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Osteoclast behaviors and surface characterization of zirconia implant discs

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-ABSTRACT-

Osteoclast behaviors and surface characterization of zirconia implant discs

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Purpose: Usage of zirconia is increased in dentistry due to its tough mechanical properties and good biocompatibility. Also zirconia shows a prospect to alternate titanium as an implant material. Osteoblast and osteoclast cells are needed to work cooperatively for bone remodeling that is crucial to the osseointegration which is a bonding structure between the bone and implant. Bone resorption related to osteoclast occurs around implant, thus it has an effect on the succession of implant. Therefore, osteoclastogenesis is needed to be investigated. In order to demonstrate whether the zirconia was able to osseointegrate, we evaluated osteoclast response on zirconia disc while comparing it to that of titanium discs.

Material and methods: 30 pieces of Y_2O_3-stabilized tetragonal zirconia polycrystals (Y-TZP) discs and 30 pieces of titanium discs with machined surface were prepared into two groups. We used titanium discs as a control group and zirconia discs as a test group. To make sure discs in both groups have similar surface roughness,

we measured surface roughness by confocal laser scanning microscopy (CLSM). Energy dispersive X-ray spectroscopy (EDS) was carried out to evaluate the chemical composition of the discs. We used murine RAW 264.7 cells as osteoclast precursor cells and cultured it on the discs of both groups in this study. We conducted Tartrate-resistant acid phosphatase (TRAP) activity assay to assess osteoclast differentiation on zirconia discs. After 3 days of culture, cells on both groups were examined through field emission-scanning electron microscope (FE-SEM) to observe osteoclast differentiation activity. We performed real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and western blot to assess mRNA and protein levels of two critical transcriptional factors, NFATc1 and c-Fos, of osteoclastogenesis. Data were analyzed by student t test at a 0.05 level of significance.

Results: There was no difference of surface roughness between two groups (p >0.05). There were no differences of TRAP activities between zirconia and titanium discs. FE-SEM exhibited that the amount of differentiated osteoclasts on both groups were similar. Even though the mRNA level of two osteoclastogenic transcriptional factors, c-Fos and NFATc1 on zirconia discs was little higher than titanium discs, there was no significant difference. There were no significant differences between both groups in protein level of these transcriptional factors as well.

Conclusion: The study showed that osteoclast responses on zirconia are similar to titanium. It seems possible that zirconia could trigger moderate osseointegration when it is used for inner bone. However further follow-up studies are needed to evaluate long-term efficiency of zirconia implants.

Key words: Zirconia disc, Osteoclast, Osteoclastogenesis, Titanium disc, Implant

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Osteoclast behaviors and surface characterization of zirconia implant discs

CONTENTS

Ⅰ.INTRODUCTION

Titanium alloy has been ideal material for dental implants for long time and their efficacy have been verified.[1] Currently Titanium are most broadly used as dental implants because of their great biocompatibility, good

mechanical characteristic, and long term follow-up in clinical success.[2,3] Branemark et al. first presented an osseointegration, which is a bonding structure between titanium implant and bone.[4] However, titanium can trigger clinical hypersensitivity and allergy in patients with dental implants.[5,6] Also titanium as a restorative material caused soft tissue to be discolored.[7] These are the major disadvantages of the titanium.

Advent of ceramic implant attracted plenty of researchers to study it in order to overcome drawbacks of titanium such as discoloration and allergy. High-stress ceramics exhibits similar bio-inert to stainless steel and show minimal ion release compared to metallic implants.[8] Osseointegration between the bone and ceramic material has been detected and these achievements imply some capability of using ceramic as dental implants.[9] Yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) which are stabilized with Y2O3 is a ceramic material used in indirect dental restorations.[10] Using Y-TZP as implant may have a capability to alter titanium implant for short-term clinical use. It indicates similar hard tissue response to a titanium implant. [11,12] It is stablished that zirconia implants showed high biocompatibility. Another advantage of zirconia is that it shows a considerably decreased plaque index, thus lowering the inflammation rate around the soft tissue.[13] Depprich et al. demonstrates that osteoblast which is primary cell to bone remodeling that is critical to osseointegration are able to attach on zirconia surface.[14] Cooperative work of osteoblast and osteoclast is required for bone remodeling that is harmony of bone formation and bone resorption after implant insertion.[15]

It is clear that the osteoclasts which are critical cells in bone resorption may take in active part, not only in the early tissue response after implant insertion, but also in the late tissue response while in function.[16] There have been many studies about the osseointegration of implant surfaces that have been focused on the bone forming osteoblasts. However, the function of osteoclasts which are the key bone resorbing cell affecting osseointegration and it' s long-term effective has less investigated relatively. It was hypothesized that osteoclast reaction on zirconia would be acceptable *in vitro* so that zirconia could be used for inner bone. So we decided to study osteoclastogenesis and differentiation of osteoclast on surface of zirconia disc. The goal of this study was to assess the osteoclast behaviors on zirconia disc and compares them with titanium disc.

