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ORIGINAL ARTICLE

Control of dental-derived induced pluripotent stem cells through modified surfaces for dental application

Hyunmin Choi[a]*, Kyu-Hyung Park[a]*, Ah-Reum Lee[a], Chin Hee Mun[b,c,d], Yong Dae Shin[b,c], Yong-Beom Park[b,c,d] and Young-Bum Park[a]

[a]Department of Prosthodontics, Oral Science Research Center, BK21 Plus Project, Yonsei University College of Dentistry, Seoul, Korea; [b]Division of Rheumatology, Department of Internal Medicine, Institute for Immunology and Immunological Disease, Yonsei University College of Medicine, Seoul, Korea; [c]Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea; [d]Severance Biomedical Institute, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

Objective: The aim of this study is to investigate the behaviour of iPSc derived from dental stem cells in terms of initial adhesion, differentiation potential on differently surface-treated titanium disc.

Materials and methods: iPSc derived from human gingival fibroblasts (hGFs) were established using 4-reprogramming factors transduction with Sendai virus. The hGF-iPSc established in this study exhibited the morphology and growth properties similar to human embryonic stem (ES) cells and expressed pluripotency makers. Alkaline Phosphatase (AP) staining, Embryoid Body (EB) formation and *in vitro* differentiation and karyotyping further confirmed pluripotency of hGF-iPSc. Then, hGF-iPSc were cultured on machined- and Sandblasted and acid etched (SLA)-treated titanium discs with osteogenic induction medium and their morphological as well as quantitative changes according to different surface types were investigated using Alizrin Red S staining, Scanning electron microscopy (SEM), Flow cytometry and RT-PCR.

Results: Time-dependent and surface-dependent morphological changes as well as quantitative change in osteogenic differentiation of hGF-iPSc were identified and osteogenic gene expression of hGF-iPSc cultured on SLA-treated titanium disc found to be greater than machined titanium disc, suggesting the fate of hGF-iPSc may be determined by the characteristics of surface to which hGF-iPSc first adhere.

Conclusions: iPSc derived from dental stem cell can be one of the most promising and practical cell sources for personalized regenerative dentistry and their morphological change as well as quantitative change in osteogenic differentiation according to different surface types may be further utilized for future clinical application incorporated with dental implant.

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Introduction

Edentulism remains as a major challenge for contemporary dentistry and continuous progress attempting to improve the osseointegration is still being made in order to achieve high success rate in implant therapy. As a consequence, there have been many *in vitro* studies to better understand the mechanisms underlying cell to implant interactions including cell adhesion, proliferation, differentiation and matrix deposition [1–3].

The conditions of limited bone healing potential due to poor bone quality and quantity may lead to increased implant failure rates and for this reason, bone augmentation is required in implant placement site when insufficient bone volume is present. Autologous bone graft has been well-documented to be one of the most reliable and effective surgical approaches and is also accepted as the gold standard treatment modality for patient with insufficient bone volume [4]. However, due to considerably high morbidity including pain, infection and loss of function, alternative tissue engineering technique based on autologous cell transplantation has been emerged to find new ways to improve the kinetics of osseointegration, ultimately increasing total bone volume around implant site [5].

Bone marrow-derived mesenchymal stem cells (BMMSCs) have long received much attention as primary cell sources for autologous cell transplantation because of their self-renewal properties and ability to differentiate into many cellular lineages, including osteocytes, adipocytes and chondrocytes [6,7]. Also, BMMSCs have been reported to exhibit potent immunomodulatory capacity [8,9], which places BMMSCs the most suitable tools for the treatment of bone defect-related disease [10,11]. Nevertheless, BMMSCs' self-renewal and proliferative ability decreases due to aging and diseases such as osteoporosis and arthritis, making it very difficult to obtain adequate cell numbers required for implantation. Moreover, current methods for obtaining BMMSCs

CONTACT Young-Bum Park drybpark@yuhs.ac Department of Prosthodontics, Oral Science Research Center, BK21 Plus Project, Yonsei University College of Dentistry, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea; Yong-Beom Park yongbpark@yuhs.ac Department of Internal Medicine, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
*These authors have contributed equally to this study.

from patients are surgically invasive, leading to substantial morbidity of donor site including pain, infection and loss of function [12]. Therefore, it is of paramount importance that alternative source of BMMSCs, which can overcome the aforementioned limitation of BMMSCs before they can be used as a standard treatment modality for cell-based bone tissue engineering.

The recent development in induced pluripotent stem cell (iPSc) offers many advantages over traditional BMMSC including easiness to generate from almost any tissue in the body as well as unlimited growth capacity which circumvents the disadvantage of BMMSCs, and many promising *in vitro, in vivo* results for application of using iPSc in regenerative medicine have been documented [13–16]. In particular, iPSc derived from dental stem cells including human gingival fibroblast (hGF) attained a special interest due to safety and easiness obtained by surgery, and relatively higher efficiency in reprogramming [17,18]. However, to date, there have been very few studies reported regarding the behaviour of iPSc derived from dental stem cells depending on different surface characteristics. Therefore, the aim of this study is to investigate the behaviour of iPSc derived from dental stem cells in terms of initial adhesion, differentiation potential on differently surface-treated titanium disc.

Materials and methods

Primary culture of hGF

Approval from the Institutional Research Ethics Committee of the Yonsei University College of Dentistry was obtained. (IRB No. 2-2014-0012). After obtaining informed consent, hGF were taken from healthy volunteers, female (Donor 1) aged 16 years old, undergoing orthodontic treatment at Yonsei University Dental Hospital. Gingival tissue was collected and prepared as previously reported [19]. Briefly, collected tissues were washed twice with 70% of ethanol then washed again with phosphate-buffered saline (PBS; Invitrogen, Waltham, MA) and digested with collagenase I (2 mg/mL; Sigma Aldrich, St. Louis, MO) and dispase II (4 mg/mL; Roche Diagnostics, Indianapolis, IN) for 2 h at 37 °C incubation and the dissociated cells were then seeded on 10-cm tissue culture dishes in a standard cell culture medium, consisting of Dulbecco's modified Eagle's medium (DMEM; Invitrogen) containing 10% foetal bovine serum (FBS; Invitrogen) and antibiotics (50 U/ml penicillin G and 50 μg/ml streptomycin (P/S); Invitrogen). These cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 10 days. *In vitro*-expanded hGFs were maintained under the same culture conditions and hGF at passage 5 was used in this study.

