Isolation, Characterization and Neural Differentiation Potential of Amnion Derived Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) derived from amnion are considered to be adult stem cells that can be easily obtained in large quantities by a less invasive method in comparison to bone marrow-derived MSCs (BM-MSCs). However, the biological properties and the differentiation capacity of amnion-derived MSCs (AM-MSCs) are still poorly characterized. The objectives of this study were to isolate, characterize and explore the potential of AM-MSCs in differentiating toward neural lineage in comparison to those of BM-MSCs. To isolate AM-MSCs, amnion was digested with trypsin-EDTA and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. The expression profiles of several MSC markers were examined by flow cytometry. AM-MSCs from passage 3-5 were used for adipogenic, osteogenic and neural differentiation assays by culturing in appropriate induction media. The expression of several neural marker genes, including MAP-2, GFAP and β-tubulin III in AM-MSCs was determined by quantitative real time-PCR. The expression of neural-specific markers, MAP-2 and β-tubulin III, was subsequently confirmed by immunocytochemistry using confocal laser microscope. The results demonstrated that AM-MSCs could be easily expanded to 18-20 passages while maintaining the undifferentiated state and exhibiting MSC markers (CD73, CD90, and CD105) but do not express the hematopoietic markers (CD34 and CD45). Similar to BM-MSCs, AM-MSCs were able to differentiate to several mesodermal-lineages including adipocytes and osteoblasts. Moreover, these cells could be induced to differentiate to neuron-like cells as characterized by cell morphology and the expression of several neural markers including MAP-2, GFAP and β-tubulin III. The present study demonstrated that AM-MSCs can be easily obtained and expanded in culture. These cells also have transdifferentiation capacity as evidenced by their neural differentiation potential. According to the results, amnion can be used as an alternative source of MSCs for stem cell therapy in neurodegenerative diseases.

Keywords: *Mesenchymal stem cell, Amnion, Neural differentiation*

J Med Assoc Thai 2010; 93 (Suppl. 7) : S183-S191
Full text. e-Journal: *http://www.mat.or.th/journal*

Mesenchymal stem cells (MSCs) are multipotent stem cells initially obtained from bone marrow (BM)[1]. They are capable of differentiating to various cells of mesenchymal origin including adipocytes, osteoblasts, chondrocytes and myocytes[2]. As there are no specific markers, MSCs are recognized on the basis of a combination of several characteristics, including the expression of several surface molecules, such as CD73, CD90, CD105 and the absence of hematopoietic cell markers such as CD34 and CD45[2]. The multilineage differentiation ability, together with their extensive proliferative capacity has generated interest about the potential use of MSCs for the treatment of degenerative diseases. In addition to their immunoregulatory properties, such as their inhibitory effect on alloreactive T lymphocyte[3], MSCs have been suggested to undergo neural differentiation under appropriate condition[4,5]. The treatment of BM-MSCs with different molecules and growth factors induced very rapid morphological changes that are typical of neuron together with the expression of neural markers,

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such as nestin, neurofilaments, MAP-2 and Neu-N[6]. The great majority of studies regarding the neural differentiation potential of MSCs have been focused on the capacity of BM-MSCs to differentiate to neurons and/or glial cells of the nervous system. A more limited number of studies have explored the neural differentiation potential of MSCs derived from tissues other than BM.

Although BM has been using as the main source for the isolation of multipotent MSCs, however, the harvest of BM is a highly invasive procedure. The number, differentiation potential, and maximal life span of BM-MSCs decline with increasing age of donors[7,8]. Therefore, the search for alternative source of MSCs for autologous and allogenic transplantation is of significant value. It has been reported that MSCs could be isolated from various tissues, such as umbilical cord[9], placenta[10] and adipose tissue[11]. Among those, amnion which can be easily obtained using non-invasive procedures and have low risk of viral contamination is considered a promising source for autologous cell therapy[9]. Many clinical studies have demonstrated that amniotic membrane transplantation promotes re-epithelialisation and angiogenesis[12]. Several growth factors produced from amniotic membrane such as epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF) have been demonstrated to involve in those processes[13]. Recent study demonstrated that placenta-derived MSCs share several embryonic stem cell surface markers such as SSEA-4, TRA-1-61, TRA-1-80 and are able to undergo neurogenic differentiation[14,15]. As BM-MSCs are the best characterized, the questions are raised whether AM-MSCs share the characteristics of BM-MSCs.

