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Effects of Niobium Ions Released from Calcium Phosphate Invert Glasses Containing Nb_2O_5 on Osteoblast-Like Cell Functions

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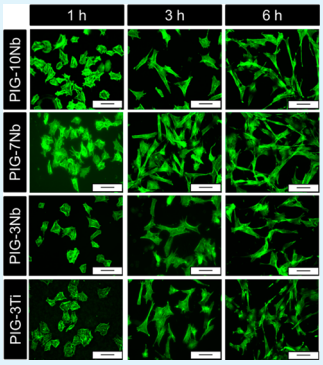
ABSTRACT: The effects of niobium ions released from $60CaO\text{-}30P_2O_5\text{-}(10\text{-}x)Na_2O\text{-}xNb_2O_5$ (mol %, x = 0−10) glasses on MC3T3-E1 cell functions were evaluated by culture tests with two systems; cell culture on glass plates, or in culture media containing glass extracts. Alkaline phosphatase (ALP) activity in the cells cultured on the glass plates containing 3 and 5 mol % of Nb_2O_5 was significantly higher than that on the Nb_2O_5-free glass, although proliferation was not enhanced on all glasses containing Nb_2O_5. Cells cultured in the medium containing 3×10^{-7} M niobium ions showed the highest ALP activity in comparison with other Nb-containing media or normal medium, regardless of the presence of osteogenic factors (ascorbic acid, β-glycerophosphate and dexamethasone) in the media. Calcium deposition by the cells cultured in the medium containing 3×10^{-7} M niobium ions was twice as high as those cultured in medium containing no niobium ions. The effects of niobium ions were thought to depend on ion concentration, and to enhance differentiation and mineralization of osteogenic cells rather than their initial adhesion or proliferation.



KEYWORDS: *niobium ion, calcium phosphate glass, osteoblast-like cell, bone tissue, bioactive glass, ionic dissolution product*

1. INTRODUCTION

Bioactive glasses based on silicate or phosphate systems have been developed for the use in medical and dental fields, and are used as bone fillers, bone tissue engineering scaffolds, bioactive coatings for bioinert metal biomaterials, and tooth fracture treatment.[1−8] These glasses can be shaped as desired and offer flexibility in the chemical components comprising the glass. Phosphate invert glasses (PIGs) have been attracting attention in the medical field because they are completely soluble and exhibit a neutral pH in aqueous media.[9−13] PIGs in the $60CaO\text{-}30P_2O_5\text{-}7Na_2O\text{-}3TiO_2$ system (PIG-3Ti) have been reported to have excellent bioactivity through in vitro and in vivo tests.[14−19] For example, a titanium alloy coated with a PIG-3Ti layer was covered with a new bone tissue after a 4-week implantation period in a Japanese rabbit, and bonelike apatite was quickly formed on PIG-3Ti in a simulated body fluid.

Various proteins have been reported to enhance tissue regeneration, and some of these have been doped into biomaterials to give implant materials that deliver proteins directly to specific regions of the body.[20−22] On the other hand, some inorganic elements also show enhancement effects, particularly with regard to bone tissue regeneration. Recently, ionic dissolution products from the bioactive glasses, such as silicon, calcium, phosphorus, magnesium, strontium, boron, vanadium, and cobalt, have been reported to stimulate osteogenic cell functions, such as proliferation, differentiation and mineralization.[23−31] The cytotoxicity of some metal ions has also been investigated by cell culture tests.[32] On the other hand, some of these elements, such as zinc and lanthanum, have a high limit of their concentration to up-regulate the osteogenic cell functions.[33,34] Silicon ions showed the dependence on the cell type to exhibit the up-regulating effects.[35] Therefore, it is important to find the optimal concentration for each element to enhance cell function and to understand the dependence of each element on cell type in order to achieve rapid bone tissue regeneration using biomaterials that release these elements. The elements are generally expected to be released from the biomaterials gradually and continuously, and glass releases elements appropriately, as it has a homogeneous chemical structure and releases the elements comprising it with its dissolution.

