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Title: Mesenchymal stem cell characteristics of apical papilla of supernumerary tooth compared to the dental pulp derived stem cells

Running Title: Stem cells from supernumerary tooth

Authors: Zeynep Burçin Gönen[1], Selami Demirci[2], Ayşegül Doğan[3], Ayşegül Sardoğan[3], Abdullah Ekizer[4], Alper Alkan[5], Fikrettin Şahin[3].

Institutions: [1]Department of Oral and Maxillofacial Surgery, Erciyes University, Genome and Stem Cell Center, Kayseri, Turkey

[2] National Heart, Lung, and Blood Institute (NHLBI), NIH, Bethesda, Maryland, USA

[3] Department of Genetics and Bioengineering, Yeditepe University Faculty of Engineering and Architecture, Istanbul, Turkey

[4] Department of Orthodontics, Medikadent Clinics, Kayseri, Turkey

[5] Department of Oral and Maxillofacial Surgery, Bezmialem University Faculty of Dentistry, , Istanbul, Turkey

Address for Correspondence: Zeynep Gönen. Department of Oral and Maxillofacial Surgery, Erciyes University, Genome and Stem Cell Center, Kayseri, Turkey

E-mail: zburcin@gmail.com

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ABSTRACT

Objective: There are several comparison studies to explain the specific properties of oral tissue derived mesenchymal stem cells. However, apical papilla stem cells from supernumerary tooth (ST-APSCs) have not been characterized for their MSC properties yet.

Methods: In the current study, ST-APSCs were isolated and characterized from a nonsyndromic male patient to evaluate the MSC characteristics. DPSCs and ST-APSCs were isolated and characterized for mesenchymal surface markers. Cells were differentiated into osteo-, chondro- and adipogenic cell types.

Results: Both cell types expressed MSC surface markers. When DPSCs and ST-APSCs were cultured in differentiation media promoting transformation to osteo-, chondro- and adipo-genic lineages, both showed calcium mineralization, chondrogenic mass formation and lipid accumulation. However, DPSCs derived from wisdom tooth demonstrated more differentiation potential to osteo- and chondro-genic cell types compared to ST-APSCs.

Conclusion: Overall, ST-APSCs were characterized by their MSC properties and able to differentiate into three cell lineages. However, they are less potent for osteo- and chondro-genic cell lineage specification compared to DPSCs derived from wisdom tooth.

Keywords: Supernumerary tooth, stem cell, dental pulp, apical papilla

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INTRODUCTION

Stem cell technology has been established as an important field in the regenerative medicine on the basis of new findings that are related with the stem cell therapy, tissue engineering and replacement. Various mesenchymal stem cells (MSCs) have been isolated, characterized and reported from different tissues in the past decades (1). Of those, MSCs from dental tissues have been shown for their significant mesenchymal characteristics as alternative stem cell sources (1).

Different MSC populations from human oral tissues have been successfully isolated such as gingival stem cells (2-5), deciduous teeth (SHED) (3), periodontal ligament derived stem cells, apical papilla stem cells (SCAP), dental follicle stem cells and dental pulp stem cells (DPSCs) from supernumerary tooth (1), wisdom tooth (4), immature and mature permanent tooth (5). Elucidating the advantages and limitations of each stem cell type obtained from different dental tissues might allow to regulate stem cell pluripotency, differentiation and growth *in vitro* and *in vivo* for creating alternative techniques in regenerative medicine. DPSCs, first isolated by Gronthos and his collegues in 2000 (2), have been obtained from deciduous and wisdom tooth (2,6,7), supernumerary (1) and human wisdom teeth germs of young adults (8). DPSCs referred as a multipotent stromal cells which can differentiate odontoblasts, osteoblasts and adipocytes after the comparison with bone marrow (9,10). Among stem cells from oral tissues, wisdom tooth DPSCs have been isolated, characterized and shown for their MSC properties in previous reports (8,11). As a young tissue remaining quiescent until the age of 6, high proliferative and

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multipotent characteristics of wisdom tooth have attracted interest in recent years. DPSCs derived from tooth germs have been proven for their significant proliferation and differentiation capacity *in vitro* (8) and dentin like matrix formation ability *in vivo* (12). Supernumerary teeth, also referred as hyperdontia, are additional teeth apart from the normal dental formula (13). They can be found in almost any region of the dental cavity. These types of teeth can be observed in both syndromic and non-syndromic patients (14, 15). DPSCs from supernumerary tooth have been reported for their MSC properties (1), differentiation capacity to osteogenic and adipogenic cell types (16,17) and immunomodulatory activities (18). However, apical papilla stem cells from supernumerary tooth have not been characterized and investigated for their MSC properties yet. The aim of current study is to isolate the apical papilla mesenchymal stem cells derived from supernumerary teeth and compare MSC surface marker expressions and differentiation capacity with dental pulp mesenchymal stem cells which obtained from third molar.