Ⅱ. MATERIAL AND METHOD

1. Disc preparation

All 30 pieces of titanium discs with machined surface were manufactured by cutting from Titanium rods (commercially pure titanium grade III) and had uniform dimensions of 1mm in thickness and a diameter of 15mm (Warantec, Seoul, Korea). 30 pieces of Y-TZP zirconia discs with 15 mm diameter and 1 mm thickness were fabricated by cold isostatic press at 200 MPa and then sintered for 2 hours at 1650° C in air. Then, the discs

were divided into two groups: (1) control group: Titanium discs, (2) test group: Zirconia discs. All discs were washed in distilled water and ultrasonically cleaned with alcohol. Finally, discs were dried and autoclaved at 120° C for 30 min.

2. Surface analysis

Surfaces of the discs were coated with a Pt/Pd film and viewed with a field emission scanning electron microscope (FE-SEM, Hitachi S-4700, Tokyo, Japan) using an acceleration voltage of 15 kV at three different magnifications (X1,000, X5,000, X20,000). Surface roughness was measured by confocal laser scanning microscopy (CLSM, LSM 5 Pascal, Zeiss, Obercochen, Germany). Energy dispersive X-ray spectroscopy (EDS, Horiba EX-250, Tokyo, Japan) analysis was also conducted to assess the chemical composition of the discs. Each evaluation was conducted with 3 discs for one group.

3. Cell culture and differentiation

Murine RAW 264.7 macrophage/monocyte cells (TIB-71; ATCC) were cultured on the surfaces of zirconia and titanium discs placed at the bottom of the 12-well plates at 37℃ in a humidified atmosphere of 5% CO_2 in Minimum Essential Medium Alpha (α- MEM, Gibco, NY, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100U/ml penicillin, and 100 μg/ml streptomycin. Cells in 250μl α- MEM with density of 2×10^4 cells/disc/well were seeded on discs and after 6 hours of cells attaching on the discs, 100 ng/ml of mouse RANKL was supplied to make RAW 246.7 cells differentiate into osteoclasts. Medium and

RANKL were exchanged every 2 days. Cells were cultured for 6 days at most. RANKL was purchased from Peprotech (Rocky Hill, NJ, USA).

4. Field emission-scanning electron microscopy (FE-SEM) analysis

Cells on each titanium and zirconia discs were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 min at room temperature after 3 days of culture, following by staining in 2% osmium tetroxide in PBS for 15 min and dehydrated in an ascending ethanol series. After critical point drying, samples were sputter-coated with 6 nm platinum and then examined in the FE-SEM. Titanium and zirconia surfaces were observed under a FE-SEM using an accelerating voltage of 15 kV at low and high magnification (x200, x2000). FE-SEM observation was performed at three different areas on 4 discs for each group.

5. Tartrate-resistant acid phosphatase (TRAP) activity assay

RAW 264.7 cells were seeded onto each titanium and zirconia discs at a density of $2X10^4$ cells/disc/well. They were cultured in α- MEM containing 10% FBS, 100 U/ml penicillin and 100 μg/mL streptomycin. After 3 and 5 days of culture, TRAP activity was measured using the TRACP assay kit (Takara, Kyoto, Japan) according to the protocol. The TRAP activity of the cultured cells was examined by a colorimetry-based assay utilizing the conversion of colorless p-nitrophenol phosphate (pNPP,

Sigma) to colored p-nitrophenol. The absorbance of the samples was measured spectrometrically at 405nm (Bio-Rad, Hercules, CA, USA). Measurements on each day were performed with 4 discs for one group (n=16).

6. Real time reverse transcriptase-polymerase chain reaction (Real time RT-PCR) analysis

Real time RT-PCR was performed to determine the gene mRNA expression levels in osteoclast cells for one reference gene, HPRT and two target genes, c-Fos and NFATc1. Total RNA was isolated from cell samples after 2 and 4 days of RANKL supplement. Cells were lysed by adding TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The homogenized samples were incubated for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. A 0.2 mL of chloroform was added to each sample and the samples were mixed vigorously and then incubated at room temperature for 3 minutes. The samples were centrifuged at 12000g for 15 min at 4℃. Following centrifugation, the mixture was separated into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA was precipitated from the aqueous phase by mixing with 0.4 mL of isopropyl alcohol. The samples were incubated at room temperature for 10 minutes and centrifuged at 12000g for 10 minutes at 4℃. The RNA precipitate forms a gel-like pellet on the side and bottom of the tube. After removing the supernate, the RNA pellet was washed with

1 mL of 75% ethanol and centrifuged at 12000g for 5 minutes at 4℃. At the end of the procedure, the RNA pellet was dried for 5 minutes. RNA was dissolved in RNase- free water and incubated at 55℃ for 10 minutes. Total RNA was quantified using a Nanodrop Spectrophotometer (ThermoScientific Nanodrop Technologies, Wilming- ton, DE, USA). 1 μg of total RNA was reverse transcribed to cDNA at 42℃ for 50 min using the Superscript II reverse transcriptase (Invitrogen) that contains both oligo(dT), 2.5 mM dNTP, 5xFS buffer, 0.1M DTT, SSII enzyme, and RNase out.

