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과잉치에서 얻은 치수 줄기세포가 골형성 세포와 지방 세포로 분화동안 나노 패턴이 미치는 영향

Comparisons between the effect of nano patterns on pulpal stem cells from supplementary tooth when differentiation into osteogenic cells and adipose cells

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Abstract

Comparisons between the effect of nano patterns on pulpal stem cells from supplementary tooth when differentiation into osteogenic cells and adipose cells

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Mesenchymal stem cells (MSCs) have several differentiation potentials that are multi-potent. Studies about cell differentiation mechanism can provide insights for regenerative therapeutics and tissue engineering. Human dental pulp stem cells (hDPSCs) are a type of mesenchymal stem cells and have the potency of cell differentiation into osteogenic and adipogenic cells. Supernumerary tooth are normally discarded but able to reuse as a resource of hDPSCs without an ethical issue. Recently, various nanoscale topographic devices are designed to control stem cell fate. Different

cell arrangements and/or contacts may change the secretion of adhesion molecules which can regulate the cell morphology, and the changes are related to cell migration, function, polarity and differentiation.

In present study, hDPSCs from supernumerary tooth were isolated and characterized, then the effect of 350-nm nanoscale ridge/groove pattern arrayed surface on osteogenic and adipogenic differentiation of hDPSCs were investigated. After 2 to 3 passaged, hDPSCs are characterized by FACS and positive for surface markers of MSCs (CD29, CD44, CD73, CD90, CD105) and negative for surface markers of hematopoietic cells (CD14, CD31, CD34, CD45, CD117, HLA-DR). To investigate the effect of the nanoscale pattern arrayed surface on osteogenic and adipogenic differentiation, lineage specific staining and Real-time PCR are conducted after differentiation induction. The results show that the nanoscale ridge/groove pattern arrayed surface have positive effect on adipogenic differentiation of hDPSC from supernumerary tooth, while have no effect on osteogenic differentiation. Interestingly, the nanoscale ridge/groove pattern arrayed surface enhances more with differentiation into adipogenic lineage at the early induction stage than late stage. In conclusion, the nanoscale ridge/groove pattern arrayed surface can be used to enhance adipogenic differentiation of DPSCs derived from supernumerary tooth. The results above provide an improved understanding of the effects of topography on cell differentiation as well as the potential use of supernumerary tooth in the field of regenerative medicine or dentistry.

keywords : Supernumerary tooth, Human dental pulp stem cells, Osteogenic cells, Adipogenic cells, Differentiation.

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Introduction

Adult stem cells (ASCs) are maintained in mature organism and have two distinct capabilities, self-renewal and differentiation into limited cell types. One of the most widely studied ASCs are mesenchymal stem cells (MSCs), which are derived from various tissues including bone marrow, adipose, adult muscle, corneal stroma [1], dental pulp, periodontal ligament. Especially, comparing with others, MSCs from bone marrow (BM-MSCs) have been more investigated for several decades and are verified to give rise to many types of differentiated cells. Dental pulp stem cells (DPSCs) are a type of MSCs and can be isolated from various dental pulp resources such as permanent, deciduous and supernumerary teeth [2-4]. It has been demonstrated that some characteristics of DPSCs show similar properties to BM-MSCs like morphology gene expression and potency of differentiation into osteogenic and adipogenic cells [5-7]. Moreover DPSCs have even reported to be able to differentiate into non-mesenchymal origins, ectodermal lineage such as neuron [3,8,9].

A supernumerary tooth is one that is additional to the normal teeth numbers and can be found in almost any region of the dental arch. Generally, supernumerary tooth are removed for esthetic or mostly functional reasons and discarded. Because of their non-functional feature, supernumerary tooth may appropriate to be better resource as stem cells comparing with deciduous or permanent teeth. Although not identical, DPSCs from supernumerary teeth are very analogous to other dental pulp resources, permanent and deciduous teeth [3,4,10].

It is reported that adhesion molecules were able to regulate the cell morphology. Recently, alteration of cell morphology was deeply connected with cell migration, function, polarity and differentiation [11-14]. It suggests that factors which can modify cell shapes may

be important to decide cell fate. Cell fate is determined by complex and elaborate cues. Some tissues have distinct nanoscale pattern arrayed surface structures in vivo [15], suggesting nanoscale constructions would be fairly important as niche which induce differentiation of stem cells. Recently, it has been demonstrated that topography environment in nanoscale cells is able to interact with cells[16] and change their conformations. Artificial micro- and nano-topographies have been confirmed to induce various effects on cells including proliferation, migration, cytoskeleton organization and gene expression [17-22]. Stem cells are also influenced by topography to differentiate into various lineages [23-26]. However, most attempts of studying nano-topography effects have been focused to pluripotent stem cells or neuronal lineage [27-31]. It is necessary to study the effect of nanoscale pattern arrayed surface on other lineages including osteogenic and adipogenic differentiation.

In the current study, we isolated and characterized of DPSCs from supernumerary teeth. And then we determined the effects of nanoscale ridge/groove on osteogenic and adipogenic differentiation of hDPSCs.