MATERIALS and METHODS

Source of Supernumerary Teeth

Multiple supernumerary teeth were identified on panoramic radiography in a healthy 16-year-old male patient **(Figure 1A)**. The patient had no significant medical and family history. The supernumerary teeth in the right maxillary region were extracted under local anesthesia and delivered to the laboratory for ST-APSCs isolation. Tooth germs were obtained from three young adults at the age between 18-20 years old and used for DPSCs isolation. Written consents

of the patients and their parents were obtained for the usage of extracted tooth according to the approval of local ethical committee (2013/508) and the guidelines of the Helsinki Declaration.

Isolation and characterization of the cells

DPSCs and ST-APSCs were isolated and characterized as described previously by our group (8, 19, 20). As cells reached to 80 % confluence at passage 2, the cells were removed via trypsin-EDTA solution (Invitrogen) and incubated with primer antibodies for 1 hour. Primary antibodies against CD271, CD90, CD146, CD44, CD73, and CD105 which obtained from BD were analyzed using Navios (Beckman Coulter, USA).

Differentiation of MSCs

In order to show MCS characteristics, the cells were induced to differentiate into three mesenchymal cell lineages (8, 19). Both types of cells were seeded onto 12-well plates at a concentration of $3x10^4$ cells/well, and pre-made differentiation mediums were added. Differentiation medium contents were shown in **Table 1**. Cells were incubated in a 5% CO_2 humidified incubator at 37°C, for 7–10 days. The differentiation media were replaced the day after.

RT- PCR analysis

Primers which synthesized by Invitrogen for Collagen type-I (Col1A1), Osteonectin (ON), Collagen type-II (Col2A1), Aggrecan (ACAN) and Fatty acid binding protein-4 (FABP4) were used in this study **(Table 2)**. Total RNAs from differentiated MSCs of both source were isolated and mRNA levels were determined using SYBR Green staining method . Maxima

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SYBR Green qPCR Master Mix was mixed with cDNAs in a final volume (20 µL). β-actin was used as a housekeeping gene for normalization of data. iCycler RT-PCR system (Bio-Rad, Hercules, CA) was used for the experiments.

Alkaline phosphatase enzyme activity

Osteogenic differentiation was confirmed by Alkaline phosphatase **(**ALP) enzyme activity assay. Cells were collected and lysis buffer containing triton-X 100 (0.2%) in PBS were used for protein extraction. Subsequently, 25 µL of protein lysate was mixed with 75 µL of ALP ligand (Randox ALP detection kit; Randox, Antrim, UK) in a 96-well plate and incubated for 15 minutes. Enzyme activity detected by measuring the absorbance at 405 nm using an ELISA plate reader (Biotek).

von Kossa Staining

von Kossa staining was performed to show mineralization and calcium deposition of differentiated cells as a marker of osteogenic transformation. Formaldehyde-fixed cells were rinsed with distilled water and stained with von Kossa kit (Polysciences Inc, Warrington, PA) which was given in manufacturer's instructions.

Alcian Blue Staining

Alcian blue staining was performed to examine chondrogenic differentiation levels of differentiated cells. Briefly, formaldehyde-fixed cells were stained with a solution which was prepared with Alcian blue in 3% acetic acid. After the incubation it was rinsed three times with PBS. Observation was performed under the light microscope (Primo Vert, Zeiss, Germany).

Oil Red Staining

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Oil red staining was conducted to show lipid vesicles to confirm the adipogenic differentiation. Formaldehyde-fixed cells were washed with PBS three times and stained with oil red diluted (6:4) in PBS for one hour. After rinsing with PBS, observation was performed under the light microscope (Primo Vert, Zeiss, Germany).

