Development of a β-type Ti–12Mo–5Ta alloy for biomedical applications: cytocompatibility and metallurgical aspects

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Ti-based biocompatible alloys are especially used for replacing failed hard tissue. Some of the most actively investigated materials for medical implants are the β-Ti alloys, as they have a low elastic modulus (to inhibit bone resorption). They are alloyed with elements such as Nb, Ta, Zr, Mo, and Fe. We have prepared a new β-Ti alloy that combines Ti with the non-toxic elements Ta and Mo using a vacuum arc-melting furnace and then annealed at 950 °C for one hour. The alloy was finally quenched in water at room temperature. The Ti–12Mo–5Ta alloy was characterised by X-ray diffraction, optical microscopy, SEM and EDS and found to have a body-centred-cubic structure (β-type). It had a lower Young's modulus (about 74 GPa) than the classical α/β Ti–6Al–4V alloy (120 GPa), while its Vickers hardness remained very high (about 303 HV). This makes it a good compromise for a use as a bone substitute. The cytocompatibility of samples of Ti–12Mo–5Ta and Ti–6Al–4V titanium alloys with various surface roughnesses was assessed *in vitro* using organotypic cultures of bone tissue and quantitative analyses of cell migration, proliferation and adhesion. Mechanically polished surfaces were prepared to produce unorientated residual polished grooves and cells grew to a particularly high density on the smoother Ti–12Mo–5Ta surface tested.

1. Introduction

Titanium alloys to be used as implants or prostheses must be highly biocompatible and have such essential properties as good resistance to fatigue and corrosion, a small modulus, and good adhesion to tissues. Many studies of biocompatibility and biofunctionality have demonstrated that the chemical composition, microstructure and surface features of metallic biomaterials must be carefully tailored to optimise the mechanical properties and minimise adverse biological effects.

The Ti–6Al–4V alloy (α/β type structure) is widely used in biomedical applications because it is mechanically stronger and resists corrosion better than Co–Cr–Mo alloys and stainless steels. However, its Young's modulus (about 120 GPa) is much higher than that of the cortical bone (max 30 GPa), which can result in bone resorption. Some alloying elements also have a negative influence on the human body: V is considered to be toxic and Al is a member of the capsule (scar tissue) group [1–3]. Consequently, many researchers have actively looked for titanium alloys with a lower Young's modulus and better cytocompatibility [4]. The most promising materials for medical implants are the β-Ti type alloys because their elastic modulus is smaller than that of α- or α/β-titanium alloys [5, 6]. As a result new β-Ti alloys using Nb, Ta, Zr, and Mo as alloying elements (β-stabiliser elements) have been developed. Some β type titanium alloys, such as Ti–Nb–Ta–Zr, Ti–Nb–Ta–Mo, and Ti–Nb–Ta–Sn compositions possess a low modulus and excellent mechanical properties, resulting in high yield and tensile strength, and good fatigue strength [7, 8].

The biocompatibility of a Ti alloy is closely associated with its resistance to corrosion and the biocompatibility of its corrosion products [9, 10]. Most of the new β-titanium alloys have excellent corrosion resistance under friction and they all are cytocompatible, even under wear test conditions in simulated physiological media.

The interaction of Ti alloy implant with its biological environment and the formation of an implant-tissue interface depend strongly on the surface features of the implant. The surface topography influences cell morphology, proliferation, differentiation and adhesion, and thus has a major influence on the survival and properties

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of the implant-tissue interface [11–16]. Therefore, it is important to determine how the various titanium surfaces influence the biological reactions at tissue–implant interface.

We have investigated the cytocompatibility and metallurgical features of a new β-metastable titanium alloy, Ti–12Mo–5Ta. We prepared alloy samples with differing surface roughnesses and determined their structure, hardness and Young's modulus. The results obtained were correlated with cell responses. The influences of alloy topography and chemistry are discussed.