Induction of iPSc

Induction of iPSc from primary hGFs via transduction of four factors was performed using a CytoTune™ Sendai Reprogramming system (Invitrogen) according to the manufactures' protocol. Using this protocol, 24 h before transduction, hGFs were seeded at 5×10^5 cells per 6-well dishes in standard medium. For the transduction, hGFs were incubated in Sendai viruses-containing medium for 24 h, followed by incubation with standard medium, which was replaced every day. Seven days later, the cells were harvested and 5×10^4 cells were placed on mitomycin-C (Sigma-Aldrich, St. Louis, MO) inactivated mouse embryonic fibroblast (MEF; CEFO bio inc, Seoul, Korea) feeder cells in a 10 cm dish in iPSc culture medium, which consisted of DMEM/F12 (Invitrogen, Carlsbad, CA), 20% serum replacement (SR, Invitrogen), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO), 1% nonessential amino acids (Invitrogen, Carlsbad, CA), 100 μM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), 1% P/S and 4ng/ml human basic fibroblast growth factor (bFGF, R&D systems, Minneapolis, MN). The medium was changed every day, and the cells were monitored daily for any morphological changes. At 30 days after transduction, colonies showing embryonic stem (ES) cell-like morphology, including a round shape, large nucleoli and scant cytoplasm, were mechanically selected for establishing iPSc cultures.

Alkaline phosphatase staining and immunocytochemistry

Alkaline phosphate (AP) staining and immunocytochemical procedure were carried out as described previously [20]. For immunocytochemistry, following isotype-specific primary antibodies were used: OCT4 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), NANOG (1:200 dilution; Millipore, San Diego, CA), SSEA4 (1:100 dilution; Millipore), SOX2 (1:100 dilution; Millipore), TRA-1-60 (1:200 dilution; Millipore) and TRA-1-81(1:100 dilution; Millipore). For the confirmation of 3-germ layer differentiation, β-tublinIII (ectoderm, 1:100 dilution; Millipore), brachyury (mesoderm, 1:100 dilution; Millipore) and alpha-feto protein (AFP, endoderm, 1:100 dilution; Millipore) were also stained. The secondary antibodies; rhodamine or alexa fluor 488 conjugated rabbit IgG, anti-mouse IgG or anti-mouse IgM (Molecular probe; Invitrogen) were used for antibody localization. Cell nuclei were counterstained with 4′-6-diamidino-2-phenylindole (DAPI) (10 μg/mL; Sigma-Aldrich).

Karyotyping

Standard G-banding chromosome analysis was performed at GenDix cytogenetics laboratory inc (Seoul, Korea) with hGF-iPSc collected at passage 15.

In vitro *differentiation of iPSc derived from dental stem cell*

Embryoid body (EB) formation followed by *in vitro* differentiation was analyzed in accordance with the protocol of Ohnuki et al. [20]. In brief, iPSc were dissociated with 4 mg/mL of Dispase I and transferred to 60mm petri-dishes in iPSc medium without bFGF. After 8 days in suspension culture, once hGF-iPSc formed ball-shaped structure, hGF-iPSc were transferred to 0.1% gelatin-coated plates to induce further differentiation for 5 days. Differentiation markers such as

AFP for endoderm, brachyury for mesoderm and β-tubulin III for ectoderm were analyzed by immunocytochemistry.

For osteogenic differentiation, hGF-iPSc were cultured in osteogenic medium containing 10 mM β-Glycerophosphate (β-GP, Sigma-Aldrich), 10 μM dexamethasone and 50 μM ascorbic acid for 21 days and fixed with 2% paraformaldehyde. Alizarin red S (Abcam, Cambridge, MA) and Von Kossa (Abcam) staining were performed to evaluate osteogenic differentiation of hGF-iPSc.

Direct differentiation on differently surface-treated titanium disc

hGF-iPSc were cultured at 1×10^5 cells on machined-and SLA-treated titanium disc with osteogenic media containing β-GP, dexamethasone, ascorbic acid and their time-dependent and surface-dependent changes were investigated for 28 days using Alizarin Red S staining, Scanning electron microscopy (SEM) and RT-PCR.

Alizarin red S staining quantification assay detection and quantification of mineralization

Measurement of alizarin red S (ARS) were performed by Osteogenesis assay kit (Millipore) according to manufacturer's guideline. Briefly, both undifferentiated cells and osteogenic differentiated iPS cells on titanium discs were washed with PBS and fixed in 10% (v/v) para-formaldehyde (Sigma–Aldrich) at room temperature for 15 min. The titanium discs were washed twice with excess dH_2O prior to addition of 1 mL of 40 mM ARS (pH 4.1) solution per well. The plates were incubated at room temperature for 20 min with gentle shaking and washed out unincorporated dye. For quantification of staining, 10% (v/v) acetic acid was added to each well, and the plate was incubated at room temperature for 30 min with shaking. The osteogenic differentiated hGF-iPSc, now loosely attached to the plate, was scraped from the plate with a cell scraper (SPL, Seoul, Korea), followed by transferring to a 1.5 mL micro centrifuge tube with 10% (v/v) acetic acid and heating to 85 °C for 10 min, and transferring to ice for 5 min. The slurry was then centrifuged at 20,000 *g* for 15 min and 500 μl of the supernatant was removed. Then, 200 μl of 10% (v/v) ammonium hydroxide was added to neutralize the acid. To make up ARS standards, each 2, 1 mM, 500, 250, 125 and 62.5 μM of ARS solution were prepared. The supernatant and standards were read in triplicate at 405 nm in 96-well format using opaque-walled, transparent-bottomed plates.