Some papers have reported that niobium shows lower cytotoxicity (IC_{50} of niobium ions for MC3T3-E1 cells is 1.47×10^{-3} mol/L) when compared with other metal ions, and that they enhance human osteoblast function.[32,36] However, it remains unclear whether there is a limit necessary to achieve these enhancing effects. On the other hand, niobium ions replace phosphorus ions in phosphate glasses, which are fabricated by a melt-quenching method, resulting in the formation of O−P−O−Nb−O type chains.[37,38] This means that bioactive phosphate glasses in which niobium ions are homogenously present could be fabricated and should release niobium ions continuously in association with dissolution. The aims of the present study were to prepare PIGs containing niobium, to evaluate their ion-releasing ability in culture medium and their cellular compatibility by culturing the

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osteoblast-like cells on PIG plates, and to examine cell functions in culture medium containing ionic dissolution products from PIG containing niobium. Nb-doping to phosphate glasses will be regarded to be useful not only for preparing the glass systems but also for enhancing their bioactivitis, if up-regulating effects of the extract from the PIGs containing niobium on cell functions, such as proliferation, differentiation, and mineralization, could be clarified in more detail. As biomaterial surface properties, such as topography, wettability and pH, are regarded to influence cell function,[39−44] these surface properties of the prepared PIGs were also characterized. A larger amount of ionic dissolution products should be present in the vicinity of the glass surface and will vary with time, because the ions are released with glass dissolution. This means that ion concentrations may change depending on distance from the glass surface. Hence, cell reactions were compared between two culture conditions; culture on the glass plate surface, and culture in medium containing ionic dissolution products. Cell reactions against PIGs containing niobium were also compared with those against PIG-3Ti, which contains no niobium and has already been confirmed to be bioactive by in vivo testing,[14] in order to evaluate their utility as bioactive materials. The effects of niobium ions released from or contained in the glass plate on the cell functions were investigated by comparing the cell reactions against different types of PIGs, giving consideration to the surface properties of each sample.

2. MATERIALS AND METHODS

2.1. Glass Preparation. PIGs containing various contents of Nb_2O_5 were prepared by the conventional melt-quenching method using stoichiometric quantities of the following precursors; $CaCO_3$, H_3PO_4, NbO_5, Na_2CO_3 and TiO_2 (Kishida Chemical CO., Ltd., Japan). The nominal compositions of the glasses are shown in Table 1.

Table 1. Nominal Compositions of Test Glasses (mol %)

code	CaO	P_2O_5	Na_2O	Nb_2O_5	TiO_2
PIG-3Nb	60	30	7	3	0
PIG-7Nb	60	30	3	7	0
PIG-10Nb	60	30	0	10	0
PIG-3Ti	60	30	7	0	3

The precursor mixture was melted in an electric furnace at 1400 °C for 30 min. After melting, the glass was rapidly quenched by pouring onto a stainless-steel plate at room temperature and allowed to cool.

2.2. pH and Ion Release Measurements for Glass Plate Sample. Powders with <50 μm in size of the 4 types glasses (Table 1) were prepared with a planetary ball mill ($P\bar{6}$, Fritsch Japan Co., Ltd., Japan) and used for examining the pH change and ion release in cell culture medium. Glass powders were dry-heated at 180 °C for 90 min for sterilization. Four milligrams of glass powders were added to 4 mL of culture medium prepared using minimum essential medium alpha modification (MEMα, Wako, Japan) containing 10% fetal bovine serum (Life Technorogies Japan Ltd., Japan) (denoted by "normal medium" hereafter), followed by incubation at 37 °C in a humidified atmosphere of 95% air, 5% CO_2. pH and ion release measurements were carried out at 1, 3, 5, and 7 days. The ion concentration and pH of the medium were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ICPS-500, Shimadzu, Japan) and pH-metry (F-51, Horiba Ltd., Japan), respectively. The pH and ion release amounts were estimated by one test.

2.3. Contact Angle Measurement for Glass Plate Samples. Glass plates that were polished using sandpaper (#600) and given a mirror finish were used for contact angle measurement. All specimens were treated with the same method to make the same surface roughness, since surface roughness is one of the important factors affecting wettability and cell functions. Distilled water (5 μL) was dropped onto the glass plates. The contact angle of the water was estimated using the average values of 5 samples for each glass.

2.4. Cell Culture Test for Glass Plate Sample. Glass plates with dimensions of 4 × 4 mm after polishing to a mirror finish and sterilization as described above were used for cell culture tests. Glass plates were placed on a 96-well plate. Observation of cell adhesion was then carried out at 1, 3, and 6 h. Mouse osteoblast-like cells (MC3T3-E1 cell) were seeded onto the glass plate with a density of 10 000 cells/well and were then incubated at 37 °C in a humidified atmosphere of 95% air, 5% CO_2. Samples for fluorescence microscopy observation were fixed in 4% paraformaldehyde phosphate buffer solution at 4 °C for 30 min and were treated with permeabilizing solution consisting of 1% of bovine serum albumin, 0.1% Triton X, and 98.9% phosphate buffer solution (PBS) at 4 °C for 25 min. Alexa Fluor 488 Phalloidin (Molecular Probes, USA) diluted with PBS was added to the sample wells. Samples were kept in a dark space for 30 min at 37 °C, and were observed under a fluorescence microscope (BIOREVO BZ9000, KEYENCE, Japan). Three plates of each glass sample were tested.

