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# The bone tissue compatibility of a new Ti–Nb–Sn alloy with a low Young's modulus

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ABSTRACT

A Ti–Nb–Sn alloy was developed as a new β-type titanium alloy which had a low Young's modulus and high strength. The Young's modulus of the Ti–Nb–Sn alloy was reduced to about 45 GPa by cold rolling, much closer to human cortical bone (10–30 GPa) than that of Ti–6Al–4V alloy (110 GPa) and other β-type titanium alloys developed for biomedical applications. The tensile strength of the Ti–Nb–Sn alloy was increased to a level greater than that of Ti–6Al–4V alloy by heat treatment after severe cold rolling. In this study the cytotoxicity of Ti–25Nb–11Sn alloy was evaluated in direct contact cell culture tests using metal disks and the bone tissue compatibility – examined using metal rods inserted into the medullary canal of rabbit femurs. The remarkable findings were that: (1) there were no significant differences in the relative growth ratio and relative absorbance ratio between cells grown with the Ti–Nb–Sn alloy, Ti–6Al–4V alloy and CP-Ti in direct contact cell culture tests; (2) there were no significant differences in the load at failure between the Ti–Nb–Sn alloy and Ti–6Al–4V alloy in pull-out metal rods tests; (3) there were no significant differences in new bone formation around metal rods between the Ti–Nb–Sn alloy and Ti–6Al–4V alloy in histological evaluations. The new Ti–Nb–Sn alloy with an elasticity closer to that of human bone is thus considered to be bioinert while also having a high degree of bone compatibility similar to that of Ti–6Al–4V alloy.



## 1. Introduction

Commercially pure titanium (CP-Ti) (α-type titanium alloy) and Ti–6Al–4V ELI (Ti–6Al–4V) alloy (α + β-type titanium alloy) are commercially available and currently used as biomaterials for bone plates, intramedullary nails and artificial joints because they have excellent specific corrosion resistance and the greatest biocompatibility with bone among the metallic biomaterials [1]. The Ti–6Al–4V alloy has cornered almost all of the market for cementless total hip arthroplasties [2] because it has excellent specific strength compared with CP-Ti. The Young's modulus of Ti–6Al–4V alloy (110 GPa) is much lower than that of Co–Cr–Mo alloy (230 GPa) and stainless steel (205 GPa) used for biomedical applications [3]. However, the Young's modulus of Ti–6Al–4V alloy is still much greater than that of human cortical bone (10–30 GPa) [3]. The stems of cementless total hip arthroplasties made with high Young's modulus alloys sometimes cause severe stress shielding in the femur and cause thigh pain when walking [4–6]. Moreover, the toxicity of the β stabilizing element of V has been previously pointed out [7]. Recently, to resolve these adverse effects of Ti–6Al–4V alloy on bone β-type titanium alloys with much lower Young's moduli than Ti–6Al–4V have been developed. In addition, V in Ti–6Al–4V has been replaced by other β stabilizing elements, for example Fe and Nb, both of which are considered to be safer for the living body than V [7]. These materials seem to be excellent biomaterials because they are expected to enhance bone healing and/or remodeling due to their low Young's moduli. In particular, less rigid β-type titanium alloys are being developed for such applications [1]. Niinomi [7] reviewed 17 titanium alloys which have already been developed for biomedical applications and showed that the Young's modulus of all β-type titanium alloys were lower than those of α- or α + β-type titanium alloys. Recently, β-type titanium alloys with low Young's moduli have been extensively investigated for potential biomedical applications. Long et al. [3] reviewed 15 titanium alloys including Ti–6Al–4V alloy, CP-Ti and 'new generation' titanium alloys developed and/or utilized as orthopedic implants with Young's moduli ranging from 55 to 113 GPa. Among them, Ti–35Nb–5Ta–7Zr alloy showed the lowest Young's modulus (55 GPa) [3]. Recently, new β-type Ti–Nb–Sn alloys have been introduced [8–17]. The Young's moduli of Ti–Nb–Sn alloys were found to be less than 55 GPa when their alloy composition and heat treatment conditions were suitably controlled. Jung et al. [17] reported that the Young's modulus of a Ti–35Nb–4Sn alloy was about 40 GPa, which was the lowest Young's modulus of all β-type titanium alloys developed for

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biomedical applications. This Young's modulus of 40 GPa is much closer to that of human cortical bone (10–30 GPa) than any other titanium alloy [18]. The tensile strength of the Ti–Nb–Sn alloy is increased to a level greater than that of Ti–6Al–4V alloy by heat treatment after severe cold rolling [13,14,16,17]. Moreover, the Ti–Nb–Sn alloy has been reported to have good corrosion resistance [19]. Therefore, the Ti–Nb–Sn alloy will have various advantages for application as orthopedic implants. For clinical applications cytotoxicity and bone tissue compatibility need to be evaluated. No studies have been reported regarding these issues to our knowledge. The purpose of this study was, therefore, to evaluate the cytotoxicity and bone tissue compatibility of this new Ti–Nb–Sn alloy as the first step towards its use for biomedical applications.