SEM analysis

hGF-iPSc were cultured at 1×10^5 cells on machined-and SLA-treated titanium disc with osteogenic media containing β-GP, dexamethasone, ascorbic acid and their time-dependent and surface-dependent morphological changes were investigated for 28 days using SEM (Hitachi, S-800, Japan)

Flow cytometric analysis

Cells were detached from the culture dish using Triple express solution (Invitrogen, Carlsbad, CA), centrifuged, rinsed and re-suspended in PBS at a concentration of 10^5 cells/mL. Re-suspended cells were incubated with 5 μL of fluorescein isothiocyanate-labelled anti-rat human osteocalcin (BD Pharmingen, San Jose, CA) in the dark at 4 °C for 30 min. After being washed twice with PBS, cytometric analysis was performed using a flow cytometer (BD FACS Verse).

Quantitative RT-PCR analysis

Total RNA was isolated using TRIzoL® reagent (Invitrogen). The quality of the RNA was evaluated using spectrophotometry and denaturing agarose gel electrophoresis. cDNA was synthesized from 1 μg of purified total RNA using a PrimeScript RT reagent kit (Invitrogen), according to the manufacturer's instructions. The used primer sequences are described in Table 1. Quantitative RT-PCR analysis was performed with SYBR green real-time PCR master mix (Thermo Fisher scientific, Waltham, MA) in a 7500 Real-Time PCR System (Thermo Fisher scientific, Waltham, MA). The PCR conditions were 95 °C for 30 s, 40 cycles of denaturation at 95 °C for 5 s and annealing and extension at 60 °C for 15 s, followed by dissociation and a standard denature action curve.

Statistical analysis

Mean values represent the mean ± standard deviation (SD). Statistical analyses were performed using ANOVA, followed

Table 1. RT-PCR primer information.

Gene name	Gene ID	Sequences	Amplicon length (bp)
Human GAPDH F	M33197.1	cgaccactttgtcaagctca	203
Human GAPDH R		aggggagattcagtgtggtg	
Human AFP F	NM_001134	agcttggtggtggatgaaac	200
Human AFP R		tccaacaggcctgagaaatc	
Human b-tublin F	NM_006086.3	cgcatcatgaacaccttcag	207
Human b-tublin R		cgataccaggtggttgaggt	
Human IBSP F	NM_004967.3	cgccaatgaatacgacaatg	196
Human IBSP R		gatgcaaagccagaatggat	
Human brachyury F	NM_003181.3	acgccatgtactccttcctg	204
Human brachyury R		tgagcttgttggtgagcttg	
Human COL1A1 F	NM_000088.3	ggcccagaagaactggtaca	200
Human COL1A1 R		cgctgttcttgcagtggtag	
Human BGLAP F	NM_199173.4	ggcagcgaggtagtgaagag	194
Human BGLAP R		agcagagcgacaccctagac	
Human RUNX2 F	NM_001015051.3	agtgccagctgcatcctatt	201
Human RUNX2 R		tgcttgaatttttcccaagg	

by Bonferroni's correction for multiple comparisons, using the SPSS program (SPSS 13.0). A value of $p < .05$ was considered statistically significant.

Results

iPSc derivation and characterization

Before transduction of the OCT3/4, SOX2, KLF4 and c-MYC, hGF displayed typical fibroblast spindle-like morphology and developed cluster-like appearance after day 2. The ES cell-like morphology became more outstanding for 14 days post transduction. (Figure 1(A)). Also, after suspension culture without inclusion of bFGF in 8 days, round-shaped EB structures were identified with the prominent germ layer formed on peri-radicular region. (Figure 1(B))

For characterization, we first analyzed undifferentiated status of hGF-iPSc using alkaline phosphate (AP) staining. Strong AP activity was observed (Figure 1(B)) and Standard Q-band chromosome analysis also exhibited the normal karyotyping of hGF-iPSc compared to hGF, showing the iPScs we produced were cytogenetically stable. (Figure 1(C)) Pluripotent stem cell markers, OCT4, NANOG, SOX2, SSEA4, TRA-1-60 and TRA-1-80 were identified by immunocytochemical analysis in all colonies collected for the analysis. (Figure 1(D))

To examine the pluripotency of hGF-iPSc, we performed *in vitro* differentiation analysis followed by EB formation. 14 days post EB culture, the expression of lineage-specific markers α-fetoprotein, brachyury and β-tubulinIII for endoderm, mesoderm and ectoderm, respectively were analyzed by immunocytochemistry. (Figure 2(A)) Also, increased lineage-specific gene expression was identified through the quantitative real-time PCR gene expression results. (Figure 2(B))

In vitro *differentiation of iPSc derived from dental stem cell*

For detection of calcium deposition, Von Kossa staining revealed osteogenic activity with mineralized nodule formation in hGF-iPSc and this was re-confirmed by the strong alizarin Red S staining and gene expression (Figure 3(A,B)). RT-PCR gene expressions revealed that pluripotent marker, OCT4 decreased in osteogenic differentiated iPSc whereas osteogenic marker, RUNX2 conversely increased in osteogenic differentiated iPSc (Figure 3(B)).

Alizarin red quantification of osteogenic differentiation

To confirm the differentiation capacity of hGF-iPSc on titanium disc, osteogenic differentiation was induced. Mid- to long-term culture (2 weeks to 4 weeks) of hGF-iPSc under the osteogenic media demonstrated significantly increased capacity to form Alizarin Red-positive compacted nodules with high levels of calcium deposition on both cell culture plate and titanium disc compared to undifferentiated hGF-iPSc (Figure 4(A)). In particular, osteogenic-induced hGF-iPSc