Cell proliferation was examined after culture for 1, 3, 5, and 7 days. MC3T3-E1 cells were seeded on the glass plate with a density of 20 000 cells/well and were then incubated at 37 °C in a humidified atmosphere of 95% air, 5% CO_2. Culture medium was changed every other day. The cell number on the glass plate was measured with a microplate reader (SUNRISE Remote, TECAN, Switzerland) using a Cell Counting Kit-8 (CCK-8, Dojindo, Japan), in accordance with the manufacturer's instructions. Cells were counted by measuring the absorbance of the resulting medium at 450 nm. Initial adhesion rate of the cells on each glass plate was also estimated by counting the cell numbers on the plate after 3 h of culture, as described above. Three plates of each glass sample were tested.

Cell differentiation was examined after culture for 5, 7, and 9 days in modified culture medium. Modified culture medium was prepared by adding 0.0025 g of ascorbic acid, 0.0073 g of β-glycerophosphate, and 1 mL of dexamethasone (100 nM), which are osteogenic factors, into 500 mL of the normal medium. These reagents were purchased from Wako (Japan). MC3T3-E1 cells were seeded on the glass plate at a density of 2000 cells/well. Culture medium was changed every other day. Alkaline phosphatase (ALP) activity in the cells was measured with a microplate reader using a kit (p-nitrophenyl phosphate tablets, Sigma-Aldrich Corporation, USA), in accordance with the manufacturer's instructions. ALP activity was estimated by measuring the absorbance of the resulting medium at 400 nm, and cell number was estimated as described above. Three plates of each glass sample were tested. The seeding number of the cells for each test was decided referring several reports and the results of our pretests using the same cells as those used in this work.

2.5. Preparation of Cell Culture Media Containing Niobium Ions. The $60CaO\text{-}30P_2O_5\text{-}10Nb_2O_5$ glass (mol %, PIG-10Nb) was used for the preparation of cell culture medium containing trace amounts of niobium ions. Glass powders of <50 μm particle size were prepared and sterilized with the same method as that in section 2.2. Glass powders (2 g) were added to normal culture medium or modified medium (200 mL), and were incubated for 5 days. The resulting medium was diluted in order to adjust the niobium ion concentration to 1×10^{-8} to 1×10^{-5} M.

2.6. pH and Ion Concentration of Media Containing Niobium Ions. pH, and calcium and phosphorus concentrations of the prepared media were measured by pH-metry and ICP-AES, respectively. pH measurement was carried out after 2 and 24 h of incubation. One sample of each medium was used for these measurements.

2.7. Cell Culture Test for Media Containing Niobium Ions. The proliferation, differentiation, and mineralization of MC3T3-E1 cells were examined after culture in the prepared Nb-containing media (derived from the normal medium). Cells were seeded in a 24-well plate at a density of 10 000 cells/well and were cultured in Nb-containing media. The proliferation test was performed using the Nb-