The following mouse specific primers were used:

Gene name	Primer sequences (F=forward; R=reverse)
HPRT	(F) 5′ -CCACAGGGACTAGAACACCTGCTAA-3′ , (R) 5′ -CTTGTGGACTGTGTGACT-3′ ;
c-Fos	(F) 5′ -CTGGTGCAGCCCACTCTGGTC-3′ , (R) 5′ -CTTTCA GCAGATTGGCAATCTC-3′ ;
NFATc1	(F) 5′ -CGGCTGCCTTCCGTCTCA TAG-3′ , (R) 5′ -CGGCTGCCTTCCGTCTCATAG-3′ .

Real-time RT-PCR was performed in an iCycler (Bio-Rad, Hercules, CA, USA) using SYBR green (Invitrogen) detection. Each reaction contained 5 μl of cDNA, 0.4 μl of the forward and reverse specific primers, 0.4 μl of Rox in a final volume of 20 μl. The amplification program consisted of a pre-incubation step for denaturation of the template cDNA (3 min 95℃), followed by 40 cycles consisting of a denaturation step (15s for 95℃), an annealing step (15s for 60℃), and an extension step (30s for 72℃). Samples were run in triplicate (n=12). Relative levels of c-Fos and NFAcT1 were normalized to HPRT.

7. Western blot analysis

Protein expression level of 2 genes, c− Fos and NFATc1 were assessed with western blot analysis. It was performed with RAW 264.7 cells grown up on titanium and zirconia discs after 1, 2 and 3 days of RANKL supplement. After washing with PBS, total cell lysates were harvested by lysing the cells with chilled RIPA buffer (10 mM Tris pH 7.2, 150 mM NaCl, 5 mM EDTA, 1 mM NaF, 2.5 mM sodium pyrophos− phate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 1% Triton X−100, 0.1% SDS and 1% deoxycholate). Total cell lysates were incubated for 20 min and centrifuged at 14,000g for 15 min at 4° C. Harvested proteins were separated by 8% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and transferred to a nitrocellulose membrane (What man GmbH, Dassel, Germany). The membrane was probed with specific antibodies and reactivity of immune complexes was detected by using enhanced chemiluminescence (ECL) reagents.

8. Statistical analysis

All data were analyzed by student *t*−test analyses (SPSS 23, IBM SPSS, USA). P−values more than 0.05 were considered statistically not significant.

Ⅲ. RESULTS

1. Surface characterization of the discs

Figure 1-1 and 1-2 show that surface of titanium and zirconia disc. CLSM was conducted to analyze the surface roughness. Figure 2 shows 3D images of surface of microstructure and figure 3 shows statistical results of surface roughness related to parameters. Based on the figure 3, it shows Ra and Sa value of 0.589±0.03 μm and 0.595 ±0.01 μm for the titanium discs. Zirconia discs resulted in Ra and Sa value of 0.659±0.093 μm and 0.707±0.086 μm. The Sdr value was 16.63% ±0.59% for the titanium discs and 23.86%±4.28% for the zirconia discs. There was no statistically significant difference between the overall roughness of titanium and zirconia surfaces. ($p > 0.05$). EDS confirmed that zirconia and titanium discs are pure which means that they contain no other composition (Figure 4).

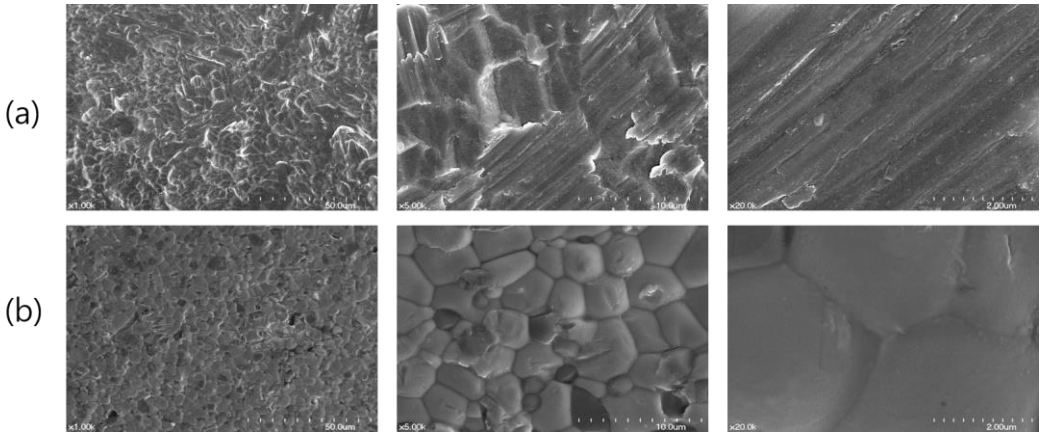

Fig 1-1. SEM images of the surface of (a) titanium disc and (b) zirconia disc (every row, left to right, x1,000, x5,000, x20,000 magnification).

