Analysis of osteogenic potential on 3mol% yttria-stabilized tetragonal zirconia polycrystals and two different niobium oxide containing zirconia ceramics

Aung Thu Hein[1], Young-Dan Cho[2], Ye-Hyeon Jo[1], Dae-Joon Kim[3], Jung-Suk Han[1]*

[1]Department of Prosthodontics, School of Dentistry, Seoul National University, Seoul, Republic of Korea
[2]Department of Periodontology, School of Dentistry and Dental Research Institute, BK21 Program, Seoul National University, Seoul, Republic of Korea
[3]Department of Advanced Materials Engineering, Sejong University, Seoul, Republic of Korea

PURPOSE. This study was performed to evaluate the osteogenic potential of 3mol% yttria-stabilized tetragonal zirconia polycrystals (3Y-TZP) and niobium oxide containing Y-TZPs with specific ratios, new (Y,Nb)-TZPs, namely YN4533 and YN4533/Al20 discs. **MATERIALS AND METHODS.** 3Y-TZP, YN4533 and YN4533/Al20 discs (15 mm diameter and 1 mm thickness) were prepared and their average surface roughness (R_a) and surface topography were analyzed using 3-D confocal laser microscope (CLSM) and scanning electron microscope (SEM). Mouse pre-osteoblast MC3T3-E1 cells were seeded onto all zirconia discs and evaluated with regard to cell attachment and morphology by (CLSM), cell proliferation by PicoGreen assay, and cell differentiation by Reverse-Transcription PCR and Quantitative Real-Time PCR, and alkaline phosphatase (*Alp*) staining. **RESULTS.** The cellular morphology of MC3T3-E1 pre-osteoblasts was more stretched on a smooth surface than on a rough surface, regardless of the material. Cellular proliferation was higher on smooth surfaces, but there were no significant differences between 3Y-TZP, YN4533, and YN4533/Al20. Osteoblast differentiation patterns on YN4533 and YN4533/Al20 were similar to or slightly higher than seen in 3Y-TZP. Although there were no significant differences in bone marker gene expression (alkaline phosphatase and osteocalcin), *Alp* staining indicated better osteoblast differentiation on YN4533 and YN4533/Al20 compared to 3Y-TZP. **CONCLUSION.** Based on these results, niobium oxide containing Y-TZPs have comparable osteogenic potential to 3Y-TZP and are expected to be suitable alternative ceramics dental implant materials to titanium for aesthetically important areas. ***[J Adv Prosthodont 2018;10:147-54]***

KEYWORDS: Dental implant; Niobium; Zirconia; Low temperature degradation (LTD); Osteogenic potential

Corresponding author:
Jung-Suk Han
Department of Prosthodontics, School of Dentistry, Seoul National University, 101, Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea
Tel. +82220723711: e-mail, proshan@snu.ac.kr
Received July 24, 2017 / Last Revision September 13, 2017 / Accepted October 30, 2017

© 2018 The Korean Academy of Prosthodontics
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

This research was supported by the Technology Innovation Program (10043164) funded by the Ministry of Trade, Industry, & Energy (MI, Seoul, Korea) and by the Bio & Medical Technology Development Program of the National Research Foundation (NRF-2014M3A9E3064466), funded by the Ministry of Science, ICT, & Future Planning.

INTRODUCTION

The replacement of missing teeth with osseointegrated dental implants has become an evidence-based treatment modality and a routine procedure in dentistry for more than four decades. Despite frequent occurrence of peri-implantitis and other complications, the survival rate for titanium implants is 90 - 95% over a period up to 20 years.[1] Commercially pure titanium and titanium alloys are gold standard dental implant materials because of their biocompatibility, excellent mechanical properties, and long term clinical success.[2-4]

Despite its great advantages, titanium exhibits grayish discoloration on the peri-implant mucosa and jeopardizes aesthetic outcomes of restoration, especially if there is

insufficient soft tissue to mask in the anterior segments.[5,6] Although the prevalence is low (0.6%), titanium allergy can be detected in dental implant patients.[7] Furthermore, titanium might induce hypersensitivity in susceptible patients and can play a critical role in implant failure.[8] Some studies have also reported corrosive behavior that occurs after titanium comes in contact with saliva and fluoride.[9,10] To compensate for the drawbacks of titanium, many researchers have tried to create tooth-colored biocompatible ceramic materials. Because of its aesthetic superiority, excellent biocompatibility and mechanical properties, ambitious efforts were made to introduce zirconia for applications in implant dentistry.[4,11]

Pure zirconia is a polymorphic crystal that can be found in three different crystalline phases depending on the temperature: monoclinic (room temperature until 1170°C), tetragonal (1170 - 2370°C), and cubic (2370°C until melting point). The transformation from the tetragonal to the monoclinic phase is associated with a 3 - 4% localized volume expansion that induces compressive stresses in the compromised areas.[12] The addition of stabilizing oxides like magnesia (MgO), yttria (Y_2O_3), and ceria (CeO_2) prevents this phase transformation and maintains a metastable tetragonal phase at room temperature. 3 mol% Y_2O_3-stabilized tetragonal zirconia polycrystals (3Y-TZP) exhibits high strength and toughness as well as tetragonal phase stability at room temperature. Based on these, 3Y-TZP has been introduced as an alternative to titanium which shows superior mechanical properties compared to other ceramics.[13,14] Many studies have been performed to compare the osseointegration of standard titanium and zirconia implants and have reported no significant differences between 3Y-TZP and titanium implants.[15-18] *In vitro* studies revealed comparable osteoblast adhesion, proliferation, and differentiation between differently treated Y-TZP disc surfaces and sandblasted/acid-etched titanium surfaces.[19,20] Several *in vivo* studies also proved that 3Y-TZP implants undergo osseointegration comparable with that of titanium implants.[21-23]