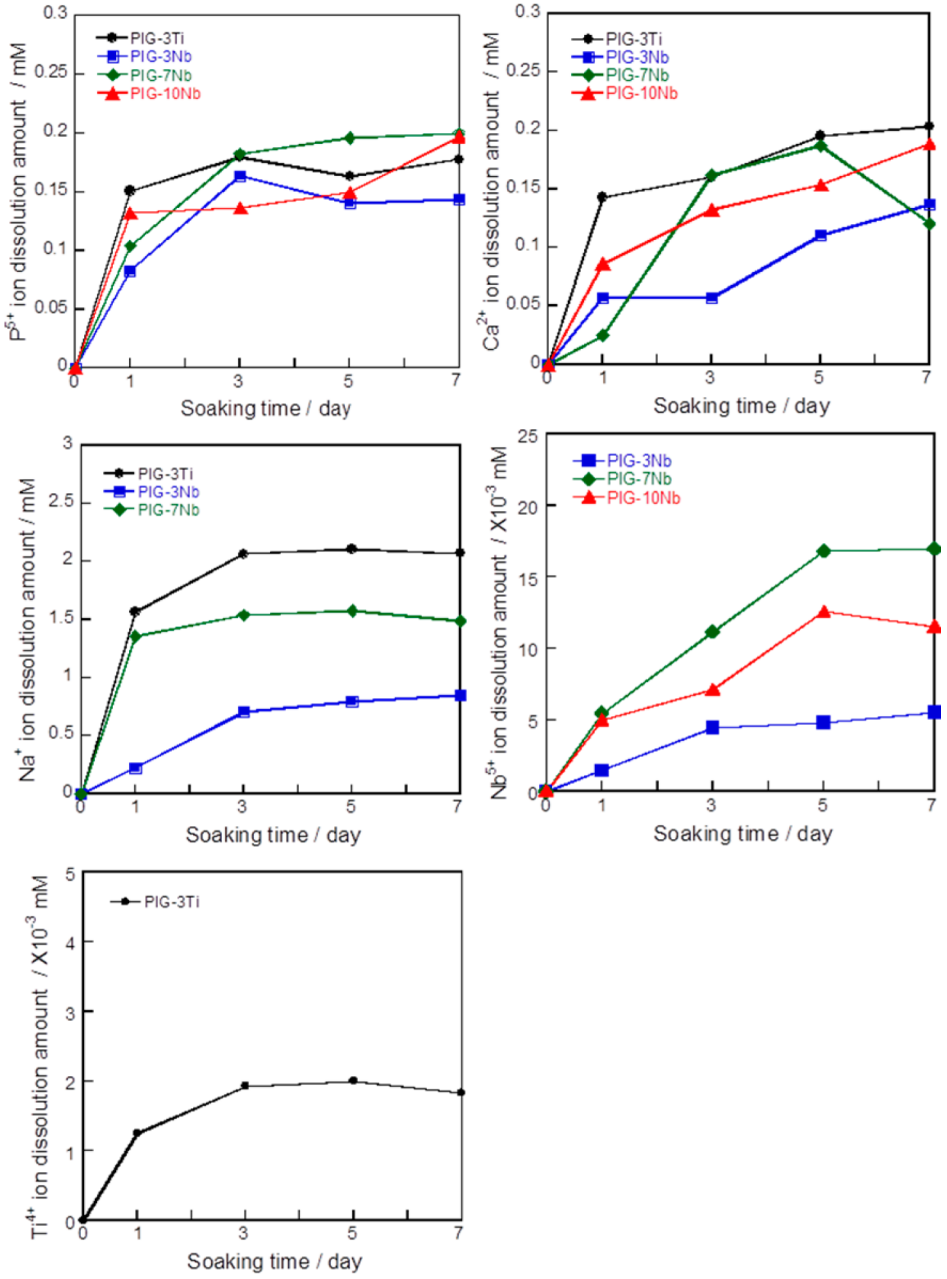

Figure 1. Ion dissolution from phosphate invert glass particles containing various contents of Nb_2O_5 in the normal medium. Soaking time "0" means the time before soaking the samples.

containing media prepared using the normal medium. The differentiation test (the ALP activity test) was performed in two different medium conditions, the Nb-containing media prepared using the normal medium and the modified one. The same seeding-number of the cells was used for these tests to compare the cell reactions in between the two different medium conditions. The cells were incubated in the same conditions as those mentioned in section 2.4. Culture medium was changed every other day. The number of cells and ALP activity were measured with the same methods as those mentioned in section 2.4. Calcium deposition by the cells was also evaluated. Cells were seeded in a 24-well plate at a density of 25 000 cells/well and were cultured for 7 days using the Nb-containing media prepared using the modified medium. After culture, 1 mL of HCl solution (2 *M*) was added to the well to dissolve the minerals generated by the cells. Calcium in the well was then measured using the Calcium E test (Wako), in accordance with the manufacturer's instructions. Calcium deposition amounts were normalized against those of controls (the modified medium containing no extract from the glass). Three plates of each glass sample were used for the proliferation, differentiation and mineralization tests.

2.8. Statistics. The results of cell culture tests are expressed as means ± SD for the number of experiments indicated, unless stated otherwise. Statistical significance was measured by ANOVA followed by Tukey's test ($p < 0.05$).