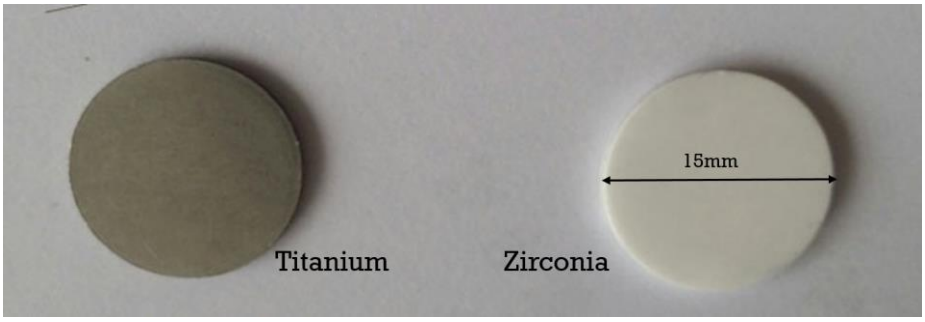

Fig 1-2. Images of the zirconia and titanium disc obtained by Digital camera. Diameter of both discs is 15mm

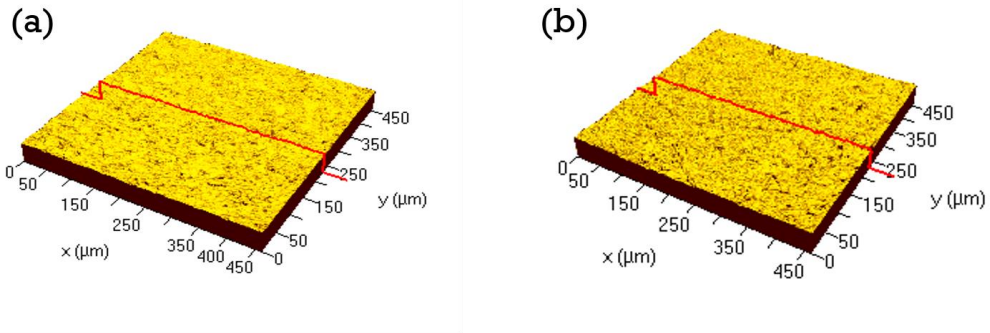

Fig 2. 3D images of surface of microstructure with dimensional range of 50 μm by CLSM: (a) titanium disc, (b) zirconia disc

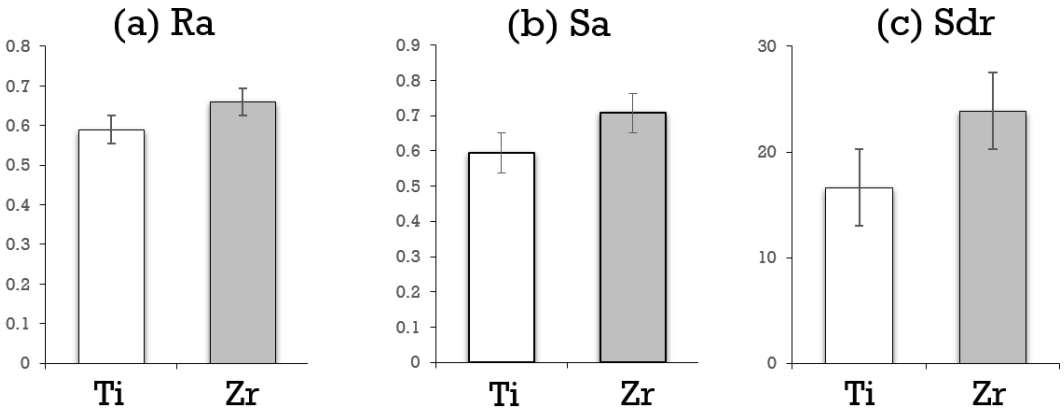

Fig 3. Surface roughness related parameters obtained by CLSM. (a) Ra: Surface roughness, (b) Sa: arithmetic mean height deviation from a mean plane and (c) Sdr: developed interfacial area ratio. No statistical difference was observed between zirconia and titanium discs on any measurement scale ($p > 0.05$; the graph bar represents mean ± SEM).