2. Materials and methods

2.1. Alloy preparation

Several ingots of Ti–12Mo–5Ta alloy (Ti–12 wt %Mo–5 wt %Ta) were prepared from pure elements in a laboratory-scale arc furnace (7400 TUBINGEN, Edmund Bühler, with a tungsten alloy electrode) under a high purity argon atmosphere (0.5 bar). Each ingot obtained (shape almost cylindrical with a diameter of about 8–10 mm and a weight of about 12 g) was annealed at 950 °C under high vacuum (10^{-6} mbar) for 1 h in a tubular furnace and then quenched in water at room temperature. This heat treatment produced the desired homogenous β-metastable microstructure alloyed with β-stabiliser (Ta and Mo). The quenched ingots were machined to a 6-mm diameter cylinder and then cut to obtain several of 1-mm thick discs suitable for biocompatibility (cytotoxicity) tests. Only one side of each sample was polished with SiC abrasive papers. As 60 samples were required for statistical analysis of the biological test results, 60 samples were polished with 400, 800, 1200, 2500, and 4000 grit SiC abrasive papers and 60 samples were polished with 80 grit SiC abrasive paper, to give two samples groups with different roughnesses. The polished samples were cleaned by immersion for 30 min in alcohol and 30 min in distilled water using an ultrasonic cleaner (GEOSON LD-050). The samples were sterilised in a Poupinel furnace before use in cytocompatibility tests.

2.2. Alloy characterization

The crystallographic structure of the heat-treated Ti–12Mo–5Ta alloy was identified by X-ray diffraction (XRD) at room temperature using a PHILIPS PW 1830/00 (X-ray generator) diffractometer ($CuK_{\alpha 1}$ radiation, 1.54060 Å wave length).

Samples were examined by optical microscopy (OM, LEICA DM/RM) and by scanning electron microscopy (SEM, JEOL JSM 6400). The samples for SEM were embedded in an electrically conducting cold-setting resin (Polyfast 485) mirror polished by standard metallographic techniques and etched with 5 % HF and 25 % HNO_3 in water. The chemical composition of the Ti–12Mo–5Ta alloy was checked by energy-dispersive spectroscopy (EDS coupled with the scanning electron microscope). Electron back-scattered diffraction (EBSD, TSL) coupled with the SEM operating at 20 kV, was used for local crystallographic analyses. The data were recorded using TSL software and provided information on the cell parameters, size, and crystallographic orientations of the alloy grains.

The surface topographies of polished samples were examined by optical microscopy and their roughness was measured using a surface profiler (alpha-step 500, Tencor Instruments). The roughness parameters usually computed by this technique are the Z range (difference between Z_{max}, the maximal amplitude of roughness, and Z_{min}, the minimal amplitude of roughness) and the root mean square (RMS) roughness (R). This RMS roughness, corresponding to the standard deviation of the Z values within the given area, is determined from Equation 1:

$$R = \sqrt{\frac{\sum (Z_i - Z_{ave})^2}{N}} \quad (1)$$

where Z_{ave} is the average of Z values within the given area, Z_i the current Z value, N the number of points within the given area.

Hardness was determined using a Vickers microhardness tester (Mituyoyo Instrument, load of 0.3 kg f). Elastic modulus (Young's modulus E) of designed alloys was determined ultrasonically from the density (ρ) and from measurements of the longitudinal V_L and the transversal V_T wave velocities. A piezo-electric transducer (10 MHz), in contact with the sample via a coupling gel, was used for these measurements. Thus, the value of the Young's modulus for the infinite mode was obtained from Equation 2,

$$E = \rho \frac{3V_L^2 - 4V_T^2}{\left(\frac{V_L}{V_T}\right)^2 - 1} \quad (2)$$

2.3. Cell culture

The tissue fragments were cultivated at the medium–air interface, so that the interactions among cells in the tissue and needed for tissue function were conserved. This technique has been used in many other studies [17, 18] and is included in AFNOR NFS 11-146 (cited as a reference in the projects of CEN 30993-5 and ISO 10993-5 norms).