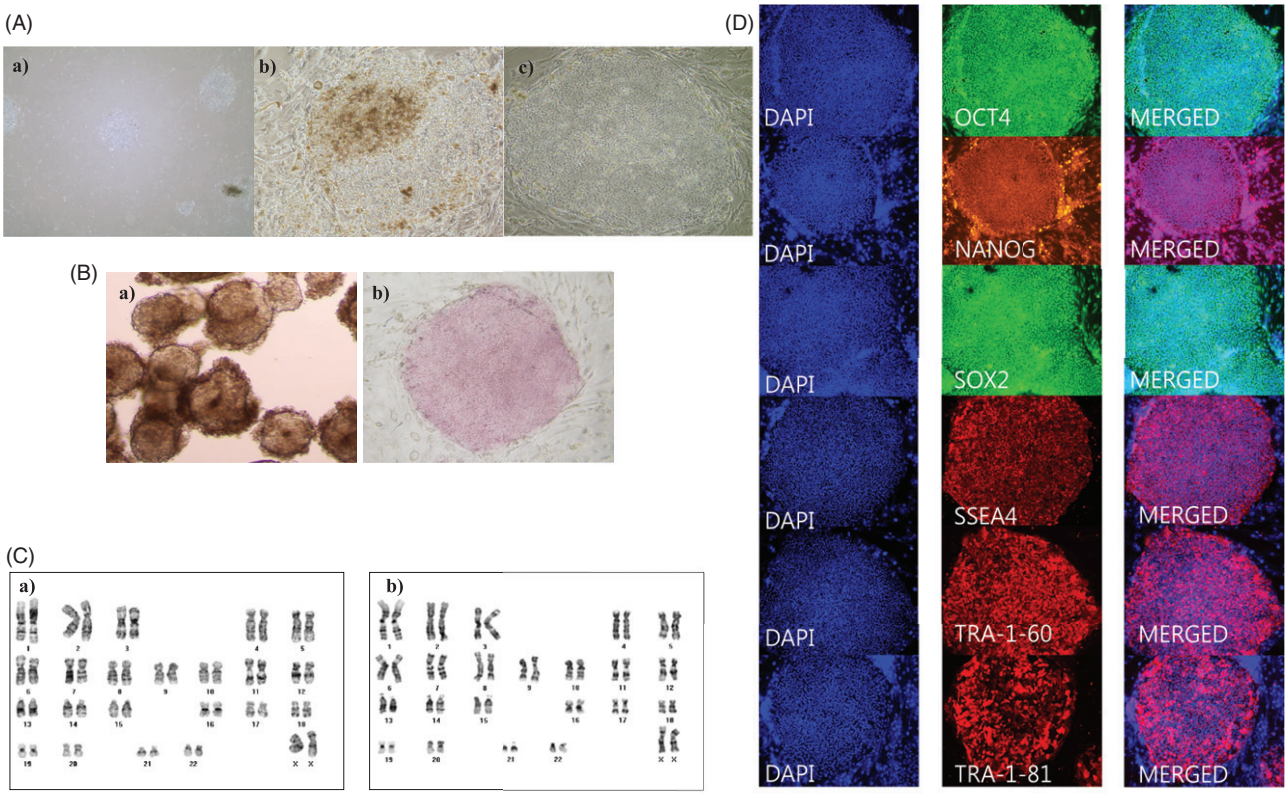

Figure 1. Characterization of hGF-derived iPSc. (A) Morphology of gingival fibroblast-iPSCs (a) Human gingival fibroblast 2 days after Sendai virus transduction (b) ES cell-like colonies after 7 days post transduction (c) more prominent ES cell-like colonies after 14 days post transduction. (B) (a) Embryonic body formation (b) E cell-like colonies stained with alkaline phosphatase. (C) Karyotyping of human gingival fibroblast-iPSCs (a) karyotyping for gingival fibroblast (b) chromosomal analysis revealed a normal karyotype for human gingival fibroblast-iPScs. (D) Expression of human embryonic stem (ES) cell-associated proteins by gingival fibroblast-iPScs; OCT4, NANOG, SOX2, SSEA4, TRA-1-60, TRA-1-81 expression were shown. 4-6-Diamidino-2-[henylindole (DAPI) was used as blue nuclear staining control.

on SLA-treated titanium disc displayed the greatest level of calcium deposition measured at 14 days post treatment. However, measurement recorded at 28 days post-treatment showed no significant difference, showing the similar level of calcium deposition regardless the type of culture surface (Figure 4(B)).

SEM analysis

The surface of machined and SLA titanium disc clearly showed the difference in cell morphology and surface characteristics. The untreated machined surface titanium disc showed a relatively flat topography while SLA titanium disc showed relatively rougher surface with shallow pits. hGF-iPSc on both surfaces adhered well from Day 1 and significant 'filopodia cell attachment' of hGF-iPSc was found on SLA-treated surface until 7 days after seeding, spreading in different layers over the whole rough surface. There was no significant difference in cell number between two groups although reduced cell numbers were observed after Day 14 in both groups. The crystalline structure on machined surface appeared on 14 days after seeding and became even more significant on Day 28, indicating the differentiation into osteoblast-like cells. In contrast, no crystalline structures were identified on SLA-treated surface. (Figure 5)

Expression of osteogenic genes of iPSc (flow cytometry and RT-PCR)

The results from flow cytometric analysis revealed that osteogenic marker, Osteocalcin, was significantly increased in osteogenic differentiated hGF-iPSc, hGF-iPSc cultured on machined disc and SLA-treated disc compared to that of undifferentiated hGF-iPSc, confirming successful osteogenic differentiation of hGF-iPSc on both cell culture plate and titanium disc. (Figure 6(A))

The expression of osteogenic genes including COL1, RUNX2, IBSP and BGLAP of hGF-iPSc cultured on differently surface-treated titanium disc were evaluated by qRT-PCR analysis on specimens collected after 14 days and 28 days of