Despite its excellent mechanical properties and biocompatibility, however, a major shortcoming of zirconia is its inherent accelerated aging and low temperature degradation (LTD). LTD is related to a lattice relaxation process induced by thermally activated oxygen vacancy diffusion.[24] It consists of a spontaneous, slow transformation of the crystals from the tetragonal phase to the monoclinic phase at low temperatures (150 - 400°C). In a humid environment, this could decrease the strength of the materials and lead to catastrophic failures over time.[25]

Various approaches to eliminate or reduce LTD have included a ceria partially stabilized zirconia/alumina nanostructured composite (NANOZIR),[26,27] alumina-toughened zirconia (ATZ),[28-30] and 3Y-TZP co-doped with niobium oxide (Y,Nb)-TZP.[24,31-34] The resistance of (Y,Nb)-TZP to hydrothermal degradation is attributed primarily to t-ZrO_2 phase stability as a result of Y-Nb ordering in the t-ZrO_2 lattice[31] as well as a reduction in the oxygen vacancy concentration in Y-TZP as a result of the substitution of Nb^{5+} for Zr^{4+}.[24,31,35] In order to utilize this advantage of niobium in dental implant treatment, it is important to analyze the osteogenic potential of niobium oxide containing tetragonal zirconia polycrystals as proper osseointegration around the implant body is a major successful criteria for implant treatment.[1-4]

Our previous study has shown that sandblasted (Y,Nb)-TZP discs have a similar osteogenic potential to that of anodized titanium.[36] However, the correct combination of each composition to achieve optimal osseointegration is still challenging for the development of new materials. In this study, we synthesized new niobium oxide containing (Y,Nb)-TZP discs with specific ratios and denoted as YN4533 and YN4533/Al20. This study was performed to evaluate the osteogenic potential of new (Y,Nb)-TZP discs, YN4533 and YN4533/Al20, and compared with that of most widely used zirconia ceramic 3Y-TZP.

MATERIALS AND METHODS

Zirconia discs containing niobium oxide were synthesized according to specific ratios. The overall composition of YN4533 is 92.2 mol% ZrO_2, 4.5 mol% Y_2O_3, and 3.3 mol% Nb_2O_5. YN4533/Al20 discs were prepared with the same concentration of YN4533 with an additional 20 vol% of Al_2O_3. YN4533 and YN4533/Al20 were test groups and 3Y-TZP used as a control. 3Y-TZP, YN4533, and YN4533/Al20 disc-shaped green compacts (15 mm diameter and 1 mm thickness) were prepared by cold isostatic pressing of the powder mixtures at 200 MPa followed by sintering for 2 hours at 1500°C for 3Y-TZP, 1450°C for YN4533, and 1600°C for YN4533/Al20. The different sintering temperatures were used because the optimum sintering temperature for each material depends on the composition of the specimens to achieve maximum strength without deterioration and based on preliminary studies.[24,31] All zirconia discs were gradually polished and finished with diamond pastes to produce mirror-like surfaces. After polishing, half of the zirconia discs in each group were sandblasted with 50-μm alumina (Al_2O_3) at 2 bar pressure for 1 minute to create rough surfaces. Mirror-like smooth surface groups were denoted as 3Y-TZP-M, YN4533-M and YN4533/Al20-M while sandblasted rough surface groups were denoted as 3Y-TZP-R, YN4533-R and YN4533/Al20-R. The average surface roughness (R_a) and surface topography were analyzed using a 3-D confocal laser microscope (LSM 5 Pascal, Carl Zeiss, Germany). The R_a values represent the mean ± SD of three independent experiments. Surface morphologies of zirconia discs were observed via a field emission scanning electron microscope (FE-SEM; HITACHI S-4700, Tokyo, Japan).

Mouse pre-osteoblast MC3T3-E1 cells were purchased from ATCC (Manassas, VA, USA). The cells were cultured in α-minimal essential medium (α-MEM, Hyclon) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and incubated in a humidified atmosphere of 95% air/ 5% CO_2 at 37°C. The osteogenic media included 10 mM β-glycerophosphate, 50 μg/mL ascorbic acid in

α-MEM with 10% FBS and 1% penicillin/streptomycin. A confocal laser scanning microscope (LSM700, Carl Zeiss, Germany) and ZEN2011 software were used to evaluate cell attachment and morphology. 24 hours after seeding onto the zirconia discs, cells that attached onto the discs were fixed with 4% formaldehyde. 4',6-diamidino-2phenylindole (DAPI, Invitrogen) was used for detection of cell nuclei and Alexa Fluor 568 phalloidin (Invitrogen) was used for detection of the cytoskeleton.

Cell proliferation was examined by a PicoGreen assay using the Quant-iT PicoGreen assay kit (Invitrogen) 1, 4, and 7 days after seeding cells on the zirconia discs. Cells adhered to the zirconia discs were washed with PBS and lysed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) to allow for formation of DNA samples. Then, 100 µl of the DNA samples was mixed with 100 µl of PicoGreen reagent. Samples were loaded in triplicate and florescence intensity was measured on a microplate reader (FLUOstar Optima, BMG LABTECH, Ortenberg, Germany). Florescence intensity was converted into DNA concentration with a DNA standard curve per the manufacturer's instructions. To evaluate osteoblast differentiation, cells were seeded on the zirconia discs and cultured in osteogenic media, which includes 10 mM β-glycerophosphate and 50 µg/mL ascorbic acid in growth media. Cells were harvested at 5, 8, and 11 days and RNA was isolated using Trizol lysis reagent (TRIzol Reagent, Invitrogen). The Primescript RT reagent kit (Takara Bio, Shiga, Japan) was used for reverse transcription and then real-time PCR was performed using Takara SYBR premix Ex Taq (Takara Bio, Shiga, Japan) on an Applied Biosystems 7500 Real Time PCR system (Foster City, CA, USA). All samples were run in triplicate. The osteoblast differentiation marker genes were alkaline phosphatase (*Alp*) and osteocalcin (*Oc*). The results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to account for variations in RNA quantitation. The marker genes were synthesized by Integrated DNA technology (IDT, Coralville, IA, USA). *Alp* activity was measured using an ALP kit (Sigma-Aldrich). Cells were seeded on the zirconia discs and cultured in osteogenic medium for 10 days. Cells were washed twice with PBS and stained as described by the manufacturer.