The culture medium was Dulbecco's Modified Eagle's medium (Gibco BRL, Invitrogen, Eragny, France) (ref. 31885-023) supplemented with 40 % foetal calf serum + 2% L-glutamine 20 mM + 0.15 % penicillin and streptomycin mixture. This complete medium was mixed v/v with buffered agar (1% Bacto–Agar Difco, Detroit, USA, in Gey's solution).

Some bone tissue fragments were taken from the tibias of 19-day-old chicken embryos. They were cut in 2 mm^3 explants and cultured in contact with the metal alloy to be tested. The tissue explants were deposited on the agar medium and covered by the metal or control samples. All the cultures were incubated for 14 days at 37 °C into 5% CO_2 humidified atmosphere.

2.4. Cell adhesion, migration and density

Plastic coverslips treated for cell culture (Thermanox®, Lux Corp.) were used as negative control samples. The cytocompatibility of each material was measured using

60 discs (1 mm thick, 6 mm diameter, see Section 2.1) all having the same chemical composition and surface roughness.

The cultured samples were stained with neutral red and the cell layers measured with a stereo-microscope fitted with a camera lucida and digitising tablet connected to a microcomputer. Some samples were fixed in 3 % glutaraldehyde solution and prepared for SEM. The surfaces of the samples were measured. They were treated with 0.025 % trypsin in EDTA and placed in an incubator at 37 °C. The cells that detached from the sample surface after incubation for 5, 10, 20, 30, and 60 min were harvested and counted. Any residual cells were removed by digestion with trypsin for one hour of enzymatic digestion. The samples were then placed in (0.25 %) trypsin-EDTA for 15 min. The cell migration area was calculated using image analysis software. Cell density and cell adhesion were assessed after removing the cells and counting them in a Coulter Multisizer (Beckman-Coulter, France).

The cell adhesion is expressed in terms of an index that represents the area between the curve of cell dissociation (% of detached cells vs. time) and the *X*-axis. This value is inversely proportional to the cell adhesion and was generally between 2000 and 6000. Cell migration and proliferation are expressed by two histograms which show the cell density (number of cells/mm^2) and the migration surface in mm^2. Cell adhesion (A) is expressed by a diagram producing three zones: strong cell adhesion ($A < 3000$), moderate cell adhesion ($3000 < A < 4500$), weak cell adhesion ($A > 4500$).

3. Results

3.1. Metallurgy

Fig. 1 shows the X-ray diffraction (XRD) profile of the Ti–12Mo–5Ta alloy after melting and quenching operations, as explained in Section 2. The expected body-centred-cubic (bcc) single β-phase was detected and the corresponding diffraction planes were well identified. The elementary cell parameter of the structure were be evaluated from this pattern by determining the position of each peak (computed by the Rietveld method, MAUD software, see Lutterotti *et al*. [19] for details); the value was 3.2625 $\pm$ 0.0008 Å. Fig. 2 shows the granular

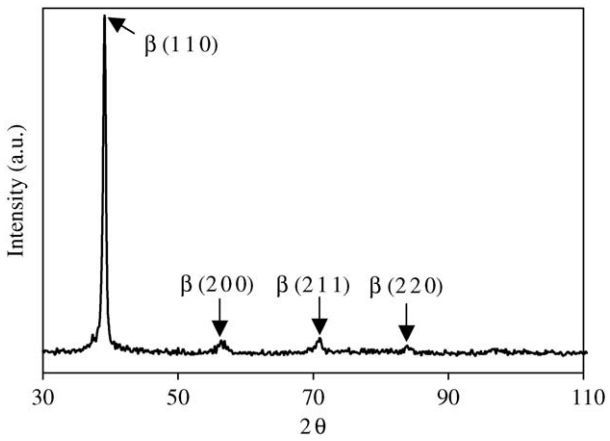

Figure 1 XRD pattern of the Ti–12Mo–5Ta alloy.