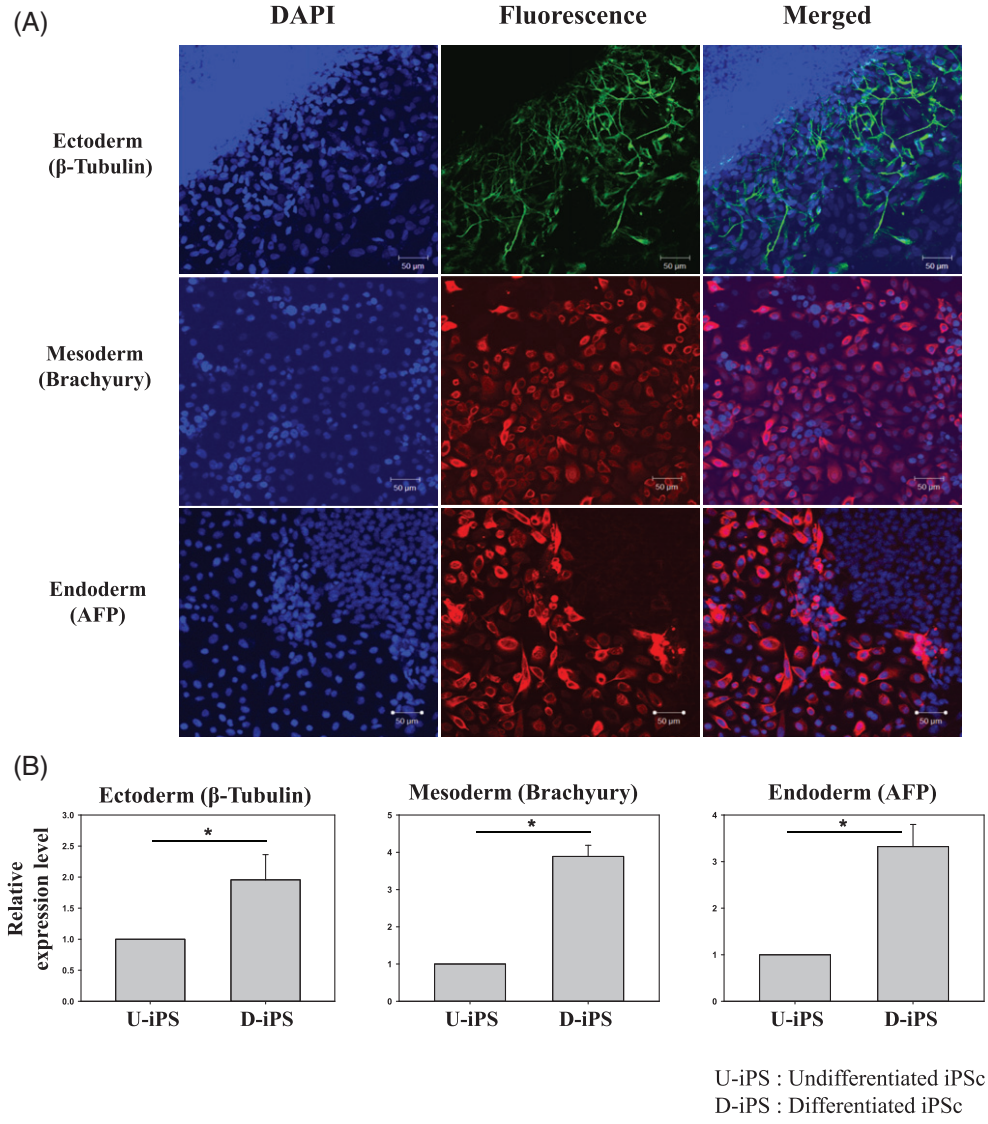

Figure 2. *In vitro* 3-germ layer differentiation of hGF-iPSc. (A) Expression of lineage-specific markers α-fetroprotein, brachyury and β-tubulinIII for endoderm, mesoderm and ectoderm, respectively by immunocytochemistry (14 days post EB culture), (B) lineage-specific gene expression obtained from qRT-PCR results.

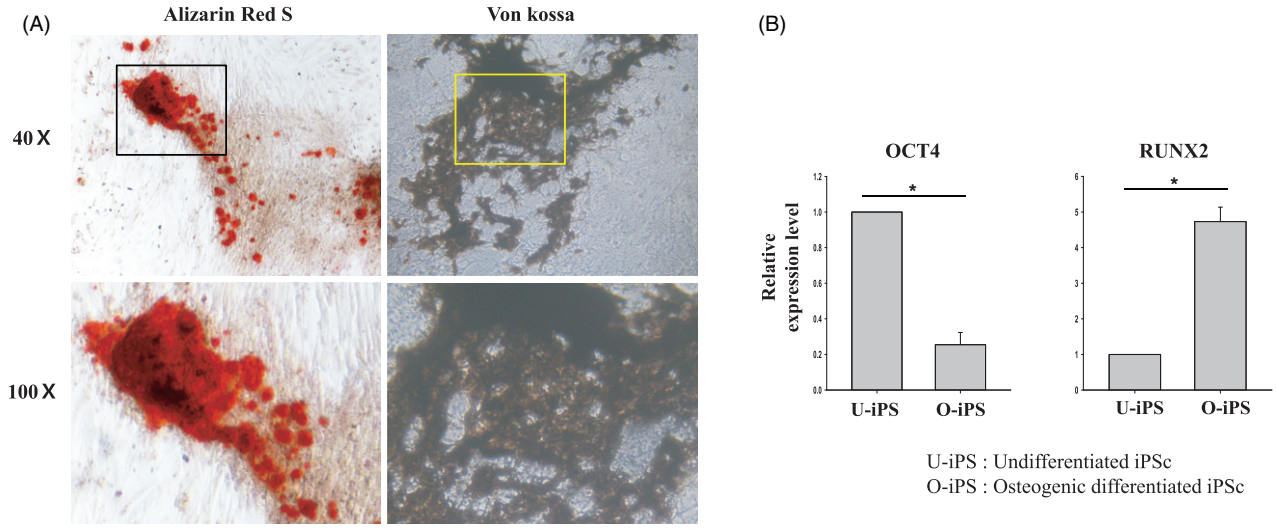

Figure 3. Osteogenic differentiation of hGF-iPSc. (A) Alizarin Red S Staining (40 ×, 100×) and Von kossa staining, (B) OCT4 and RUNX2 gene expression level of U-iPS and O-iPS.