All quantitative data are presented as the mean ± SD and each experiment was performed at least three times. The data analysis was performed using two-way ANOVA-test and Tukey post hoc test. Differences were considered as being significant at $P < .05$.

RESULTS

The average roughness values (R_a) and topographies of all zirconia discs under three-dimensional confocal laser scanning microscopy (3D-CLSM) are shown in Fig. 1. The R_a values of the mirror-like surface of 3Y-TZP, YN4533, and YN4533/Al20 were 0.09 ± 0.01 µm, 0.09 ± 0.01 µm, and 0.08 ± 0.02 µm, respectively. The surface roughness of the mirror-like surface discs was similar. To increase roughness, we sandblasted the zirconia discs with alumina particles. After sandblasting, the roughness of all zirconia discs increased significantly. As a result, the R_a values of the rough surfaces of 3YTZP, YN4533, and YN4533/Al20 were 0.62 ± 0.05 µm, 0.72 ± 0.04 µm, and 0.71 ± 0.07 µm,

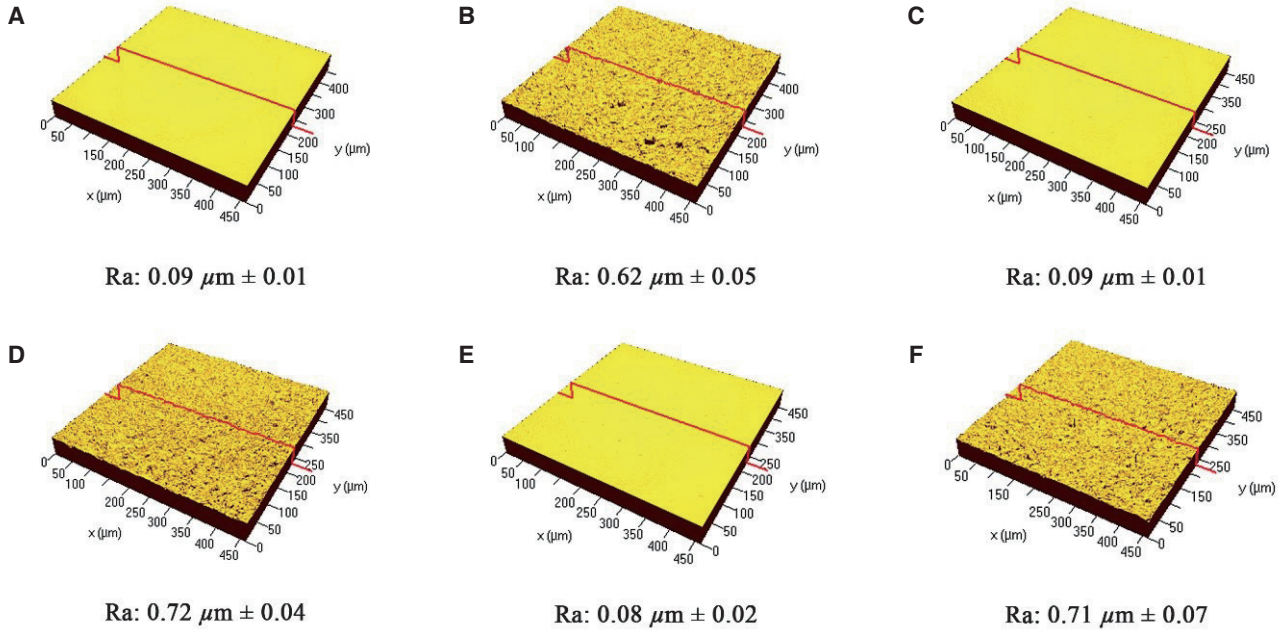

Fig. 1. Three-dimensional confocal laser scanning microscopy (3D-CLSM) images show the roughness R_a values of zirconia discs (A) 3Y-TZP-M, (B) 3Y-TZP-R, (C) YN4533-M, (D) YN4533-R, (E) YN4533/Al20-M, (F) YN4533/Al20-R.

respectively. Although there was no significant difference between the rough surface discs, slightly higher R_a values were noted for modified zirconia discs.

The surface morphologies of zirconia discs were analyzed by using scanning electron microscopy (SEM) (Fig. 2). Mirror-like zirconia surfaces showed a smooth and fine dotted pattern, which is assumed to be from the process of sintering. After sandblasting with alumina particles, all zirconia discs exhibited irregular rough patterns. The surface morphologies of 3Y-TZP and new (Y,Nb)-TZP discs did not differ significantly and were in good agreement with their R_a values (Fig. 1).

Fig. 3 shows MC3T3-E1 pre-osteoblast cells after 24 hours of culture on the mirror-like surface and the rough surface of zirconia discs. Cell attachment and morphology were analyzed with confocal laser scanning microscopy. The cells on the mirror-like surface of all zirconia discs showed regular size and morphology and a widely spread cytoskeleton. However, cells on the rough surface exhibited some morphologic irregularities, with a thin cytoskeleton and a less-stretched appearance on both 3Y-TZP and new (Y,Nb)-TZPs. All newly modified (Y,Nb)-TZP discs displayed good cell attachment similar to 3Y-TZP, and cell to cell contacts were observed on all zirconia discs regardless of surface roughness.