Immunocytochemical Analysis

4% paraformaldehyde was a fixation agent for the cells. Permeabilization was obtained with 0.1% Triton-X 100/phosphate-buffered saline (PBS) solution. 1% bovine serum albumin (BSA) was used as blocking reagent. Cells were incubated with anti-COL1A1 (Santa Cruz Biotechnology#59772), anti-OCN (Santa Cruz Biotechnology # 30044), anti-COL2A1 (Santa Cruz Biotechnology #28887), anti-FABP4 (Santa Cruz Biotechnology #271) overnight. Then, AlexaFluor 488 conjugated secondary antibodies were used for 1 h at room temperature. DAPI staining was performed and then, samples were visualized under a fluorescence microscope (Zeiss Primo Vert, Göttingen, Germany).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 6.00 for windows (GraphPad Software, San Diego California, the USA). Histogram, q-q plots and Shapiro-Wilk's test were applied to assess the data normality. Levene test was used to test variance homogeneity. The data were statistically analyzed by using Student's *t*-test. The values of p value <0.05 were considered as statistically significant.

RESULTS

Isolation and characterization

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DPSCs and ST-APSCs were successfully isolated, expanded and demonstrated fibroblast-like cell morphology **(Figure 1B**). Freshly isolated DPSCs and ST-APSCs were exerted MSC characteristics according to surface protein expression profile. Both cells were expressed CD73, CD90, CD105, and CD44 high whereas low expression of CD146, and CD271 (**Figure 1C-D)**. There was not a significant difference in all markers except CD146. ST-APSCs expressed 16% of CD146, while DPSCs are negative for this marker.

Stainings

DPSCs and ST-APSCs were differentiated into the three cell lineages to prove and compare MSC characteristics. von Kossa staining indicate the osteogenic differentiation by calcium deposition. The results revealed that DPSCs exerted relatively increased calcium mineralization with respect to ST-APSCs indicating the osteogenic transformation capacity of both cells (**Figure 2A**). Chondrogenic differentiation was confirmed by staining the mucopolysaccaharydes and glycosaminoglycans with Alcian Blue. DPSCs exhibited more intense staining for chondrogenic transformation indicating remarkable cartilage differentiation capacity (**Figure 3A**). Lipid vesicles were visualized with oil red staining as the result of adipogenic differentiation. No significant difference in adipogenic transformation capacity was detected. Both cells were successfully differentiated into adipocyte-like cells and lipid vesicles were stained with Oil red in both experimental groups (**Figure 4A**).

ALP acitivity in differentiating stem cells

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ALP as an osteogenic marker is found in bone and teeth at high concentrations and involved in mineralization. ALP level was significantly increased in DPSCs compared to ST-APSCs at the end of the osteogenic differentiation (**Figure 2B**).

Immunostaining Analysis

In order to confirm the differentiation of cells, both cell types were labeled with specific antibodies against markers of osteogenic (COL1A1, OCN), chondrogenic (COL2A1), and adipogenic (FABP4) cell types. The results indicate that DPSCs expressed higher levels of COL1A1 and OCN proteins (**Figure 2C and D**). COL2A1, a marker of chondrogenesis, was increased as 2 folds in DPSCs compared to ST-APSCs (**Figure 3B and C**). On the other hand, FABP4 was expressed in similar amounts in adipogenic transformation (**Figure 4B and C**).

RT-PCR analysis

mRNA levels for COl1A1 and ON were upregulated in DPSCs compared to ST-APSCs after osteogenic differentiation **(Figure 2E)**. COl1A1 and ACAN gene levels were high in differentiated DPSCs compared to ST-APSCs **(Figure 2D).** Adipogenic differentiation results revealed that DPSCs and ST-APSCs expressed FABP4 gene at equal amounts **(Figure 3D).**

DISCUSSION

Identification of MSCs in the adult body has led to the development of treatment regimen involving stem cell therapy applications. BMSCs, as primary source of adult stem cells, are well characterized and have been proven to display multipotent differentiation capacity.

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However, painful surgical procedures during resection, contamination risk and inadequate cell number yield restrict clinical applications. Therefore, isolation of adult MSCs from alternative sources that are enabling the generation of high number of proliferative cells is of great interest in recent years.

