิทักษ์, ชัยรัตน์ ตัณฑราวัฒน์พันธ์, ภาคภูมิ เขียวละม้าย, ดวงรัตน์ ตันติกัลยาภรณ์, ศิริกุล มะโนจันทร์

วัตถุประสงค์: เซลล์ต้นกำเนิดมีเซนไคม์จากรกเป็นเซลล์ต้นกำเนิดที่สามารถคัดแยกได้ง่ายไม่ก่อให้เกิดความเจ็บปวดต่อผู้บริจาค และมีศักยภาพที่จะนำไปประยุกต์ใช้ในทางคลินิก อย่างไรก็ตามในปัจจุบันการคัดแยกและการเพาะเลี้ยงเซลล์ต้นกำเนิดมีเซนไคม์ จำเป็นต้องใช้น้ำยาที่มีส่วนผสมของซีรัมลูกวัว ซึ่งการใช้ซีรัมลูกวัวอาจก่อให้เกิดความเสี่ยงต่อการติดเชื้อจากสัตว์สู่คน รวมทั้งการเกิดปัญหาทางด้านปฏิกิริยาภูมิคุ้มกันต่อโปรตีนจากซีรัมลูกวัว จากรายงานการศึกษาในระยะหลังได้มีการนำซีรัมมนุษย์มาใช้ในการเพาะเลี้ยงเซลล์ต้นกำเนิดมีเซนไคม์เพื่อนำไปใช้ในทางคลินิก พบว่าเซลล์ดังกล่าวมีคุณสมบัติไม่แตกต่างจากเซลล์ที่เพาะเลี้ยงด้วยน้ำยาที่ผสมซีรัมลูกวัว อย่างไรก็ตามยังไม่มีรายงานการนำซีรัมมนุษย์มาใช้ในการเพาะเลี้ยงเซลล์ต้นกำเนิดมีเซนไคม์จากรก การศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาคุณสมบัติของเซลล์ต้นกำเนิด มีเซนไคม์จากรกที่เพาะเลี้ยงโดยใช้ซีรัมมนุษย์เปรียบเทียบกับเซลล์ต้นกำเนิดมีเซนไคม์จากรกที่เพาะเลี้ยงโดยใช้ซีรัมลูกวัว

วัสดุและวิธีการ: เซลล์ต้นกำเนิดมีเซนไคม์จากรก ถูกนำมาเพาะเลี้ยงในน้ำยาเลี้ยงเซลล์ที่มีส่วนผสมของซีรัมลูกวัวหรือซีรัมมนุษย์ และนำมาตรวจลักษณะรูปร่าง การแสดงออกของโมเลกุลที่จำเพาะต่อเซลล์ต้นกำเนิดมีเซนไคม์ ความสามารถในการเจริญพัฒนา และความสามารถในการแบ่งตัวเพิ่มจำนวน

ผลการศึกษา: เซลล์ต้นกำเนิดมีเซนไคม์ที่เพาะเลี้ยงโดยใช้ซีรัมมนุษย์ มีคุณสมบัติเช่นเดียวกับเซลล์ต้นกำเนิดมีเซนไคม์ที่เพาะเลี้ยงโดยใช้ซีรัมลูกวัว ทั้งในด้านรูปร่าง การแสดงออกของโมเลกุลบนผิวเซลล์ รวมทั้งความสามารถในการเจริญพัฒนาเป็นเซลล์กระดูกและเซลล์ไขมันที่สำคัญคือ เซลล์ต้นกำเนิดมีเซนไคม์ ที่เพาะเลี้ยงโดยใช้ซีรัมมนุษย์มีความสามารถในการแบ่งตัวเพิ่มจำนวนได้มากกว่าเซลล์ต้นกำเนิดมีเซนไคม์ที่เพาะเลี้ยงโดยใช้ซีรัมลูกวัว

สรุป: ข้อมูลที่ได้จากการศึกษาในครั้งนี้เป็นองค์ความรู้ใหม่ในด้านการเพาะเลี้ยงเซลล์ต้นกำเนิด มีเซนไคม์จากรกโดยใช้ซีรัมมนุษย์ทดแทนซีรัมลูกวัว เป็นประโยชน์ในด้านความปลอดภัยในการนำเซลล์ดังกล่าวไปประยุกต์ใช้ในทางการแพทย์โดยเฉพาะในด้านของเวชศาสตร์ฟื้นฟูสภาวะเสื่อม