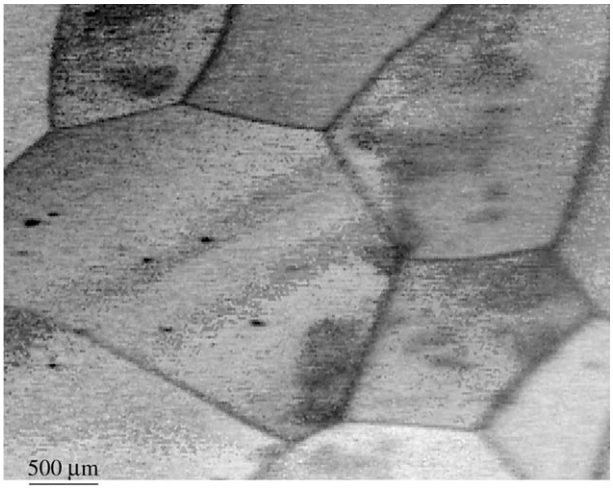

Figure 2 EBSD map from the Ti–12Mo–5Ta alloy showing β-type grains.

β-type microstructure (SEM, cartography of grain orientations using EBSD). The average grain size, determined by XRD (Rietveld method [19]) and by direct observation, was about 800 $\pm$ 100 µm. EBSD also showed the local crystallographic structure (of grains) [20]; an electron diffraction pattern showing the Kikuchi bands is shown in Fig. 3. The pattern, obtained from one single grain, fits the diffraction atomic planes (dotted lines) detected by XRD quite well, confirming the bcc β-phase structure. The different bandwidths of this kind of pattern are connected to the elementary cubic cell parameter and a similar result was obtained by comparison with that evaluated from XRD.

The Vickers hardness of the Ti–12Mo–5Ta alloy was measured, and an example of the indentation obtained is shown in Fig. 4 (optical microscopy). The Young's modulus, *E*, was also evaluated ultrasonically (see Table I). This table also shows published data for pure Ti (α-type structure), Ti–6Al–4V (α/β-type structure) and other β-type Ti-based alloys [9, 21–23].

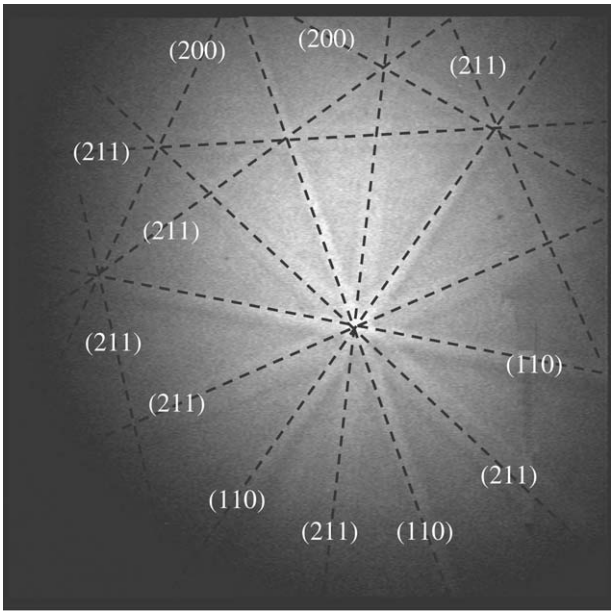

Figure 3 EBSD Kikuchi pattern from the Ti–12Mo–5Ta alloy. Pattern indexed as β-phase is indicated (dotted lines with corresponding diffracted planes).