DPSCs obtained from wisdom teeth of young adults have been characterized by our group in previous reports and their remarkable multipotent differentiation capacity to osteo-, odonto-, chonro-, adipo- and neuro-genic cell lineages were shown (8, 19, 20). In addition to easy isolation of highly proliferative and multipotent stem cells, using the pulp tissue as a cell source might provide enough number of cells for a functional stem cell therapy. Such sources of dental stem cells are valuable for regenerative medicine applications and should be well-characterized for improvements of stem cell based therapy applications.

Supernumerary teeth, residing in the oral cavity instead of normal permanent teeth, are generally recognized by radiographic examinations for routine dental applications (21-23). These type of extra teeth might cause several complications including prevention of permanent teeth eruption, diastemas, dentigerous cysts, rotations, crowding and root resorption problems (14,15). In order to avoid such complications, supernumerary teeth should be removed by standard dental treatment applications. The patient in the current investigation had multiple supernumerary teeth without any systemic conditions, which is uncommon. Although multiple supernumerary teeth, in most cases, associated with different syndromes such as cleidocranial dysplasia, familial adenomatous polyposis, Chondroectodermal dysplasia, Trichorhino phalangic syndrome, and Robinow syndrome (24-27), multiple supernumerary teeth without

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syndrome have been reported with previous studies (14, 22). In the current study, supernumerary teeth from non-syndromic patient were used to isolate apical papilla stem cells and these cells were compared with the DPSCs derived from wisdom tooth pulps for their MSC characteristics.

Colony forming ability and differentiation capacity of supernumerary tooth derived stem cells have been shown in previous reports (1, 17). Supernumerary tooth derived dental pulp stem cells were compared with SHED in a previous study (17). Similar results were obtained for immunophenotypic characteristics and differentiation abilities. ST-APSCs and DPSCs have a similar surface marker expression for all MSC markers. The only differentially expressed surface antigen is CD146 which was shown to be associated with vascular smooth muscle commitment (28). CD146 level was high in ST-APSCs compared to DPSCs which should be analyzed with further studies. The cells were also compared for their differentiation capacity by inducing osteo-, chondro- and adipogenic cell transformation. The current investigation proved that DPSCs obtained from young adults of tooth germs were found superior to ST-APSCs in terms of osteo-and chondro-genic differentiation. High levels of calcium mineralization and osteogenic gene and protein expression was observed in DPSCs indicating higher bone formation capacity compared to ST-APCSs. Chondrogenic differentiation when both cells were stained with Alcian blue showed similar in both cell source. More prominent chondrogenic masses appeared in the DPSCs group. COL2A1 as a chondrogenic marker protein expressed in low levels in ST-APSCs as well as chondrogenic genes.

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On the other hand both cell types exerted similar characteristics for adipogenic differentiation. The present study clearly indicated that supernumerary tooth can be used for MSC isolation with some limitations. Although ST-APSCs have differentiation ability to three cell lineages: osteo-, chondro- and adipogenic; originated from mesenchyme, they are less potent adult stem cells compared to DPSCs derived from wisdom tooth.

CONCLUSION

In conclusion this report described the isolation of apical papilla stem cells from supernumerary tooth for the first time and their stem cell potential was compared with DPSCs derived from wisdom tooth. As waste materials of dental applications, supernumerary teeth could be used to isolate MSCs.

References

1- Huang AHC, Chen YK, Lin LM, Shieh TY, Chan AWS. Isolation and characterization of dental pulp stem cells from a supernumerary tooth J Oral Pathol Med 2008; 37:571-74.

2- Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 2000; 97:13625-30.

3- Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth Proc Natl Acad Sci USA 2003; 100:5807-12.

4- Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;10 16;364:149-55.

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5- Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT-J. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008;34:166-71.

6- d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 2007; 14:1162-71.

7- Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G,Papaccio G. A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res 2005;20(8):1394-402.

8- Yalvac ME, Ramazanoglu M, Rizvanov AA, Sahin F, Bayrak OF, Salli U, Palotás A,Kose GT. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-,adipo- and neurogenesis. Pharmacogenomics J 2010;10(2):105-13.

9- Karaöz E, Demircan PC, Sağlam Ö, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol 2011; 136:455-73.

10- Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 2003;18:696-704.

