



Nanocrystalline β -Ti alloy with high hardness, low Young's modulus and excellent in vitro biocompatibility for biomedical applications

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ABSTRACT

High strength, low Young's modulus and good biocompatibility are desirable but difficult to simultaneously achieve in metallic implant materials for load bearing applications, and these impose significant challenges in material design. Here we report that a nano-grained β -Ti alloy prepared by high-pressure torsion exhibits remarkable mechanical and biological properties. The hardness and modulus of the nano-grained Ti alloy were respectively 23% higher and 34% lower than those of its coarse-grained counterpart. Fibroblast cell attachment and proliferation were enhanced, demonstrating good in vitro biocompatibility of the nano-grained Ti alloy, consistent with demonstrated increased nano-roughness on the nano-grained Ti alloy. Results suggest that the nano-grained β -Ti alloy may have significant application as an implant material in dental and orthopedic applications.

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1. Introduction

Pure Ti and Ti alloys are attractive materials for biomedical applications due to their light weight, high strength, relatively low Young's modulus and good biocompatibility. Currently Ti–6Al–4V (Ti64) is the most widely used commercial Ti alloy for dental and orthopedic applications [1–3]. Ti64 consists of both hexagonal close-packed α and body-centered cubic β phases, with a Young's modulus of ~ 110 GPa. Although Ti64 exhibits only half the Young's modulus of either stainless steel or Co–Cr alloys, it is still about 4 times stiffer than cortical bone (20–30 GPa) [1,4–6]. The difference in the modulus between artificial biomedical alloys and cortical bone creates a 'stress shielding' effect that undermines normal bone remodeling and maintenance and results in low bone density, loosening of implants, implant failure, and an increased likelihood for revision surgery [1,7]. Furthermore, the passive film of Ti64 can slowly leach-out toxic V ions [8], which have been linked to lower in-vitro cultured cell viability compared with pure Ti [9]. Therefore, the current research sought to

design a new generation of Ti alloys that has similar strength, but lower Young's modulus and better biocompatibility than Ti64.

Ti alloys consisting of mainly the β phase have recently drawn substantial attention because they exhibit Young's moduli ranging between 55 GPa and 90 GPa, and thus result in less stress shielding [9–13]. In addition, these Ti alloys contain only non-toxic elements such as Nb, Zr, and Ta [9,10,14], from which improved biocompatibility would be expected. Unlike Ti64 where V can leach out from the surface passive oxide film, the addition of Nb stabilizes the film, thus improving the passivation and corrosion resistance of Ti alloys in the body. Although high hardness and low Young's modulus are desirable qualities, they rarely coexist in this group of materials. This is because the single phase β -Ti alloys, which exhibit the lowest Young's modulus, are generally obtained after solution treatment, and so are relatively soft. Substantial strengthening can be achieved by aging treatments that induce a fine and uniform precipitation of ω and α phase components, but this inevitably increases the Young's modulus of the alloy [9,10,15,16]. Consequently, there is a critical need to devise strategies to produce β -Ti alloys that exhibit low Young's modulus, and high strength, and are thus more suitable for use in dental and orthopedic applications.

In this study, we demonstrate that it is possible to produce high strength of β -Ti alloys without long-time aging treatment, by reducing

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the grain size of the material to the nanometer range using high-pressure torsion [17,18], and that this is achieved without a concomitant increase in Young's modulus. The nanocrystalline β -Ti alloy prepared in our studies displayed attractive mechanical properties, such that the hardness and Young's modulus of the nano-grained Ti alloy are respectively 23% higher and 34% lower than those of the coarse-grained counterpart. Furthermore, we demonstrated that the nanocrystalline β -Ti has increased surface nano-roughness, considered a plausible explanation for enhanced in vitro biocompatibility.

2. Experimental approach

The coarse-grained Ti alloy (Ti–36Nb–2.2Ta–3.7Zr–0.3O (at.)) was produced by arc melting and extruded into a long cylinder with a diameter of ~ 20 mm. Disks with a thickness of ~ 1.7 mm were sliced and then processed using high pressure torsion for 10 revolutions under 6 GPa at room temperature to obtain the nanocrystalline Ti alloy. Hardness tests were carried out from the center to the edges of specimens along radial directions on the disks using a Leco LV700AT hardness tester under a load of 10 kg with 15 s dwell time. Nanoindentation was performed using a Hysitron TriboIndenter under a load of 10 mN with a loading/unloading time of 10 s and dwell time 5 s.

Samples for transmission electron microscopy (TEM) investigation were ground and polished down to ~ 100 μm thickness, punched into 3 mm diameter disks and then electropolished using 8 vol.% H_2SO_4 in methanol at -40 $^\circ\text{C}$. In order to study the nature of the oxide forming on the coarse-grained and nanocrystalline Ti surfaces, cross sectional samples were also prepared. Ti alloys were mechanically polished to a mirror-like surface in 1 μm and then 200 nm colloidal silica suspension solutions at the final polishing step. Strips of materials were cut from the disks and the mirror-like surfaces were glued together. Specimens were then placed on a tripod such that the mirror-like surfaces were perpendicular to the grinding plane and mechanically ground and polished on a series of diamond lapping papers. The cross-sectional TEM specimens were attached to Cu rings and finally thinned down to electron transparency by ion milling at an incident angle of 6° using 4 keV argon ions at -20 $^\circ\text{C}$. Structural characterization was performed on a JEOL 3000F TEM operating at 300 kV.

For atomic force microscopy (AFM) and in vitro cell attachment/proliferation tests, coarse-grained and nanocrystalline Ti were mechanically polished to a mirror-like surface in the way described above. Ethanol was used to rinse away any residual colloidal silica. Surface tomography of the polished samples was evaluated by an AFM (PicoSPM) in tapping mode and four 5×5 μm^2 areas were scanned for each specimen.