Materials and methods

Chemicals

All inorganic and organic compounds were obtained from Sigma-Aldrich Korea (Yong-in, Korea) unless indicated in the text.

Fabrication of polyurethane acrylate (PUA) mold

Fabrication of PUA mold was performed as previously described [17]. Briefly, the PUA mold for nanoscale patterning was fabricated by curing PUA pre-polymer (311RM, Minuta Technology, Osan, Korea) on silicon master molds prepared by photolithography. The ultraviolet (UV)-curable PUA mold material consists of a functionalized precursor with an acrylate group for cross-linking, a monomeric modulator, a photo-initiator and a radiation-curable releasing agent for surface activity. To fabricate a sheet-type mold, the liquid precursor was drop-dispensed onto a silicon master mold, and then a flexible, transparent polyethylene terephthalate (PET) film was brought into contact with the precursor surface. Subsequently, the mold was exposed to UV light (λ = 200 - 400 nm) for 20 s through the transparent backplane (dose = 100 mJ/cm^2).After UV curing, the mold was peeled from the master and additionally cured over night to terminate the remaining active acrylate groups prior to use as a first replica. The resulting PUA mold used in the experiment was a thin sheet with a thickness of~50μM.

Fabrication of the nanoscale ridge/groove-patterned surface

Fabrication of the nanoscale ridge/groove-patterned surface PUA micro- and nanoscale ridge/groove pattern arrays were fabricated on glass coverslips using UV-assisted capillary force lithography. The glass coverslip was rinsed with ethanol in an ultrasonic bath for 30 min, washed in a flow of distilled water and dried in a drying oven.

To increase the adhesion between the PUA nanostructures and the glass interface, an adhesion promoter (phosphoric acrylate: isopropyl alcohol 1.25:10, v/v) was coated onto the glass substrate. A small amount of the PUA precursor (~ 0.1 - 0.5 ml) was drop-dispensed onto the substrate, and a first-replicated PUA mold (same material but without active acrylate groups) was directly placed onto the surface. The PUA precursor spontaneously moved into the cavity of the mold by means of capillary action and was cured by exposure to UV light (λ = 250 - 400 nm) for 30 s through the transparent backplane (dose = 100 mJ/cm^2). After curing, the mold was peeled from the substrate using sharp tweezers.

Isolation of human dental pulp cells (DPCs) and cell culture

To isolate human dental pulp tissue, mesiodens (maxillary central supernumerary teeth) (n=8) were extracted from children at the Department of Pediatric Dentistry in Dental Hospital of Seoul National University according to the guidelines provided by ethics committee. The extracted teeth were cut around the cemento-enamel junction using cutting disk to expose the pulp tissue as previously described [16]. The pulp tissue was gently separated from the crown and root using sterile endodontic file and digested in 1% (w/v) collagenase type I to generate single-cell suspensions. The digested cells were seeded into 24-well culture dishes with DPSC culture medium supplemented α-MEM containing 10% (v/v) fetal bovine serum (FBS; Life technologies, NY, USA) and 100 IU/ml penicillin-100 μg/ml streptomycin (Life technologies) and then incubated at 37°C in humidified atmosphere containing 5% CO_2. The culture medium was

refreshed once every 3days to allow further growth. The adherent cells grown to more than 70% confluence were named as passage zero(P0) cells. When cells were grown 70% confluence, they were sub-cultured at 1/5 dilution for later passaging. The culture media was replaced every 3 days till growing to proper confluence.

Culture of human DPCs on the nanostructured pattern surface

The 250-nm nanoscale ridge/groove pattern arrays were immersed in a 0.1% gelatin solution for 12 h and rinsed with PBS. Human DPCs (5 to 7 passages; 3,000 cells/cm^2) were plated on the nanoscale pattern surfaces with DPSC culture medium, then attached cells were cultured in each differentiation media for3,7and14days[17].

Characterization of human dental pulp stem cells by FACS

For fluorescence-activated cell sorting (FACS) analysis, hDPSCs (passage 5) were de-attached and resuspended in ice cold PBS containing 0.5% bovine serum albumin (BSA) at a concentration of 1.0×10^6 cells/ml. Then the cells were incubated for 20 min on ice with monoclonal antibodies against CD14-fluorescein isothiocyanate(FITC), CD29-Phycoerythrin(PE), CD31-FITC, CD34-PE, CD44-FITC, CD45-PE, CD73-PE, CD90-PE, CD105-Allophycocyanin(APC), CD117-PE and HLA-DR-APC. Analysis were performed by

flowcytometry(FACSAria®;BDBiosciences,Erembodegem,Belgium) using the CellQuest software(BDBiosciences).