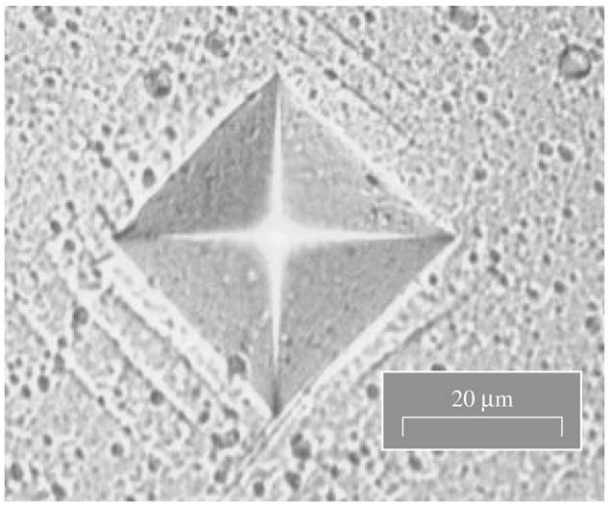

Figure 4 Indentation obtained by Vickers hardness test.

The topography and roughness profiles (amplitude vs. distance) of polished samples (see Section 2) are shown in Fig. 5. Fig. 5(a) shows an optical microscopy micrograph of a Ti–12Mo–5Ta sample polished with 400–4000 grit SiC abrasive (with the corresponding roughness profile below), while Fig. 5(b) shows a sample polished with 80 grit SiC. Both surface morphologies had randomly oriented scratches. The root mean square roughness, R, (calculated from Equation 1 for over 10 profiles) of samples polished with 400–4000 grit SiC paper was $0.80 \pm 0.08\,\mu m$, while the R value after polishing with the 80 grit SiC abrasive paper was $5.5 \pm 0.5\,\mu m$.

3.2. Cytocompatibility

Tissue layers were clearly visible on each surface of treated samples incubated with cells (Section 2.3) (Fig. 6). Fig. 6(a) shows a SEM micrograph of a bone explant surrounded by tissue in contact with the Ti–12Mo–5Ta alloy, while Fig. 6(b) shows the relatively thick continuous layer of tissue at a higher magnification.

Fig. 7 shows the cytocompatibility results, such as cell adhesion (Fig. 7(a)), cell migration (Fig. 7(b)) and cell density (Fig. 7(c)) for samples of Ti–12Mo–5Ta alloy with different roughnesses (5.5 and 0.8 μm; using 2×60 discs, see Section 2.1). The results are compared with data for two references, Thermanox® (surface roughness of 1.477 nm measured by AFM analysis) and Ti–6Al–4V

TABLE I Vickers hardness and Young's modulus of Ti-based alloys

Structural type Chemical composition (wt %)	Vickers hardness (HV)	Young's modulus E (GPa)
β-structure		
Ti–12Mo–5Ta	303 ± 10 [this study]	74 ± 3 [this study]
Ti–12.5Mo	340 [21]	85 [21] by bending test
Ti–35Nb–7Zr–5Ta	?	55 [9]
Ti–29Nb–13Ta–4.6Zr	≈ 185 [22]	?
Ti–15Zr–4Nb–4Ta	≈ 330 [23]	90 [23]
α/β-structure		
Ti–6Al–4V	≈ 330 [this study]; ≈ 340 [23]	≈ 120 [this study]; ≈ 110[9]
α-structure		
Pure cp-Ti	156 [21]; ≈ 180 [23]	92 [21] by bending; 105 [9]

