Evaluation of cytocompatibility and osteoconductivity of Zr-14Nb-5Ta-1Mo alloy with MC3T3-E1 cells

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The cytocompatibility and osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy were investigated using a mouse osteoblastic cell line (MC3T3-E1) to promote the application of this newly developed alloy in dental/medical treatment. The initial cell-attached morphology was visualized by fluorescent staining, and cells cultured on the Zr alloy showed similar cell adhesion behavior to cells cultured on titanium (Ti). In our 5-day proliferation investigation, similar cell numbers were obtained with both Zr alloy and Ti. These results indicate that the cytocompatibility of Zr alloy is similar to that of Ti. In addition, the similar results in the evaluation of alkaline phosphatase (ALP) activity and staining of deposited calcium using alizarin red S with both Zr alloy and Ti indicated that the osteoconductivity of the Zr alloy is similar to that of Ti. Our results prove the good cytocompatibility and osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy, enabling its promotion for use in dental/medical applications.

Keywords: Zirconium alloy, Cytocompatibility, Osteogenic differentiation, Calcification

INTRODUCTION

Zirconium (Zr) alloys have recently been investigated for application in medical devices and implants. Zr and titanium (Ti) belong to the fourth group of the periodic table and have similar properties. Additionally, there is no evidence that Zr is toxic. The cytotoxicity of 43 metal salts was tested using two types of cell lines; Zr in the form of $ZrCl_4$ was categorized as the safest element⁽¹⁾. The corrosion properties, cell morphology, size, proliferation, and mitochondrial activity of MC3T3-E1 and GM7373 cells were evaluated on polished Zr. The biocompatibility range was investigated and showed following order of the investigated metals: Nb>Ta, Ti, Zr>aluminum (Al)>316 L stainless steel (SS)>molybdenum (Mo)⁽²⁾. Zr showed excellent cell proliferation and a variety of alkaline phosphatase (ALP) activities in SaOS₂ cells⁽³⁾. The bone quality formed on Zr implants is at least as good as that on Ti when evaluated by the mineralization level, mineral composition, and alignment and order of mineral particles⁽⁴⁾.

Magnetic resonance imaging (MRI) that is a used medical imaging diagnostic method even in dentistry is not suitable for patients with metal implants or other non-removable metals inside their bodies, as MRI uses a strong magnetic field for scanning. Unacceptable artifacts and image defects appear around metallic devices owing to the differences in magnetic susceptibility between

metals and surrounding tissues⁽⁵⁻⁷⁾, and the additional magnetocaloric effect precludes these patients from undergoing an MRI examination safely. However, it is difficult to alter the inherent properties of metals, for example, to reduce the magnetic susceptibility of metals for a safe MRI examination of patients who have metal implants or other non-removable metal devices. Therefore, considering the demands of dental and medical use, the development of new alloys with relatively low magnetic susceptibility is urgently needed.

The MRI artifact volume of metal is proportional to its magnetic susceptibility⁽⁹⁾. Pure Zr shows low magnetic susceptibility and high corrosion resistance but poor yield and tensile strength. Therefore, Zr alloys strengthened with solid-solution strengthening elements have been developed to decrease the artifact volume of implant devices. Zr-Nb, Zr-Ta, and Zr-Mo system alloys are promising candidates because the magnetic susceptibilities of Nb, Mo, and Ta are low, and all these elements are safe. To date, many attempts have been made to develop new Zr alloys with low magnetic susceptibility, including Zr-Nb⁽⁸⁻¹⁰⁾, Zr-Ta^(11,12), Zr-Mo⁽¹³⁻¹⁵⁾, and Zr-Ag alloys⁽¹⁶⁾. According to these reports, Zr-14Nb and Zr-1Mo alloys of decreased magnetic susceptibility have been developed. Also, as reported previously, dental casings or dental porcelains were fabricated using Zr-14Nb⁽¹⁷⁻²⁰⁾ and Zr-1Mo alloys and used in additive manufacturing to produce MRI-compatible device fimbriation by three-dimensional printing⁽²¹⁻²⁵⁾. For the Zr-14Nb alloy, the dental casting ability and mechanical

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properties of the cast are acceptable¹⁷⁾, and the bonding strength of dental porcelain is good enough¹⁸⁾. In addition, a white oxide layer consisting predominantly of monoclinic ZrO₂, tetragonal ZrO₂, and Nb₂O₅ is formed by heating that can be used for the preparation of the dental implant abutment²⁶⁾.