A PicoGreen assay was performed to examine cellular proliferation. Fig. 4 shows cellular proliferation on the zirconia discs for 1, 4, and 7 days. Cells proliferated well on all zirconia discs and the proliferation rate increased as time went on. Mirror-like surfaces had higher cell proliferation than rough surfaces, and this was highest at day 7. This indicates that MC3T3-E1 cells proliferate well on the smooth surface and match well with cell morphologies. Significant differences were found only between day 4s of 3Y-TZP mirror and YN4533 rough surface groups and days 7s of 3Y-TZP mirror and YN4533/Al20 rough surface groups. There was no statistically significant difference between cells grown on 3Y-TZP, YN4533, and YN4533/Al20, within the same surface roughness groups.

Quantitative real time polymerase chain reaction (RT-PCR) was performed to evaluate mRNA expression levels after 5, 8, and 11 days of culture. Fig. 5 (A and B) show the mRNA expression patterns of alkaline phosphatase (*Alp*) and osteocalcin (*Oc*), which are marker genes of osteoblast differentiation. Although the morphology of cells cultured on the rough surface appeared smaller and less stretched, cell differentiation between smooth and rough new (Y,Nb)-TZPs did not differ significantly. Osteoblast differentiation patterns of new (Y,Nb)-TZP discs were not influenced by the surface roughness, however rough 3Y-TZP discs showed more cellular differentiation than smooth 3Y-TZP discs. Significant differences were found when compared with the 3Y-TZP mirror surfaces. Both mirror and rough new (Y,Nb)-TZP discs showed significant *Alp* activities at all experiment days except day 5 of YN4533 mirror, while significant *Oc* levels were seen at all experiment days. Moreover, *Alp* gene expression level of both mirror and rough new (Y,Nb)-TZP discs showed significantly higher than that of both mirror and rough 3Y-TZP discs at experiment day 8, while osteocalcin level

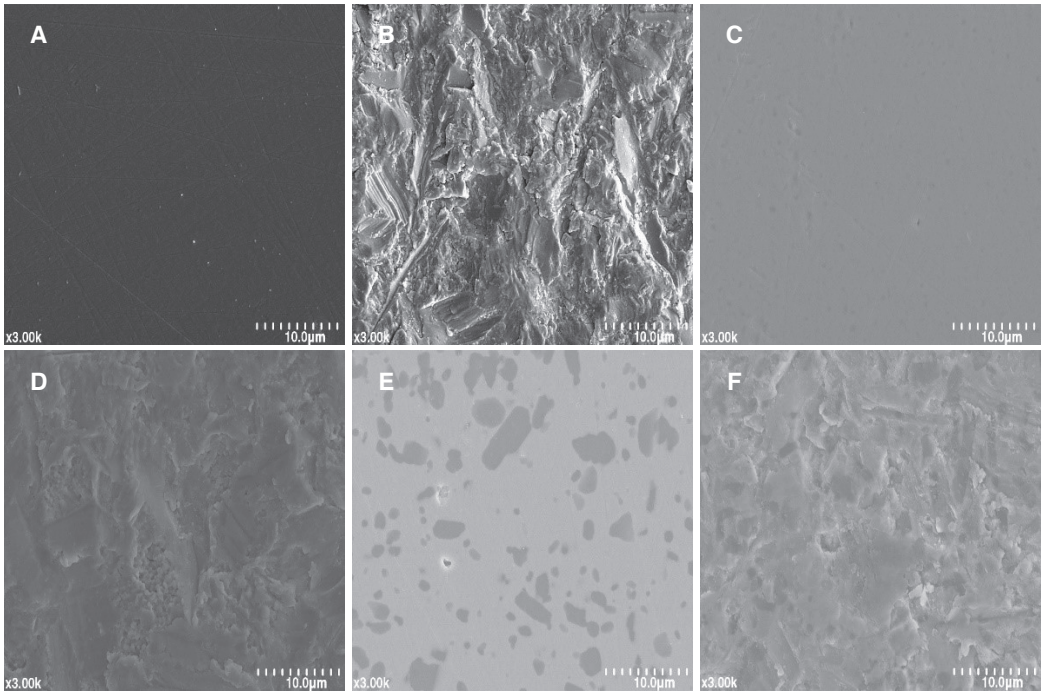

Fig. 2. Scanning electron microscopy (SEM) images of zirconia discs (A) 3Y-TZP-M, (B) 3Y-TZP-R, (C) YN4533-M, (D) YN4533-R, (E) YN4533/Al20-M, (F) YN4533/Al20-R. ×3000 magnification.

