# Comparison of the Stem Cell Characteristics among Periodontium-derived Human Mesenchymal Stem Cells

# การเปรียบเทียบคุณสมบัติของเซลล์ต้นกำเนิดชนิดมีเซนไคม์จากเนื้อเยื่อปริทันต์ในมนุษย์

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## ABSTRACT

Objective: To investigate and compare the characteristics of human mesenchymal stem cells (MSCs) among 3 different stem cell sources in periodontium; periodontal ligament (PDL) tissue, marginal gingival tissue (MG), and supra-crestal gingival connective tissue (SG). Materials and Methods: PDL-MSCs, SG-MSCs, and MG-MSCs were obtained from 4 subjects with healthy periodontal condition. The characteristics of 3 types of MSCs were compared in terms of an expression of MSCs markers, colony-forming ability (CFA), cell proliferation, alkaline phosphatase (ALP) activity, osteogenic and adipogenic potential Results: Flow cytometry revealed a similar pattern of MSCs marker expression among 3 cells types with positive expression of CD29, CD44, CD73, CD90, CD105, CD146, and STRO-1. SG-MSCs showed the highest CFA with difference compared with PDL-MSCs ($P<0.001$) but not different from MG-MSCs. The proliferation rate of SG-MSCs was higher than that of the PDL-MSCs ($P=0.003$) but not different from MG-MSCs. The ALP activity and osteogenesis were highest in PDL-MSCs followed by SG-MSCs and MG-MSCs, respectively. Between the gingival tissue-derived MSCs, SG-MSCs showed higher osteogenesis compared to MG-MSCs ($P=0.025$). No differences found in adipogenesis among 3 cell types. Conclusions: This study found the distinct stem cell characteristics among MSCs derived from 3 parts of periodontium. SG-MSCs demonstrated the notable CFA with a higher osteogenic potential compared to MG-MSCs and appeared to have a closer stem cell potential to PDL-derived MSCs.

## บทคัดย่อ

วัตถุประสงค์ เพื่อตรวจสอบคุณสมบัติเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จากอวัยวะปริทันต์ 3 ส่วน คือ เนื้อเยื่อเหงือกส่วนเหนือขอบกระดูกเบ้าฟัน เนื้อเยื่อส่วนขอบเหงือก และเอ็นยึดปริทันต์ การทดสอบพบว่าเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จากเนื้อเยื่อทั้ง 3 ส่วนมีคุณสมบัติที่แตกต่างกัน เนื้อเยื่อเหงือกส่วนเหนือขอบกระดูกเบ้าฟันมีความสามารถในการสร้างกลุ่มโคโลนีและแบ่งตัวเจริญเติบโตที่เหนือกว่า คุณสมบัติในการเจริญพัฒนาไปเป็นเซลล์ชนิดอื่นพบว่าเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จากเอ็นยึดปริทันต์สามารถเปลี่ยนแปลงไปเป็นเซลล์สร้างกระดูกได้ดีที่สุด เมื่อเปรียบเทียบระหว่างเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จากเนื้อเยื่อเหงือกสองส่วน พบว่าเนื้อเยื่อเหงือกส่วนเหนือขอบกระดูกเบ้าฟันสามารถเปลี่ยนแปลงเป็นเซลล์สร้างกระดูกได้ดีกว่าส่วนขอบเหงือกอย่างมีนัยสำคัญทางสถิติ และไม่พบความแตกต่างในการเปลี่ยนแปลงเป็นเซลล์สร้างไขมันระหว่างเซลล์ทั้ง 3 ชนิด การศึกษานี้ชี้ให้เห็นว่าเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จากเนื้อเยื่อเหงือกส่วนเหนือขอบกระดูกเบ้าฟันมีคุณสมบัติที่ใกล้เคียงกับเอ็นยึดปริทันต์ และมีคุณสมบัติของความเป็นเซลล์ต้นกำเนิดซึ่งสามารถนำไปใช้ประโยชน์ในการพัฒนางานวิศวกรรมเนื้อเยื่อต่อไป

