The influence of temperature during water-quench rapid heat treatment on the microstructure, mechanical properties and biocompatibility of Ti[sbnd]6Al[sbnd]4V ELI alloy

J.A. Chafino, K. Yamanaka, F. Mercier, P. Rivory, S. Balvay, D.J. Hartmann, A. Chiba, D. Fabregue

► To cite this version:

J.A. Chafino, K. Yamanaka, F. Mercier, P. Rivory, S. Balvay, et al.. The influence of temperature during water-quench rapid heat treatment on the microstructure, mechanical properties and biocompatibility of Ti[sbnd]6Al[sbnd]4V ELI alloy. Journal of the mechanical behavior of biomedical materials, 2019, 96, pp.144-151. 10.1016/j.jmbbm.2019.04.024 . hal-02405430

HAL Id: hal-02405430

<https://hal.science/hal-02405430>

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial| 4.0 International License

The influence of temperature during water-quench rapid heat treatment on the microstructure, mechanical properties and biocompatibility of Ti-6Al-4V ELI alloy

J.A. Chafino^a, K. Yamanaka^b, F. Mercier^a, P. Rivory^c, S. Balvay^c, D.J. Hartmann^c,
5 A. Chiba^b, D. Fabregue^a

^aUniv Lyon, INSA-Lyon, MATEIS, UMR CNRS 5510, 20 Avenue Einstein, 69621
Villeurbanne, France

^bInstitute for Materials Research, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-
8577, Japan

10 ^cUniv Lyon, Universite Claude Bernard Lyon1, MATEIS, UMR CNRS 5510, 8 avenue
Rockefeller, 69373 Lyon, France

Abstract

This study investigates the influence of a rapid heat treatment followed by water-
15 quenching on the mechanical properties of Ti-6Al-4V ELI alloy to improve its strength
for use in implants. Prior to the experiment, a dilatometry test was performed to
understand the progressive α - to β -phase transformation taking place during heating.
The results were then used to carry out heat treatments. Microstructure was analysed
using SEM, EBSD, EDX and XRD techniques. Vickers micro-hardness, tensile and
20 high cycle rotating bending tests were used to analyse the influence of the α -
phase fraction on the strength of the studied alloy. Results show that this process can
provide a Ti-6Al-4V ELI alloy with a better Yield Strength (YS)/uniform
deformation(ϵ_u) ratio and improved high cycle fatigue strength than those observed in
the current microstructure used in medical implants. Lastly, cytotoxicity tests were
25 performed on two types of human cells, namely MG63 osteoblast-like cells and
fibroblasts. The results reveal the non-toxicity of the heat-treated Ti-6Al-4V ELI alloy.

Keywords: Biomaterial, Ti-alloy, Ti-6Al-4V, ELI, Phase Transformation, Martensite,
 α '-phase, Dilatometry, Microstructure, Mechanical Properties, Fatigue, Cytotoxicity.

2010 MSC: 00-01,99-00

30

1. Introduction

Titanium has exhibited high biocompatibility in laboratory tests since the outset of the titanium industry in 1940 [1, 2, 3]. Initially designed for use in the turbine blades of aircraft jet-engines, the Ti-6Al-4V alloy and its derivate ELI (Extra Low Interstitial) grade were rapidly recognized for their balanced combination of mechanical properties and their excellent biocompatibility and were rapidly adopted by the ASTM (American Society for Testing Materials) as a standard material for biocompatibility applications [4].

The equiaxed microstructure (which offers high strength and good ductility) required by the ASTM norm for biomedical applications is already provided by the microstructure optimization of this alloy, which was designed for use in aeronautical structures with relative high temperatures of $>400^{\circ}\text{C}$. Nevertheless, an improvement in the mechanical properties of Ti-6Al-4V has always been desired. Researchers have studied how the equiaxed microstructure can be refined to improve its mechanical properties. Costly and complicated processes can be used to obtain a smaller microstructure, the so-called UFG (Ultra Fine Grain) microstructure. L.R. Saitova *et al.* used the ECAP (Equal Channel Angular Pressing) technique to produce the desired UFG equiaxed microstructure in Ti-6Al-4V ELI [5, 6], and obtained an improvement of 9% in the HCF (High Cycle Fatigue) strength compared to the initial microstructure. Using a different technique, S. Zherebtsev *et al.* produced the desired UFG microstructure through multiaxial forging, increasing the HCF strength of the Ti-6Al-4V ELI alloy by 15% [7].