3. RESULTS

3.1. Glass Characterization. Figure 1 shows the ion levels after dissolution of each glass powder in culture medium. Calcium, phosphorus and sodium ions are present in the normal medium, but the amounts of these ions increased after dissolution of the glass, and except for sodium ions, they eventually reached similar levels as in PIG-3Ti. The amounts of sodium and niobium ions released from the glasses increased with their contents in each glass system. The solubility of these

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glasses has not checked, but these ion release behaviors are expected to well reflect it, since niobium ion replaces phosphorus ion in phosphate glasses and could be present homogenously in them. Figure 2 shows the pH changes in the culture media after dissolution of glass powders. pH reached 8.2−8.4 at day 1, regardless of glass composition.

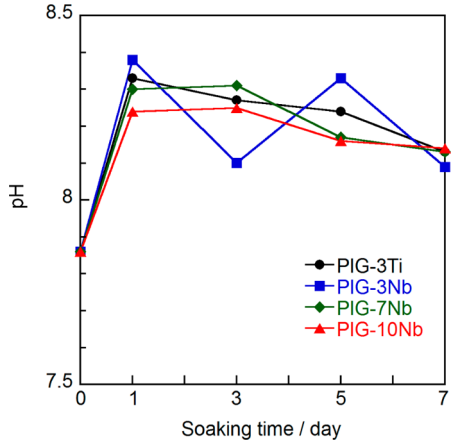

Figure 2. pH changes in the normal medium after incubation with phosphate invert glass plates containing various amounts of Nb_2O_5. Soaking time "0" indicates time before soaking with samples.

The contact angles of the glass plate samples described in Table 2 showed almost no significant differences among the

Table 2. Contact Angles for Glass Plate Samples (mean ± SD)

	PIG-3Ti	PIG-3Nb	PIG-7Nb	PIG-10Nb
angle (deg)	85 ± 3	88 ± 6	88 ± 3	95 ± 4

prepared samples, with the values reaching approximately 85−95°. Contact angles of the glass plates were not considered to be a serious factor for comparing cell reactions among the glasses, since there were no significant differences in the values ($p < 0.05$). To add Nb_2O_5 to PIG glasses, however, may influence their hydrophilicity, since the mean value of PIG-10Nb was 10° larger than that of PIG-3Ti.

3.2. Cell Reactions on the Glass Plate Samples. Cell adhesion at earlier stages of culture was observed by fluorescence microscopy. Figure 3 shows actin filaments (green) in the cells after culture for 1, 3, and 6 h. There were no obvious differences in the cell morphologies cultured on the glass plate samples in the results of visual comparison, with the cells showing round and spindle shapes after culture for 1 and 6 h, respectively. The cells spread wider as culture period increased. Table 3 shows the initial adhesion rates estimated by counting the cells on each glass sample after 3 h of culture. There were no significant differences in the rates among the samples, with the rates being 30−40%.

Figure 4 shows the cell numbers on each glass plate. The numbers of PIG-3Nb at day 5 and 7 were significantly smaller than those of PIG-3Ti, although they of PIG-7Nb and −10Nb were almost the same as those of PIG-3Ti. Figure 5 shows the ALP activity of the cells after culture for 5, 7, and 9 days using modified culture medium. The activity values of all samples peaked on day 7. The values for PIG-3Nb and −7Nb were significantly larger than those of PIG-3Ti on day 7.

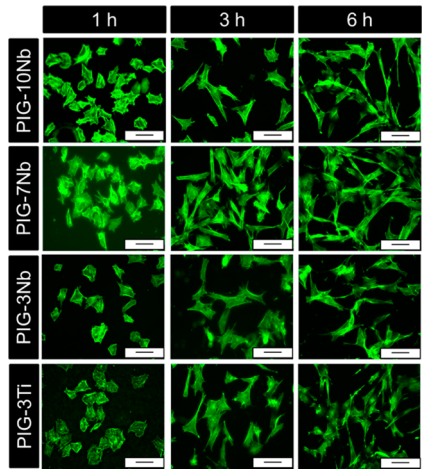

Figure 3. Fluorescence microscopic images of MC3T3-E1 cells cultured on phosphate invert glass plates containing various amounts of Nb_2O_5. Actin filaments are indicated with green color. Scale bar 50 μm.