Osteogenic differentiation of human DPCs

To determine the differentiation potential, the basal medium was replaced with an osteogenic medium consisting of α-MEM supplemented with 0.1 μM dexamethasone, 0.05 μM ascorbate-2-phosphate, 10 mM β-glycerophosphate, 100 U/ml penicillin, 100 mg/ml streptomycin and 10% FBS. The osteogenic medium was replaced every other day. After 3, 7 and 14 days of induction culture, osteogenic differentiation was assessed by the expression of osteogenic marker genes, Osteocalcin (OC) and Runx2, and Alizarin red staining. For Alizarin red staining, the cells were fixed in 10% formalin for over 1 h, and were stained with alizarin red solution (2%, pH 4.2) for 15 min. The stained cells were dehydrated in pure acetone, washed in acetone-xylene (1:1) solution, cleared with xylene [16].

Adipogenic differentiation of human DPCs

To determine differentiation potential, the basal medium was replaced with an adipogenic medium consisting α-MEM supplemented with 2 mM L-glutamine, 0.5 μM dexamethasone, 0.5 mM isobutylmethylxantine, 50 μM indomethacin, 100 U/ml penicillin, 100 mg/ml streptomycin and 20% FBS. The adipogenic medium was replaced every other day. After 3, 7 and 14 days, adipogenic differentiation was assessed by the expression of adipogenic marker

gene, peroxisome proliferator activated receptor-ɣ (PPAR- ɣ), and Oil Red O staining. For Oil Red O staining, the cells were fixed in 10% formalin for over 1 h and stained with fresh Oil Red O solution for 2 h [18].

RNA isolation and Real-time (Quantitative) PCR

Total RNA from ESCs or EBs was extracted using the RNeasy mini kit (Qiagen), and M-MLV Reverse Transcriptase was used to synthesize cDNA according to the manufacturer's instructions. Real-time PCR was performed with an Applied Biosystems 7500HT system (Foster City, CA) using SYBR Premix Ex Taq (Takara). PCR volume was 20 μl, containing 1 μl reverse transcript product. Cycling conditions were 1 cycle of 95°C for 30 s and 40 cycles of 95°C for 5 s and 60°C for 30 seconds. The $^{\Delta\Delta}$Ct method was used to determine relative quantitation of mRNA expression in samples, and fold change was determined as $2^{-\Delta\Delta Ct}$. The specific primer sequences representing pluripotency and three-germ layer differentiation marker genes are listed in Table 1.

Results

Characterization of human dental pulp stem cells

Primary hDPSCs were isolated from a supernumerary tooth and cultured in vitro. Under light microscopy, the mixed cell populations

were observed (Fig. 1A). After 3 passages, the adhered and expanded homogenous population of the cells revealed fibroblastoid morphology (Fig. 1B). To confirm the differentiation potential of hDPSCs, the cells were cultured for 28 days with adipogenic and osteogenic induction media. Each the differentiated cell groups exhibited round bubble-like adipocyte appearance (Fig. 1C) and osteoblast producing osteoid materials (Fig. 1D). To further demonstrate properties of hDPSCs, DPSCs at P5 were characterized using FACS. As shown in Fig. 2, the hDPSCs were expressed MSC markers (CD29, CD44, CD73, CD90, CD105), while only small number of the hematopoietic and endothelial markers (CD14, CD31, CD34, CD45, CD117, HLA-DR) were expressed in the cells.

Effects of 350-nm nanoscale ridge/groove pattern arrayed surface on osteogenic and adipogenic differentiation

To investigate the role of the 350-nm nanoscale ridge/groove pattern arrayed surface on differentiation of hDPSCs, the nanoscale pattern arrayed surface were coated with gelatin and DPSCs were seeded onto both the nanoscale pattern arrayed surface and the gelatinized surface of the conventional culture dish. After seeding, the morphology of attached cells from both experimental groups was compared. The cells on nanoscale pattern group showed linear arrangement shape (Fig. 3A), while the cells on conventional surface placed irregularly (Fig. 3B).

In order to determine whether a ridge/groove pattern arrayed surface could influence differentiation, hDPSCs were cultured on nanoscale

pattern arrayed surface and conventional gelatinized surface. In both cases with adipogenic or osteogenic induction media. Alizarin red staining was conducted to compare the level of osteogenic differentiation on 3, 6 and 9 days of culture (Fig. 4). The conventional surface group was observed Alizarin red staining positive cells from Day 3 to 9, whereas the nanoscale pattern arrayed surface group was showed the staining positive cells from Day 6 to 9. Moreover, there were no differences of Alizarin red staining positive cells between two the different surface groups after Day 9 in osteogenic induction. Next, in order to compare the level of adipogenic differentiation, Oil red O staining was conducted to measure accumulated intracellular lipid droplets on 3, 6 and 9 day of culture (Fig. 5). Different from osteogenic induction result, Oil red O staining results showed that the cells on nanoscale pattern arrayed surface showed remarkable increase in Oil red O staining during differentiation induction. Interestingly on Day 3, the differences of Oil red O stained cells rate between two of the different surface groups were larger than the results from Day 6 or Day 9. Although the effect of nanoscale pattern arrayed surface on differentiation of hDPSCs was measured, the lineage specific staining technology was not enough to confirm the effects of the pattern on differentiation precisely.

Analysis of quantitative gene expression

To further determine the effect of nanoscale pattern arrayed surface, lineage specific genes were analyzed by quantitative Real-time PCR. Runt-related transcription factor 2 (Runx-2) and lipoprotein lipase (LPL) are related to osteogenesis and adipogenesis respectively.