Ti-6Al-4V alloy is an extremely versatile material due to its ability to change its microstructure and consequently its mechanical properties. In addition to the equilibrium phases (α and β), this alloy is able to form metastable phases such as the hexagonal martensite (α'). However, the time during which this phase can be studied is limited by the ASTM standard and the decomposition of the alloys at moderate temperatures.

R.G. Sherman *et al.* presented the first results showing possible use of the martensite phase to improve the mechanical properties of this alloy. Their work shows that unlike martensite in steels, the presence of α' -martensite in titanium results in a high-strength and ductile material [8]. M.A. Imam *et al.* identified the use of water-quench heat

treatment after 10 min. at 900°C as the most appropriate treatment to obtain a fourfold improvement in torsion fatigue properties compared to those obtained in the annealed state, previously thought to possess the best fatigue performance [9]. Authors claimed that the fatigue improvement was due to a TRIP (Transformation Induced Plasticity) deformation mechanism from a retained β -phase in the martensite phase. The microstructure of this optimized heat treatment was composed of a mixture of 50% of α_p , 25% of α' and 25% of β . J.R. Kennedy carried out a similar study to that of M.A. Imam *et al.* In his study, a 1hr heat treatment was carried out on three samples of different sizes. One of the most relevant results was the non-reproducibility of heat treatments for the different sample sizes in terms of mechanical properties, although an improvement of fatigue strength was obtained in comparison with its annealed state. J.R. Kennedy also explained the fatigue improvement by a TRIP deformation mechanism, even if the XRD measurement on the quenched samples did not detect the retained β -phase.

Most recently, C. Formanoir *et al.* also proposed martensite as a suitable method to increase the mechanical properties of Ti-6Al-4V produced by EBM (Electron Beam Melting) [10]. In this study, small samples were treated for 2hrs before quenching. As a result, the work hardening of almost all samples was improved. The effect of martensite on the cold-rolling ability of Ti-6Al-4V has also been reported by H. Matsumoto *et al.* [11, 12].

To conclude this review of the previous results found in literature, the high potential of Ti-6Al-4V martensite to optimize mechanical properties is therefore evident. However, it was hard to draw clear conclusions due to discrepancies in the results. Despite the different proposals on the interest of the α' -phase, a systematically study of the influence of phase fractions on static and dynamic mechanical properties has not been performed to date.

It should also be noted that titanium and its alloys possess low thermal conductivity; for this reason, the heating time and sample size acutely influence the resulting properties and lead to the observation of the discrepancies in the bibliography results.

In this study, rapid heat treatment was applied to the Ti-6Al-4V ELI alloy, leading to a dual-phase microstructure after quenching. The resulting microstructure and its mechanical properties were subsequently measured.

95 2. Materials and methods

The material used in this investigation is an ELI grade Ti-6Al-4V alloy (for composition, see Table 1). Its composition respects the ASTM F136 standard for surgical applications [4]. The $\alpha+\beta$ microstructure, described in Figure 1, is the result of an annealing treatment at 704°C for two hours, followed by air cooling after a rolling
100 process. The average grain size is 1.15 μm and 0.28 μm for the α - and β -phases, respectively. The product certification indicates β -transus at 973°C.

The dilatometry test used to study the phase transformation during heating was carried out on a Gleeble 3500 coupled to a mechanical extensometer. The schematic sample dimensions used for testing is presented in Figure 2. A type-K thermocouple
105 was used to control the 20°C/min heating and cooling rate of the specimen. The sample was heated to 1050°C, exceeding the β -transus temperature. To avoid oxidation and nitrogen diffusion, which both cause great change in phase transformations and mechanical properties in titanium alloys [13], the whole test was performed under argon atmosphere after a previous vacuum.

110 Once the experimental plan had been designed, the heat treatments were carried out using a muffle furnace under argon atmosphere. Three different temperatures were tested, leading to three different phase fractions: 923°C, 948°C and 1030°C.

The first microstructure analysis was carried out using XRD (X-Ray Diffraction). A PANalytical X'Pert X-ray diffractometer equipped with a monochromator detector
115 and a 1/32° slit was used to analyse a range between 34° and 42°. Scan parameters were set to a step size of 0.002 and an acquisition rate of 600s/step.