Table 3. Initial Cell Adhesion Rates for Glass Plate Samples (mean ± SD)

	PIG-3Ti	PIG-3Nb	PIG-7Nb	PIG-10Nb
rate (%)	41 ± 1.4	33 ± 6.6	41 ± 2.5	35 ± 2.9

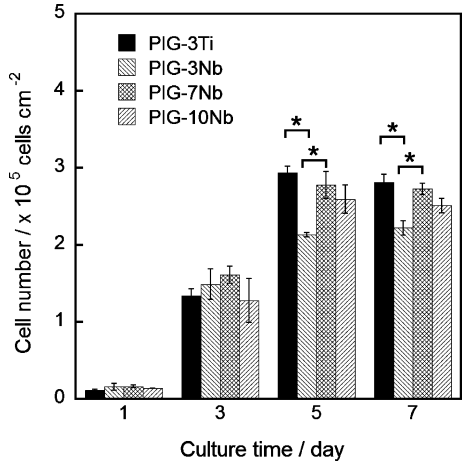

Figure 4. Numbers of MC3T3-E1 cells cultured on phosphate invert glass plates containing various Nb_2O_5 contents. Error bar means SD. Three samples of each sample were used. (*$p < 0.05$).

3.3. Characterization of Nb-Containing Media. Table 4 shows the niobium ion concentrations in the prepared Nb-containing media. There were almost no differences in the calcium and phosphorus ions among the media, including a control (the normal medium), since the values of increased concentrations of the two ions in the Nb-containing media were very small; they were ~0.1 mM for each ion. All the media contained 2.7−2.8 mM calcium ions and 1.3−1.4 mM phosphorus ions. The changes in the concentrations of the two ions were regarded not to be serious factors for comparing the cell functions. The pH values of the prepared Nb-containing media were measured after incubation for 2 and 24 h in the CO_2 incubator. The pH values were 7.4−7.5 at all time points.

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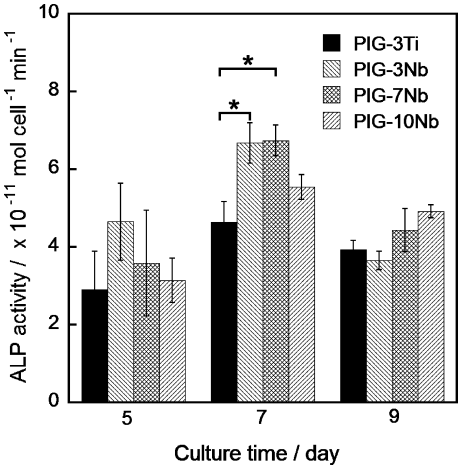

Figure 5. ALP activities in MC3T3-E1 cells cultured on phosphate invert glass plates containing various amounts of Nb_2O_5. Modified culture medium (supplemented with osteogenic factors) was use. Error bar means SD. Three samples of each sample were used. (*p < 0.05).

3.4. Cell Reactions in Nb-Containing Media. Figure 6 shows the numbers of cells cultured in Nb-containing media or normal medium (Control) for 7 days. Cells successfully proliferated in all the media through the culture period. There were no significant differences in the numbers among the samples at all time points. The ALP activity in the cells shown in Figure 7 demonstrated that differentiation was enhanced in Nb 10^{-7}. All media used for this test were derived from normal medium, i.e., it was not supplemented with osteogenic factors. ALP activity in all media peaked on day 14, except for $Nb10^{-5}$. Activity in $Nb10^{-7}$ was significantly higher when compared with $Nb10^{-8}$, $Nb10^{-5}$ and Control by day 14. Only $Nb10^{-5}$ had a significantly higher activity than Control on day 21.

Figure 8 shows the ALP activity in cells cultured in various media derived from the modified culture medium (supplemented with the osteogenic factors). The activity was measured in different time scale from that of Figure.7, because our pretest using the same cells showed the activity reached a maximum quite earlier in the modified medium than the normal one. Since the activities in all the media decreased after day 5, they were expected to reach a maximum at day 5 or before. The activity in $Nb10^{-7}$ was significantly higher than when compared with $Nb10^{-8}$, $Nb10^{-5}$ and Control on day 5. The activity in $Nb10^{-5}$ was significantly higher than that in Control on day 15. Figure 9 shows the calcium deposition by cells cultured in various media derived from the modified culture medium (supplemented with osteogenic factors) for 7 days. Calcium deposition was only seen in $Nb10^{-7}$, $Nb10^{-6}$ and Control, and the value in $Nb10^{-7}$ was twice that in Control.

4. DISCUSSION

The mineralization process of MC3T3-E1 cells is generally categorized into three stages; proliferation and expression of collagen-I, maturation characterized by expression of ALP and osteopontin, and mineralization characterized by expression of

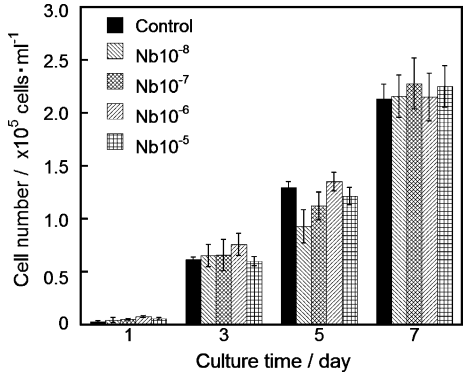

Figure 6. Numbers of MC3T3-E1 cells cultured in media containing various amounts of niobium ions. Error bar means SD. Three samples of each sample were used.