11- Collignon AM, Castillo-Dali G, Gomez E, Guilbert T, Lesieur J, Nicoletti A, Acuna-Mendoza S, Letourneur D, Chaussain C, Rochefort GY, Poliard A. Mouse Wnt1-CRE-Rosa(Tomato) dental pulp stem cells directly contribute to the calvarial bone regeneration process. Stem Cells. 2019 Jan 23. doi: 10.1002/stem.2973.

12- Takeda T, Tezuka Y, Horiuchi M, Hosono K, Iida K, Hatakeyama D, Miyaki S,Kunisada T, Shibata T, Tezuka K. Characterization of dental pulp stem cells of human tooth germs. J Dent Res 2008;87(7):676-81.

13- Cobourne MT, Sharpe PT. Making up the numbers: The molecular control of mammalian dental formula. Semin Cell Dev Biol 2010; 21(3):314-24.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Gönen ZB, Demirci S, Doğan A, Sardoğan A, Ekizer A, Alkan A, Şahin F. Mesenchymal stem cell characteristics of apical papilla of supernumerary tooth compared to the dental pulp derived stem cells. Erciyes Med J 2019; DOİ: 10.14744/etd.2019.62592.

14- Diaz A, Orozco J, Fonseca M. Multiple hyperodontia: report of a case with 17 supernumerary teeth with non syndromic association. Med Oral Patol Oral Cir Bucal 2009; 14: 229-31.

15- Ferrés-Padró E, Prats-Armengol J, Ferrés-Amat E. A descriptive study of 113 unerupted supernumerary teeth in 79 pediatric patients in Barcelona Med Oral Patol Oral Cir Bucal 2009; 14:46-52.

16- Kim D, Kim J, Hyun H, Kim K, Roh S. A nanoscale ridge/groove pattern arrayed surface enhances adipogenic differentiation of human supernumerary tooth-derived dental pulp stem cells in vitro. Arch Oral Biol 2014;59(8):765-74.

17- Lee S, An S, Kang TH, Kim KH, Chang NH, Kang S, Kwak CK, Park HS. Comparison of mesenchymal-like stem/progenitor cells derived from supernumerary teeth with stem cells from human exfoliated deciduous teeth. Regen Med 2011;6(6):689-99.

18- Makino Y, Yamaza H, Akiyama K, Ma L, Hoshino Y, Nonaka K, Terada Y, Kukita T, Shi S, Yamaza T. Immune therapeutic potential of stem cells from human supernumerary teeth. J Dent Res 2013;92(7):609-15.

19- Doğan A, Yalvaç ME, Şahin F, Kabanov AV, Palotás A, Rizvanov AA. Differentiation of human stem cells is promoted by amphiphilic pluronic block copolymers Int J Nanomedicine 2012;7:4849-60.

20- Abbas OL, Özatik O, Gönen ZB, Öğüt S, Özatik FY, Salkın H, Musmul A.Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Adipose Tissue, and Dental Pulp as Sources of Cell Therapy for Zone of Stasis Burns. J Invest Surg 2018; 14: 1-14.

21- Inchingolo F, Tatullo M, Abenavoli FM, Marrelli M, Inchingolo AD, Gentile M,Inchingolo AM, Dipalma G. Non-syndromic multiple supernumerary teeth in a family unit with a normal karyotype: case report. Int J Med Sci 2010;7(6):378-84.

22- Kawashita Y, Saito T. Nonsyndromic multiple mandibular supernumerary premolars: a case report. J Dent Child (Chic) 2010;77(2):99-101.

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23- Yagüe García J, Berini Aytés L, Gay Escoda C. Multiple supernumerary teeth not associated with complex syndromes: a retrospective study. Med Oral Patol Oral Cir Bucal 2009; 1:331-6.

24- Kreiborg S, Jensen BL. Tooth formation and eruption - lessons learnt from cleidocranial dysplasia. Eur J Oral Sci 2018;126 Suppl 1: 72-80.

25- Ma D, Wang X, Guo J, Zhang J, Cai T. Identification of a novel mutation of RUNX2 in a family with supernumerary teeth and craniofacial dysplasia by whole-exome sequencing: A case report and literature review. Medicine (Baltimore) 2018;97(32):e11328.

26- Lubinsky M, Kantaputra PN. Syndromes with supernumerary teeth. Am J Med Genet A 2016;170(10):2611-6.