Recently, a new beta-type Zr-14Nb-5Ta-1Mo alloy was prepared according to the d-electron alloy design method²⁷⁾ showing magnetic susceptibility of $16.96 \times 10^{-9} \text{ m}^3 \text{ kg}^{-1}$, approximately one-fifth that of Co-Cr-Mo alloys and one-third that of Ti alloys. Its high strength (798 MPa), elongation (15%), and low Young's modulus (61 GPa) suggest great potential for dental treatment; these properties together with low magnetic susceptibility, Young's modulus, large strength, and elongation are not achieved by Ti alloys. It showed that the Zr-14Nb-5Ta-1Mo alloy is a promising material in dentistry and medicine.

However, the cytotoxicity and hard-tissue compatibility of the Zr-14Nb-5Ta-1Mo alloy are crucial factors for use in dental and medical applications. In this study, the cytocompatibility and osteoconductivity of this Zr alloy were investigated using a commonly used mouse preosteoblast cell line (MC3T3-E1). Our investigation into Zr-14Nb-5Ta-1Mo alloy properties could not only promote this hard-tissue compatible alloy's use in dental implant fixtures, but also other potential oral treatments, including maxillofacial prostheses.

MATERIALS AND METHODS

Specimen preparation

The theoretical design of the Zr-14Nb-5Ta-1Mo alloy was described in a previous report²⁷⁾. The starting material in the present study was an as-cast Zr alloy ingot with a diameter and weight of 130 mm and 6 kg, respectively, which was formed by induction skull melting from weighed commercially pure Zr crystal bars (99.8%), Nb chips (99.8%), Ta chips (99.95%), and a piece of Mo wire (99.95%). The chemical composition of the alloy is presented in Table 1. Then, the ingot was subjected to hot forging at 1,323 K to form homogenized rods with

a diameter of 32 mm, and the diameter was decreased to 24 mm with peeling of the oxidized shell by lathe machining. Subsequently, the forged Zr-14Nb-5Ta-1Mo rods with 8 mm in diameter were processed using wire electrical discharge machining. The rod was cut into disks with a thickness of 1.5 mm. The disk specimens were mechanically polished with a series of waterproof SiC abrasive papers and mirror-polished using a colloidal SiO₂ suspension. After surface polishing, the specimens were rinsed two times in acetone and one time in ethanol.

In addition, a commercial-pure Ti rod (grade 2, diameter of 8 mm, The Nilaco, Tokyo, Japan) was purchased and similarly surface mirror-polished and used as a control.

Surface roughness evaluation

Surface morphology and surface roughness parameters (Sa; arithmetic average height) of Zr-14Nb-5Ta-1Mo alloy and Ti specimens were measured by scanning electron microscopy (SEM; S-3400NX, Hitachi, Tokyo, Japan, not shown) and a three-dimensional measurement laser microscope (LM; LEXT OLS4000, Olympus, Tokyo, Japan), respectively. Five different areas were randomly selected for each specimen, and Sa was evaluated using bundled photo analysis software with ISO25178. Five specimens were evaluated for each metal.

Cell culture and induction of osteogenic differentiation

A mouse calvaria-derived preosteoblast cell line, MC3T3-E1, was purchased from RIKEN BioResource Center (Tsukuba, Japan) and used directly. As described in previous reports²⁸⁻³⁰⁾, cells were cultured at 37°C in a humidified atmosphere of 5% CO₂ in air and maintained in a growth medium composed of the minimum essential medium Eagle with alpha modification (α -MEM, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and 100 U L-glutamine- penicillin-streptomycin solution (G1146, Sigma-Aldrich).