During osteogenic differentiation, the expression of Runx-2 was gradually increased for 9 days in each group. However, comparing with nanoscale pattern arrayed surface, Runx-2 expression was slightly higher to the conventional surface group on the whole period (Fig. 6). During adipogenic differentiation, LPL expression was also increased for 9 days in both groups. Different from osteogenic differentiation, however, the expression of LPL was higher in nanoscale pattern arrayed surface group than conventional surface group. Interestingly, the increase rate in the pattern arrayed surface group was highest at 3 days (about 7 times) and the rate was decreased (Fig. 7).

Discussions

In present study, we investigated that the effects of 350-nm nanoscale ridge/groove pattern arrayed surface on hDPSCs differentiation into osteogenic and adipogenic lineage cells.

DPSCs are considered to be an alternative source of adult stem cells because they were able to culture in vitro easily and exhibited a high proliferation rate compared with MSCs which are from bone marrow and major resource of adult stem cells, according to the literature [33]. Moreover, DPSCs can differentiate into multiple cell lineages, neurogenic, adipogenic, and osteogenic cells [34,35]. Induction of cells differentiation from stem cells are very important in stem cells researches because sometimes stem cells can be differentiated cells that we do not want and even unexpected type of cells such as teratoma. Previously, chemicals are broadly used for stem cell differentiation [4,10]. Recently, it is known that nano scale patterned surfaces on facbricated materials can influence stem cell

differentiation [20,27,36]. Thus, geomorphology and physical stress of these niche are significant factors for stem cell differentiation.

In current study, we determined whether nanoscale ridge/groove pattern arrayed surface that is known for the effects on neurons [27] is able to influence on osteogenic and adipogenic differentiations of hDPSCs. Firstly, we isolated dental pulp cells from supernumerary tooth and observed their morphology. The hDPSCs showed small-sized and linear shapes after several passages. These morphologic characteristics of our hDPSCs were in close agreement with those found in the earlier experimental studies [4,10]. To investigate the state of hDPSCs, surface markers were analyzed by FACS. The established hDPSCs stained positively with MSCs markers but were not stained with hematopoietic markers (Fig. 2). These properties of our hDPSCs corresponded well with those found in the earlier experimental studies of hDPSCs [10,37,38].

To verify the effects of nanoscale ridge/groove pattern arrayed surface on differentiation of hDPSCs, Alizarin red and Oil red O were conducted to measure the level of differentiation. As our results, the cells stained by Alizarin red were gradually increased both on nanoscale pattern arrayed surface and conventional surface but there was no big difference between both groups on Day 9 (Fig. 4). During adipogenic differentiation, the cells stained by Oil red O were also increased on nanoscale pattern arrayed surface and conventional surface. Different from osteogenic differentiation, However, Oil red O stained cells on nanoscale pattern arrayed surface were rapidly revealed and showed remarkably increased numbers on Day 3 comparing with conventional surface, suggesting that the nanoscale pattern arrayed surface may effect on adipogenic differentiation and the effect is started at the early stages of differentiation.

To better evaluate the effects of nanoscale pattern arrayed surface on hDPC differentiation, we conducted Real-time PCR. Runx-2 and LPL were chosen as gene markers for the osteogenic and adipogenic phenotype separately [39]. During osteogenic differentiation, although Runx-2 expression was increased on whole differentiation period, the expression in nanoscale pattern arrayed surface group was lower than in convention surface group. Interestingly, Runx-2 expression was temporarily decreased on Day 3. It suggests that the physical stimulus of nanoscale pattern arrayed surface may not influence or inhibit the osteogenic differentiation (Fig. 6). Moreover, our Alizarin red staining analysis may support the results (Fig. 4).

On the other hand, during adipogenic differentiation, the expression of LPL was higher in nanoscale pattern arrayed surface group than conventional surface group during the whole period (Fig. 7). Excitingly, the increase rate of the LPL expression was the highest on Day 3, which shows that the nanoscale pattern arrayed surface may influence or induce adipogenic differentiation rapidly comparing with conventional surface. The results were also supported by Fig. 5. Together, these results indicate that the nanoscale pattern arrayed surface in current study enhances to differentiate adipogenic lineage, especially initial differentiation period.

It has been demonstrated that a correlation of proliferating adipose tissue with declined bone mass in bone marrow and increased osteogenesis at the expense of adipogenesis during MSCs differentiation [40,41]. In addition, Runx-2 and LPL also involved in WNT signaling which regulate differentiation pathway into osteogenesis and adipogenesis [42,43]. It suggests that excessive differentiation of adipogenic lineage would results in repression of osteogenesis. In this study, nanoscale pattern arrayed surface group showed more inducement into adipogenic differentiation than

osteogenic. It is supposed that topographic factors of nanoscale pattern arrayed surface would influence the cell fate into adipogenic lineage from hDPSCs by inhibition of osteogenesis.

