Proliferation, Differentiation and Calcification of MC3T3-E1 Cells on Zr-14Nb-5Ta-1Mo Alloy

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Research Background & Our Purposes

Potential problem (1) during visual inspection

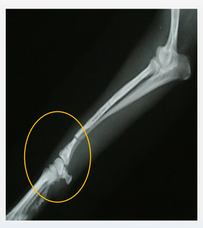
Artifact with increasing magnetic field and magnetic susceptibility of dental implant

Metallic biomaterial with low magnetic susceptibility is necessary.

Reference:杉村和郎：現場で役立つ臨床MRIシリーズ1MRIの原理と撮像法, メジカルビュー, (2000), p.90

Potential problem (2) during clinical treatment

Re-fracture due to stress shielding



Metallic biomaterial with low Young's modulus is necessary.

Reference:ストレスシールディングによる再骨折の例 through http://jvrm.jp/kvh/cap/cap3/02.html

Table. The mechanical properties of the new Zr alloy we developed previously.

Composition	Young's modulus, E / GPa	Ultimate tensile strength, σ_{UTS} / MPa	0.2% proof strength, $\sigma_{0.2\%}$ / MPa	Elongation to fracture, ε / %	Vickers hardness, HV	Mass magnetic susceptibility, χ_g / $10^{-9}m^3 \cdot kg^{-1}$
As-forged Zr-14Nb-5Ta-1Mo alloy	**64±1**	**651±13**	**632±13**	**17±7**	**213±8**	**17.5±0.4**
As-cast Zr-14Nb-5Ta-1Mo alloy	53±1	796±64	754±57	15±4	208±1	17.3±0.2
Zr-14Nb (as cast)	70	784	686	12	275	17.0
Zr-1Mo (as cast)	98	970	855	3	---	14.2
Ti-6Al-4V ELI	100	980	920	14	320	39.8
Ti-6Al-7Nb	114	933	817	7	325	35.3
Co-Cr-Mo	200	980	680	11	370	94.5

Purpose: To study the potential usage of new designed Zr-14Nb-5Ta-1Mo (ZNTM) alloy with medical and dental applications, we investigated the responses of mouse osteoblastic cells (MC3T3-E1) to this ZNTM alloy.

Materials & Methods

1. Preparation of ZNTM alloy specimens

Preparation of Large scale melting of Zr alloy

Success of melting to obtain 5-kg ingot

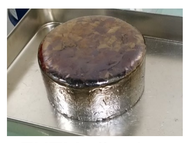


Fig. ZNTM alloy ingot

Formation of homogenized rods with diameter of 32 mm with hot forging at 1323 K

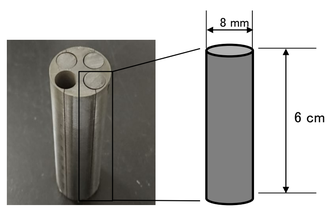


Fig. Photo of rods prepared with electric discharge machining

Specimens cutting

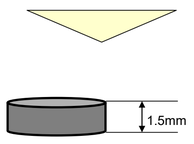


Fig. Schematic diagram of metal test specimen

Surface polishing

Mechanical polished with the SiC waterproof emery papers and mirror-finished with a colloidal silica suspension.

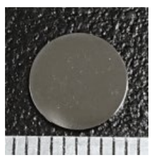


Fig. Photo of mirror-finished specimen

2. Cell culture

Cell-line: A mouse calvaria-derived preosteoblast cell line, MC3T3-E1

Proliferation Medium: Minimum Essential Medium Eagle Alpha Modification (α-MEM)
+ 10% Fetal Bovine Serum (FBS)
+ 100 U mL-1 Penicillin
+ 100 mg mL-1 Streptomycin