To induce osteogenic differentiation of the MC3T3-E1 cells, the growth medium was shifted with a differentiation induction medium after 100%

Table 1 Concentrations of elements in Zr-14Nb-5Ta-1Mo alloy and CP Ti and the surface roughness (Sa) after polishing

Specimen	Concentrations of elements (wt%)											Sa (μm)
	Zr	Nb	Ta	Mo	Fe	Ti	C*	O**	N***	H**		
Zr-14Nb-5Ta-1Mo alloy	Bal.	13.5	4.96	1.00	<0.01	<0.01	<0.01	0.037	0.014	0.009	Mukai-Tanko	0.008±0.001
CP Ti (Grade 2)	—	<0.01	—	—	0.034	Bal.	0.004	0.109	0.004	0.001	Aichi Steel	0.012±0.001

* Determined by infrared absorption spectroscopy after combustion in a high-frequency induction furnace with a LECO CSLS-600;

** Determined by an inert gas fusion-thermal conductivity detection using LECO TCH-600;

*** Determined by an inert gas fusion-infrared absorption spectroscopy using LECO TCH-600;

Other elements were determined by inductively coupled plasma atomic emission spectroscopy (OPTIMA 8300, PerkinElmer).

The values of Sa are presented as mean±standard deviation values (n=10).

confluence³¹⁻³³), and the differentiation induction medium consisted of growth medium supplemented with 50 mg mL⁻¹ L-ascorbic acid (Wako, Osaka, Japan) and 2 mM β -glycerophosphate (Wako). The differentiation induction medium was replaced every three days.

Before cell seeding, the Zr-14Nb-5Ta-1Mo alloy and Ti specimens were sterilized by immersion in 70% ethanol and then fully rinsed with ultra-pure water.

Fluorescent staining

The extension morphology of cells on both specimens was visualized with fluorescent staining obtained after 3, 6, and 24 h of cell seeding. Briefly, specimens with cell attachment were rinsed and fixed with 4% paraformaldehyde (Taab Laboratory Equipment, Reading, UK). Filamentous actin (F-actin) was stained with rhodamine phalloidin (Cytoskeleton, Denver, CO, USA). The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Invitrogen, Carlsbad, CA, USA). Digital images were acquired using a fluorescence microscope (IX71; Olympus). ImageJ was used to count the number of attached cells on each surface by evaluating the number of DAPI-stained nuclei. Average data were calculated from three specimens for each testing condition, and five randomly selected locations on each specimen were detected.

Cell counting

The cellular proliferation of each specimen was evaluated by the number of attached cells on both specimens using the Cell Counting Kit-8 (CKK-8, Dojindo Laboratories, Kumamoto, Japan) for five consecutive days. The absorbance of each specimen was measured at 450 nm using a microplate reader (ChroMate 4300, Awareness Technology, Palm City, FL, USA) according to manufacturer's instructions²⁸⁻³⁰. In addition, a tissue-culture polystyrene dish (TCPS) was employed as a control.

Alkaline phosphatase (ALP) activity analysis

ALP activity is widely used to determine the cellular potential for osteogenic differentiation³¹⁻³⁴. In this study, the LabAssay™ ALP (Wako) was used to evaluate the ALP activity of cells with both metallic surfaces for 6, 8, 10, 12, and 14 days of osteogenic differentiation induction. The ALP activity of cells on each specimen was measured using ChroMate 4300 at 405 nm as described in our previous reports^{31,33}.

Alizarin red S staining and quantitative analysis

To evaluate the calcification behavior of MC3T3-E1 cells cultured on both specimens, the calcium deposited by MC3T3-E1 cells was stained with alizarin red S (Sigma-Aldrich) after 14, 21, and 28 days of osteogenic differentiation induction, according to the approach reported previously³¹⁻³⁵. In brief, all specimens were fixed with 10% formalin (Sigma-Aldrich), fully rinsed with ultrapure water, and stained with 1% alizarin red S solution. After staining, all samples were rinsed to remove excess dye and dried at room temperature. The

red-stained calcium deposition was photographed, and the percentage of calcified areas of the total surface was evaluated using ImageJ software.

In addition, to quantify the amount of calcium produced by MC3T3-E1 cells on both metals, the stained dye was extracted from specimens using 10% acetic acid (Sigma-Aldrich) and the absorbance was measured at 405 nm using ChroMate 4300, according to previous reports^{34,35}.

Statistical analysis

All data in this study were obtained at least three times independently and are shown as mean±standard deviation. The observed values were analyzed by one-way analysis of variance followed by a Student's *t*-test using KaleidaGraph4.0 (Synergy Software). Statistical significance was set at *p*<0.05.