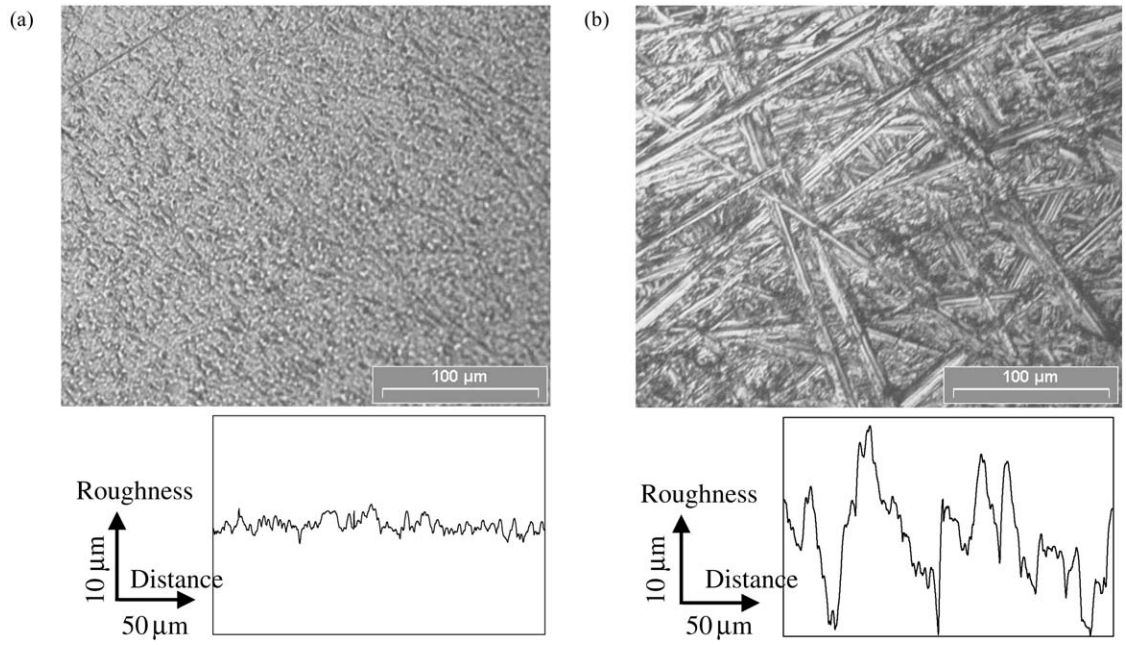

Figure 5 Optical microscopy micrographs and corresponding rouhgness profiles (below) on Ti–12Mo–5Ta alloy surfaces. (a) shows the roughness obtained by polishing the surface successively with 400, 800, 1200, 2500, and 4000 grit SiC abrasive paper and (b) by polishing only with the 80 grit SiC paper.

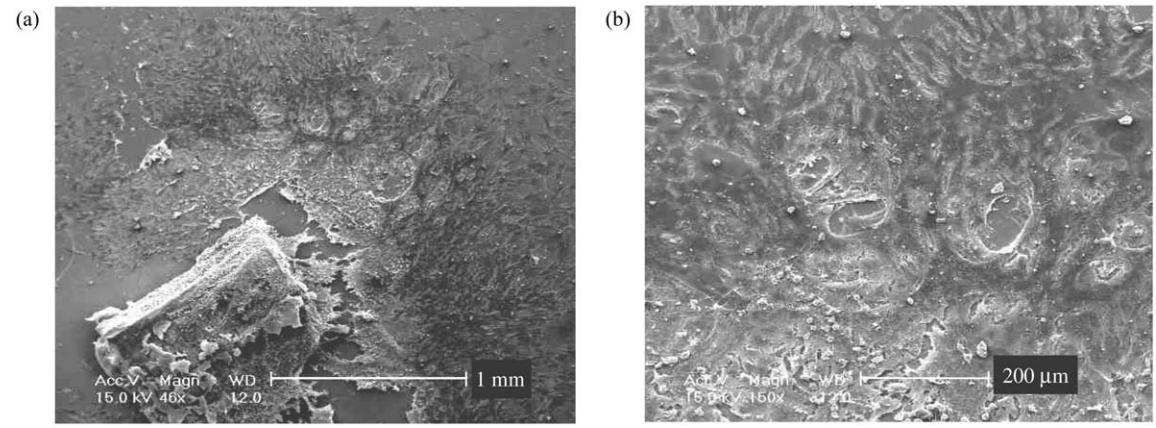

Figure 6 Example of SEM observations of bone tissue cultured on Ti–12Mo–5Ta surface. The micrograph in (a) shows the explant at low magnification and (b) shows the tissue layer at higher magnification.