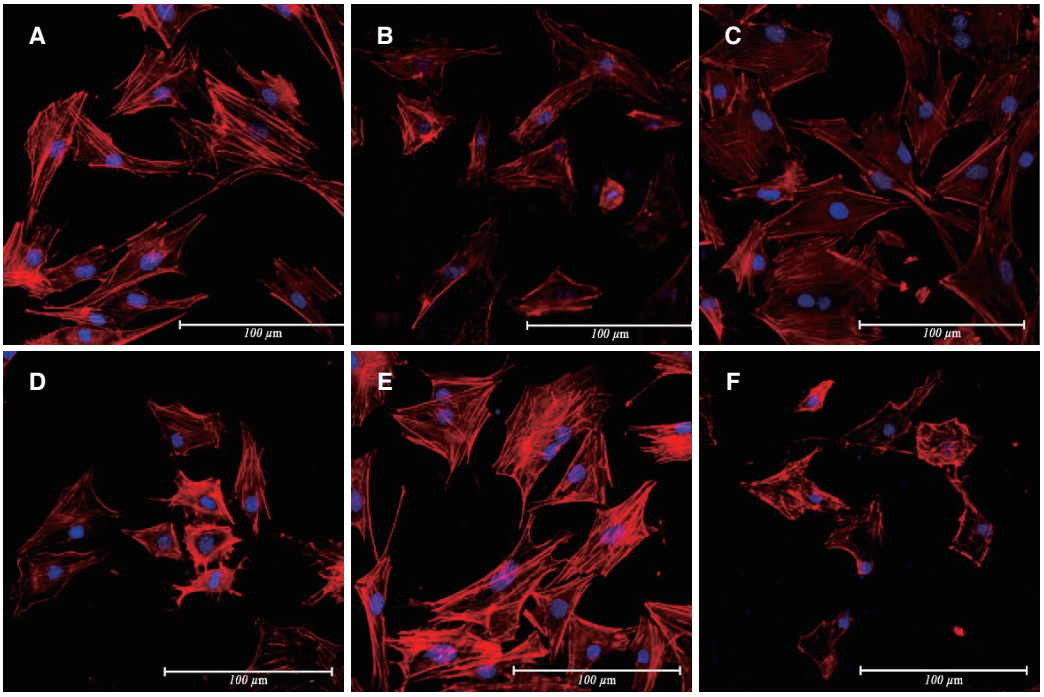

Fig. 3. Microscope observation 24 h after MC3T3-E1 cells were seeded onto the zirconia discs (A) 3Y-TZP-M, (B) 3Y-TZP-R, (C) YN4533-M, (D) YN4533-R, (E) YN4533/Al20-M, (F) YN4533/Al20-R. Original magnification is ×200 and the scale bar is 100 μm.

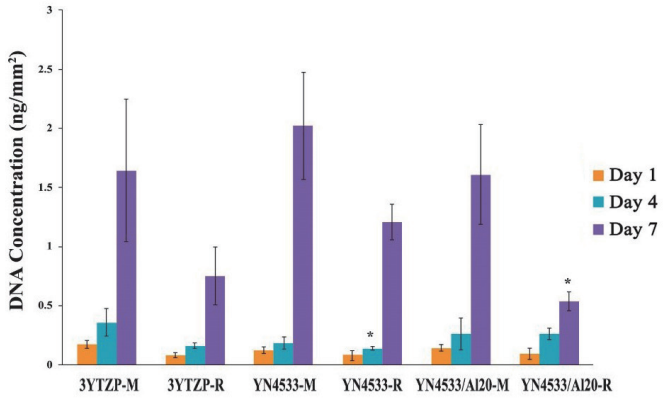

Fig. 4. Cellular proliferation (PicoGreen assay) of MC3T3-E1 on the zirconia discs at days 1, 4, and 7. Data are expressed as the mean ± standard deviation (SD) of three independent experiments. Significant differences (*) were denoted by Tukey and two-way analysis of variance (ANOVA) tests at $P < .05$.

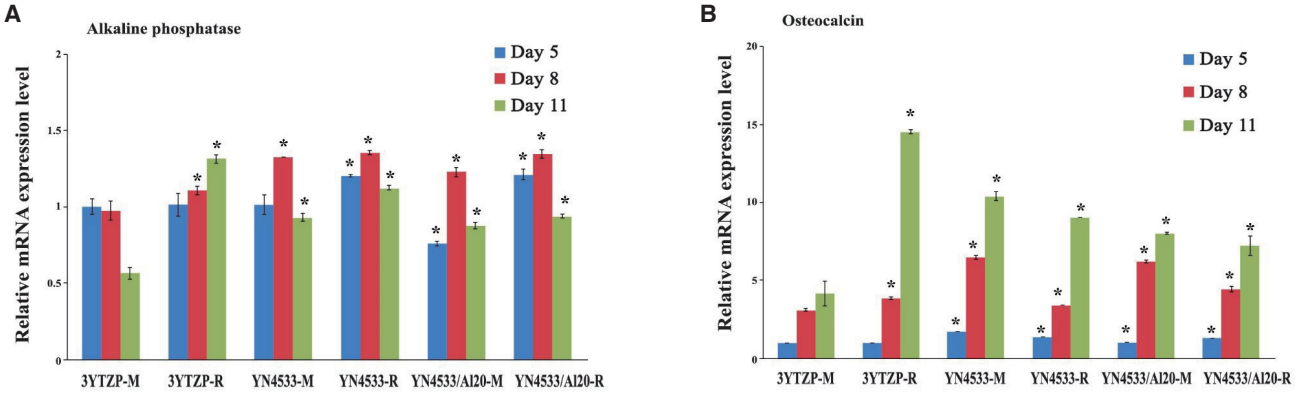

Fig. 5. Real-time PCR analysis of MC3T3-E1 cells on the zirconia discs after 5, 8, and 11 days of culture in osteogenic medium for both (A) Alkaline phosphatase (*Alp*) and (B) Osteocalcin (*Oc*). Data are expressed as the mean ± standard deviation (SD) of three independent experiments. Significant differences (*) were evaluated using Tukey and two-way analysis of variance (ANOVA) tests at $P < .05$.