Therefore, the first goal of the present study was to verify whether cells with MSCs characteristics can be isolated from amnion using the culture condition that have been used to isolated BM-MSCs. The second goal is to compared the characteristics of AM-MSCs in term of cell morphology, immunophynotypes, proliferative capacity as well as their neural differentiation capacity with those of BM-MSCs. The results obtained from this study might provide the valuable information regarding AM-MSCs and their potential for future cell-base therapeutic applications.

Material and Method

Collection of specimen

The collection of human specimen was approved by the Ethical Committee of the Faculty of Medicine, Thammasat University. All subjects participated in the study after giving written informed consents.

Isolation and culture of MSCs from bone marrow

Bone marrow of approximately 5 ml were aspirated from sternum or iliac crest of normal healthy volunteers. Mononuclear cells (MNCs) were isolated from bone marrow using Ficoll-Hypaque (GE Healthcare) density gradient centrifugation (100xg, 20°C, 20 min) and washed twice with phosphate buffered saline (PBS). MNCs (1×10^5 cells/cm^2) were cultured on 25-cm^2 tissue culture flasks (Corning) in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) containing 10% fetal bovine serum (FBS; Cambrex) under humidified atmosphere of 5% CO_2 in air at 37°C. After culture for 3 days, non-adherent cells were removed and fresh medium was added. Thereafter, the medium was replaced every 3 days for the entire culture period. Culture flasks were observed continuously for the developing colonies of adherent cells. The adherent cells were passaged using 0.25% trypsin-EDTA (Invitrogen) when the cell density reached 90% confluence. Some batches of cultured cells were cryopreserved using 90% FBS and 10% DMSO (Sigma) and stored in liquid nitrogen for future use.

Isolation and culture of MSCs from amnion

Amniotic membrane was separated from chorion through blunt dissection. Pieces of amnion (AM) were minced into small pieces (approximately 1-2 mm^2) and extensively washed with PBS. Subsequently, the tissue was digested with 0.5% trypsin-EDTA solution (Invitrogen) for 30 min. The pellet was then washed with PBS, resuspended in DMEM supplement with 10% FBS and plated into 25-cm^2 tissue culture flasks. Culture was maintained at 37°C under humidified atmosphere of 5% CO_2 in air. The medium was replaced every 3-4 days for the entire culture period. Culture flasks were observed continuously for the developing colonies of adherent cells. The adherent cells were passaged using 0.25% trypsin-EDTA when the cell density reached 90% confluence. Some batches of cultured cells were cryopreserved using 90% FBS and 10% DMSO and stored in liquid nitrogen for future use.

Immunophenotyping of culture cells

To evaluate cell-surface marker expression, cell suspensions were incubated for 30 min at 4°C with

fluorescein isothiocyanate (FITC) or phycoerythrin (PE) -conjugated antibodies specific for human markers associated with mesenchymal and hematopoietic lineages. The antibodies used were CD34 (BD Bioscience), CD45 (BD Bioscience), CD73 (BD Bioscience), CD90 (AbD Serotec) and CD105 (AbD Serotec). The samples were analyzed using flow cytometer (FACScalibur™, Becton Dickinson) and CellQuest software (BD Bioscience).

Proliferation assay

For the assessment of growth characteristics of AM-MSCs in comparison to BM-MSCs, 6 x 10^3 culture-expanded MSCs (passage 2-5) were seeded in 24-well culture plate (Corning) containing 1 ml of DMEM supplemented with 10% FBS. The cells were then harvested at culture day 2, 4, 6 and 8 to determine cell number by hemacytometer. The mean value of total cell number at each time point was plotted against culture time to generate a growth curve.

In vitro differentiation studies

At the third passage, AM-MSCs were induced to differentiate into three different types of cell including adipocytes, osteoblasts and neural cells. The differentiation studies were performed in parallel with BM-MSCs.

Adipogenic differentiation

MSCs were cultured in NH AdipoDiff Medium (Miltenyi Biotec) according to the manufacturer's instruction. Briefly, the cells were seeded at a density of 8 x 10^3 cells/cm^2 and cultured under humidified atmosphere of 5% CO_2 in air at 37°C with complete change of medium every 3 days. After 3 weeks of culture, cytoplasmic inclusions of neutral lipids were stained with Oil Red O (Sigma). Control cultures without the differentiation stimuli were carried out in parallel to the experiments and stained in the same manner.