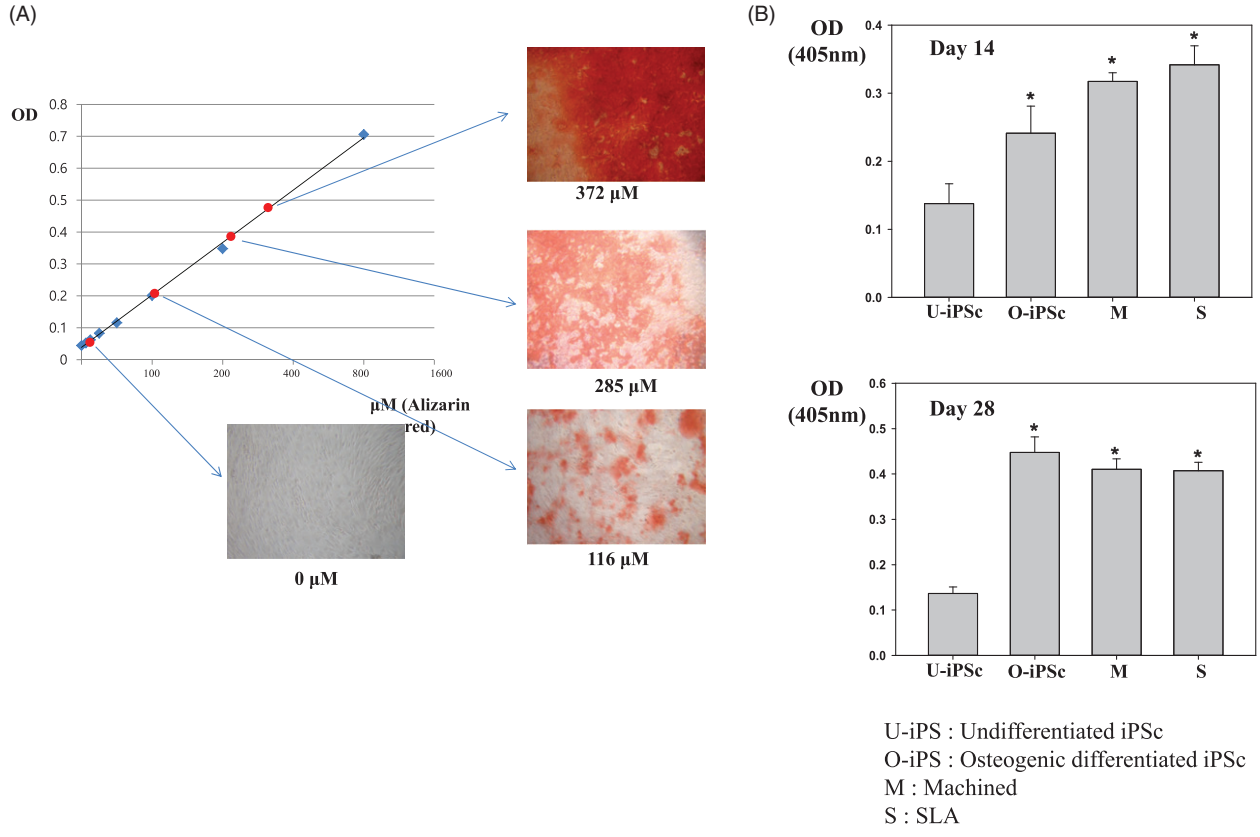

Figure 4. Alizarin Red quantification of osteogenic differentiation. (A) Alizarin Red S standard curve. (B) Quantitative results of the Alizarin Red staining measured at day 14 and day 21.

initial cell culture. Expression levels were evaluated against a control consisting of hGF-iPSc cultured on conventional cell plate as well as hGF-iPSc cultured without osteogenic induction medium. It was clearly noticed that hGF-iPSc cultured on surface-treated titanium disc, measured at both 14 and 28 days post cell culturing, demonstrated the greater bone-related gene expressions than hGF-iPSc cultured on conventional cell plate. However, when comparing gene expression between SLA-treated titanium disc and machined titanium disc, SLA-treated titanium disc showed statistically significantly increased gene expression (*COL1*, *RUNX2*). Although statistically not significant, IBSP and BGLAP expression of hGF-iPSc culture on SLA-treated titanium disc also found to be greater than machined titanium disc. In addition, it was also identified *COL1* and *RUNX2* expression, measured at 14 days of initial cell culture, and was rather decreased on