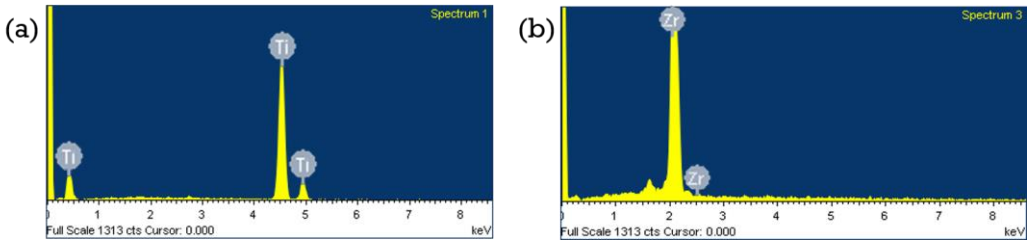

Fig 4. EDS patterns of zirconia discs compared with the titanium discs: (a) titanium discs, (b) zirconia discs. The pattern shows that zirconia and titanium discs contain no other composition which means that discs are clear.

2. FE-SEM analysis

FE-SEM was used to visualize and compare the distribution of differentiated osteoclasts on titanium and zirconia surfaces. Figure 5 shows that similar amount of osteoclasts observed on both titanium and zirconia discs. High magnification FE-SEM image (x2,000) confirmed characteristic morphology of differentiated osteoclasts on both group (Figure 5b, 5d).

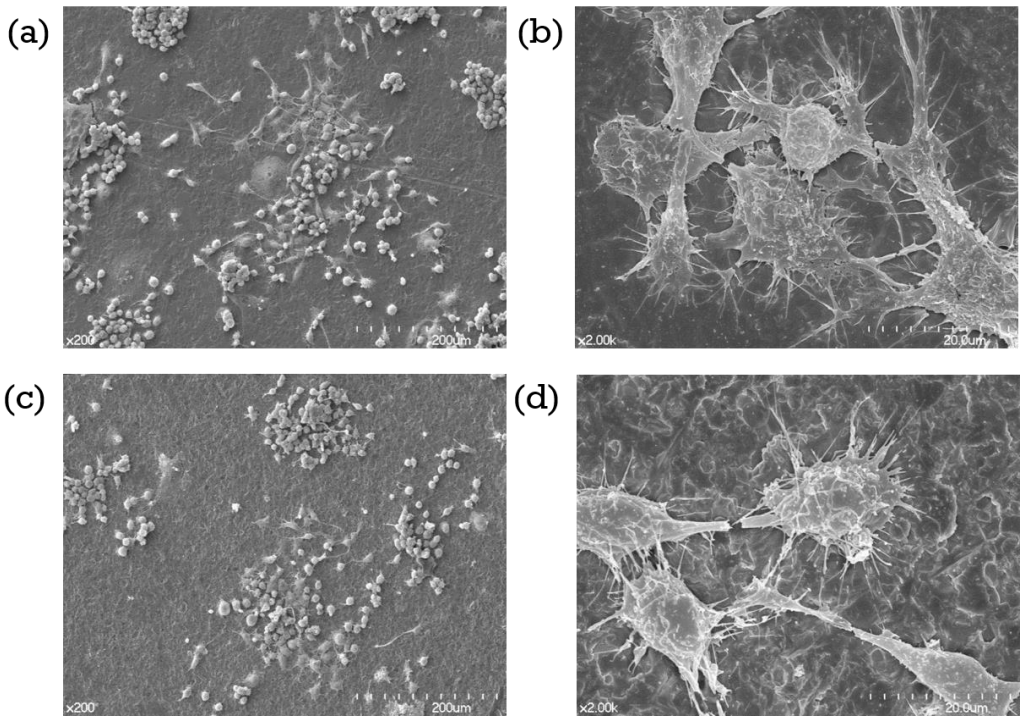

Fig 5. FE-SEM images of Osteoclast differentiated from RAW 264.7 cells cultured on: (a) titanium surface with low magnification (x200), (b) titanium surface with high magnification (x2000), (c) zirconia surface with low magnification (x200), (d) zirconia surface with high magnification (x2000).

3. TRAP activity assay

TRAP enzyme activities on zirconia discs were measured to evaluate osteoclast differentiation activity quantitatively. 3day O.D value of titanium discs was 0.301±0.016 whereas that of zirconia disc was 0.286±0.024 (Figure 6a). Data at day 5 revealed O.D value of 0.461±0.043 on the titanium discs and O.D value of 0.461±0.026 on the zirconia discs (Figure 6b). At 3day and 5day of culture, there were no significant differences between the TRAP activities of cells grown on zirconia and titanium discs (Figure 5).