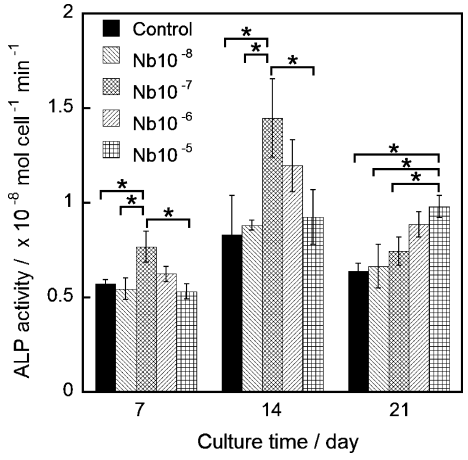

Figure 7. ALP activity in MC3T3-E1 cells cultured in media containing various amounts of niobium ions. Normal medium (containing no osteogenic factors) was used. Error bar means SD. Three samples of each sample were used. (*p < 0.05).

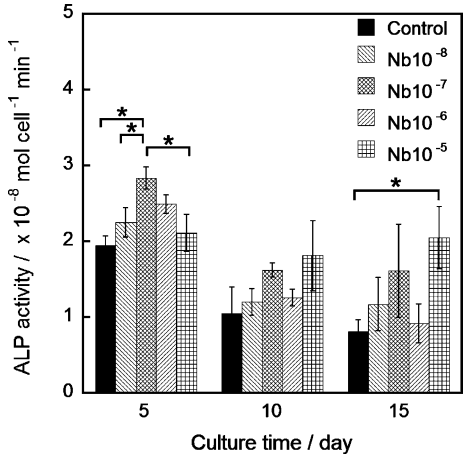

Figure 8. ALP activity in MC3T3-E1 cells cultured in media containing various amounts of niobium ions. Modified culture medium (supplemented with the osteogenic factors) was used. Error bar means SD. Three samples of each sample were used. (*p < 0.05).

Table 4. Niobium Ion Concentrations in Each Medium

	$Nb10^{-8}$	$Nb10^{-7}$	$Nb10^{-6}$	$Nb10^{-5}$	control
conc (mol/L)	3.97×10^{-8}	3.44×10^{-7}	2.33×10^{-6}	1.78×10^{-5}	N.D.

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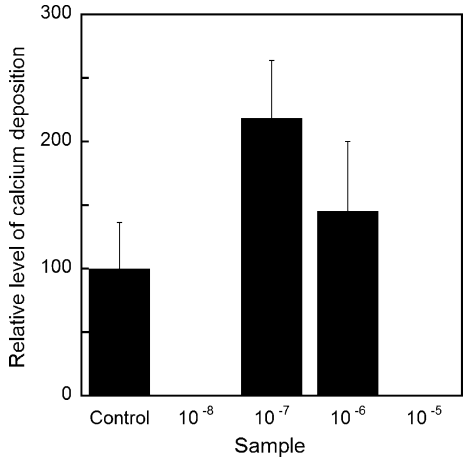

Figure 9. Calcium deposition by MC3T3-E1 cells cultured in media containing various amounts of niobium ions. Modified culture medium (supplemented with the osteogenic factors) was used. Error bar means SD. Three samples of each sample were used.

osteocalcin and bone nodule.[45] Any gene expression was not investigated in the present work but we supposed from the results of ALP activity tests, in the case of the Nb-containing media, the transition point from the second stage to the third stage was thought to be at day 14 for culture tests using media derived from normal culture medium (Figure 7) and at day 5 for tests using media derived from modified medium (Figure 8). The transition point was thought to be at day 7 for tests using media derived from modified medium and PIGs containing Nb_2O_5 (Figure 5). ALP activity in the Nb-containing media with higher concentrations of niobium ions and on the PIGs containing larger amounts of Nb_2O_5 remained high for longer periods, regardless of the medium type (Figures 5, 7, and 8). As a result, the transition points for these samples were unclear. One reason for this may be the relationship between niobium ions and ALP expression. ALP activity is reportedly associated with the maturation of the matrix, but its major role is cell metabolism before bone formation.[46−48] ALP activity generally decreases when calcification starts, and the timing of maximum activity depends on culture conditions.