27- Kantaputra P, Miletich I, Lüdecke HJ, Suzuki EY, Praphanphoj V, Shivdasani R, Wuelling M, Vortkamp A, Napierala D, Sharpe PT. Tricho-rhino-phalangeal syndrome with supernumerary teeth. J Dent Res 2008;87(11):1027-31.

28- Espagnolle N, Guilloton F, Deschaseaux F, Gadelorge M, Sensébé L, Bourin P. CD146 expression on mesenchymal stem cells is associated with their vascular smooth muscle commitment. J Cell Mol Med 2014;18(1):104-14.

Figure legends

Figure 1. Isolation and surface marker analysis of DPSCs and ST-APSCs. (A) Panoramic radiography of the 16 year old male patient. White arrows show the supernumerary teeth on panoramic radiography. (B) Fibroblastic cell morphology of DPSCs and ST-APSCs. (C) Percentage of MSC surface marker expression. (D) Immunophenotypic characteristics of

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DPSCs and ST-APSCs. Surface marker expressions were represented in percentage expression levels. Scale bar: 100µm

Figure 2. Osteogenic differentiation of DPSCs and ST-APSCs. (A) von Kossa staining results. Scale bar: 100µm (B) ALP activity measurements. (C) COL1A1 and OCN protein expression. Scale bar: 50µm (D) Intensity measurements of COL2A1 and OCN immunocytochemistry. (E) COL1A1 and ON gene expression analysis. *$P < 0.05$

Figure 3. Chonrogenic differentiation of DPSCs and ST-APSCs. (A) Alcian blue staining of chondrogenic masses Scale bar: 100µm (B) COL2A1 protein expression. Scale bar: 50µm (C) Intensity measurements of COL2A1. (D) COL2A1 and ACAN gene expression analysis. *$P < 0.05$

Figure 4. Adipogenic differentiation of DPSCs and ST-APSCs. (A) Oil red staining staining of lipid droplets after adipogenesis Scale bar: 100µm (B) FABP4 protein expression. Scale bar: 50µm (C) Intensity measurements of FABP4. (D) FABP4 gene expression analysis. *$P < 0.05$

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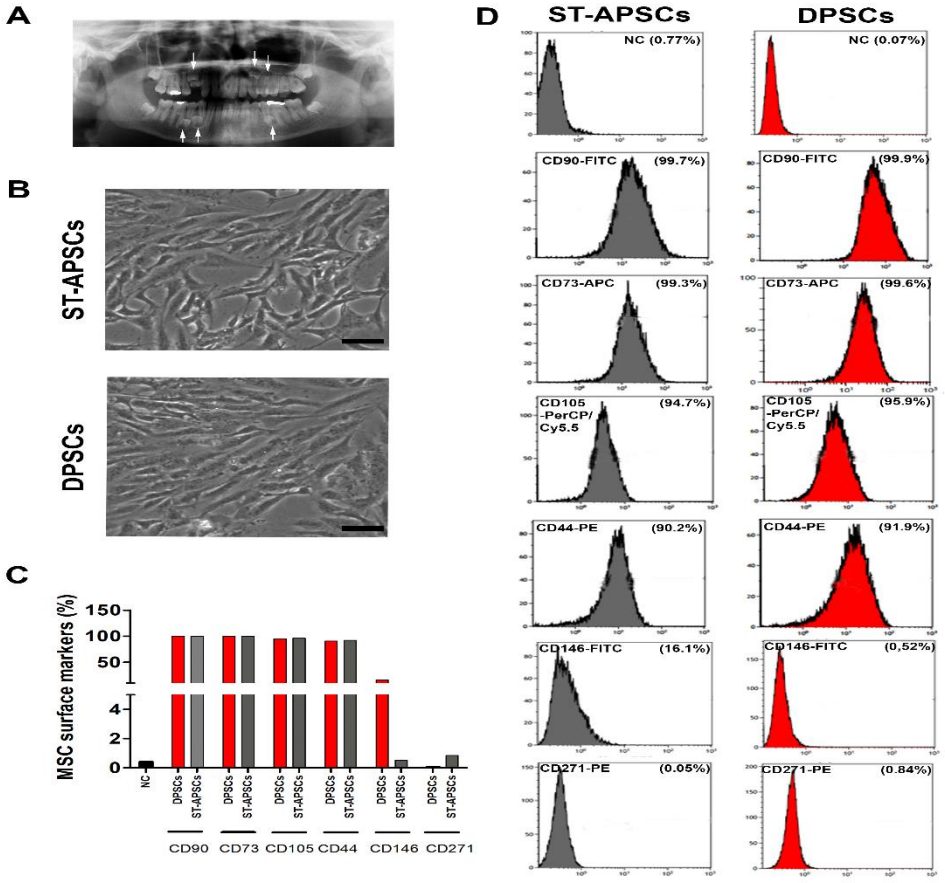