In conclusion, we characterized hDPSCs from supernumerary teeth and verified the effect of nanoscale pattern arrayed surface on hDPSCs differentiation. During the differentiation of hDPSCs, the pattern enhanced differentiation into adipogenic lineage especially at the beginning of the differentiation, while the result from osteogenic differentiation on nanoscale pattern arrayed surface group was weak or showed no relation. The results of current study provide us with a better understanding of the effects of topography on cell differentiation as well as the potential use of supernumerary tooth in the field of regenerative medicine or dentistry.

	Forward primer (5'-3')	Reverse primer (5'-3')
Runx-2	GATTACAGATCCCAGGCAGAC	CAGAGGCAGAAGTCAGAGGT
LPL	ATGGATGGACGGTGACAGGA	CCAAGACTGTACCCTAAGAGGTG
GAPDH	GCTCTCTGCTCCTCCCTGTTCTAG	TGGTAACCAGGCGTCCGAT

Table 1. The primers used for Real-time polymerase chain reaction

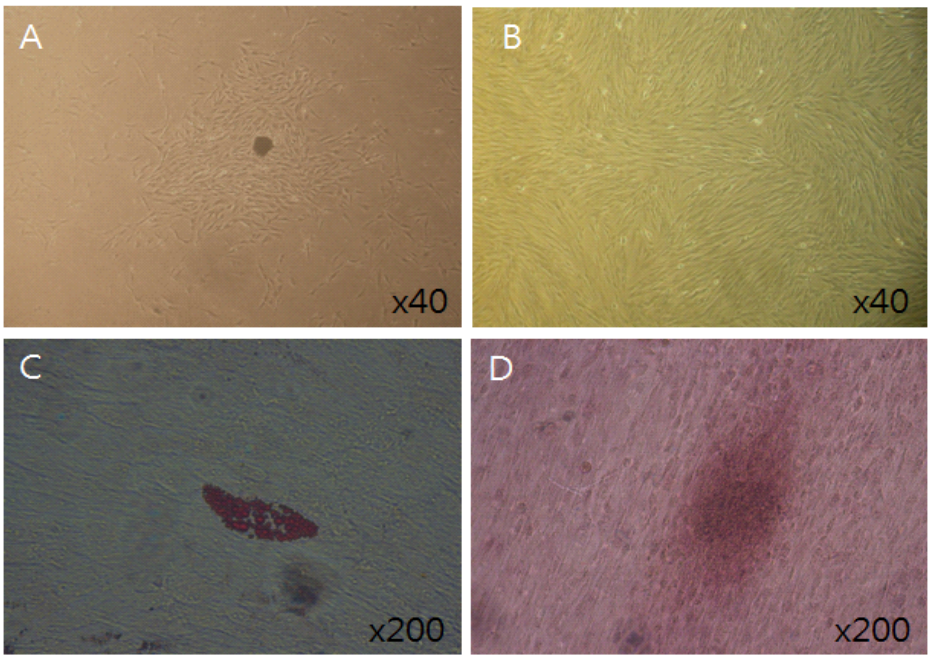

Figure1. Morphology and differentiation properties of dental pulp cells (DPCs). (A) Primary human DPCs derived from pulp tissue of a supernumerary tooth. (B) *In vitro* cultured DPCs at passage 3. (C) Differentiated DPCs stained with Oil red O after 28 days of adipogenic induction. (D) Differentiated DPCs stained with Alizarin red after 28 days of osteogenic induction.

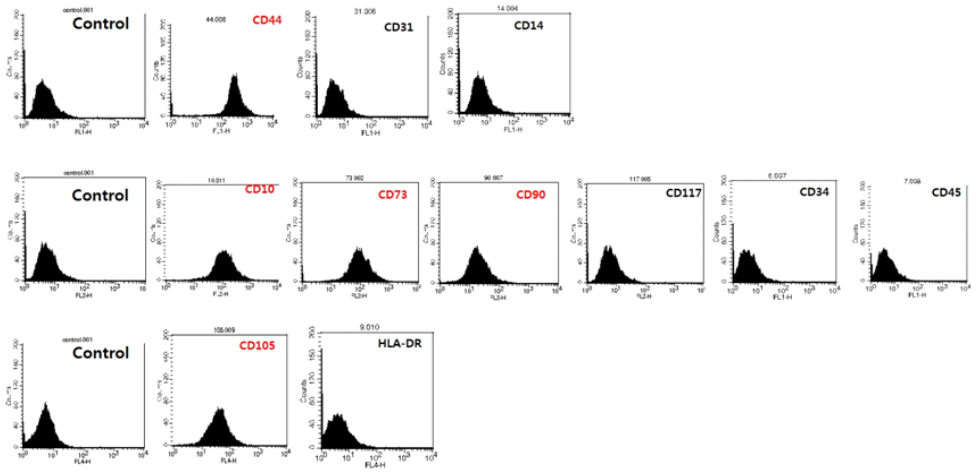

Figure 2. Characterizations of human dental pulp stem cells (hDPCSs) from supernumerary teeth by FACS analysis. After 2 to 3 passages, hDPSCs are characterized by FACS analysis using specific surface markers of mesenchymal stem cells (MSCs; CD29, CD44, CD73, CD90, CD105) and hematopoietic cells (CD14, CD31, CD34, CD45, CD117, HLA-DR). More than 80% of the cells were positive to markers for MSCs (CD29, CD44, CD73, CD90, CD105) whereas only small number of the cells were positive to hematopoietic and endothelial markers (CD14, CD31, CD34, CD45, CD117, HLA-DR)