## 2. Materials and methods

### 2.1. Alloy preparation

β-Type Ti–Nb–Sn alloys show Young's moduli lower than 55 GPa for various compositions. Ti–25Nb–11Sn (wt.%) alloy was selected in this study, since it has been found that a near net shape stem could be fabricated by cold die forging of similar to this alloy composition [16]. Ti–16Nb–5.5Sn alloy was prepared by a high frequency induction melting process using pure Ti (99.9 wt.%), pure Nb (99.9 wt.%) and pure Sn (99.9 wt.%). The melted ingot was cut into several pieces and vacuum arc remelted to homogenize the chemical composition. The remelted ingot was forged at 1100 °C to a cross section of 35 × 35 mm and then hot and cold rolled to 15 mm diameter. Disk samples of 12.5 mm diameter and 1.8 mm thickness were prepared from the 15 mm diameter rod for determination of cytotoxicity. For the direct contact tests 10 disks were made from the following five different materials; Ti–Nb–Sn alloy, Ti–6Al–4V alloy (ASTM F136), CP-Ti (ASTM F67 grade 2) and pure vanadium (V) (99.9 wt.%). The surfaces of the disks were ground using a series of water-resistant emery papers with various sizes up to 1500 grit, and then polished on a velvet cloth using $Al_2O_3$ powders of 0.06 μm suspended in water to finally obtain a mirror finish. All disks were sterilized in an autoclave prior to cell culture. The remaining part of the 15 mm diameter rod was further rolled to an 8 mm diameter rod to prepare rod samples for in vivo studies. Solid cylindrical rods measuring 4.5 mm in diameter and 32 mm in length were prepared from the 8 mm diameter rod by turning on a lathe. The machined surface had a roughness of $R_a \leqslant 0.4$ μm. The rods made of the Ti–Nb–Sn and Ti–6Al–4V alloys were inserted into the medullary canal of the distal femur of rabbits. Dynamic Young's moduli of the Ti–Nb–Sn and Ti–6Al–4V rods were 45.6 and 116 GPa, respectively, along the long axis, as determined by the free resonance vibration method (ASTM E1875-08, JE-LT, Nihon Techno-Plus Corp., Japan). Each implant had a protruding portion (4.0 mm diameter, 6 mm length) with a transverse hole for mechanical testing and a capped tapering portion on the other end (3 mm end diameter, 1 mm length) so as to be smoothly inserted into the medullary canal. The rods were produced with a machined surface finish and stabilized with $HNO_3$. All implants were sterilized in an autoclave prior to implantation.

### 2.2. Evaluation of cytotoxicity

L929, A murine fibroblast cell line, and MC3T3-E1, a murine osteoblast cell line, were obtained from the RIKEN cell bank. 24-well plates were used for contact cell culture. Ten wells containing each metal disk were used (described in the alloy preparation section). Ten wells without metal disks were used as controls. Each cell line was seeded at $1.0 \times 10^4$ cells per well. The L929 cells were seeded in 1000 μl of MEM containing 5% fetal bovine serum and antibiotics, while MC3T3-E1 cells were seeded in 1000 μl of MEMα containing 10% fetal bovine serum and antibiotics in each well. After incubation for 72 h we counted the number of cells in each well. Cell viability was evaluated using the WST-1 assay (Dojindo Laboratories, Japan) at an absorbance of 450 nm with an ELISA reader. The WST-1 assay is based on the fact that tetrazolium salts are cleaved to formazan by succinate dehydrogenase located in the mitochondrium. A reduction in the number of viable cells (with constant cell metabolism) or in the metabolic activity (within the same number of cells) results in decreased overall activity of mitochondrial dehydrogenases in the sample. This decline in the enzyme activity leads to a decreased amount of formazan dye, correlating directly with a reduced viability of metabolically active cells in the culture.

The relative growth ratio and the relative absorbance ratio were calculated, which were compared with the controls for both L929 and MC3T3-E1 cells.

### 2.3. Animal experiments

36 Adult male Japanese white rabbits weighing 3.0–3.5 kg were used. After premedication with ketamine (25 mg $kg^{-1}$) by intramuscular injection, the animals were anesthetized with ketamine (10 mg $kg^{-1}$) and xylazine (3 mg $kg^{-1}$) by intravenous injection. Cefazolin (30 mg $kg^{-1}$) was administered by intravenous injection before surgery. The knee of the rabbit was opened through a medial parapatellar arthrotomy and a 3.0 mm Kirschner wire was inserted into the intercondylar notch of the distal femur to open the medullary canal. The distal medullary canal was then gently reamed. After washing the medullary canal with saline solution, all implants were advanced proximally within the medullary canal until the distal portion of the implant was at the same level as the entry hole. Metal rods were put into both femurs in each rabbit. All rabbits could use their operated limbs completely freely without any limitations after operation. We labeled the bones using tetracycline (Sigma, Germany) and calcein (Dojindo Laboratories, Japan). Tetracycline (20 mg $kg^{-1}$) was injected for double labeling 7 and 2 days before operation, and calcein (10 mg $kg^{-1}$) injected 7 and 2 days before death. The animals, in three groups (six rabbits in each group), and killed using pentobarbital (120 mg $kg^{-1}$) 3, 6 and 12 weeks after rod implantation. The bilateral femurs were removed, wrapped with saline-moisturized gauze and cooled in ice. The right femur was used for the pull-out test and the left femur for histological examination. This animal experiment was approved by the committee for the care and use of laboratory animals of the Center for Laboratory Animal Research, Tohoku University.

### 2.4. Pull-out test

The distal end of the femoral condyle was excised and the protruding portion of the rods for attachment to the testing machine were exposed (Autograph, Shimadzu Corporation, Japan). A universal connecting device was made to allow the testing machine to always continue to pull the rod vertically (Fig. 1). The failure load was recorded, and compared them between the Ti–Nb–Sn alloy and Ti–6Al–4V alloy at 3, 6 and 12 weeks.

### 2.5. Histological evaluation

The femurs with the implants were fixed in 70% ethanol immediately after death. They were stained with Villanueva bone stain for 6 days, dehydrated in ascending grades of ethanol, defatted in an acetone/methyl methacrylate monomer mixture (1:3) and embedded in methyl methacrylate (Wako Chemicals, Japan) without decalcification. Cross-sections (200 μm thick) were cut with a

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