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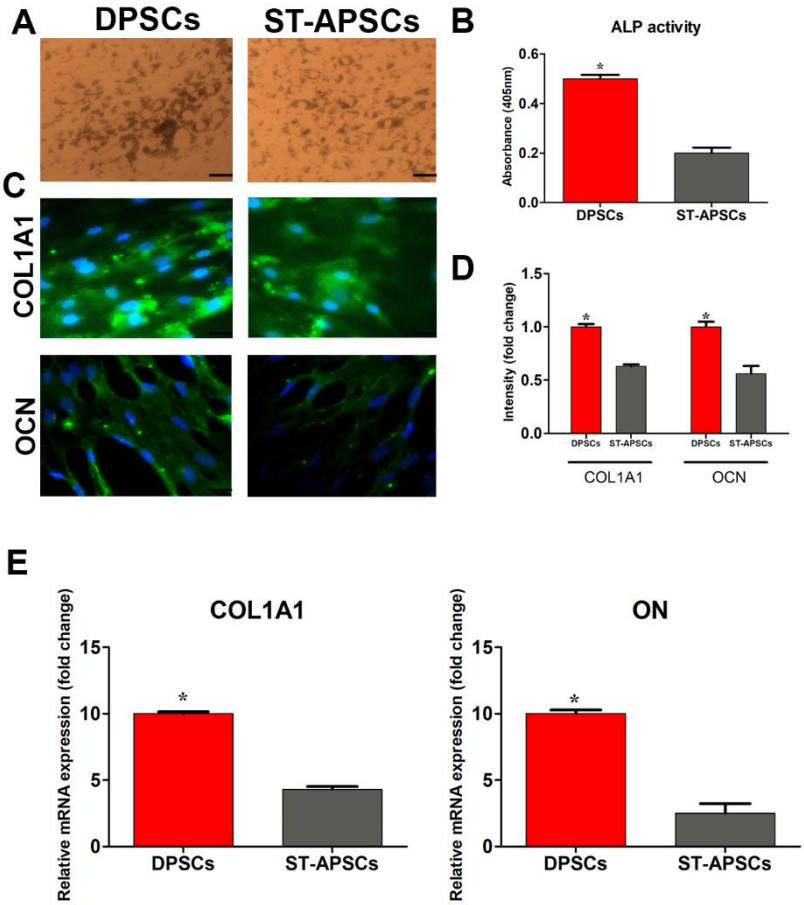