RESULTS

Surface roughness

The surface morphology of prepared specimens was determined using SEM and LM. SEM images (not shown) revealed that, with a mirror-finishing process, both specimens possessed smooth surfaces. There was no significant difference between two specimens. In addition, Sa was automatically calculated from the observed surface morphology images by LM. Similar Sa values were observed for both specimens (Table 1), with no significant difference in Sa between the Zr-14Nb-5Ta-1Mo alloy (12±1 nm) and Ti specimens (8±1 nm).

Cell adhesion

To virtualize the cellular morphology attached to both specimens, the cellular cytoskeleton and nuclei under investigation were labeled with red and blue fluorescent dyes, respectively (Fig. 1a). Similar cellular morphologies were observed in cells cultured on both specimens after 3, 6, and 24 h of cell seeding. In addition, the attached cells' number was counted based on the stained nuclei. Although there was no significant difference between the attached cells' numbers of two specimens, a slightly larger cell number was detected with the Zr-14Nb-5Ta-1Mo alloy compared to that with Ti, after 3 and 6 h of cell adhesion (Fig. 1b). Based on these results, it was shown that the cell adhesion behavior of MC3T3-E1 cells was similar in both Zr-14Nb-5Ta-1Mo alloy and Ti.

Cell proliferation

Cell proliferation was evaluated for 5 consecutive days (Fig. 2). The results showed that the number of adherent cells on all specimens increased with increasing culture time. Although larger cell numbers were observed for the Zr alloy compared with that for Ti and TCPS, the results of the statistical analysis showed that similar numbers of MC3T3-E1 cells were obtained from both the Zr alloy and Ti specimens. This result indicated that the cell proliferation behavior of MC3T3-E1 cells was similar on both Zr-14Nb-5Ta-1Mo alloy and Ti.

Fig. 1 Initial adhesion of MC3T3-E1 cells attached on Zr-14Nb-5Ta-1Mo alloy and Ti specimens after 3-h, 6-h, and 24-h incubations with fluorescent staining.

(a) Fluorescent photos of cellular morphologies on specimens, where nuclei and F-actin were visualized with blue and red, respectively. Scale bar: 200 μ m. (b) Attached cell numbers on specimens after 3-h, 6-h, and 24-h incubations.

Fig. 2 Proliferation of MC3T3-E1 cultured on Zr-14Nb-5Ta-1Mo alloy, Ti, and TCPS.

The numbers of attached cells were counted by WST-8. Results were statistical analyzed, where $*p < 0.05$; n.s., non-significant.

Osteogenic differentiation

To evaluate the potential osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy, the expression level of

Fig. 3 Osteogenic differentiation in MC3T3-E1 cultured on Zr-14Nb-5Ta-1Mo alloy and Ti specimens through ALP activity levels detected after incubation of 6-d, 8-d, 10-d, 12-d, and 14-d differentiation induction.

intracellular ALP activity was determined, as shown in Fig. 3. A similar expression tendency of ALP activity was observed in MC3T3-E1 cells cultured on both the Zr alloy and Ti specimens. Compared with the Zr alloy, significantly higher ALP expression levels were detected in cells cultured on Ti specimens at an early stage, with 6-d and 8-d differentiation induction. A similar high ALP expression level was observed in cells cultured on both specimens after a 10-d differentiation induction. With further induction of osteogenic differentiation, the expression level decreased in both specimens. This result indicated that Ti showed an advantage in osteoconductivity at the initial induction period, and a similar ALP activity trend and expression level was detected in MC3T3-E1 cells cultured on both specimens after 10 days.

Calcification

To evaluate the calcification of mature osteoblasts cultured on the Zr-14Nb-5Ta-1Mo alloy, the deposited calcium on both specimens were stained with alizarin red S in red color after 14, 21, and 28 days of osteogenic differentiation induction (Fig. 4). All specimens showed a red-stained area on the surfaces, and the stained area on both specimens increased with increasing induction time from 14 to 28 days. To quantitatively evaluate this result, ImageJ was used to calculate the calcified deposit area (Fig. 4b). Similar increased extracellular calcified deposit areas were obtained by cells cultured on both specimens, although a slightly larger average area value was observed in cells cultured on Ti compared with those cultured on Zr-14Nb-5Ta-1Mo alloy. Moreover, the total amount of deposited calcium was investigated by evaluating the absorbance of the extracted dye (Fig. 4c). A similar trend was observed in the absorbance of the extracted calcium-bonded Alizarin red S dye for both specimens. Although a higher value of absorbance

Fig. 4 Calcification of MC3T3-E1 cultured on Zr-14Nb-5Ta-1Mo alloy and Ti specimens after 14-d, 21-d, and 28-d osteogenic differentiation induction stained with alizarin red S.