In the present study, cells were cultured in two types of culture medium; normal and modified (supplemented with osteogenic factors; ascorbic acid, β-glycerophosphate, and dexamethasone). These osteogenic factors are considered to induce osteogenic differentiation and mineralization of osteoblasts and mesenchymal stem cells.[49,50] On the other hand, inorganic elements, such as silicate ions, have been also reported to induce these processes without osteogenic factors.[27−29] In the case of niobium ions, Tamai et al. reported that ALP activity and calcium deposition in normal human osteoblasts were enhanced in medium containing β-glycerophosphate and $>1 \times 10^{-5}$ M niobium ions.[36] However, there have been no reports showing whether the ions are able to stimulate osteoblasts to undergo osteogenic differentiation and mineralization without any osteogenic factors. In the present study, the ALP activity of MC3T3-E1 cells was significantly higher in $Nb10^{-7}$ when compared with Control or other Nb-containing media, despite none of the media containing osteogenic factors (Figure 7). This may indicate that the niobium ions not only enhance but also induce the osteogenic differentiation, because no difference was found in the calcium and phosphorus ion concentrations or pH of among all the media. The ALP activity test has not been performed for the cells cultured on the PIGs glass plates, but their differentiation may be induced, since the cells were stimulated to differentiate in the Nb-containing media. Further investigation will be necessary to clarify the mechanism of the stimulatory effects of niobium ions on cell functions.

Yttrium ions were reported to stimulate osteogenic cell functions, such as proliferation, differentiation and mineralization, although the stimulatory effects depended on ion concentration in the culture medium.[51] Silicon and zinc ions have been also reported to show similar trends in their stimulatory effects.[30,33] In addition, in the case of these two ions, their effects were reported to depend on characteristics such as tissue origin.[33,35] In this study, the effects of niobium ions on cell functions depended on their concentration in the culture medium or in the glass system; ALP activity and calcium deposition in $Nb10^{-7}$ were higher than those in other media. On the other hand, activity and deposition by normal human osteoblasts were reported to be enhanced after culture Nb-containing media derived from $NbCl_5$ with $>1 \times 10^{-5}$ M niobium ions, but no enhancement was seen in media with 1×10^{-7} to 1×10^{-6} M. Thus, the effects of niobium ions also appear to affect induction of differentiation and mineralization of osteogenic cells, rather than initial adhesion or proliferation, depending on the ion concentration.

The pH of the culture medium reached 8.3 after soaking the glass plate samples. pH levels of >8, which exceed the standard physiological value of 7.4, are reported to be good for cell proliferation and differentiation.[40] PIG-3Ti has been already been shown to induce no inflammatory reactions with natural bone tissue in in vivo tests using rabbits.[14] Therefore, PIGs containing Nb_2O_5 were not considered to inhibit cell functions or to have any inflammatory effect on the natural tissue because of pH increases. ALP activity in the cells cultured on PIG-3Nb and PIG-7Nb was significantly higher than those cultured on PIG-3Ti and PIG-10Nb at day 7 (Figure 5). The exact concentration of niobium ions in each medium could not be estimated, because the medium was exchanged with fresh medium (medium containing no niobium ions) every other day throughout the culture period. Thus, PIGs containing smaller amounts of Nb_2O_5 are expected to be better candidates as materials to enhance osteogenic cell functions.

5. CONCLUSION

The effects of niobium ions released from glasses in the $60CaO\text{-}30P_2O_5\text{-}(10\text{-}x)Na_2O\text{-}xNb_2O_5$ (mol %, x = 0−10) system on MC3T3-E1 cell functions were evaluated using two types of culture system: culture of cells on glass plates containing Nb_2O_5; and culture in medium containing extracts from the glass. ALP activity in the cells cultured on glass plates containing 3 and 5 mol % of Nb_2O_5 was significantly higher than that on the glass containing no Nb_2O_5, although proliferation was not enhanced on glasses containing Nb_2O_5. Cells cultured in medium containing 3×10^{-7} M niobium ions showed the highest ALP activity in comparison with the other Nb-containing media or normal medium, regardless of whether medium was supplemented with osteogenic factors. The effects of niobium ions are thought to depend on ion concentration and to induce differentiation and mineralization of osteogenic cells rather than initial adhesion or proliferation.

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Notes

The authors declare no competing financial interest.

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