**Keywords:** Periodontal ligament, Gingiva, Mesenchymal stem cell

**คำสำคัญ:** เอ็นยึดปริทันต์ เหงือก เซลล์ต้นกำเนิดชนิดมีเซนไคม์

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**Introduction**

Chronic periodontitis is an inflammatory condition that causes pathological destruction in periodontium, including gingival tissue, periodontal ligament, cementum, and supporting alveolar bone, which resulting in tooth loss (Pihlstrom et al., 2005). The major goal of periodontal treatment is to regenerate all component of the periodontium to its original function and architecture (Wang et al., 2005). As current therapies, guided tissue regeneration (GTR) has shown variable and unpredictable outcomes for periodontal regeneration (Needleman et al., 2006). One problems is that periodontal infection may reduce the number of periodontal progenitor cells. Therefore, to ensure a sufficient number of progenitor cells, treatment focus has been shifted to stem cell-based therapy.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells with an ability of self-renewal, multilineage differentiation potential, and hypo-immunogenic response (Aggarwal and Pittenger, 2005; Pittenger et al., 1999). MSCs can be obtained from several adult tissues that originated from mesodermal origin. MSCs exist in several tissues, such as skeletal muscle, umbilical cord blood, liver, adipose tissue, lung, tendons, placenta, skin, and dental tissue. MSCs from dental tissue have been introduced and applied to cell therapies due to an ease of collection and availability (Liu et al., 2015).

PDL-derived MSCs have been characterized for the first time in human PDL tissue and can be generated cementum/PDL-tissue *in vivo* (Seo et al., 2004). These cells are able to differentiate into osteoblast, adipocyte, chondrocyte and expressed bone-associated markers (Wada et al., 2009; Yang et al., 2009). Furthermore, in animal studies, PDL-MSCs could regenerate new periodontium in all defect types through various delivery techniques (Bright et al., 2015). One human clinical study has performed an autologous transplantation of human PDL progenitor cells into deep intrabony defects and suggested a good benefit from this approach (Feng et al., 2010). Since an isolation of PDL-MSCs need an extraction of healthy tooth which impractical for a clinical situation. Therefore, the look for alternative source of MSCs is of necessary.

Gingival tissue-derived MSCs (G-MSCs) have been reported as having multi-differentiation potential (Tomar et al., 2010; Zhang et al., 2009) and immunomodulatory properties (Mitrano et al., 2010; Tang et al., 2011; Zhang et al., 2009). G-MSCs have the proliferation ability similar to PDL-MSCs but differ in multipotent differentiation potentials. Previous studies demonstrated that PDL-MSCs showed more significant differentiation potential to become the osteoblasts compared to G-MSCs, whereas, G-MSCs showed higher differentiative ability to become adipocyte than PDL-MSCs (Yang et al., 2013). In animal models, G-MSCs were able to enhance a formation of new periodontium when being transplanted in periodontal defects (Fawzy El-Sayed et al., 2012; Yu et al., 2013). G-MSCs become more attractive than the other dental originated MSCs an ease of obtaining from the routine dental procedures and provide tissue healing without scar after tissue biopsy (Mitrano et al., 2010; Tomar et al., 2010). These special properties make gingival tissue become recognizable as an ideal candidate cell source for periodontal regeneration.

In many previous studies, the location for harvesting gingival tissue sample was not clearly described. Interestingly, due to a proximity of the periodontal anatomy, some parts of gingival fibers overlap with the alveolar crest fiber from periodontal ligament (Nanci and Bosshardt 2006), may show divergent genotypic profile to marginal gingival tissue portion. It is reasonable to hypothesize that supra-crestral gingival connective tissue-derived MSCs (SG-

MSCs) may show the genotypic profile and differentiation potential closer to PDL-MSCs than that of the MSCs from marginal gingival tissue (MG-MSCs). This study will provide an important information regarding the potential sources of MSCs derived from periodontium which will be useful and beneficial to the field of periodontal tissue engineering.

**Objectives of the study**

To investigate and compare the characteristics of MSCs among three different stem cell sources in periodontium; PDL tissue, marginal gingival tissue, and supra-crestal gingival connective tissue.

**Materials and methods**

**1. Isolation of primary cells from human gingiva and periodontal ligament**

This study was approved by the ethics committee of Faculty of Dentistry, Chulalongkorn University, and informed consent was obtained from all patients. Human gingival tissue and periodontal ligament were obtained from four healthy patients (21-30 years old). The designated teeth had been scheduled to extract with orthodontic reasons or irreversible pulpitis. The tooth affected by periodontal disease or the tooth with periapical lesion was excluded.