Osteogenic differentiation

MSCs were cultured in NH OsteoDiff Medium (Miltenyi Biotec) according to the manufacturer's instruction. Briefly, the cells were seeded at a density of 5 x 10^3cells/cm^2 and cultured at 37°C under humidified atmosphere of 5% CO_2 in air. The medium was replaced every 3 days. To visualize osteogenic differentiation, cells were stained for alkaline phosphatase (AP) activity. Control cultures without the differentiation stimuli were carried out in parallel to the experiments and stained in the same manner.

Neural differentiation

To induce neural differentiation, MSCs were cultured in AdvanceSTEM™ Neural Differentiation Medium (HyClone) according to the manufacturer's protocol. Briefly the cells were plated on cultured dish at a density of 2.5 x 10^3 cells/cm^2 and cultured at 37°C under humidified atmosphere of 5% CO_2 in air. The medium was replaced every 2 days until the neural like cells were observed. The cells were then processed for immunocytochemistry or quantitative real-time PCR.

Immunocytochemistry

To analyze neural differentiation, AM-MSCs and BM-MSCs, cultured on glass cover-slips with or without neural differentiation medium, were rinsed in PBS for three times and fixed with 4% paraformaldehyde in PBS, pH 7.4 for 10 min, at room temperature. After washing twice in PBS and incubating with 0.1% Triton X-100 in PBS for 10 min at room temperature, the cells were incubated for 30 min in a blocking solution containing 4% BSA before incubation with the primary antibody [Anti-microtubule-associated protein 2 (Anti-MAP-2; Sigma) and Anti-β-tubulin III (Sigma)]. Thereafter, primary antibodies were labeled with appropriate secondary antibodies. After immunostaining, cover-slips were mounted on microscopic slides using Antifade mounting medium (Vectra Shield). Confocal obtained using an Olympus laser scanning confocal microscope. The images were analyzed using Olympus software.

Quantitative real-time PCR

Total RNA from BM-MSCs and AM-MSCs cultured with or without neural differentiation medium were prepared using RNA mini Kit (Invitrogen). For cDNA synthesis, 1 μg of total RNA for each sample was reverse transcribed to cDNA in 20 μl volume using iScript™ Select cDNA Synthesis Kit (Bio-Rad Laboratories) according to the manufacturer's instruction. Quantitative real-time PCR was carried out using standard protocols with the SYBR® PCR Master Mix (Applied BioSystems). The PCR mix contained SYBR® Green PCR Master Mix, 1 ng DNA template, 1 μM primers, and nuclease free water to reach a final volume of 20 μl. Quantitative real-time PCR was performed using the StepOnePlus™ Real-Time PCR System (Applied BioSystems). The primer sets used for the quantitative real-time PCR were microtubule-associated protein 2 (MAP-2); forward primer: 5′-CCAATGGATTCCCATACAGG-3′, reverse primer: 5′-CTGCTACAGCCTCAGCAGTG-3′, β-tubulin III;

forward primer: 5′-AACGAGGCCTCTTCTCACAA-3′, reverse primer: 5′-CCTCCGTGTAGTGACCCTTG-3′ and glial fibrillary acidic protein (GFAP); forward primer: 5′-CCAGTTG CAGTCCTTGACCT-3′, reverse primer: 5′-ATCTCGT CCTTGAGGCTCTG-3′. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. A melt curve analysis was performed at the end of each reaction. The gene expression levels were normalized to individual GAPDH (internal control). The profile was obtained by plotting relative gene expression levels compared to undifferentiated MSCs.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). The ANOVA test was used to assess the significance of differences between observed data. P-value of less than 0.05 was considered to be statistically significant.