Human gingival fibroblasts (HGF, passages 5–6, 8–9) and human dental follicular cells (HDFC, passages 4–5) were used to evaluate the in vitro biocompatibility of coarse-grained-Ti and the nanocrystalline β -Ti alloy. Mirror-like surface β -Ti alloy substrates were sterilized in 70% ethanol and under ultraviolet light for 20 min. Triplet cultures were made for each β -Ti alloy specimen in 6-well culture plates. Cells were then seeded and cultured in M199 with 10% bovine calf serum with antibiotics penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and amphotericin B (2.5 $\mu\text{g}/\text{ml}$), and incubated at 37 $^\circ\text{C}$, 5% CO_2 with 100% relative humidity. Control experiments were conducted on the coarse-grained β -Ti alloy in conventional tissue culture plates. For the cell attachment test, HGF were seeded at a density of 14,000 cells/ cm^2 and cultured for 30 min. For the cell proliferation tests, HGF and HDFC were seeded at a density of 4800 cells/ cm^2 and 2000 cells/ cm^2 , and cultured for 5 and 9 days respectively, changing medium every 3 days. For scanning electron microscopy (SEM) observations, cells were rinsed with phosphate-buffered solution, fixed with 2% glutaraldehyde and 1% osmium, and then dehydrated in graded alcohols and finally subjected to critical point drying. Samples were coated with 10 nm gold film and

subsequently observed using a field emission SEM (Zeiss Ultra). For fluorescent microscopy observations, cells were rinsed with PBS, fixed with 4% paraformaldehyde and stained using DAPI and phalloidin. Samples were observed under a reflective fluorescent microscope (Olympus BX61). The number of attached cell was counted in 4 randomly selected areas (1.5 mm^2 each) for each specimen. ANOVA and one tail t-tests were performed, with $p < 0.05$ considered statistically significant.

3. Results

Fig. 1a shows a typical bright-field TEM image of the as-extruded coarse-grained Ti alloy. The average grain size of the coarse-grained-Ti alloy was ~ 2 μm . Inset in Fig. 1a is a corresponding $<110>_\beta$ zone axis selected area electron diffraction (SAED) pattern. The strong diffraction spots in the SAED pattern arise mainly from the β -Ti phase, while the weak diffuse diffraction spots (circled, Fig. 1a) are from the ω -Ti phase, indicating the co-existence of these two phases [19,20]. Fig. 1b shows a dark-field TEM image obtained using the circled diffraction spot in Fig. 1a, demonstrating that the ω phase (the bright areas in the figure) presents in the material as very fine precipitates distributed uniformly throughout the β phase matrix. Severe plastic deformation during high pressure torsion processing assists the formation of a nanocrystalline microstructure and the average grain size of the material was reduced to ~ 10 nm (Fig. 1c). A typical SAED pattern obtained from the nanocrystalline Ti alloy (Fig. 1d) reveals diffraction rings exclusively from the β -Ti phase (solid arrows). The dashed arrows indicate the positions for diffraction rings from the ω phase: the absence of diffraction rings at these positions indicates that the ω phase is absent in the nanocrystalline Ti alloy. Details on the high pressure torsion-induced phase evolution of the alloy have been reported elsewhere [21]. Fig. 2 shows the TEM images of both coarse-grained and nanocrystalline Ti samples in cross sectional view, revealing the nature of the oxide layer on mirror-like polished surfaces. An amorphous oxide layer with a thickness of 3–4 nm has developed on both samples with no crystalline form of oxides observed. Regular linear atomic arrays are seen in the coarse-grained material as opposed to disrupted arrays of atoms in nanocrystalline Ti as expected.

The roughness of the mirror-like polished surfaces of both coarse-grained and nanocrystalline Ti alloys was measured by AFM and results with corresponding Fourier modulus density diagrams are presented in Fig. 3. Scan areas of $\sim 1 \times 1$ μm^2 were cropped from larger scan areas (5×5 μm^2) to more clearly illustrate surface nano-roughness. From visual inspection, the coarse-grained Ti alloy (Fig. 3a) has a less rough surface compared to the nanocrystalline counterpart (Fig. 3b), although both specimens were polished in an identical way to achieve mirror-like surfaces. Surface roughness was also investigated quantitatively by comparing the corresponding Fourier modulus density diagrams of both surfaces. There is a hump (marked by the black arrow) present in the Fourier modulus density diagram in the nanocrystalline Ti in the range of 0.04 – 0.08 nm^{-1} in reciprocal space, indicating that the surface of nanocrystalline Ti has a large number of nano-crests/troughs 12.5–25 nm apart in real space. This however was not a prevalent feature on the coarse-grained Ti surface. Although there was a minor peak at 0.04 nm^{-1} (25 nm in real space) observed in the coarse-grained Ti Fourier modulus density diagram, it was much less prominent compared to the nanocrystalline Ti surface. It is also noteworthy that the surface roughness on coarse-grained and nanocrystalline Ti differs in frequency but not in magnitude. Multiple lines were drawn across AFM micrographs and the magnitude of surface roughness was measured to be $\sim \pm 2$ nm for both surfaces.

Hardness values were obtained by micro-indentation testing. The nanocrystalline β -Ti alloy reached 320 ± 6.2 Vicker's hardness (HV), compared to $\sim 260 \pm 5.2$ HV from the as-extruded coarse-grained material. The Young's moduli of both materials were measured using a nano-indentation test. Fig. 4 depicts typical nanoindentation

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