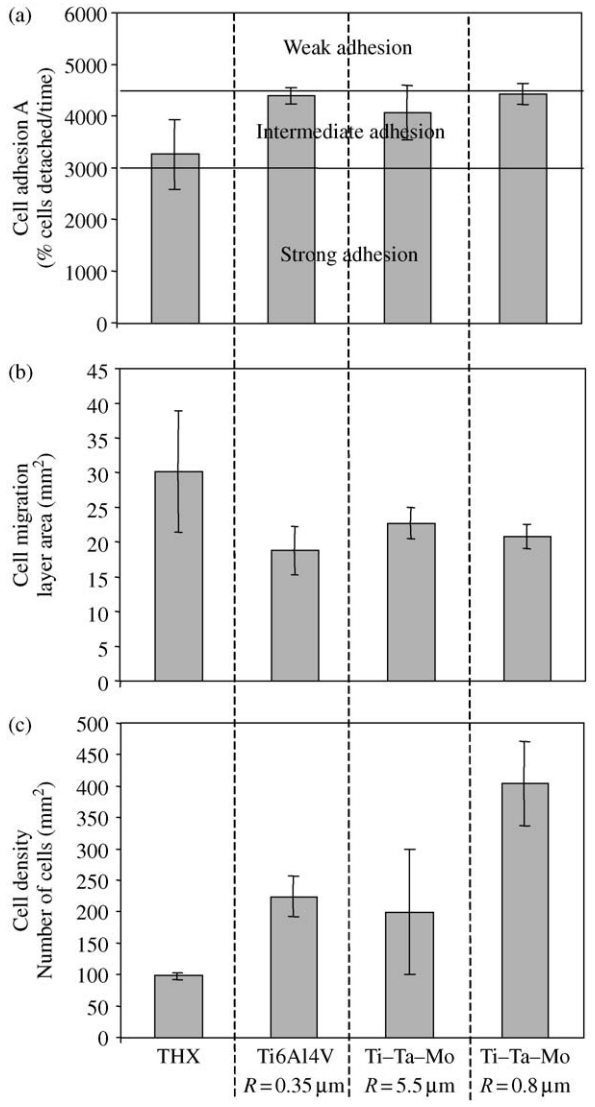

Figure 7 Cytocompatibility results obtained on the Ti–12Mo–5Ta alloy for two different roughness (5.5 and 0.8 µm) and compared with two references: Thermanox® (THX) and Ti–6Al–4V. (a) represents the cell adhesion on each studied material surface, (b) the cell migration and (c) the cell density variation.

alloy (60 discs each, surface roughness of 0.35 ± 0.04 µm measured by using a surface profiler).

Cytocompatibility was better than that of the control material (Thermanox®). But the cell adhesion (Fig. 7(a)) and cell migration (Fig. 7(b)) on Ti–6Al–4V and Ti–12Mo–5Ta were not significantly different. On the other hand, measurements of cell densities (Fig. 7(c)) that expressed the cell proliferation showed that cell densities were considerably better on Ti-based alloys than on Thermanox®. There were twice as many cells on Ti–6Al–4V and Ti–12Mo–5Ta as on Thermanox® (roughness: 5.5 µm), and four times as many cells on a smoother sample of Ti–12Mo–5Ta alloy (0.8 µm). Thus the surface topography has more influence than the chemical composition (because of the TiO_2 surface layer) under our experimental conditions.

4. Discussion

The XRD profile (Fig. 1) of the Ti–12Mo–5Ta alloy showed bcc β-phase microstructure. The beta-stabilizer equivalence ($\%Mo_{eq}$.) was 13.1 wt %, more than 10.0 wt %, which makes this alloy a β-metastable one. The elementary cell parameter of the bcc β-phase (3.2625 Å) is smaller than that of pure titanium (3.3060 Å). This difference is due to the difference between the atomic radius of pure titanium (1.47 Å) and the atomic radius of the alloy elements Ta (1.49 Å) and Mo (1.39 Å).