Marginal gingival tissue was collected by external bevel incision followed by accessing the supra-crestal connective tissue of gingiva by making an internal bevel incision. PDL tissue was separated from the middle 1/3 of root by scraping method. In order to remove the epithelium layers, gingival tissue sample were enzymatically de-epithelialized with 1200 PU/mL dispase (Roche Life Science, Tokyo, Japan) and kept overnight at 4°C. The tissue samples were digested in 3 mg/mL collagenase type I (Gibco BRL, Carlsbad, CA, USA) and 4 mg/mL dispase for 1 h at 37°C. Single-cell suspensions was obtained with a 70 µm cell strainer (Falcon BD, Franklin Lakes, NJ, USA). To separate cells expected to be stem cells, we allowed suspensions of $1\times10^4$ cells to attach to 100 mm dishes (NUNC, Roskilde, Denmark) in alpha modification of Eagle's medium ($\alpha$-MEM; Gibco BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in humidified atmosphere with 5% $CO_2$. The culture medium was changed every 3-4 days and cells from the third to sixth passage (P3-P6) were used in this study.

**2. Flow cytometry analysis**

Flow cytometry (BD FACSCelesta™; BD Biosciences, San Jose, CA, USA) was used to analyze MSCs surface markers. SG-MSCs, MG-MSCs and PDL-MSCs were adjusted to a concentration of $1\times10^5$/50 µL Dulbecco's phosphate-buffered saline (PBS) containing 10% FBS. Fluorescence-conjugated mouse anti-human CD29, CD73, CD90, CD146 (R&D systems, Minneapolis, USA), CD44, CD105 (ImmunoTools, Friesoythe, Germany), STRO-1, and CD31 (BioLegend Inc., San Diego, CA, USA) were used. Antibody were diluted in 1:10 concentration. Cells were incubated with antibodies at 4 °C for 30 minutes in the dark, washed with PBS, and added PBS in up to 500 µL. Dead cells were stained with PI dye before performing the flow cytometry analysis.

**3. Colony-forming ability**

MG-MSCs, SG-MSCs, and PDL-MSCs were plated separately onto culture dishes in $\alpha$-MEM at a density of 100 cells/60 $cm^2$ and cultured in completed medium for 7-10 days. The cells were stained with 0.5% crystal violet

in methanol for 5 mins, and then washed with distilled water and dried for evaluation. The colony greater than 2 mm in diameter were counted.

**4. MTT assay**

MG-MSCs, SG-MSCs, and PDL-MSCs were plated separately in 96-well plates at a density of 1 $x10^3$ cells/well and cultured in complete medium for 24 h. 20 µL MTT solution [3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium, inner salt] were added to each well, and the plate was incubated for 4 h at 37 $^oC$. Then, the absorbance was measured at 490 nm by a microplate reader (EPOCH, Bio Tek instruments Inc., Highland Park, Winooski VT, USA).

**5. Osteogenic differentiation**

MG-MSCs, SG-MSCs, and PDL-MSCs were plated separately in culture dish (100cells/60 $cm^2$) and incubated in complete medium for 14 days. Then, the medium was changed to osteoinductive medium: 82 µg/ml ascorbic acid (Sigma–Aldrich, St Louis, MO, USA), 10 mM/L β-glycerophosphate (Sigma-Aldrich) and 10 mM/L dexamethasone (Sigma–Aldrich). The calcified nodules were identified after 21 days of osteoinduction by staining with 1% alizarin red solution.

**6. Adipogenic differentiation**

MG-MSCs, SG-MSCs, and PDL-MSCs were plated separately in culture dish (100 cells/60 $cm^2$) and incubated in complete medium for 14 days. Then, the medium was changed to adipogenic inductive medium containing 10 mM/L dexamethasone, 0.5 mM/L isobutyl-1-methyl xanthine (Sigma-Aldrich), and 50 µM/L indomethacin (Sigma-Aldrich). After 21 days, the cells were stained with fresh oil red O solution and oil red O positive colonies were counted.