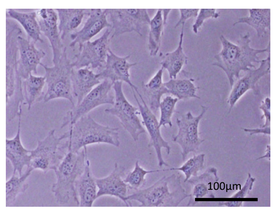


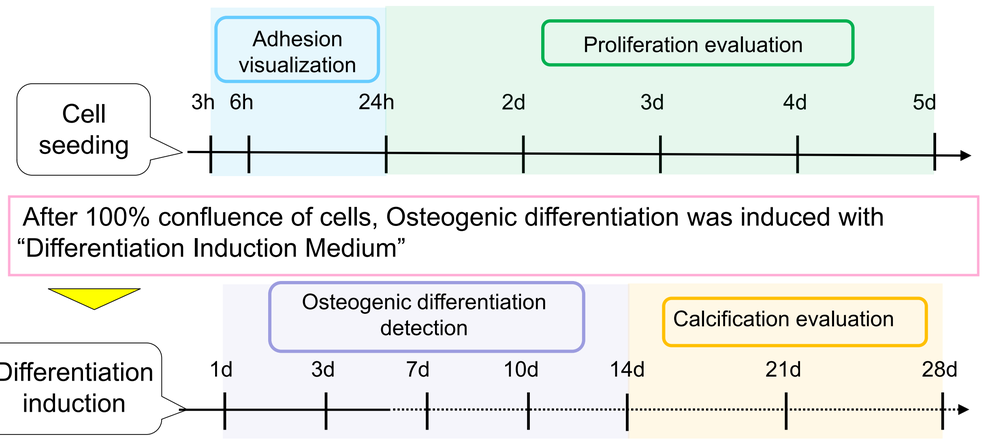
Fig. Opical photo of MC3T3-E1 cell

Differentiation Induction Medium: Proliferation Medium
+ 50 mg mL^{-1} L-ascorbic acid
+ 2 mM β-glycerophosphate

The induction medium was replaced every three days.

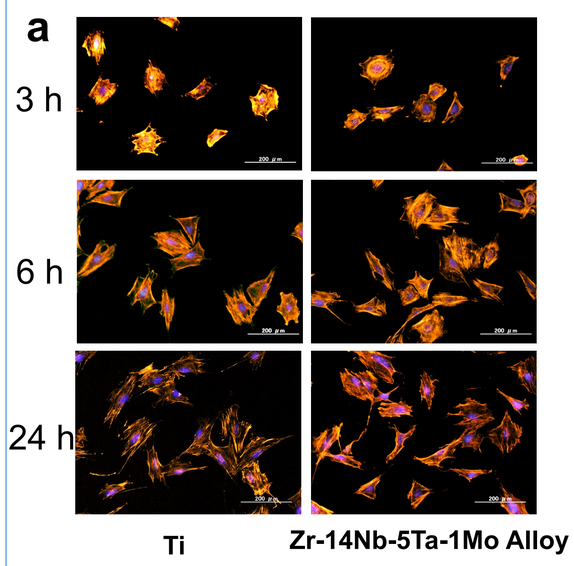
3. *in vitro* evaluation

Adhesion visualization: Fluorescent staining
Proliferation evaluation: Cell Counting Kit-8
Osteogenic differentiation detection: Alkaline phosphatase (ALP) activity analysis
Calcification evaluation: Alizarin red s staining and quantitative analysis



Results & Discussion

1. Adhesion visualization



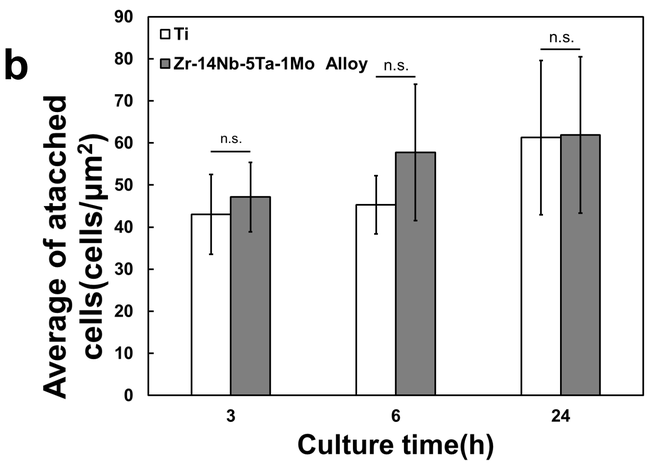


Fig. Initial adhesion of MC3T3-E1 cells attached on Zr-14Nb-5Ta-1Mo alloy and Ti specimens after 3-h, 6-h, and 24-h incubations with fluorescent staining. (a) Fluorescent photos of cellular morphologies on specimens, where nuclei and F-actin were visualized with blue and red, respectively. Scale bar: 200μm.

2. Proliferation evaluation

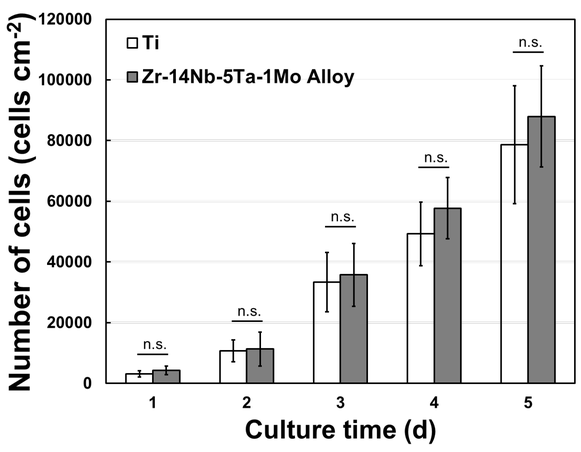


Fig. Proliferation of MC3T3-E1 cultured on Zr-14Nb-5Ta-1Mo alloy and Ti specimens. The numbers of attached cells were counted by WST-8. The "n.s." represents non-significance.