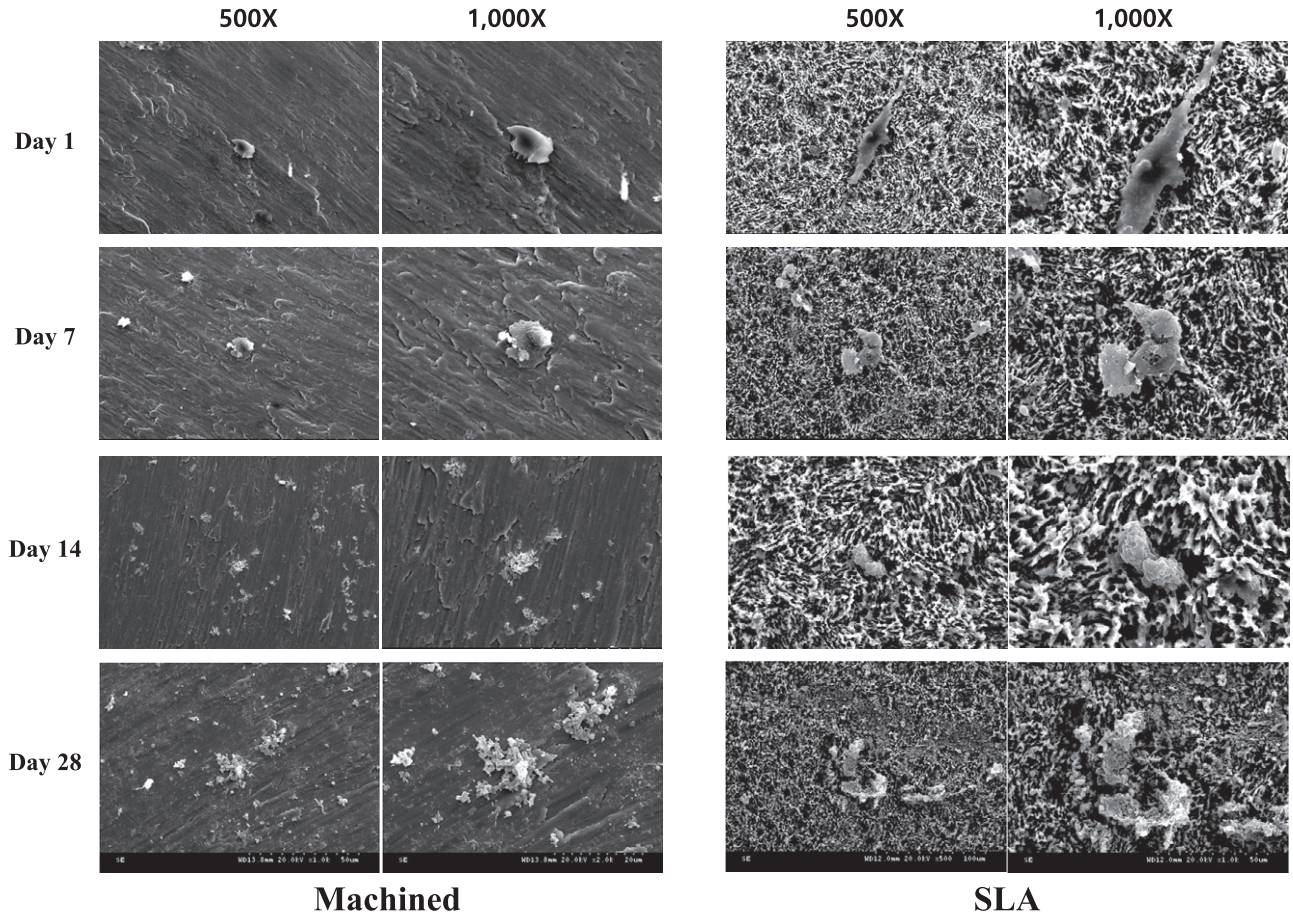

Figure 5. SEM images of hGF-iPSc cultured with osteogenic media on Machined- and SLA-treated titanium surfaces at 1,7,14 and 28 days after initial culturing.

machined titanium disc compared to hGF-iPSc cultured on conventional cell plate (Figure 5(B)).

Discussion

The ability to control the fate of stem cell is of major relevance in regenerative dentistry as well as tissue engineering, providing more rational approach for the design of next-generation implants. In particular, osteogenic response from these stem cells is favourably desired, stimulating rapid new bone formation and enhanced osseointegration, which will, in turn, lead to increase early-stage stability of the implant, reducing in healing times after implantation. It is now well accepted that implant surface topographies ranging from nano- to micro-scale have a significant impact on cellular attachment, proliferation and even differentiation and consequently, there has been several studies investigating the effect of surface topography on the fate of different stem cells including MSCs which has been used as a gold standard for regenerative medicine [21,22]. However, very few studies reported bone regeneration by osteogenic response from iPSc derived from dental stem cells although iPSc showed a greater possibility of being utilized in many aspects over MSCs as aforementioned. Therefore, in this study, we aim to investigate the behaviour of iPSc derived from dental stem cells in terms of initial adhesion, differentiation potential using differently surface-treated titanium disc.

In this study, we demonstrated the generation of iPSc lines from hGF using the Yamanaka cocktails as previously reported [23]. The established hGF-iPSc share the similar characteristics with the ES cells as shown in the results of the current study; a strong AP activity, the same pluripotent stem cell markers (OCT4, NANOG, SOX2, SSEA4, TRA-1-60, TRA-1-80) detected, possession of pluripotency to differentiate into three germ layers (endoderm, mesoderm and ectoderm), and normal karyotyping. We also observed strong osteogenic activity of hGF-iPSc observed by Alizarin red S and Von Kossa staining. Although, Yan et al. reported that hGF other than other dental stem cell lines were poor cell sources to reprogram into iPSc, judging from the results of the current study, we have confirmed that hGF could be an efficient cell sources for the generation of iPSc, thereby suggesting that hGFs are good alternative sources for iPSc banking in replacement of bone marrow stem cell due to easy accessibility during simple, routine dental treatment. Also, while many other multiple adult stem cell lines taken far away from dental origin only shows limited potential, iPSc was reported to contain an epigenetic memory of their tissue of origin, contributing to a greater differentiation potential into desired cell lineage [24]. Therefore, it can be suggested that our studies using iPSc derived from dental stem cells