**7. Alkaline phosphatase activity**

MG-MSCs, SG-MSCs, and PDL-MSCs were separately seeded in 96-well plate at a density of $1x10^4$ cells/well and cultured in complete medium for 48 h. Then, the medium was changed to complete medium with or without osteoinductive supplements. After additional 3-day culture, cells were washed once with PBS, and alkaline phosphatase (ALP) activity was evaluated using LapAssay™ ALP (Wako). The enzyme activity was optically measured at 405 nm wavelength using microplate reader (EPOCH, Bio Tek instruments Inc., Highland Park, Winooski VT, USA)

**8. Statistical analysis**

All experiments were performed in triplicate. Results were presented in mean ± standard deviation (SD). Normal distribution was tested with the Kolmogorov–Smirnov test. For parametric data, ANOVA test was used to evaluate the mean different among three groups. In case of parametric data, Friedman test was used to evaluate the mean different between two groups. *P*-values less than 0.05 ($P<0.05$) was considered significant.

## Results

**1. Determination of surface markers in MG-MSCs, SG-MSCs and PDL-MSCs by flow cytometry analysis**

Flow cytometry analysis revealed that MG-MSCs, SG-MSCs, and PDL-MSCs were uniformly positive for CD44, CD29, CD73, CD90, and CD105 markers (all above 95%) and were negative for CD31. The percentage of positive cells varied among three different stem cell sources, particularly for the markers CD146 and STRO-1.

**Table 1** The mean expression levels of the surface markers by PDL-MSCs, MG-MSCs, and SG-MSCs

| SURFACE MARKERS (%) | CD29 | CD44 | CD73 | CD90 | CD105 | STRO-1 | CD146 | CD31 |
|---|---|---|---|---|---|---|---|---|
| PDL-MSCS | 99.76 | 99.89 | 99.96 | 99.52 | 99.59 | 35.40 | 54.78 | 0.58 |
| MG-MSCS | 99.92 | 99.35 | 99.91 | 99.67 | 99.66 | 45.95 | 30.92 | 0.70 |
| SG-MSCS | 99.77 | 99.45 | 99.87 | 99.21 | 99.60 | 30.87 | 51.14 | 0.65 |

**2. The Colony-forming ability**

The colony forming ability of MG-MSCs, SG-MSCs, and PDL-MSCs showed no difference morphological characteristics of 3 cells types. SG-MSCs possessed the highest colony-forming ability (57.7%), followed by MG-MSCs (54.4%) and PDL-MSCs (48%). The number of colony forming unit of SG-MSCs was significantly higher than that of the PDL-MSCs (*P*<0.001) but not different from MG-MSCs.

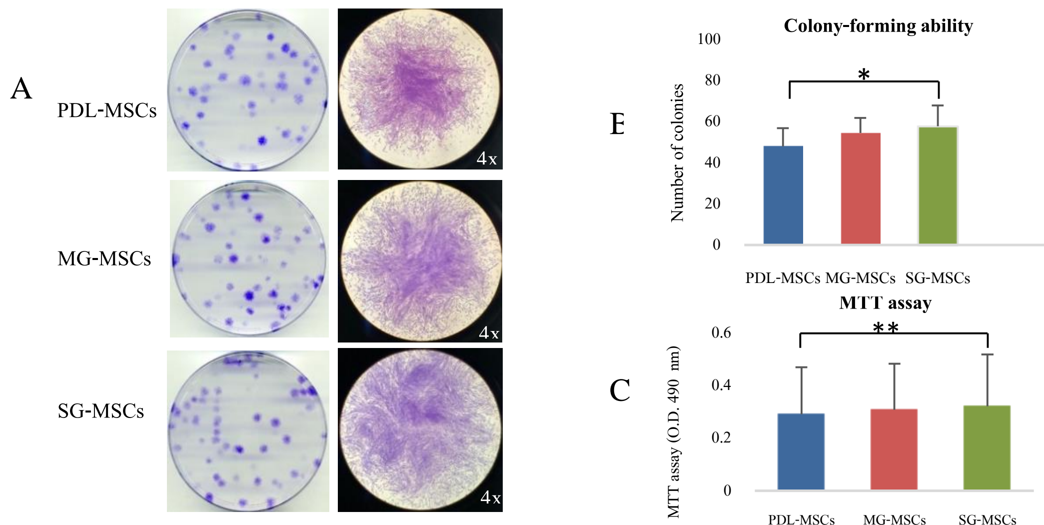


**Figure 1** (A) Representative image of Colony-forming unit of PDL-MSCs, MG-MSCs, and SG-MSCs (B) The number of colonies generated by PDL-MSCs, MG-MSCs, and SG-MSCs[1], (C) Proliferation of PDL-MSCs, MG MSCs, and SG-MSCs by MTT assay[2].