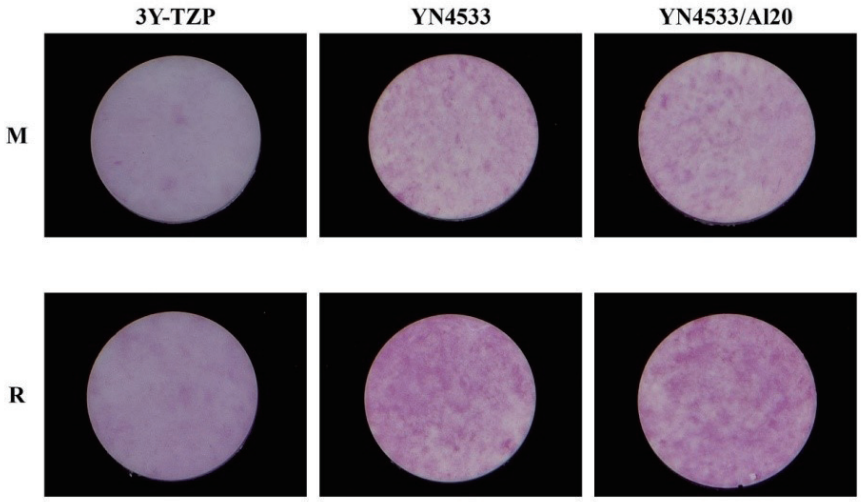

Fig. 6. *Alp* staining 10 days after cells were seeded on the zirconia discs and cultured in osteogenic medium.

showed significantly higher at experiment day 5. Osteoblast differentiation patterns on new (Y,Nb)-TZP discs were similar to or slightly higher than that of 3Y-TZP. We performed *Alp* staining to confirm the differentiation capacity of modified zirconia. Cells were stained at differentiation day 10. As shown in Fig. 6, new (Y,Nb)-TZPs had a higher differentiation capacity than 3Y-TZP, regardless of surface roughness. Although there were no significant differences in bone marker gene expression, *Alp* staining showed better osteoblast differentiation on new (Y,Nb)-TZPs than 3Y-TZP.

DISCUSSION

Modified zirconia newly combined with yttrium, niobium, and aluminum oxides were developed in this study to overcome the drawbacks of 3Y-TZP. Several researchers have already shown that niobium has higher biocompatibility and osteoconductivity than titanium.[37-39] Other previous studies revealed that the LTD phenomenon in zirconia was substantially reduced by the addition of Nb_2O_5.[31-33,40,41] In order to utilize this advantage of niobium in dental implant treatment, we analyzed the osteogenic potential of niobium oxide containing tetragonal zirconia polycrystals and compared with that of most widely used zirconia ceramics 3Y-TZP.

There is ample evidence that the increased surface roughness of commercially pure titanium results in a higher percentage of bone-to-implant contact and removal torque values, or faster osseointegration. This principle is the same for zirconia surfaces. However, it is difficult to modify a dense, hard zirconia surface to achieve sufficient roughness and this may adversely affect its mechanical strength. The sandblasting technique is the most commonly used technique to increase surface roughness of zirconia.[42] In this study, we sandblasted all zirconia discs with alumina particles (Al_2O_3). Albrektsson and Wennerberg[43] classified implants into four different categories depending on their surface roughness (R_a): smooth (R_a < 0.5 μm), minimally rough (R_a between 0.5 and 1.0 μm), moderately rough (R_a between 1.0 and 2.0 μm), and rough (R_a > 2.0 μm). Most currently used titanium implants have a moderately rough surface to facilitate osseointegration.[43,44] Several studies revealed that zirconia, (3Y-TZP) and titanium implants have comparable biocompatibility and osseointegration.[15-18] Although the average roughness values of zirconia discs used in this study (0.62 ± 0.05 μm, 0.72 ± 0.04 μm, and 0.71 ± 0.07 μm) were less than that of current titanium implants, they have a comparable osteogenic potential to titanium.

We found that MC3T3-E1 cells attach more weakly to rough surfaces than to smooth ones, and this was consistent with the cell morphologies on these two surfaces (Fig. 2 and Fig. 3). Cellular proliferation was predominant on the mirror-like surfaces and there was no significant difference between 3Y-TZP and new (Y,Nb)-TZP discs, YN4533 and YN4533/Al20, regardless of surface roughness (Fig. 4). Cell proliferation rates increased as time went on and highest at day 7 for all zirconia discs. Significant differences were found only when compared with the 3Y-TZP mirror surfaces, between day 4s of 3Y-TZP mirror and YN4533 rough surface and day 7s of 3Y-TZP mirror and YN4533/Al_2O rough surface groups. These results are in agreement with a previous study that showed that cells on polished surfaces proliferated more rapidly than those on the rough surfaces,[36] but was not consistent with another study that stated that cell proliferation was significantly greater on rough zirconia surfaces than on smooth surfaces.[45] The sample discs used in this study were minimally rough, while samples from Taniguchi's study[45] were moderately rough. When performing zirconia surface roughing, it is important to achieve the minimum effective roughness without jeopardizing the mechanical properties. In our study, cell morphology and cellular proliferation were associated with and influenced by

the surface roughness of zirconia discs.

Cell differentiation of 3Y-TZP increased with surface roughness. However, although the differentiation patterns of all new (Y,Nb)-TZP discs were increased, their osteogenic responses were not influenced by surface roughness. Statistically significant differences were found when compared with 3Y-TZP mirror discs. Moreover, *Alp* gene expression level of both mirror and rough new (Y,Nb)-TZP discs showed significantly higher than that of both mirror and rough 3Y-TZP discs at experiment day 8, while osteocalcin level showed significantly higher at experiment day 5 (Fig. 5). This indicates that new (Y,Nb)-TZPs have comparable osteogenic potential to 3Y-TZP discs. On the basic of the available data from systematic reviews, osseointegration of 3Y-TZP implants might be comparable to that of titanium implants, however, they are prone to low temperature degradation.[15-18] Our tested bioceramics, new (Y,Nb)-TZP, has the potential to solve this problem. Bosshardt[18] stated that yttria-stabilized zirconia can be toughened by adding alumina and our study revealed that addition of 20 vol% Al_2O_3 into YN4533 does not affect its osteogenic potential. It was important to note that although osteocalcin levels of new (Y,Nb)-TZPs increased as time went on, alkaline phosphatase activities decreased at day 11. In addition to RT-PCR, we also performed *Alp* staining to confirm the osteogenic potential of modified zirconia (Fig. 6). *Alp* staining showed that the osteogenic potential of all zirconia discs increased with surface roughness. *Alp* staining also revealed that new (Y,Nb)-TZP discs have superior osteogenic potential compared to 3Y-TZP, and these are biomaterials that have been widely used and already proven for use in medical and dental restorations.[46,47] This also indicates that niobium may improve the biocompatibility of zirconia.