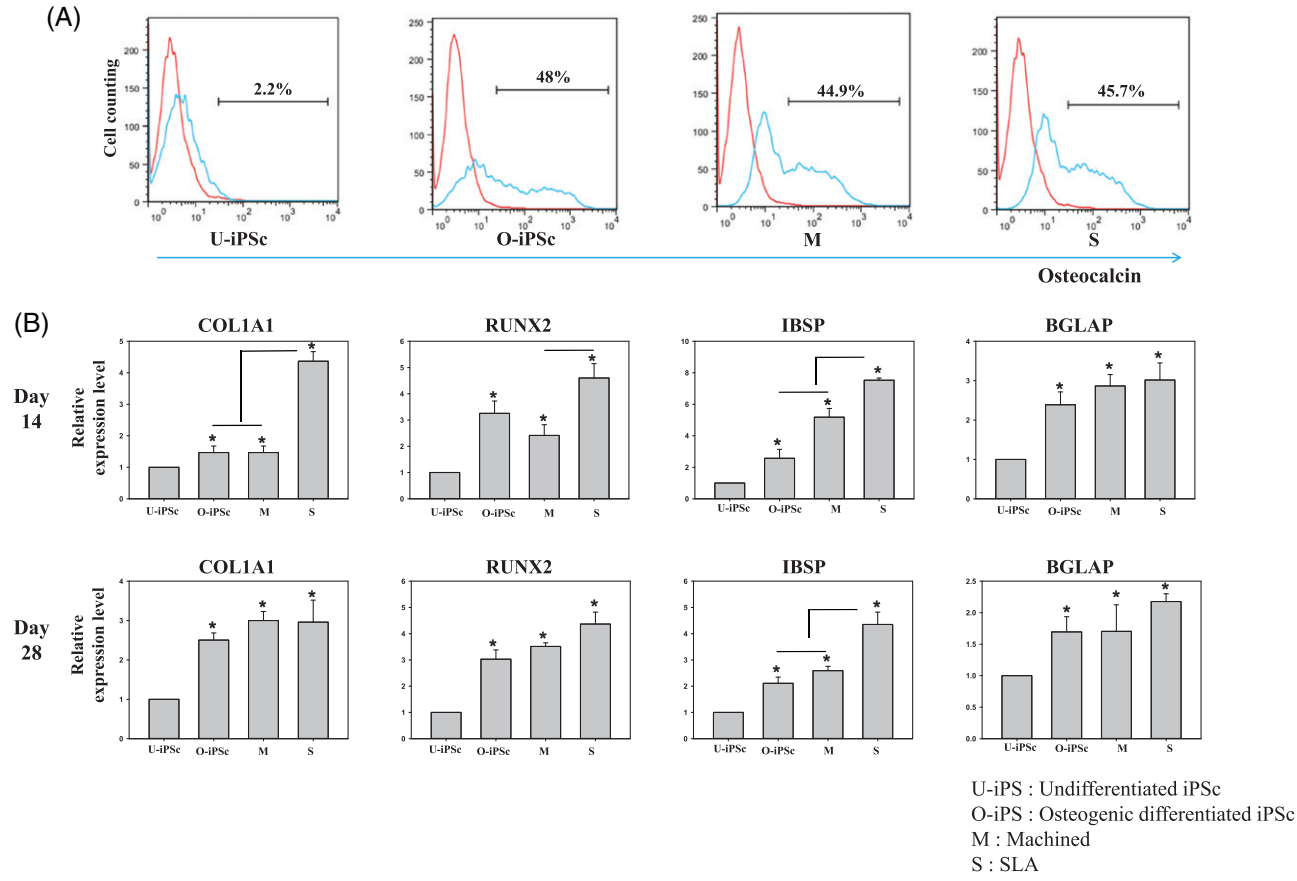

Figure 6. Molecular quantification of osteogenic differentiation. (A) Flow cytometric analysis (Osteocalcin marker expression) (B) RT-PCR results (Day 14 and Day 21) of U-iPSc (Control), iPSc-derived osteoblast (O-iPSc), iPSc cultured on SLA surface (S) and iPSc cultured on machined surface (M).

represent the significant advancement in utilizing patient-specific tissue regenerative treatment for implant or implant-related disease. Nevertheless, due to the use of viruses during the cell reprogramming process, safer application of iPSc to clinic has been very limited. Therefore, the importance of further study investigating long-term behaviour of iPSc for clinical application cannot be more emphasized.

In this study, we also aimed to evaluate the behaviour of iPSc derived from dental stem cells depending on different surface characteristics. Based on the previous studies indicating there is a strong correlation between early cell response to surfaces and cell's behaviour, behaviour of hGF-iPSc in terms of initial adhesion, differentiation potential were investigated. The results from SEM analysis revealed that the surface of machined and SLA titanium disc clearly showed the difference in cell morphology and surface characteristics. Although hGF-iPSc cultured on both surfaces seemed to be adhered well from Day 1, philophodia cell attachment of hGF-iPSc was found on SLA-treated surface until 7 days after seeding, adhering more tightly over the entire rough surface. While the presence of influences of the surface topography to bone response at the cellular level is considered controversial, it is generally accepted that the rougher the implant surface, the more active cellular response in terms of adhesion and differentiation into osteoblast [25,26]. Inzunza et al. [27] reported that early osteoblast adhesive response was accelerated on the rougher nano-porous coated structure, promoting osteogenic differentiation of mesenchymal stem cell with spontaneous mineral nodule formation. It was also reported that embryonic stem (ES) cell also showed increased osteogenic differentiation and increased expression of collagen type I, RUNX2 and osteocalcin when cultured on rougher nanosurface-modified scaffold [28]. Therefore, it may be anticipated that iPSc, which shares the similar characteristic with ES cell represent similar cellular response to the certain surface morphology and this was confirmed by the quantitative RT-PCR results of the current study. The quantitative RT-PCR results clearly revealed that COL1 and RUNX2, indicative of early osteogenic markers, were significantly increased with hGF-iPSc culture on the SLA-treated titanium disc compared to machined titanium disc ($p < .05$). Also, the level of IBSP and BGLAP were greater in iPSc cultured on the SLA-treated titanium disc although significance between two groups was not found ($p > .05$). These results were in accordance with previous study, investigating the effect of surface type on osteogenic differentiation potential of human MSCs [29]. Although cell lines investigated were different, multiple osteogenic makers including Alkaline phosphatase(ALP), and Osteocalcin(OC), Osteoprotegerin(OPG) and Transforming growth factor beta 1(TGF-β1) were significantly increased with MSCs cultured on the SLA-treated surface [29–31]. Therefore, we can assume that this result is particularly meaningful, since our results displayed promising results with iPSc by replicating the results from MSCs, showing the

potential use of hGF-iPSc in the field of cell-based bone tissue engineering. As MSCs have been regarded as a key cell type required for osseointegration and bone healing [6,7], the results of this study clearly demonstrated that even iPSc which can circumvent the disadvantage MSCs can also be utilized as standard treatment modality for possible reduction in healing times after dental implantation. Indeed, further study investigating the anti-inflammatory effect of differentiated iPSc in order to improve the potential use of iPSc in clinic and comparing the level of osseointegration with MSCs *in vivo* to confirm the iPSc as a new treatment modality for implant-related disease may be required.

In conclusion, within the limitation of the study, our study clearly represents that behaviour iPSc derived from dental origin can be directed by implant topographic change, indicating potential use of iPSc derived from dental origin as patient-specific tissue regenerative treatment modality for dental implant or implant-related diseases.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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ORCID

Ah-Reum Lee http://orcid.org/0000-0003-0441-8415

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