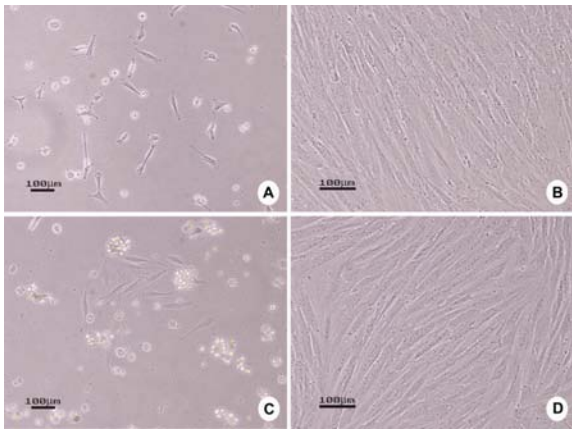

Fig. 1 Morphology of BM-MSCs and AM-MSCs. A: Spindle-shaped of BM-MSCs at day 7 after seeding, B: BM-MSCs was reached 100% confluence at day16, C: Spindle-shaped of AM-MSCs at day 7 after seeding, D: AM-MSCs was reached sub-confluence at day 25. Scale bar = 100 μm

Results

The characteristics of cultured AM-MSCs

The MNCs isolated from bone marrow were cultured in DMEM supplemented with 10% FBS at a density of 1 x 10^5 cells/cm^2. After plating, MNCs had spherical shape and floated over the culture flask. Cultures were monitored daily and between 7-10 days of culture, small outgrowth colonies consisted of 20-50 rapidly proliferative cells were observed (Fig. 1A, B).

The cells isolated from amnion were also cultured in the same condition as those isolated from bone marrow. At the beginning, only a few cells attached to the surface of the culture flasks (Fig. 1C). The media were changed every 3-4 days and the number of non-adherent cells was steadily diminished over the course of approximately 3 weeks. AM-MSCs appeared as spindle-shaped cells which were similar to those of BM-MSCs (Fig. 1D). Further passages of MSCs from these sources were done whenever the cell density reached 90% confluence. These MSCs were also able to freeze, thaw, and subsequently passage. AM-MSCs can be propagated up to 18-22 passages and can be revived after frozen without significant cell death. At passage 22, the cells showed signs of replicative senescence and stop growing. In contrast to AM-MSCs, BM-MSCs can only be propagated up to passage 10 when they reach the stage of replicative senescence.

The proliferative characteristics of AM-MSCs

The growth characteristics of AM-MSCs and BM-MSCs were determined during 8 days of culture. The results showed that the proliferative capacity of AM-MSCs were significantly lower than that of BM-MSCs in all passages examined ($p < 0.05$) (Fig. 2).

Immunophenotype of AM-MSCs

Immunophenotype of AM-MSCs and BM-MSCs were determined by flow cytometry with the use of phycoerythrin (PE)-conjugated or fluorescein isothiocyanate (FITC)-conjugated antibodies against CD34, CD45, CD73, CD90 and CD 105. AM-MSCs exhibited the similar immunophenotype to those of BM-MSCs which express high levels of MSC markers (CD73, CD90 and CD105) but did not express hematopoietic markers (CD34, CD45) (Fig. 3).

Osteogenic differentiation potential of AM-MSCs

The osteogenic differentiation potential of AM-MSCs was compared to BM-MSCs. After 10 days of induction, AM-MSCs had the appearance of refringent crystals in the cells similar to BM-MSCs. By the end of culture, most cultured AM-MSCs expressed alkaline phosphatase-activity similar to BM-MSCs (Fig. 4B, D). The untreated control cultures growing in regular medium without osteogenic differentiation stimuli did not expressed alkaline phosphatase-activity even after 3 weeks of culture (Fig. 4A, C).