The results of this study indicate that niobium oxide-combined zirconia has significant potential for use as an implant biomaterial. Niobium oxide, contained in modified zirconia discs, has shown excellent biocompatibility and osteogenic potential.[37,39] Besides, oxygen ions in niobium oxide may stabilize the tetragonal structure, resulting in enhanced crack resistance and biaxial strength in addition to resistance to low temperature degradation. This study revealed that new niobium oxide containing (Y,Nb)-TZP discs, YN4533 and YN4533/Al20 have comparable or better osteogenic response than 3Y-TZP when considering alternative titanium bioceramics implants. However, further studies might be necessary to confirm good osteogenic potential, proper peri-implant soft tissue integration, and the mechanical strength of new (Y,Nb)-TZP bioceramics (YN4533 and YN4533/Al20) in the form of implant fixtures.

CONCLUSION

Based on these results, niobium oxide containing Y-TZPs have comparable osteogenic potential to 3Y-TZP and are expected to be suitable alternative ceramics dental implant materials to titanium for aesthetically important areas.

ORCID

Aung Thu Hein *https://orcid.org/0000-0002-6925-8654*
Jung-Suk Han *https://orcid.org/0000-0002-9439-1465*

REFERENCES

1. Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res 2008;10:207-17.
2. Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990;5:347-59.
3. Steinemann SG. Titanium-the material of choice? Periodontol 2000 1998;17:7-21.
4. Depprich R, Zipprich H, Ommerborn M, Naujoks C, Wiesmann HP, Kiattavorncharoen S, Lauer HC, Meyer U, Kübler NR, Handschel J. Osseointegration of zirconia implants compared with titanium: an in vivo study. Head Face Med 2008;4:30.
5. Heydecke G, Kohal R, Gläser R. Optimal esthetics in single-tooth replacement with the Re-Implant system: a case report. Int J Prosthodont 1999;12:184-9.
6. Sailer I, Zembic A, Jung RE, Hämmerle CH, Mattiola A. Single-tooth implant reconstructions: esthetic factors influencing the decision between titanium and zirconia abutments in anterior regions. Eur J Esthet Dent 2007;2:296-310.
7. Sicilia A, Cuesta S, Coma G, Arregui I, Guisasola C, Ruiz E, Maestro A. Titanium allergy in dental implant patients: a clinical study on 1500 consecutive patients. Clin Oral Implants Res 2008;19:823-35.
8. Siddiqi A, Payne AG, De Silva RK, Duncan WJ. Titanium allergy: could it affect dental implant integration? Clin Oral Implants Res 2011;22:673-80.
9. Nakagawa M, Matsuya S, Udoh K. Effects of fluoride and dissolved oxygen concentrations on the corrosion behavior of pure titanium and titanium alloys. Dent Mater J 2002;21: 83-92.
10. Tschernitschek H, Borchers L, Geurtsen W. Nonalloyed titanium as a bioinert metal-a review. Quintessence Int 2005;36: 523-30.
11. Osman RB, Swain MV. A Critical review of dental implant materials with an emphasis on titanium versus zirconia. Materials (Basel) 2015;8:932-58.
12. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials 1999;20:1-25.
13. Zarone F, Russo S, Sorrentino R. From porcelain-fused-to-metal to zirconia: clinical and experimental considerations. Dent Mater 2011;27:83-96.
14. Zembic A, Bösch A, Jung RE, Hämmerle CH, Sailer I. Five-year results of a randomized controlled clinical trial comparing zirconia and titanium abutments supporting single-implant crowns in canine and posterior regions. Clin Oral Implants Res 2013;24:384-90.
15. Wenz HJ, Bartsch J, Wolfart S, Kern M. Osseointegration and clinical success of zirconia dental implants: a systematic re-