**3. MTT assay**

The proliferation rate of MG-MSCs, SG-MSCs, and PDL-MSCs were compared by MTT assay. The data showed that cell proliferation rate in SG-MSCs was significantly higher than that of the PDL-MSCs (*P*=0.003) but not different from MG-MSCs.

[1] * $P< 0.05$ by Paired T test

[2] ** $P< 0.05$ by Wilcoxon Signed Ranks test

**4. Alkaline phosphatase activity**

After being induced in an osteogenic inductive medium for 3 days, the ALP activity of MG-MSCs, SG-MSCs, and PDL-MSCs were evaluated. The results showed that the ALP activity of both PDL-MSCs and SG-MSCs were significantly increased, while the MG-MSCs showed no difference. The ALP activity of PDL-MSCs was significantly higher than SG-MSCs (*P*=0.003) and MG-MSCs (*P*=0.002), respectively. In addition, SG-MSCs expressed higher levels of ALP than that of the MG-MSCs (*P*=0.002).

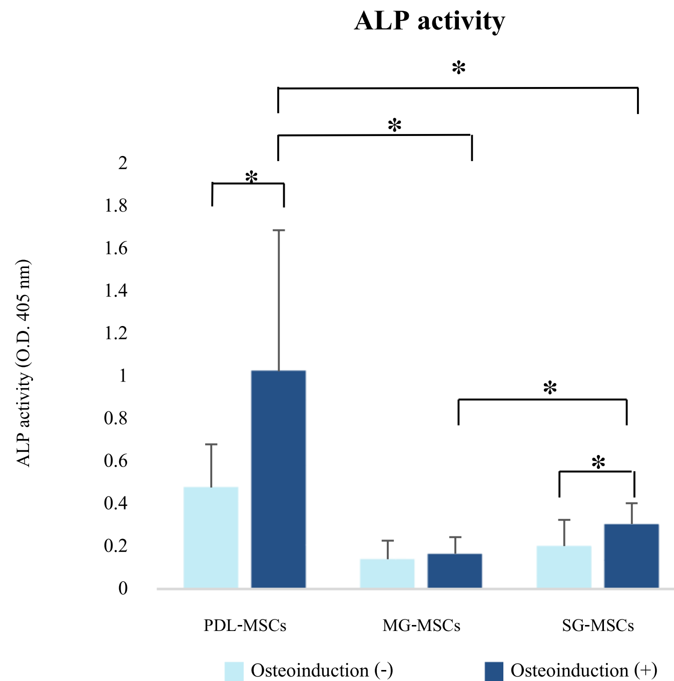


**Figure 2** ALP activity of PDL-MSCs, MG-MSCs, and SG-MSCs[3]

**5. Osteogenic differentiation**

An osteogenic differentiation potential was determined after being induced with osteogenic induction medium for 21 days. The Alizarin Red-positive colony-forming efficiency observed in PDL-MSCs was significantly higher than those of the MG-MSCs (*P*=0.001) and the SG-MSCs (*P*=0.010), respectively. Between the gingival tissue derived MSCs, SG-MSCs showed significantly stronger and higher number of positive colony compared with MG-MSCs (*P*=0.025).