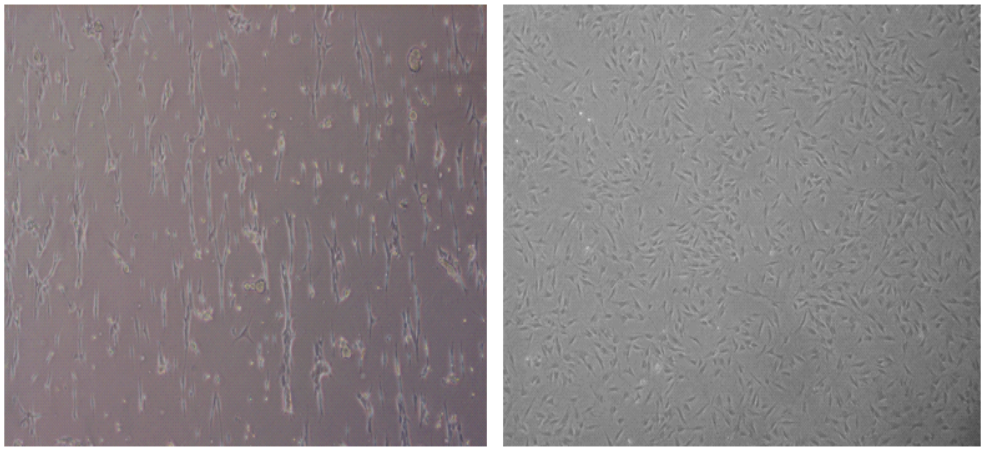

Figure 3. Human dental pulp stem cells (hDPCSs) cultured on the 350-nm nanoscale ridge/groove pattern arrayed surface. (A) hDPSCs cultured on nanoscale pattern arrayed surface showing linear arrangement shape (B) DPCs cultured on gelatinized conventional dish surface showing irregular distribution.

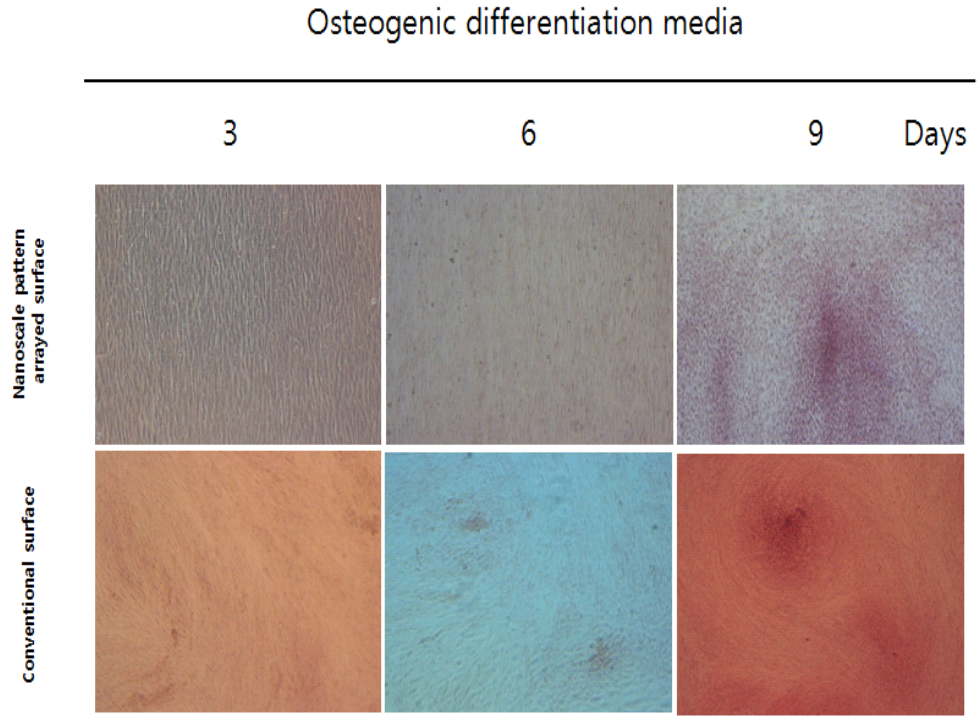

Figure 4. Osteogenic differentiation of dental pulp cells (DPCs) cultured on nanoscale ridge/groove pattern arrayed surface. DPCs were plated on either nanoscale pattern arrayed or conventional surface and pre-cultured in a dental pulp stem cell culture medium for 24 h. Then the medium was replaced with osteogenic medium and the cells were cultured for additional 3, 6 and 9 days. The differentiated cells were stained with Alizarin red. Conventional surface tended to have more red stained (presumptive osteogenic) cells on Day 9.