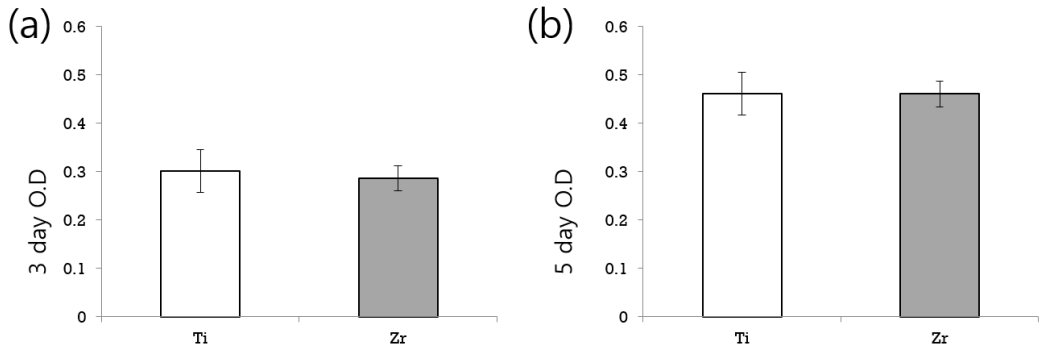

Fig 6. TRAP activities on titanium and zirconia surfaces: The expression of TRAP was measured among the both group. (a) At 3day, there was no significant statistical difference between both discs. (b) At 5day, there was no significant difference between groups as well ($p > 0.05$; the graph bar represents mean ± SEM)

4. RT-PCR analysis

To compare c-Fos mRNA level, which is expressed at the early phase of differentiation, real time RT-PCR of c-Fos and NFATc1 was performed at day 2 and day 4 after RANKL supplement. We set mRNA expression level of c-Fos and NFATc1 on titanium disc at day 2 as a control value of 1.00. Expression value of c-Fos for zirconia at day 2 was 1.303± 0.125. At day 4, c-Fos mRNA expression of cells grown on zirconia was 2.308 ±0.261, slightly higher than the value of 1.652±0.092 on titanium. However, there was no statistically significant difference between them ($p > 0.05$) (Figure 7a). NFATc1 mRNA level on zirconia at day 2 was 1.009 ±0.047. At day 4, NFATc1 mRNA expression on each of titanium and zirconia were 1.906±0.149 and 3.150±0.440. However, they did not reach significant difference ($p > 0.05$) (Figure 7b).

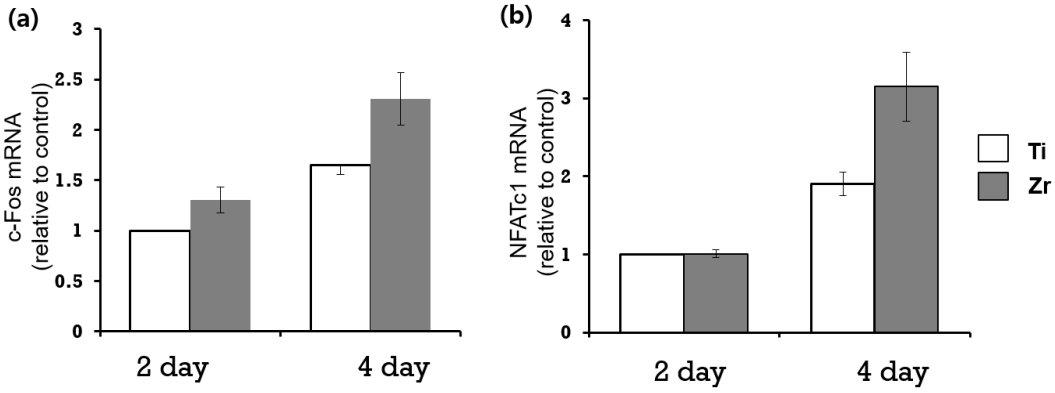

Fig 7. The mRNA expression level of osteoclastogenic transcriptional factors. (a) c-Fos mRNA levels at day 2 and 4. (b) NFATc1 mRNA levels at day 2 and 4. There were no significant differences between the titanium and zirconia discs. ($p > 0.05$; the graph bar represents mean ± SEM).

5. Western blot analysis

Measuring the c-Fos protein level and NFATc1 protein level on both discs have been done at day 1, 2 and day 3 after RANKL supplement. Figure 8 shows the protein level variances. C-fos protein level on zirconia discs on day 2 is slightly higher than that of titanium discs. But at day 3, c-fos protein level on zirconia and titanium discs have become similar. No significant difference in NFATc1 protein was observed between the zirconia and titanium discs. β-actin was used as a loading control.