[3] * $P < 0.05$ by Wilcoxon Signed Ranks test

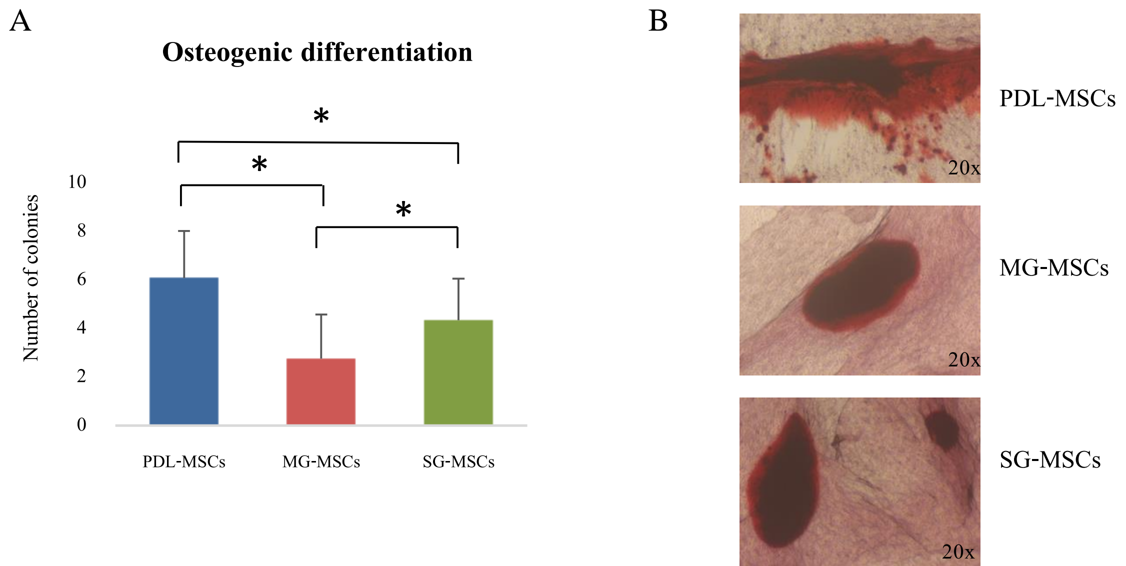


**Figure 3** (A) The number of Alizarin Red-positive colonies generated by PDL-MSCs, MG-MSCs, and SG-MSCs[4]

(B) Mineralized nodules formation by PDL-MSCs, MG-MSCs, and SG-MSCs

**6. Adipogenic differentiation**

The MG-MSCs, SG-MSCs, and PDL-MSCs were induced to differentiate toward adipogenic lineage with an adipogenic inductive medium for 21 days. The Oil Red O positive colony-forming efficiency was more pronounced in SG-MSCs than PDL-MSCs and MG-MSCs, but no statistical significant difference found in adipogenic differentiation potential among 3 cell types.

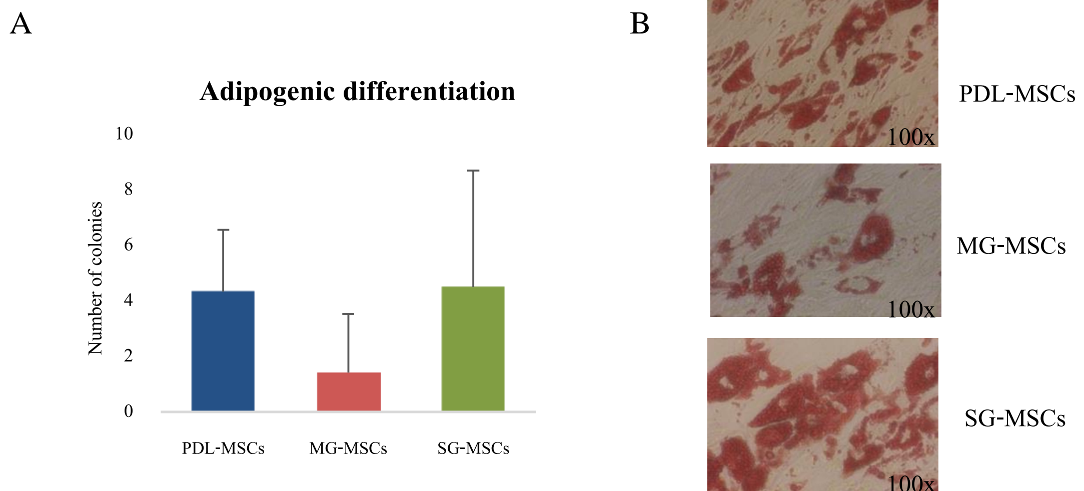


**Figure 4** (A) The number of Oil Red O positive colonies by PDL-MSCs, MG-MSCs, and SG-MSCs

(B) Oil red O staining of PDL-MSCs, MG-MSCs, and SG-MSCs.