view. Int J Prosthodont 2008;21:27-36.
16. Manzano G, Herrero LR, Montero J. Comparison of clinical performance of zirconia implants and titanium implants in animal models: a systematic review. Int J Oral Maxillofac Implants 2014;29:311-20.
17. Cionca N, Hashim D, Mombelli A. Zirconia dental implants: where are we now, and where are we heading? Periodontol 2000 2017;73:241-58.
18. Bosshardt DD, Chappuis V, Buser D. Osseointegration of titanium, titanium alloy and zirconia dental implants: current knowledge and open questions. Periodontol 2000 2017;73:22-40.
19. Bächle M, Butz F, Hübner U, Bakalinis E, Kohal RJ. Behavior of CAL72 osteoblast-like cells cultured on zirconia ceramics with different surface topographies. Clin Oral Implants Res 2007;18:53-9.
20. Hempel U, Hefti T, Kalbacova M, Wolf-Brandstetter C, Dieter P, Schlottig F. Response of osteoblast-like SAOS-2 cells to zirconia ceramics with different surface topographies. Clin Oral Implants Res 2010;21:174-81.
21. Scarano A, Di Carlo F, Quaranta M, Piattelli A. Bone response to zirconia ceramic implants: an experimental study in rabbits. J Oral Implantol 2003;29:8-12.
22. Hoffmann O, Angelov N, Gallez F, Jung RE, Weber FE. The zirconia implant-bone interface: a preliminary histologic evaluation in rabbits. Int J Oral Maxillofac Implants 2008;23:691-5.
23. Gahlert M, Roehling S, Sprecher CM, Kniha H, Milz S, Bormann K. In vivo performance of zirconia and titanium implants: a histomorphometric study in mini pig maxillae. Clin Oral Implants Res 2012;23:281-6.
24. Kim DJ, Jung HJ, Cho DH. Phase transformations of Y2O3 and Nb2O5 doped tetragonal zirconia during low temperature aging in air. Solid State Ionics 1995;80:67-73.
25. Kelly JR, Denry I. Stabilized zirconia as a structural ceramic: an overview. Dent Mater 2008;24:289-98.
26. Nawa M, Nakamoto S, Sekino T, Niihara K. Tough and strong Ce-TZP/alumina nanocomposites doped with titania. Ceram Int 1998;24:497-506.
27. Takano T, Tasaka A, Yoshinari M, Sakurai K. Fatigue strength of Ce-TZP/Al2O3 nanocomposite with different surfaces. J Dent Res 2012;91:800-4.
28. Andreiotelli M, Kohal RJ. Fracture strength of zirconia implants after artificial aging. Clin Implant Dent Relat Res 2009; 11:158-66.
29. Kohal RJ, Wolkewitz M, Mueller C. Alumina-reinforced zirconia implants: survival rate and fracture strength in a masticatory simulation trial. Clin Oral Implants Res 2010;21:1345-52.
30. Chappuis V, Cavusoglu Y, Gruber R, Kuchler U, Buser D, Bosshardt DD. Osseointegration of zirconia in the presence of multinucleated giant cells. Clin Implant Dent Relat Res 2016;18:686-98.
31. Kim DJ, Jung HJ, Jang JW, Lee HL. Fracture toughness, ionic conductivity, and low-temperature phase stability of tetragonal zirconia codoped with Yttria and Niobium Oxide. J Am Ceram Soc 1998;81:2309-14.
32. Ray JC, Panda AB, Saha CR, Pramanik P. Synthesis of niobium(V)- stabilized tetragonal zirconia nanocrystalline powders. J Am Ceram Soc 2003;86:514-6.
33. Gutiérrez-González CF, Moya JS, Palomares FJ, Bartolomé Gómez JF. Low-temperature aging degradation-free 3Y-TZP/Nb composites. J Am Ceram Soc 2010;93:1842-44.
34. Li P, Chen IW, Penner-Hahn JE. Effect of dopants on zirconia stabilization - An X-ray absorption study: III, charge-compensating dopants. J Am Ceram Soc 1994;77:1289-95.
35. Guo X, Wang Z. Effect of niobia on the defect structure of yttria-stabilized zirconia. J Euro Ceram Soc 1998;18:237-40.
36. Cho YD, Shin JC, Kim HL, Gerelmaa M, Yoon HI, Ryoo HM, Kim DJ, Han JS. Comparison of the osteogenic potential of titanium- and modified zirconia-based bioceramics. Int J Mol Sci 2014;15:4442-52.
37. Johansson CB, Albrektsson T. A removal torque and histomorphometric study of commercially pure niobium and titanium implants in rabbit bone. Clin Oral Implants Res 1991;2: 24-9.
38. Matsuno H, Yokoyama A, Watari F, Uo M, Kawasaki T. Biocompatibility and osteogenesis of refractory metal implants, titanium, hafnium, niobium, tantalum and rhenium. Biomaterials 2001;22:1253-62.
39. Godley R, Starosvetsky D, Gotman I. Bonelike apatite formation on niobium metal treated in aqueous NaOH. J Mater Sci Mater Med 2004;15:1073-7.
40. Kim DJ. Effect of Ta2O5, Nb2O5, and HfO2 alloying on the transformability of Y2O3-stabilized tetragonal ZrO2. J Am Ceram Soc 1990;73:115-20.
41. Kim DJ, Lee MH, Lee DY, Han JS. Mechanical properties, phase stability, and biocompatibility of (Y,Nb)-TZP/Al2O3 composite abutments for dental implant. J Biomed Mater Res 2000;53:438-43.
42. Ewais O, Al Abbassy F, Ghoneim MM, Aboushelib MN. Novel zirconia surface treatments for enhanced osseointegration: Laboratory characterization. Int J Dent 2014;2014: 203940.
43. Albrektsson T, Wennerberg A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004;17:536-43.
44. Quirynen M, Van Assche N. RCT comparing minimally with moderately rough implants. Part 2: microbial observations. Clin Oral Implants Res 2012;23:625-34.
45. Taniguchi Y, Kakura K, Yamamoto K, Kido H, Yamazaki J. Accelerated Osteogenic Differentiation and Bone Formation on Zirconia with Surface Grooves Created with Fiber Laser Irradiation. Clin Implant Dent Relat Res 2016;18:883-94.
46. Raigrodski AJ, Chiche GJ, Potiket N, Hochstedler JL, Mohamed SE, Billiot S, Mercante DE. The efficacy of posterior three-unit zirconium-oxide-based ceramic fixed partial dental prostheses: a prospective clinical pilot study. J Prosthet Dent 2006;96:237-44.
47. Sailer I, Pjetursson BE, Zwahlen M, Hämmerle CH. A systematic review of the survival and complication rates of all-ceramic and metal-ceramic reconstructions after an observation period of at least 3 years. Part II: Fixed dental prostheses. Clin Oral Implants Res 2007;18:86-96.