3. Osteogenic differentiation detection

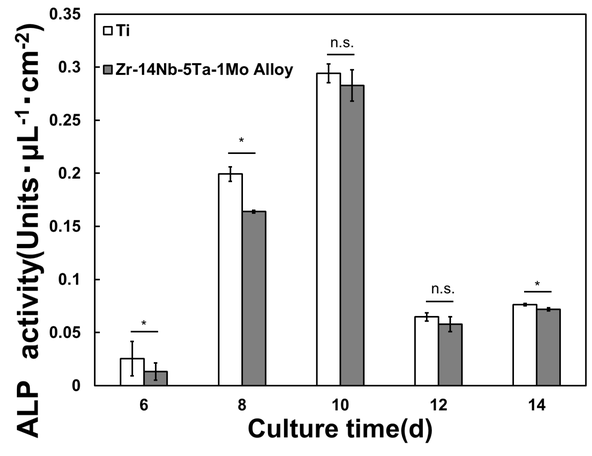
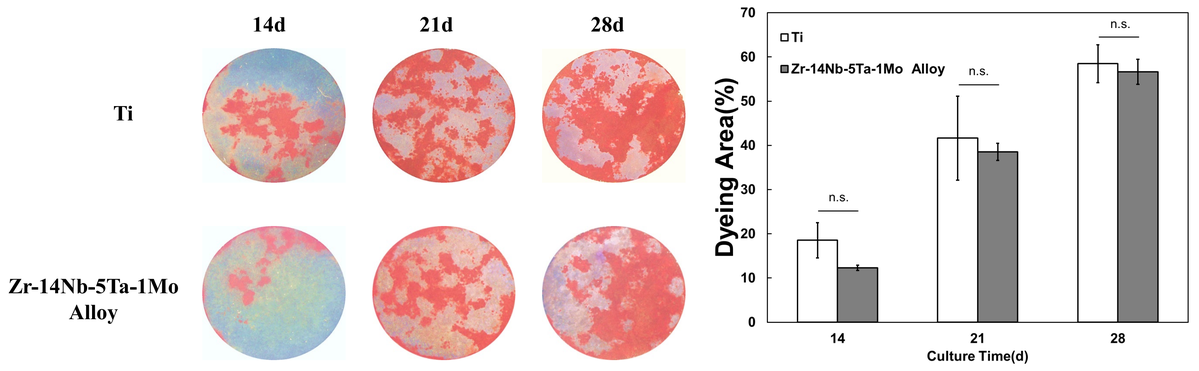


Fig. Osteogenic differentiation in MC3T3-E1 cultured on Zr-14Nb-5Ta-1Mo alloy and Ti specimens through ALP activity levels detected after incubation of 6-d, 8-d, 10-d, 12-d, and 14-d differentiation induction.

4. Calcification evaluation



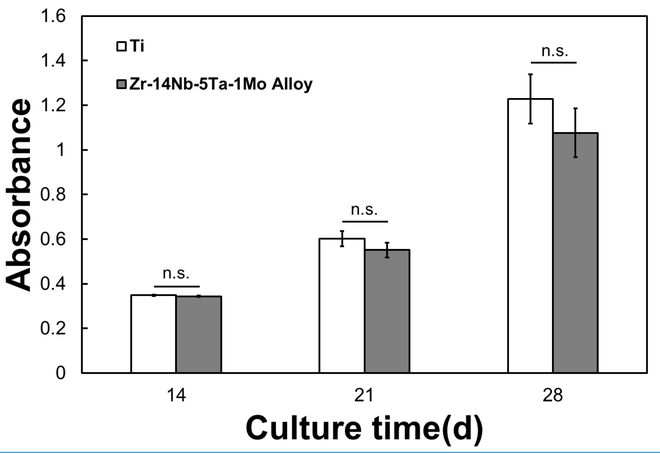


Fig. Calcification of MC3T3-E1 cultured on Zr-15Nb-5Ta-1Mo alloy and Ti specimens after 14-d, 21-d, and 28-d osteogenic differentiation induction stained with alizarin red s. (a) Photos of calcified deposits on specimens. Scale bar: 4 mm. Histograms of quantitative analysis of calcium formation by MC3T3-E1 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.

Conclusions

In this study, cytocompatibility and osteoconductivity of developed ZNTM were investigated. A similar good biocompatibility of ZNTM and Ti was obtained based on cellular adhesion and proliferation results. For cellular osteogenic differentiation and calcification, an insufficient osteoconductivity of ZNTM was obtained comparing with Ti. This work is expected to promote the MRI-compatible ZNTM to be used for medical applications, in particular, fabrication of bone fixation devices.

Acknowledgment

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