(a) Photos of calcified deposits on specimens. Scale bar: 4 mm. Histograms of quantitative analysis of calcium formation by MC3T3-E1 cultured on both specimens: (b) the proportion of the calcified area to total area of the specimens, which was calculated by ImageJ and (c) the amount of calcium deposited on specimens evaluated with the extracted stain through measuring the optical density at 405 nm. Results were statistical analyzed, where $*p < 0.05$; n.s., non-significant.

was observed for Ti compared with that of Zr-14Nb-5Ta-1Mo alloy, the results of statistical analysis showed that there was no significant difference between the two groups. This result indicated that a similar calcification behavior of MC3T3-E1 cells was obtained by both the

Zr-14Nb-5Ta-1Mo alloy and Ti specimens.

DISCUSSION

The Zr-14Nb-5Ta-1Mo alloy showed an improved balance of mechanical properties with low magnetic susceptibility ($16.96 \times 10^{-9} \text{ m}^3 \text{ kg}^{-1}$) and Young's modulus (61 GPa)²⁷, which strongly implies its potential application as implants. Although the safety or promotion of osteogenic differentiation *in vitro* for each additional element, niobium^{36,37}, tantalum³⁸, and molybdenum^{39,40}, respectively, has been reported, the lack of detailed information about the cytocompatibility and osteoconductivity of Zr-14Nb-5Ta-1Mo alloy limits its dental and medical applications.

To avoid the potential effects of the surface morphology of the specimens, mirror finishing of both specimens was carried out. Due to the difference in mechanical properties, a slightly rougher surface was determined for Zr-14Nb-5Ta-1Mo alloy specimens ($12 \pm 1 \text{ nm}$) compared with that of Ti specimens ($8 \pm 1 \text{ nm}$); however, this difference was not significant (Table 1).

To evaluate the cytocompatibility of the Zr-14Nb-5Ta-1Mo alloy using MC3T3-E1 cells, the initial cell adhesion and five continuous days of cellular proliferation were investigated. After 3-h of cell attachment, a similar cell morphology was observed on both the specimens. With increasing culture time, the typical cell morphology was observed on both metallic surfaces; a slightly larger cellular extension could be seen for cells on Ti compared with cells on the Zr-14Nb-5Ta-1Mo alloy (Fig. 1a). On the other hand, a larger attached cell number was determined for the Zr alloy after 3-h and 6-h of incubation, which suggested a satisfactory biocompatibility of the Zr alloy for initial cell attachment (Fig. 1b). After 24-h incubation, similar attached cell numbers were detected for both specimens. The above data indicated that the cell adhesion presented by the Zr alloy and Ti were similar, especially early, at 3 and 6 h. On the surface of the Zr alloy, MC3T3-E1 cells showed equal or slightly better cell proliferation than cells cultured on Ti specimens (Fig. 2). The Zr-14Nb-5Ta-1Mo alloy was found to have good cell compatibility similar to Ti *in vitro*.

To evaluate the osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy with MC3T3-E1, cells were induced to differentiate into osteoblasts, which is called osteogenesis, and mature osteoblasts induce calcium phosphate formation on the specimens, which is called calcification. Among the currently used dental implant materials, superior osseointegration of Ti and its alloys is widely known, given the promotion of osteoconductivity of pre-osteoblasts cultured on them^{41,42}. In addition, the cellular expression level of ALP has been recognized as an important criterion for evaluating the osteoconductivity of test specimens^{43,44}. After approximately one week of osteogenesis, a relatively lower level of cellular ALP activity was observed by Zr-14Nb-5Ta-1Mo alloy compared with that by Ti specimens (Fig. 3), which indicated a potential advantage of Ti in osteoconductivity during initial osteogenic differentiation stage,

comparing with that displayed by Zr-14Nb-5Ta-1Mo alloy. Undifferentiated data were then analyzed. This slight advantage in cellular osteogenesis influences the calcification of mature osteoblasts, as shown in Fig. 4. Interestingly, through long-term observation, this advantage was not obvious, although Ti still showed a larger amount of deposited calcium on its surface after 28 days of culture (Fig. 4c). This phenomenon could be explained by the subsequent calcification occurring on the initial calcification layer during the first two weeks³². Based on these results, the Zr-14Nb-5Ta-1Mo alloy showed good cytocompatibility and osteoconductivity similar to Ti.