Adipogenic differentiation potential of AM-MSCs

The adipogenic differentiation potential of

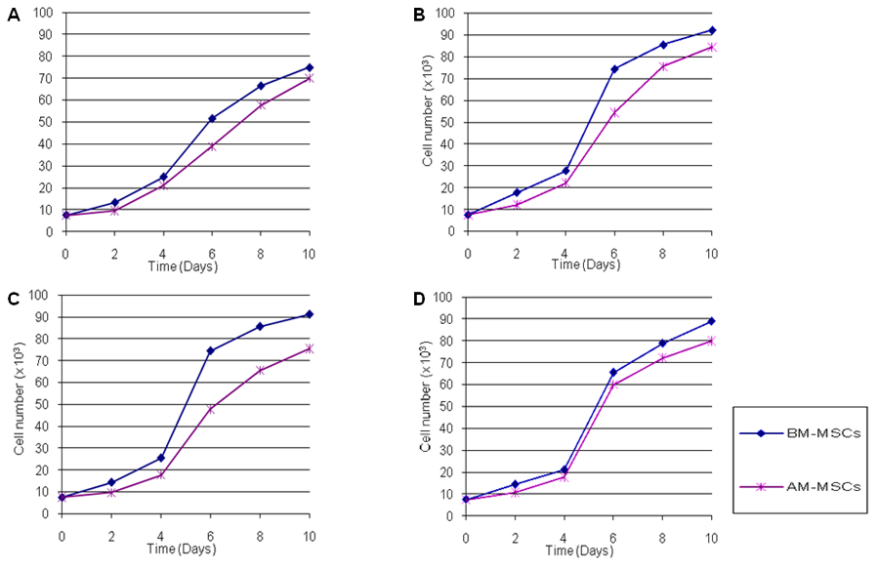

Fig. 2 Growth curves of BM-MSCs and AM-MSCs. A: At passage 2, B: At passage 3, C: At passage 4, D: At passage 5.

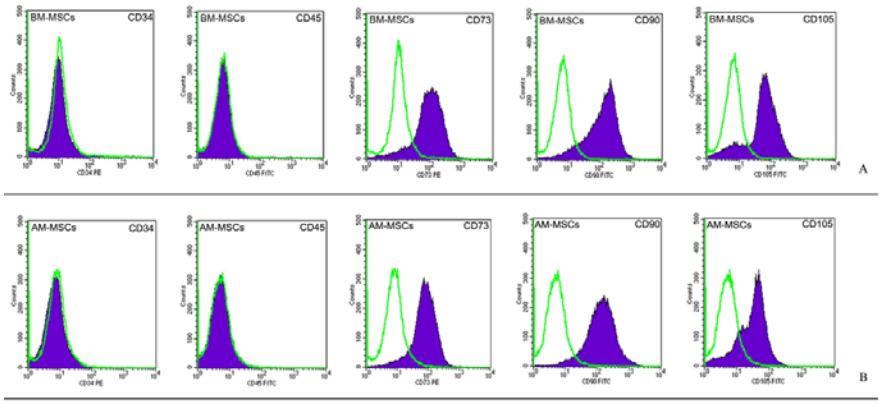

Fig. 3 Flow cytometric analysis of surface-marker expression on BM-MSCs and AM-MSCs. The green line shows the profile of negative control. The data shown are representative of those obtained in three different experiments.

AM-MSCs was compared to BM-MSCs. After 3 weeks of induction, AM-MSCs became large cells containing numerous lipid droplets in their cytoplasm similar to that of differentiating BM-MSCs. These lipid droplets were positive for Oil Red O staining (Fig. 5B, D) while the MSCs from untreated control cultures did not have any lipid droplets in their cytoplasm (Fig. 5A, C).

Differentiation of MSCs after neural induction

At the beginning, both BM-MSCs and AM-MSCs used for neural differentiation were phenotypically homogeneous. After exposure to neural induction medium, both of them exhibited very rapid morphological changes: most cells retracted their cytoplasm, forming spherical cell body and exhibited cellular protrusions, as compared to MSCs in control conditions. At the end of induction process, BM-MSCs (Fig. 6E) and AM-MSCs (Fig. 6I) from induction group appeared as sharp, elongated bi-or-tripolar cells with primary and secondary processes, which express several neural markers including MAP-2 (Fig. 6G, K) and β-tubulin III (Fig. 6H, L). In contrast, MSCs from untreated control cultures did not have any morphologically reminiscent of neurons (Fig. 6A, C)

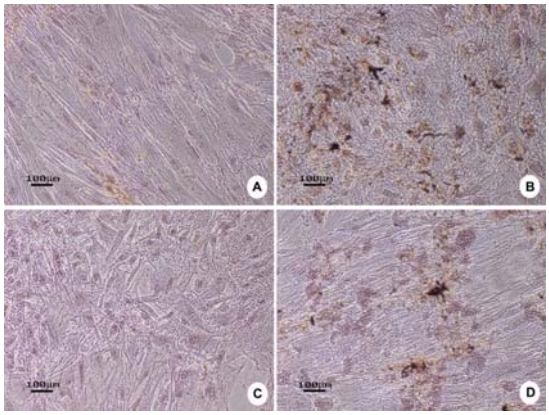

Fig. 4 Representative photomicrographs of osteogenic differentiation of BM-MSCs (A,B) and AM-MSCs (C,D). Osteogenic differentiation was evidenced by the formation of alkaline phosphatase-positive aggregates in cytoplasm after osteogenic induction using NH OsteoDiff Medium (B,D). No alkaline phosphatase-positive aggregates was found in cytoplasm of BM-MSCs (A) and AM-MSCs (C) cultured in DMEM supplemented with 10% FBS. Scale bar = 100 μm