The microstructures of the initial and heat-treated samples were imaged using a Backscattered Electron (BSE) detector and an Electron Backscatter Diffraction (EBSD) detector (NordlysF+ model from Oxford Instruments) in a Zeiss Supra 55 Scanning
120 Electron Microscope (SEM) operated at an accelerating voltage of 20kV. Following a Monte Carlo simulation showing that absorption is lower at this voltage than the β grain size for this experimental set up, EDS (Energy Dispersive X-ray Spectroscopy) evaluation was carried out at a voltage of 8kV.

The Vickers micro-hardness test was performed on a Buehler Micromet 5140
125 machine. Indentation was achieved by applying a 1kg load for 10s (10 measures per sample).

An Instron 5967 tensile machine, equipped with an optical extensometer, was used to test three samples for each condition at a displacement rate of 0.2mm/s. Samples were machined using the EDM (Electron Discharge Machining) technique, according to the diagram presented in Figure 3.

Rotating bending fatigue tests were carried out on a Walter+Bai UBM 5-200Nm. A fully reversed ($R=-1$) test was performed at 50Hz on the high cycle fatigue range (10^7 cycles), testing 10 samples from each condition. A diagram of the fatigue sample design is shown in Figure 4.

A colorimetric MTT assay was used to assess cytotoxicity on two different human cell types (obtained from adult skin explants), namely MG-63 osteoblast-like cells and the fibroblast. The cells were cultured in 24-well plates, with RPMI 1640 (Dutscher, France, ref L0498-500) supplemented with 10% of foetal bovine serum (ref Dutscher, France, ref P040637100) and 5% of antibiotic/antimycotic solution (ref Dutscher, France, ref SV30079.01) at 37°C in a humidified atmosphere of 5% CO₂.

The discs of the studied material were sterilised by ethanol 70% bath during 30min, rinsed with distilled water and dried. Then the discs were placed in the well of 24-well plate (Corning USA ref 3524) (one sample per well). Finally, the tested material was compared to the control group (culture dish surface - polystyrene treated for cell culture). 100µL of cells suspension were directly deposited on disc of the studied material, with exactly 250,000 cells/mL of MG-63 osteoblast-like cells and 300,000 fibroblasts/mL. The samples thus seeded were then incubated in a humid atmosphere for 2hrs at 37°C and 5% CO₂ to allow cell adhesion before the addition of 2mL of culture medium, for culture until first cytotoxic test at D3.

Each material was evaluated in triplicate and each test was replicated three times.

The cytotoxicity evaluation was performed through a colorimetric MTT assay: yellow salt of tetrazolium was reduced by NAD(P)H-dependent cellular oxidoreductase enzymes to insoluble Formosan of purple colour. After lysis of cells and solubilisation of crystals of Formosan by DMSO, optical density was measured using an INFINITE F 200 Pro fluorimeter (Tecan) to wavelength of 570nm. The DO was directly proportional of number of living cells present in the well.

3. Results & Discussion

A dilatometry test was performed prior to the design of the heat treatments carried out in this study. Due to the different crystallography of α and β -phase, the phase transformation is accompanied by a change in volume. The dilatometry test allows us to quantify the α/β phase transformation during heating. The martensitic phase results from the diffusionless transformation of the high temperature β -phase; the equivalent martensitic phase fraction can therefore be calculated using the dilatometry data. The resulting dilatometry curve is shown in Figure 5.

It should be noted that the dilatometry technique only quantifies the α/β transformation. It is thus necessary to take the initial β -phase fraction into account to correctly evaluate changes in the β -phase during heating. Microscopic analysis was used to obtain this value.

A BSE detector was used to identify the different chemical compositions of each phase. As the β -phase is richer in vanadium than the α -phase, it appears brighter than α -phase, as shown in Figure 6a. The initial β -phase fraction was also determined by EBSD (Figure 1) to compare the results.

The β -phase fraction was also calculated using image software analysis. Analysis using BSE images calculated the average of 3 zones and concludes that the initial microstructure is composed of 6% β -phase, compared to 4% when the EBSD technique is used. The initial β -phase fraction was therefore considered to be an average of 5% in order to allow for the possible errors of both methods.