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[4] * $P< 0.05$ by Paired T test

**Discussion and Conclusions**

Chronic periodontitis is an inflammatory condition which damages the periodontal tissue and results in tooth loss in advanced cases (Pihlstrom et al., 2005). Although GTR has been introduced to regenerate the previously deprived periodontal tissues for decades, it demonstrated highly variable and unpredictable outcomes for periodontal regeneration (Needleman et al., 2006). The reduction of periodontal progenitor cells at the periodontal defect site due to periodontal infection could explain such precariousness. Thus, the stem cell therapy is currently in focus for periodontal treatment as a promising technic to restore the periodontal progenitor cells. Periodontal ligament-derived MSCs (PDL-MSCs) have been previously characterized and considered as the strongest potential cell source for periodontal tissue regeneration (Iwata et al., 2010; Seo et al., 2004). However, to obtain PDL-MSCs for regeneration, the tooth extraction is required. This is in turn impractical for a clinical application. On the other hand, the gingival tissue-derived MSCs (G-MSCs), another periodontal tissue-derived stem cells, is known as easily accessible and abundant (Fournier et al., 2010; Zhang 2009). Although the MSCs isolated from gingival tissue have been characterized in several previous studies, it was reported that these cells possessed the lower stem cell potentials compared to the PDL-MSCs (Yang et al., 2013). Noticeably, the location of an obtained gingival tissue for an isolation of G-MSCs appeared to be varied among these studies which may result in an unclear characteristic of this cell source.

Anatomically, some part of the gingival fibers overlaps with the alveolar crest fibers of the PDL at the superior part of an alveolar crest, which known as "supra-crestal gingival connective tissue" (Nanci and Bosshardt 2006). The gingival fibers located at this area may show a distinct and divergent genotypic profile to the marginal gingival tissue, the portion that often used in many previous studies. As the supra-crestal gingival connective tissue locates adjacent to the PDL tissue, MSCs isolated from this area may show similar characteristics to the PDL-MSCs. In this study, we investigated and compared the characteristics of MSCs among three different stem cell sources in periodontium; PDL tissue, marginal gingival tissue, and supra-crestal gingival connective tissue

We successfully isolated and characterized 3 types of stem cells populations derived from PDL tissue (PDL-MSCs), marginal gingival tissue (MG-MSCs), and supra-crestal gingival connective tissue (SG-MSCs). These stem cells showed no difference in morphology. They all exhibited spindle shape and fibroblast-like cells with plastic adherence. The analysis of MSCs surface markers demonstrated no differences in their phenotypes properties. There were positive for CD29, CD44, CD73, CD90, and CD105 markers and were negative for the hematopoietic marker, CD31. These results correspond with the minimal criteria of MSCs definition proposed by the ISCT (Dominici et al., 2006). Interestingly, FACs analysis of all 3 cell types demonstrated that the percentage positivity for CD146 and STRO-1 was in various levels. CD146 is an endothelial cell marker and highly expresses in the vascular endothelial cells, smooth muscle cells, and pericytes. In the present study, the MSCs from 3 tissue areas demonstrated moderate levels (30-55%) expression. These data supported the hypothesis that MSCs may reside in a perivascular location, although it remained possible that MSCs may originate in other sources (Chen et al., 2006; Crisan et al., 2011). In terms of STRO-1, one of the most well-known markers for MSCs, its identity remained unclear with the varying percentage of expression in PDL-MSCs and G-MSCs in many previous studies (Fournier et al., 2010; Gao et al., 2014; Nagatomo et al., 2006; Tang et al., 2011; Wang et al., 2011a; Wang et al., 2011b). In vitro, STRO-1 could identify a subpopulation of

mesenchymal stem cells that were capable of forming clonogenic fibroblast colonies (Simmons and Torok-Storb 1991). In the present study, STRO-1 was detected in 30-46% of 3 types of MSCs. The differences in the percentages showed in the studies could be explained by the different flow cytometric devices, samples preparation technic and biodiversity.