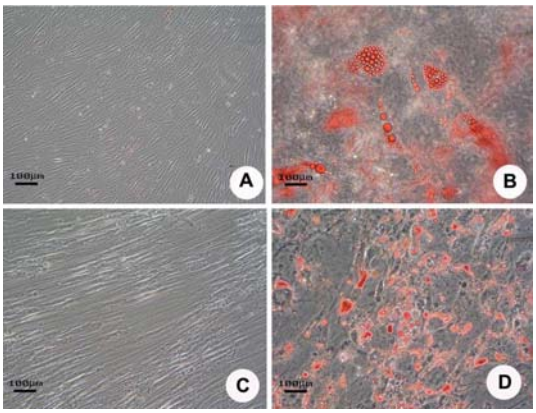

Fig. 5 Representative photomicrographs of adipogenic differentiation of BM-MSCs (A,B) and AM-MSCs (C,D). Adipogenic differentiation was evidenced by the formation of lipid droplet (Oil Red O positive) in cytoplasm after adipogenic induction using NH AdipoDiff Medium (B,D). No lipid droplet was observed in the cytoplasm of BM-MSCs (A) and AM-MSCs (C) cultured in DMEM supplemented with 10% FBS. Scale bar = 100 μm

and did not express any neural markers (Fig. 6B, D). Moreover, expression profile of *MAP-2, β-tubulin III* and *GFAP* genes were observed to be up-regulated at day 3 after neural induction. The comparison of *MAP-2, β-tubulin III* and *GFAP* mRNA expression levels in both BM-MSCs and AM-MSCs showed that the relative in neural induced BM-MSCs/non-induced BM-MSCs were 1.22, 1.68 and 3.27 and neural induced AM-MSCs/non-induced AM-MSCs were 1.78, 1.74 and 3.63, respectively (Fig. 7).

Discussion

The BM-MSCs have been extensively studied by several groups because of their multilineage differentiation potential. Despite these appealing properties, the cell source may become an issue for broad clinical application of cell therapy, because age and disease state may affect the collection of sufficient healthy autologous bone marrow for transplantation[8]. Thus, seeking for alternative sources would have obvious biomedical implication. This study demonstrated that amnion is the alternative sources of MSCs. AM-MSCs could be isolated through mechanical and enzymatic digestion as previously reported[16]. Recent studies are providing increasing convincing evidence that this tissue can be used as a source of stem cells that can undergoes differentiation towards all three germ layers[17], thus reinforcing their potential for clinical applications in tissue regeneration. The morphological characteristics of AM-MSCs were similar to those described for BM-MSCs[18], and included plastic adherence and fibroblast-like cells.

Immunophenotypic characterization of AM-MSCs demonstrated the presence of markers described for BM-MSCs (CD73, CD90 and CD105) and the absence of hematopoietic markers (CD34, CD45), consistent with data previously reported in cells also isolated from other regions of the placenta[19,20]. These findings suggest that our isolation procedures can effectively yield MSCs from amnion, thus expanding the possible sources of MSCs of placental origin. Amniotic tissue is the only source of MSCs without any contamination from hematopoietic and endothelial cells usually present in placenta derived cells, since amniotic tissue is truly devoid of any vasculature[21]. The multi-lineage differentiation capacity of AM-MSCs was study through the osteogenic and adipogenic differentiation as previously reported[10]. Similar to BM-MSCs, AM-MSCs were capable of differentiating toward osteogenic and adipogenic lineages.