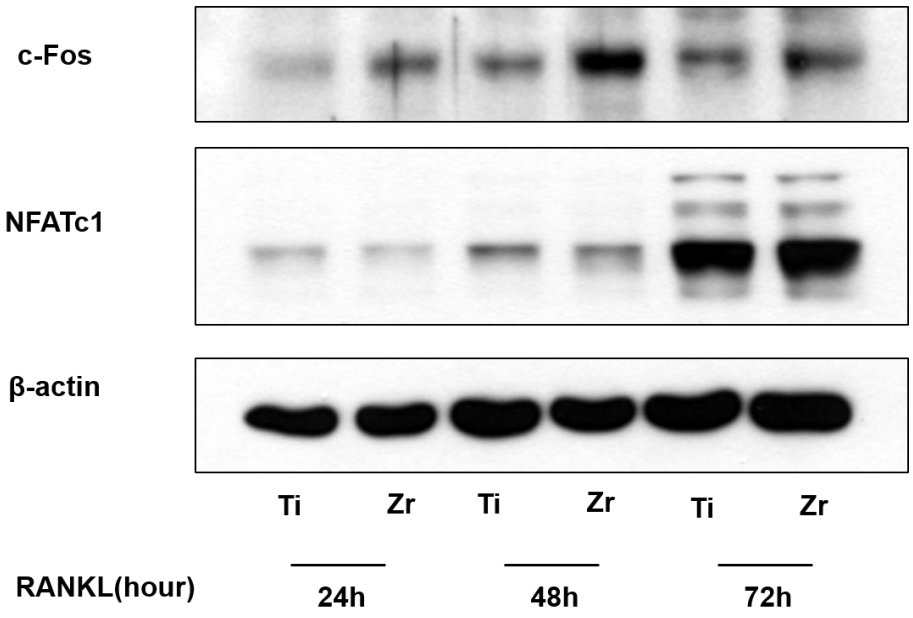

Fig 8. The protein expression of NFATc1, c-Fos and β-actin. NFATc1 protein expression level was observed similarly on both discs. Also in case of c-Fos protein, there was no significant difference between zirconia and titanium discs.

Ⅳ. DISCUSSION

Osseointegration is critical to survival of the implant inserted in the body, which is described as a structural and functional complex that occur between the implant surface and inner bone.[17] For many decades, titanium has been used as a material for dental implant. Unexplained titanium implant failures have pushed dental clinicians to study the other materials which could replace titanium. In biomaterial field, ceramic materials are getting popular with a great speed due to their high strength, enhanced fracture resistance and low wear characteristic and excellent biocompatibility.[18] Zirconia is presented to the dental ceramics family recently, which is a polymorphic material that appears in three different forms: monoclinic (M), tetragonal (T) and cubic (C).[19] Making zirconia metastable at tetragonal phase can be obtained at room temperature by supplying stabilizing oxides such as yttria, ceria and magnesia. It seems to be a proper material for dental implants due to their tooth-like color, their good mechanical properties and excellent biocompatibility.[20] Zirconia could probably be used as non-metal implant alternating titanium despite the lack of long-term clinical results of zirconia dental implants.[21] There have been no specific studies like this that conducted direct comparison of osteoclast cell responses between titanium and zirconia. It is likely that formation of osteoclast is involved in a complex process that causes peri-implant osteolysis.[22] That is why we selected osteoclast cell as an experimental target.

We used zirconia discs without surface treatment and titanium discs with machined surface. And the images of disc surface were obtained from SEM observations with a high and low magnification. At first, in order to

measure the cells cultured on titanium and zirconia discs under same conditions, we used CLSM to measure the roughness of both discs to confirm that the surfaces have similar roughness. This is because that it is noted that surface topography of discs may determines the osteoclast behavior.[23-25] Thus, we confirmed that zirconia and titanium contain no other composition by using EDS. The study that compared osteoblast responses on zirconia and titanium showed the abilities of osteoblasts such as attaching, proliferating and differentiating on zirconia.[14] We assumed that osteoclast would be the same. Osteoclasts are multinucleate giant cells that cause mineralized tissues to be resorbed.[16] We used RAW 264.7 cells, the murine bone marrow macrophage cell line as osteoclast precursor cells for *in vitro* studies of osteoclastogenesis. Mature osteoclasts, having ability of resorbing bone is elicited by stimulation with receptor activator nuclear factor-κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF).[26] In this study, we supplied RANKL on the cell culture-well in order to trigger osteoclast differentiation after 6 hours of RAW 264.7 cells seeding on discs. TRAP is one of the lysosomal enzymes that osteoclasts secrete to perform their specialized resorption process. Acid phosphatase is an enzyme which hydrolyzes aliphatic and aromatic phosphate esters giving rise to the phosphates release in the acid pHs. Potent acid phosphatase activity is found in the osteoclast and it is suitable biochemical probes for osteoclasts function.[27,28] Kim et al. assessed TRAP activities of osteoclast on titanium disc at 3 day and 6 day after supplying RANKL.[29] In this study, assessing day 3 and day 5 was appropriate. This is because O.D value of the 6 day was saturated according to the pilot study we have done previously. TRAP activities of osteoclasts on titanium and zirconia showed similar values. NFATc1 and

c-Fos strongly induced by RANKL stimulation, are master transcription factors required for the terminal differentiation of osteoclasts.[30] Previous study confirmed mRNA level of c-Fos at day 1 and NFATc1 at day 3 after RANKL supplement.[29] The other study evaluated mRNA level at day 7 after RANKL supplement.[31] We conducted RT-PCR to assess mRNA level of each of these 2 transcriptional factor, NFATc1 and C-fos at day 2 and day 4 after RANKL supplement. At day 2, there were no difference of mRNA level between two groups. The increase in mRNA expression level was detected on zirconia compared to titanium at day 4. However, it showed no statistical significant differences between zirconia and titanium discs.