Regarding the self-renewal ability, SG-MSCs showed the higher numbers of colony forming unit and proliferation rate than those of PDL-MSCs and MG-MSCs, although not statistically different from MG-MSCs. Consistently, the previous studies also reported that G-MSCs showed a higher self-renewal ability compared to PDL-MSCs (Gao et al., 2014; Yang et al., 2013). This may be due to the fetal-like phenotype of gingival tissue in which associates with the fast wound healing without scar formation and a higher regenerative ability compared to periodontal ligament (Fournier et al., 2013). The unique characteristic can be explained by the high levels expression of migration stimulating factor (MSF), which found only in fetal skin (Schor et al., 1988).

In terms of differentiation potential, the results showed that when PDL-MSCs and G-MSCs were induced by osteogenic induction medium, mineralized nodules were formed and an ALP activity was enhanced. These results were in accordance with the study by Yang et al in 2013, which PDL-MSCs demonstrated higher osteogenic potential compared to G-MSCs (Yang et al., 2013). These results were also supported in an mRNA level by RT-PCR analyses in previous studies (Gao et al., 2014; Yang et al., 2013), which revealed that the expression level of osteogenesis-related genes such as, Runx2, ALP, osteocalcin, and collagen I in PDL-MSCs was higher than that of G-MSCs. Interestingly, when the two types of G-MSCs were compared, SG-MSCs showed significantly higher osteogenic differentiation potential compared to MG-MSCs. The location where these 2 types of cells reside specifically could possibly explain such distinct differentiation potential between them. As the SG-MSCs resides closely to the PDL tissue, the closer or similar characteristic of SG-MSCs to PDL-MSCs would be expected. Furthermore, we demonstrated that PDL-MSCs and G-MSCs could be induced by an adipogenic induction medium to form lipid droplets as confirmed by Oil Red O staining that coincide with previous studies (Fournier et al., 2010; Wada et al., 2009; Wang et al., 2011a; Wang et al., 2011b). PDL-MSCs formed more Oil Red O positive colonies compared to MG-MSCs and SG-MSCs but there was no significant difference among 3 cell types. This is in line with the previous observation (Yang et al., 2013) showed that adipogenic potential of G-MSCs and PDL-MSCs was comparable. In mRNA levels, there was no significant difference in the expression of the adipogenic gene marker; PPAR$\gamma$ found between these GMSCs and PDL-MSCs. The further study on gene expression profiles is of needed to identify the distinct stem cell characteristics among these 3 types of MSCs in an mRNA levels and to warrant our results of the functional assays.

Our study first time declared and brought up the benefit of the juxtaposition of PDL fibers and supra-crestal connective tissue fibers of gingiva in order to use as the stem cell source for periodontal regeneration. As the data suggested that the SG-MSCs showed the similar phenotypic profile and differentiation potential to the PDL-MSCs. Therefore, SG-MSCs could be considered as a good candidate cell type for periodontal regeneration which can overcome the limitation when using PDL-MSCs by which a tooth extraction is not required. In addition, our results notified the importance of the harvesting area for gingival tissue sampling. The results demonstrated that despite being considered as the gingival tissue, the characteristics of stem cells derived from a supra-crestal gingival connective tissue and marginal gingival tissue were different regarding a self-renewal capacity and an osteogenic differentiation potential.

Therefore, the specific location of gingival tissue for isolating the MSCs should be carefully selected for a future experiment and application.

Still, this study carried some limitations. Because of an in vitro feature of this study, yet the results could not be applied clinically. Therefore, the future animal models should be performed to confirm the stem cell properties and cellular characteristics of MSCs derived from the supra-crestal gingival connective tissue. According to the study protocol that handled with human tissue samples, the number of the study sample is somewhat limited. The larger sample size maybe of necessary to provide the higher level of significant results of this study.

In conclusion, this study revealed the distinct and specific characteristic of the MSCs derived from periodontal tissue, including PDL tissue, marginal gingival tissue, and supra-crestal gingival connective tissue. MSCs derived from the supra-crestal gingival connective tissue demonstrated the notable colony-forming ability with a higher osteogenic potential compared to MG-MSCs and appeared to have a closer stem cell potential to the PDL-derived MSCs. Stem cells derived from the supra-crestal gingival connective tissue may be considered as a candidate cell for the periodontal regeneration and provide a future direction for clinical application in periodontal therapy.

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