Evidence of neural differentiation has been obtained by several groups using BM-MSCs, with the acquisition of morphological, phenotypic and functional features of neural cells[6,22]. In addition, the

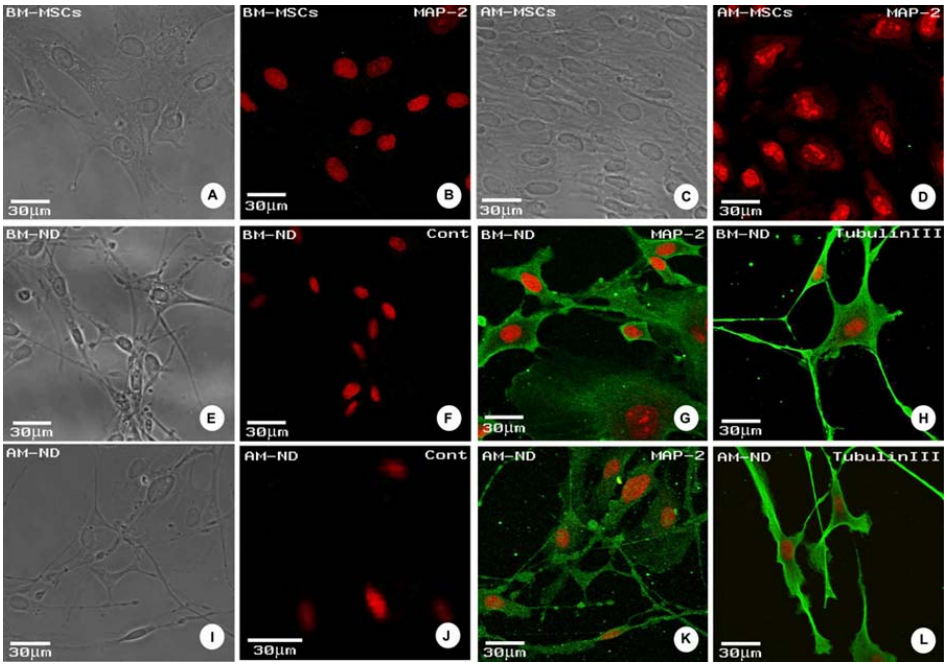

Fig. 6 Representative photomicrographs of neural differentiation of AM-MSCs and BM-MSCs. Spindle-shaped MSCs from amnion (C) showed similar morphology to MSCs derived from bone marrow (A) in basal conditions. Immunostaining for MAP-2 on AM-MSCs (D) and BM-MSCs (B) in basal medium reveals negative staining. Dramatic changes after the exposure to neural differentiation medium in terms of morphology and phenotype were observed on MSCs from amnion (E-H) and bone marrow (I-L). Background immunostaining obtained with the omission of primary antibody on MSCs stimulated with neural differentiation medium (F, J). Scale bar = 30 µm

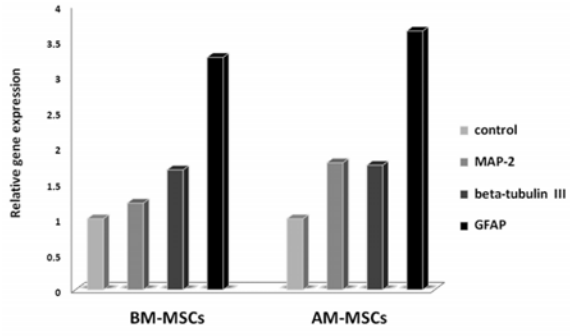

Fig. 7 Quantitative real-time PCR detected the expression of neural specific mRNA, *MAP-2*, *β-tubulin III* and *GFAP*, in differentiated and undifferentiated BM-MSCs and AM-MSCs at day 3 of induction. BM-MSCs and AM-MSCs cultured in basal condition served as a control.

same finding have been shown with MSCs from adipose tissue[23]. The same induction medium with BM-MSCs was applied to induced neural differentiation of AM-MSCs. The purpose was to assess whether cells with neural characteristic could be achieved from tissues that differ from BM in terms of development and functions. The results demonstrated that amnion has a reservoir of MSCs with the same differentiation properties into lineages of mesodermal origin of those in BM; in addition, AM-MSCs could differentiate into cells with morphological characteristics of ectodermal origin.

The present study demonstrated that amnion is an invaluable source of MSCs. Understanding the molecular mechanisms responsible for neural differentiation of these cells will ultimately yield a readily available source of neural cells for cellular therapies ranging from gene therapeutics to neural reconstruction in neurodegenerative diseases, stroke, and trauma.

Conclusion

In conclusion, the results obtained from this study demonstrated that amnion is an alternative source of MSCs that can be easily expanded in culture. AM-MSCs and BM-MSCs are similar in terms of growth requirements, biological properties and differentiation potential. Their inducible neurogenic potential may

open a new therapeutic approach to treating various forms of neurodegenerative diseases. Taken together; AM-MSCs could provide a novel and non-invasive source of MSCs for future clinical applications.