We also implemented western blot analysis to obtain data of protein levels of two transcriptional factor, NFATc1 and C-fos. In this experiment, we obtained protein levels at day 1,2 and day 3 after RANKL supplement which was as same day as Kim et al.[29] had done. C-Fos protein expression level on zirconia at day 2 was higher than that on titanium. But at day 3, both discs showed same protein expression level. In case of NFATc1, there were no differences of protein expression level between zirconia and titanium discs at all experimental day. These results (TRAP activity, SEM and CLSM observation, osteoclastogenesis-related gene and protein expression) have consistently shown that zirconia induces similar osteoclast cell response to titanium. Some study indicate that zirconia has a genuine ability to become a reliable implant material.[32] However, it needs much more work to clarify being recommended for daily use in dental practices

V.CONCLUSION

TRAP activities on zirconia discs were similar to titanium discs. There were no significant differences. FE-SEM observation showed that the distribution of differentiated osteoclasts was similar on both titanium and zirconia discs. Both mRNA and protein expression of two osteoclastogenesis marker, NFATc1 and c-Fos, showed no significant differences between titanium and zirconia discs. According to these data obtained from the experiment, osteoclast cells are observed to proliferate and differentiate in similar feature on titanium and zirconia discs. It seems that ability of osteoclast' s behavior on zirconia are comparable to that of titanium discs *in vitro*, suggesting that osseointegration that zirconia could trigger would be similar to titanium when zirconia is used as an implant material. Further experiments should be focused on long-term clinical studies.

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국문초록

지르코니아 임플란트 디스크 표면에서의 파골세포 반응 및 표면 분석

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볼드바얄

목 적: 지르코니아는 좋은 기계강도와 인체적합성 때문에 치과에서의 사용범위가 더욱 넓어지고 있다. 이는 임플란트 재료로서 타이타늄을 대체할 가능성또 보인다. 골 리모델링이 파골세포와 조골세포의 조화를 필요로 하며 이는 골과 임플란트의 결합을 일으키는 osseointegration의 결정적인 요소이다. 파골세포로 인한 골흡수가 임플란트 주변에 일어나며 이는 임플란트 성공여부에 영향을 미친다. 따라서 파골세포의 분화능력과 활성화를 분석할 필요가 있다. 본실험의 목적은 지르코니아의 osseointegration 형성능력을 보기 위해서 지르코니아 디스크위에서의 파골세포 반응을 타이타늄과 비교하여 분석하는 것이다.

재료 및 방 법: 30 개의 Y_2O_3-stabilized tetragonal zirconia polycrystals

(Y-TZP) 지로코니아 디스크 와 30 개의 기계 가공된 타이타늄 디스크를 준비하여 두 개의 군으로 나누었다. 대조군으로 타이타늄 디스크, 실험군으로 지르코니아 디스크가 사용되었다. 두 군의 디스크 표면 거칠기를 confocal laser scanning microscopy (CLSM) 로 분석하였다. 표면 조성을 Energy dispersive X-ray spectroscopy (EDS) 로 확인하였다. Murine RAW 264.7 cell을 파골세포 전구세포로 사용하여 두 가지 디스크 표면에서 최대 6일까지 배양하였다. 지르코니아 표면에서의 파골세포 분화활성을 평가하기 위해 TRAP (Tartrate-resistant acid phosphatase) activity assay를 시행하였다. 지르코니아 표면에서의 세포 분포를 보기 위해 FE-SEM (Field emission-scanning electron microscopy)을 사용하였다. 또한 real time RT-PCR 과 western blot을 각각 실행하여 osteoclastogenesis에서 결정적인 두 가지 전사인자인 NFATc1과 c-Fos의 mRNA 및 단백질 발현 수준을 조사하였다.

결 과: 두 군의 디스크간에 표면거칠기의 유의미한 차이는 나지 않았다 ($p > 0.05$). TRAP activity는 지르코니아 디스크와 타이타늄 디스크간에 유의한 차이가 없었다 ($p > 0.05$). FE-SEM 촬영하여 봤을 때 지르코니아 디스크 표면에서의 분화된 파골세포의 분포도는 타이타늄 디스크와 비슷하였다. 두 가지 osteoclastogenesis marker인 c-Fos와 NFATc1 의 mRNA의 발현은 지르코니아 디스크에서 조금 증가하는 추세를 보였으나 유의성 있는 차이는 없었다 ($p > 0.05$). c-Fos와 NFATc1의 단백질 발현은 두 디스크 간에 비슷한 결과를 보였다.

결론: 지르코니아 디스크 표면에서의 파골세포의 반응은 타이타늄 디스크와 비슷하였다. 이는 지르코니아가 골속에서 사용되었을 때 적절한 osseointegration 을 일으킬 수 있을 거라고 보여진다. 그러나 지르코니아 임플란트의 장기적인 효능을 보기 위해서 follow-up 연구가 필요로 하다.

주요어 : 지르코니아 디스크, 파골세포, Osteoclastogenesis, 타이타늄 디스크, 임플란트

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