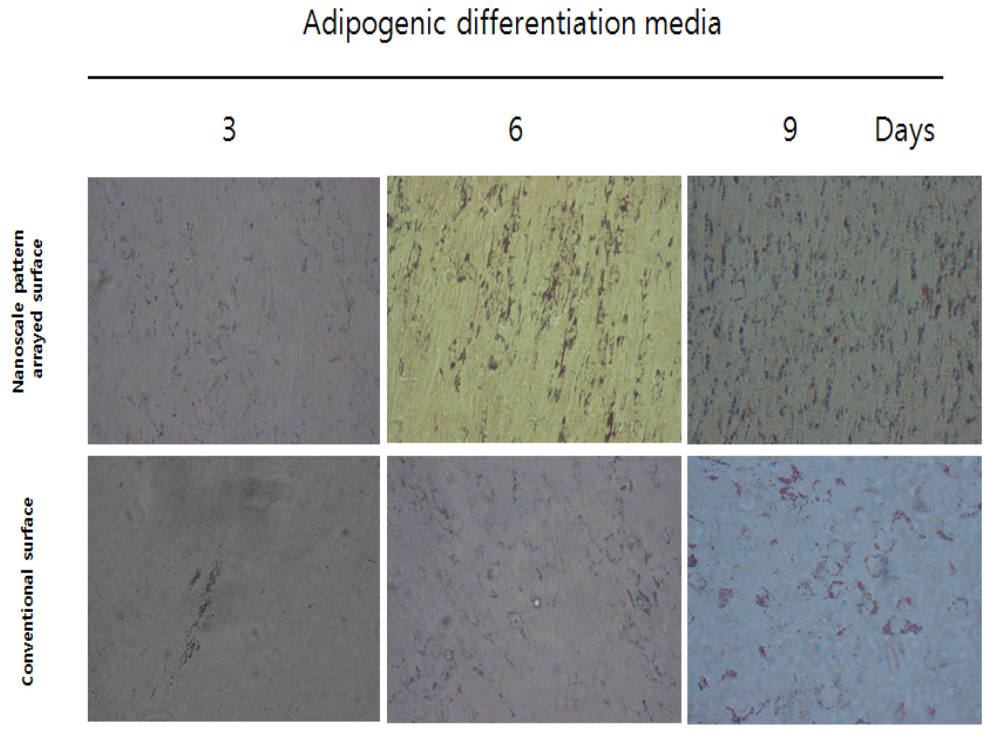

Figure 5. Adipogenic differentiation of dental pulp cells (DPCs) cultured on nanoscale ridge/groove pattern arrayed surface. DPCs were plated on either nanoscale pattern arrayed or conventional surface and were pre-cultured in dental pulp stem cell culture medium for 24 h. Then the medium was replaced with adipogenic medium and the cells were cultured for additional 3, 6 and 9 days. The differentiated cells were stained with Oil red O. The cells on nanoscale pattern arrayed surface showed remarkable increase in Oil red O staining on Day 9.

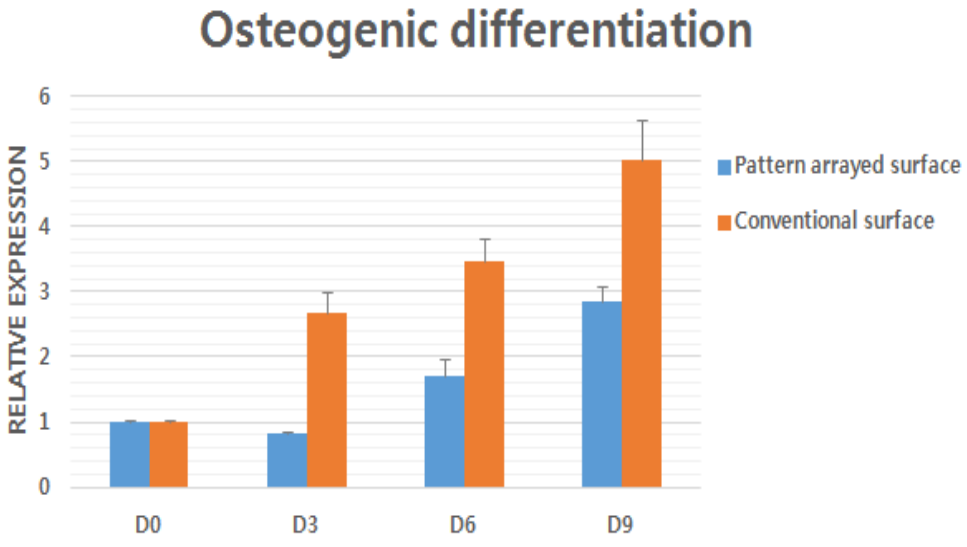

Figure 6. The effect of nanoscale ridge/groove pattern arrayed surface on osteogenic differentiation of human dental pulp stem cells (hDPSCs). Runx-2 expression on nanoscale pattern arrayed surface was decreased at the initial stage but increased gradually after Day 3. While the expression of Runx-2 on conventional surface was steadily increased on whole period.