β-titanium alloys (metastable or stable) are particularly useful in biomedical applications because of their strength, excellent cold formability, and hardness. The Ti–12Mo–5Ta alloy studied here has very large β grains (a few hundred µm, Fig. 2), produced by the high temperature annealing (in the stable β-phase domain) followed by very fast quenching at room temperature. Studies on the microstructure/properties relationships of new beta-titanium alloys [8] indicate that the average grain size has an important effect on the deformation modes in tensile deformation and on the creep strain at ambient temperature. Consequently, reducing the grain size will further enhance the (303 HV) microhardness of Ti–12Mo–5Ta (Hall–Petch law) and we plan to develop a thermo-mechanical process to give such a β-metastable Ti-based alloy. Our objective is to optimise mechanical properties required for biomedical applications, such as hardness, wear resistance, and ductility.

β-type Ti-based alloys also combine a low elastic modulus with superior corrosion resistance [9, 24–26], which is particularly important for bone substitutes (hip prostheses). The modulus of Ti–12Mo–5Ta (74 GPa) is

very similar to the moduli of recently developed β type alloys (55–90 GPa) [5, 27] (see Table I). The elastic modulus of an alloy with fixed fractions of different phases depends mainly on its chemical composition [6, 28]. It has been shown recently that the value of the Young's modulus decreases with increasing bond strength (between Ti and alloying elements) and the metal d-orbital energy (which is correlated with electronegativity and the metallic radius elements) [8]. The Young's modulus of Ti-12Mo-5Ta is not as low as that of Ti-based alloys with a high niobium content (Ti–35Nb–7Zr–5Ta), but it is quite acceptable compared to Ti–12.5Mo and Ti–15Zr–4Nb–4Ta alloys (Table I). On the other hand, Ti–12Mo–5Ta is much harder than high-niobium alloys (Ti–29Nb–13Ta–4.6Zr). Consequently, this alloy is a good mechanical compromise between high hardness and low Young's modulus, making it well suited for use in bone implants.

Surface roughness determines the shear strength of the implant-bone interface [29–31], and this is very important for long-term implant bonding [11]. The topography of the implant also influences cell adhesion, morphology, proliferation and differentiation [32–35] and many recent studies have demonstrated that roughness has a great influence on cell responses [15, 16, 36–38]. We prepared two surface roughnesses and found better cell proliferation on the smoother Ti–12Mo–5Ta surface ($R = 0.8\,\mu m$). Our results agree with many other studies, but some have reported the opposite effects, depending on the method (*in vivo* or *in vitro*), the cell type (osteoblastic, fibroblastic, or epithelial), the surface topography preparation (grinding, sand-blasting, micro-machining) used. Surface preparation may be critical for implant survival (more than roughness itself, the surface profile can greatly influence cell responses). This will be the subject of future work.

5. Conclusions

We have investigated the metallurgical properties and cytocompatibility of a new β-type structure Ti–12Mo–5Ta alloy by X-ray diffraction, EBSD, SEM, optical microscopy, measuring roughness, Vickers hardness and Young's modulus, and biological tests (cell adhesion, migration and proliferation).

The new alloy has a bcc structure (β-phase) and the interstitial β-stabiliser alloying elements Mo and Ta make the elementary cell parameter smaller than that of pure β-Ti.

The microhardness of the new alloy (about 303 HV), which can be enhanced still further by appropriate thermomechanical treatment, and the relatively low Young's modulus (about 74 GPa) provide a good compromise for use as a bone substitute (hip prostheses).

The biological tests indicate that the Ti–12Mo–5Ta alloy provides better cell adhesion, migration and proliferation than Thermanox® and Ti–6Al–4V alloy.

The studies on the surface topography show that cells respond to surface roughness. Two surface roughnesses were tested and cells proliferation was better on the smoother Ti–12Mo–5Ta surface ($R = 0.8\,\mu m$). Thus surface topography is an important feature of orthopedic implants, which can mitigate negative effects and stimulate bone formation and function. Consequently, surface characteristics must be optimised. Surface engineering methods are under investigation and will be presented in a future report.

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