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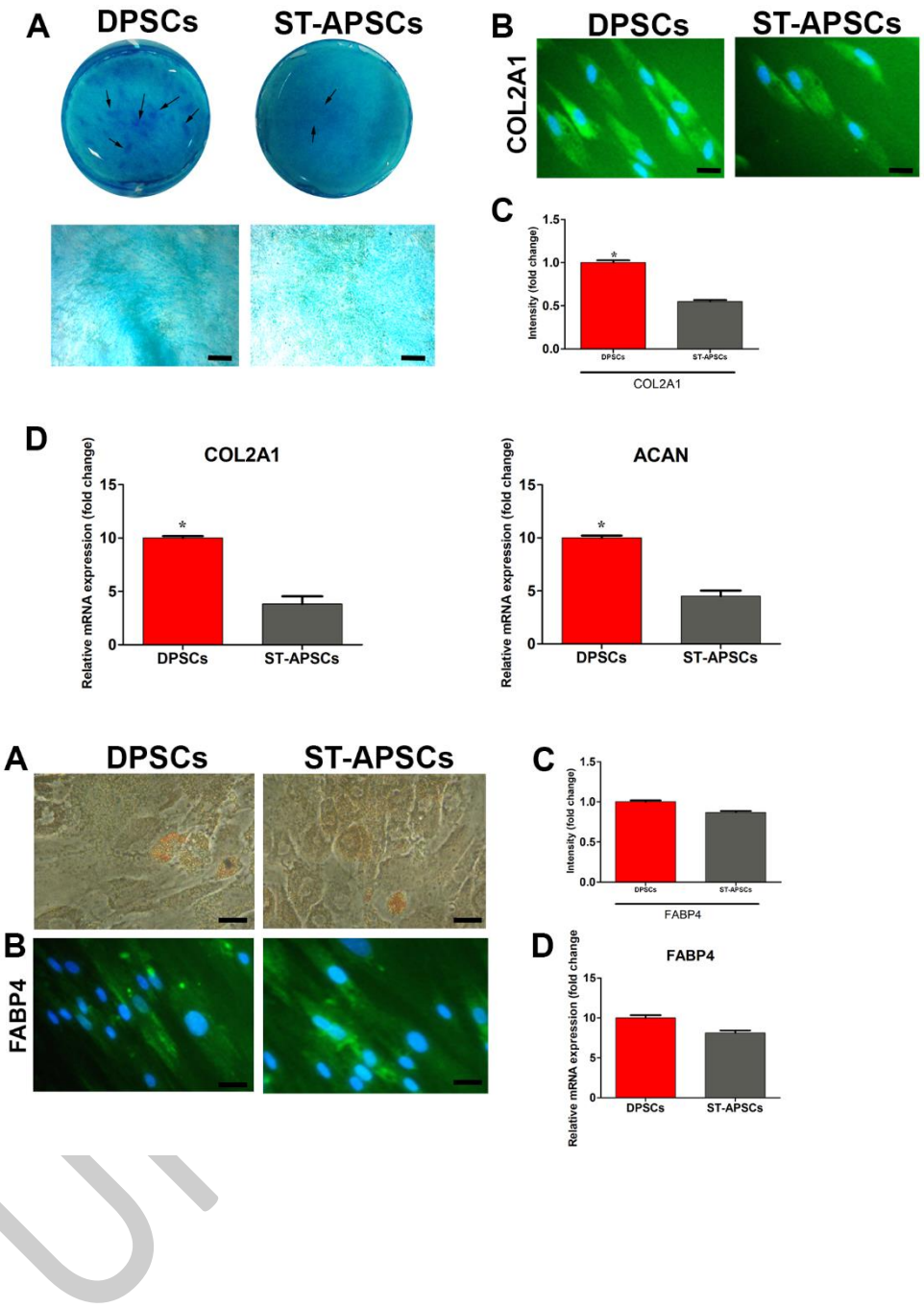

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Table1. Differentiation medium contents used to differentiate stem cells to osteo-, chondro-adipogenic cell lineages

Differentiation	**Content**
Osteogenic medium	100nM Dexamethason e, 10mM β-Glycerophosphate, 0.2mM Ascorbic acid
Chondrogenic medium	1x Insulin-Transferrin-Selenium (ITS -G), 100nM Dexamethasone, 100ng/ml TGF-β 14µg/ml Ascorbic acid 1mg/ml BSA
Adipogenic medium	100nM Dexamethasone, 5µg/ml Insulin 0.5mM 3-Isobutyl-1-methylxanthine (IBMX) 60µM Indomethacin

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Table 2. The list of primers used in RT-PCR

Gene	Sequences
COL1A1	5′-CCACGCATGAGCGGACGCTAA-3′ 5′-ATTGGTGGGATGTC TTCGTCTTGG-3′
COL2A1	5′-GTGTGGAAGCCGGAGCCCTG-3′ 5′-GGTCCTGGTTGCCCACTGGC-3′
ON	ATGAGGGCCTGGATCTTCTT CTGCTTCTCAGTCAGAAGGT
ACAN	ACTGCTGCAGACCAGGAGGT TCCTCGGGGGTGACGATGCT
FABP4	5′-GGGTCACAGCACCCTCCTGA-3′ 5′-TGGTGGCAAAGCCCACTCCTAC-3′
β-actin	5′- GACAGGATGCAGAAGGAGATTACT -3′ 5′- TGATCCACATCTGCTGGAAGGT-3′

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