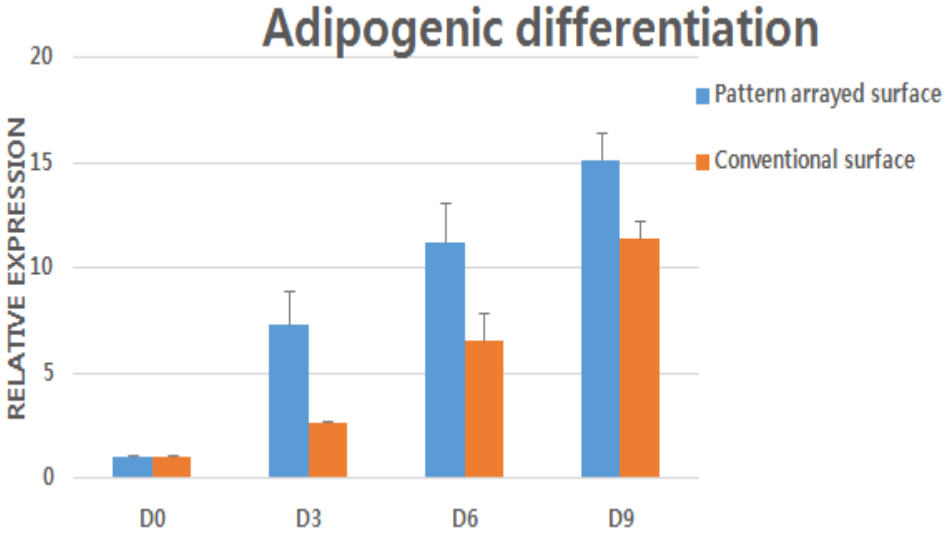

Figure 7. The effect of nanoscale ridge/groove pattern arrayed surface on adipogenic differentiation of human dental pulp stem cells (hDPSCs). LPL expression **was higher in** nanoscale pattern arrayed surface group on whole period comparing with in conventional surface group. Interestingly, the increase rate was the highest on Day 3.

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논 문 초 록

과잉치에서 얻은 치수 줄기세포가 골형성 세포와 지방 세포로 분화동안 나노 패턴이 미치는 영향

Comparisons between the effect of nano patterns on pulpal stem cells from supplementary tooth when differentiation into osteogenic cells and adipose cells

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중간엽 줄기세포는 몇 가지의 종류로 분화할 수 있는 다분화능을 가졌다. 이와 관련된 분화 기전에 대한 여러 연구들은 재생성 의학분야와 조직 공학에 대한 통찰력을 준다. 인간 치수 줄기세포는 골형성 세포와 지방 세포로 분화하는 능력을 가진 중간엽 줄기세포 중의 하나이다. 과잉치는 대부분 발치 후 버려지지만, 윤리적인 문제없이 인간 치수 줄기세포를 얻을 수 있는 좋은 자원이 된다. 부착 분자들은 세포의 형태를 조절하고 세포의 이동, 기능, 극성, 분화를 변화시키는 역할을 한다. 최근에는, 나노스케일의 인공 표면들이 줄기세포의 운명을 결정짓는데 사용되고 있다. 이 실험에서 우리는 과잉치에서 얻은 인간 치수 줄기세포의 특성을 확인하고 350-nm의 융기/고랑 패턴의 인공 표면이 인간 치수 줄기

세포의 골형성 세포나 지방 세포로의 분화에 영향을 미치는지를 확인하였다. 2~3회의 패세지 후에 인간 치수 줄기세포는 중간엽 줄기세포의 표면 마커(CD44, CD10, CD73, CD90, CD105)에 양성반응을, 조혈 세포의 표면 마커(CD14, CD34, CD45, CD117)에 음성반응을 보임을 FACS를 통해 확인 하였다. 나노 스케일 패턴이 골형성 세포와 지방 세포로의 분화에 미치는 영향을 확인하기 위해 RT-PCR과 각 세포들에 특정적인 염색법을 시행하였다. 실험 결과 나노스케일 융기/고랑 패턴이 과잉치에서 얻은 인간 치수 줄기세포의 지방세포로의 분화에는 영향을 미치고, 골형성 세포로의 분화에는 영향력이 없는 것으로 밝혀졌다. 나노스케일 융기/고랑 패턴은 인간 치수 줄기세포의 지방세포로의 분화에서 초기 단계를 후기 단계 보다 더 많이 증가 시켰다. 결과적으로, 나노스케일 융기/고랑 패턴이 과잉치에서 얻은 치구 줄기세포의 지방세포로의 분화를 증진시키는데 사용될 수 있다. 이러한 실험 결과들은 세포의 분화에 나노스케일의 인공 표면들이 영향을 미치는 것에 대한 이해를 증진시키고 과잉치가 치과계의 재생성 의료 분야에서의 잠재적인 역할을 가짐을 의미한다.

주요어 : 인간 치수줄기세포, 과잉치, 골형성세포, 지방세포, 나노구조 표면
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