Acknowledgements

The authors would like to thank Assoc. Prof. Korakot Sirimai, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University for providing the amniotic tissues. This study was supported by grant from the Faculty of Medicine, Thammasat University.

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การคัดแยก คุณสมบัติ และศักยภาพในการเจริญพัฒนาเป็นเซลล์ประสาทของเซลล์ต้นกำเนิดมีเซนไคม์จากถุงน้ำคร่ำ

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เซลล์ต้นกำเนิด mesenchyme (MSCs) จากถุงน้ำคร่ำ (amnion derived MSCs หรือ AM-MSCs) เป็นเซลล์ต้นกำเนิดร่างกายที่สามารถคัดแยกได้ง่ายด้วยวิธีที่ไม่ก่อให้เกิดความเจ็บปวดกับผู้บริจาคเหมือนกับ MSCs ที่คัดแยกจากไขกระดูก (bone marrow derived MSCs หรือ BM-MSCs) อย่างไรก็ตามรายงานการศึกษาที่ระบุถึงคุณสมบัติทางชีววิทยารวมถึงความสามารถในการเจริญพัฒนาของ AM-MSCs โดยเปรียบเทียบกับ BM-MSCs ยังมีอยู่น้อยมากส่งผลให้การนำ AM-MSCs ไปใช้ในการศึกษาวิจัยและรักษาผู้ป่วยทำได้ยาก การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาคุณสมบัติของ AM-MSCs รวมทั้งความสามารถของเซลล์ดังกล่าวในการเจริญพัฒนาไปเป็นเซลล์กระดูก เซลล์ไขมัน และเซลล์ประสาท โดยเปรียบเทียบกับ BM-MSCs กระบวนการแยก AM-MSCs จากถุงน้ำคร่ำนั้นเริ่มจากการนำถุงน้ำคร่ำมาตัดเป็นชิ้นเล็ก ๆ และนำไปย่อยด้วยเอนไซม์ trypsin ก่อนนำเซลล์ที่แยกได้ไปเพาะเลี้ยงในน้ำยา Dulbecco's Modified Eagle's Medium (DMEM) ที่เติม 10% fetal bovine serum AM-MSCs ที่แยกได้จะถูกนำมาตรวจสอบการแสดงออกของ MSC marker ด้วยวิธี flow cytometry รวมถึงความสามารถในการเจริญพัฒนาไปเป็นเซลล์กระดูก เซลล์ไขมัน และเซลล์ประสาท ภายหลังการเหนี่ยวนำด้วยน้ำยาเลี้ยงเซลล์ที่จำเพาะ AM-MSCs ที่ได้รับการเหนี่ยวนำให้เป็นเซลล์ประสาทจะถูกนำมาตรวจสอบการแสดงออกของ neural marker (MAP-2, GFAP และ β-tubulin III) ด้วยวิธี quantitative real-time PCR และ immunocytochemistry ผลการทดลองพบว่า AM-MSCs สามารถเพิ่มจำนวนภายนอกร่างกายได้ถึง 20 passage ในขณะที่ BM-MSCs สามารถเพิ่มจำนวนภายนอกร่างกายได้เพียง 10 passage โดย AM-MSCs ดังกล่าวมีการแสดงออกของ MSC marker (CD73, CD90 และ CD105) และสามารถเจริญพัฒนาไป เป็นเซลล์กระดูกและเซลล์ไขมันได้เช่นเดียวกับ BM-MSCs นอกจากนี้ยังพบว่าภายหลังได้รับการเหนี่ยวนำ AM-MSCs สามารถเจริญพัฒนาไปเป็นเซลล์ที่มีลักษณะคล้ายเซลล์ประสาททั้งทางด้านรูปร่างและการแสดงออกของ neural markers คือ MAP-2, GFAP และ β-tubulin III จากผลการทดลองทั้งหมดสามารถสรุปได้ว่า AM-MSCs มีคุณสมบัติเช่นเดียวกับ BM-MSCs และสามารถเหนี่ยวนำให้เจริญพัฒนาเป็นเซลล์ประสาทได้ดังนั้น AM-MSCs จึงจัดเป็นแหล่งของเซลล์ที่มีศักยภาพในการนำมาใช้ในการศึกษาวิจัย และรักษาผู้ป่วยในอนาคตต่อไป