Other potential reasons why Zr-14Nb-5Ta-1Mo alloy displays good cytocompatibility and osteoconductivity similar to Ti may include the following two points: First, although the ability of pure Zr to form calcium phosphate is inferior to that of Ti⁴⁵, cell compatibility with the Zr alloy is as good as that of Ti due to potent corrosion resistance of the zirconium alloy, and strong immobility of the film⁴⁶. Secondly, as mentioned in the introduction and a previous report²⁷, during the new alloy design stage, only non-toxic elements were selected for incorporation into Zr alloys. The current experimental results confirm our concept of alloy design.

On the other point of view, we hypothesize that the similar good cytocompatibility and osteoconductivity detected with Zr alloys, as Ti does, benefits from their surface passive oxide film. It was reported that, compared sputter-deposited Au, a similar cellular behaviors, including proliferation and osteogenic differentiation, was detected by cells cultured on sputter-deposited Ti and Zr, where a thin titanium oxide film and zirconium oxide film were detected on the specimens, respectively³². The high expression levels of *Runx2*, *Col1a1*, and *Akp2* in MC3T3-E1 cells cultured on sputter-deposited Ti and Zr indicated a faster osteogenic differentiation and calcification. It is worth noting that the both deposited Ti and Zr through sputtering are amorphous structure. On the other hand, as an additional component, it reported in several literatures, that the cytocompatibility and osteoconductivity of the composites improved with the addition of zirconium oxide⁴⁷⁻⁵⁰ or addition of zirconium oxide nanoparticles into nanocomposites directly⁵¹. Although the up-regulation of osteogenic genes were determined^{32,50}, the induction mechanisms is still not fully clear. One potential osteoconductivity of related cellular signaling pathways was thought to be the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERKs)^{52,53}. Among the different stimuli activates this MAPK/ERK pathway, the regulation of gene expression of *BMP2* and its receptors was recognized important one. It was reported that the addition of the zirconium ions could be another effective to improve the cellular cytocompatibility⁵⁴. Even, it was reported that zirconium ions increased the proliferation and gene expression of *BMP2* and BMP receptor and associated with increased phosphorylation of SMAD1/5 of human osteoblasts (HOBs), which indicated the zirconium ions promote the cellular proliferation and

osteogenic differentiation through up-regulate the BMP/SMAD signaling pathway⁵⁵.

The satisfactory cytocompatibility and osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy will not only strongly promote the use of this hard-tissue compatible alloy with relatively low magnetic susceptibility in dental implant fixtures, but also in other oral treatments, including maxillofacial prostheses. The low elastic modulus of this Zr alloy is thought to be another advantage supporting application in maxillofacial prosthesis plates, where the quotient force is not directly conducted to the mandible, and stress shielding will be prevented.

CONCLUSIONS

In this study, the cytocompatibility and osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy were investigated using a mouse preosteoblast cell line, MC3T3-E1. After the mirror-finishing polishing process, a smooth surface was obtained. The visualized cellular initial extension morphology showed a similar good cell adhesion behavior of both the Zr-14Nb-5Ta-1Mo alloy and Ti. The cell proliferation investigation showed similar cell numbers on the Zr alloy and Ti, indicating good cytocompatibility of the alloy, similar to Ti. In addition, the osteoconductivity of the Zr-14Nb-5Ta-1Mo alloy was investigated through ALP activity evaluation and alizarin red S staining calcification. The results showed that, although a slower osteogenic differentiation induction was observed at an early stage by cells on the Zr-14Nb-5Ta-1Mo alloy compared with those on Ti, a similar good osteoconductivity of Zr quaternary alloy and Ti was observed at the following stage. Our study provides proof of the good cytocompatibility and osteoconductivity of the newly designed Zr-14Nb-5Ta-1Mo alloy; these results can enable the promotion of the application of Zr alloys in dental/medical treatment.

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