The dilatometry curve shows three different distinct zones:

1. From room-temperature to approximately 600°C, a linear behaviour appears that corresponds to the thermal expansion of $\alpha+\beta$ initial microstructure.
2. From 600°C to 980°C, a drastic change occurs, corresponding to α - to β -phase transformation.
3. At temperatures over 980°C, a linear behaviour corresponding to β -phase thermal expansion is restored.

These distinct zones make it possible to apply the Lever rule (see Figure 5) to calculate the β -phase fraction transformation during the heating process. Figure 7 represents the correct β -phase fraction evolution, taking the initial phase fraction into account.

190 It is well known that the Ti-6Al-4V alloy is stable until it reaches moderate temperatures (400°C), and this can be observed on the dilatometry curve. Indeed, Figure 7 shows the beginning of the α/β transformation at 500°C and the β -transus occurring at 980°C, a value close to the 973°C noted on the product certification. These results validate the experiment and the calculation of the β -phase fraction.

195 Once the relationship between the temperature and phase fraction transformation had been plotted, three heat treatments were chosen to represent the total α' -phase region (from 900°C to β -transus temperature [14]).

The microstructure of the different heat-treated samples was analysed by SEM using a BSE detector, which identifies the chemical difference between the α' -phase and α -phase. The resulting images and the initial microstructure are presented in Figure 6. The phase fraction was evaluated using image software analysis. Comparative values are given in Table 2.

200 The differences observed between the two techniques may be due to the heating rate. It was previously reported by J.W. Elmer *et al.* that the heating rate influences the phase fraction transformation in Ti-6Al-4V alloy [15]. Their study shows that an increase in the heating rate decreases the percentage of the transformed β -phase for a given temperature. In the present study, 51 minutes were needed to reach a temperature of 1030°C in the dilatometry test carried out at 20°C/min, while the same temperature was reached in under 15min in the muffle furnace, as the 100% α' alloy microstructure shows. The higher heating rate on the muffle furnace therefore explained the lower values of phase transformation compared to those obtained from the dilatometry test. Below, the different samples will be referred to by their martensitic phase fraction, calculated by SEM microstructure analysis.

210 The activation energy of the grain growth (Q) is calculated to identify how the main alloying elements influence the α/β transformation during holding. In Figure 8, the β grain size (measured from SEM images) is represented according to the holding temperature.

215 Basic calculations predicting the grain size are based on the equation for ideal grain growth, see Equation 1:

$$D - D_0 = K t^n$$

where D is the actual grain size, D_0 is the initial grain size at the considered temperature; K is the proportional constant, t is the holding time at the temperature and n is its exponent ($n = 1$). This equation presumes the absence of defects and precipitates and supposes that grain growth is controlled by diffusion alone.

225 Although this equation represents an isothermal condition, the relative rapidity of the heating stage would logically limit any growth during heating. D_0 can therefore be considered as the grain size of the initial material state at room temperature.

Equation 2, or the *Arrhenius equation*, defines the relation between the proportionality constant K and temperature T at which the grain grows.

230

$$K = K_0 \exp\left(-\frac{Q}{RT}\right)$$

In Equation 2, T is the holding temperature, Q is the activation energy necessary for grain growth, K_0 is the total pre-exponential constant and R is the gas constant.

235 The proportional constant K can be calculated from Equation 1 before being applied to Equation 1 to identify the activation energy (Q) value through a linear regression line.

The activation energy value obtained is 176KJ/mol; this is close to the value of 173.3KJ/mol found in the literature for vanadium diffusion in titanium [16]. This result
240 leads to the conclusion that the α to β transformation is governed by the diffusion of vanadium.

To obtain a better insight into the transformation phase resulting from heat treatments, XRD analysis was carried out on the initial and heat-treated samples. The results are presented in Figure 9.

245 One example of β/α' martensitic transformation is the disappearance of the initial β -phase in the heat-treated samples. This is clearly seen in the different diffractograms. The lattice parameters of the α -phase and α' -phase can be observed on the $\alpha+\alpha'$ samples in the form of double peaks in the initial α -phase peak. The evolution of these parameters is also observed on the heat-treated samples, mainly thought to be due to
250 their chemical evolution (vanadium diffusion).

The XRD diffractograms were used to calculate the lattice parameters of the initial and heat-treated alloys. Table 3 shows the different values of the α -phase and α' -phase.

Different studies report varying α -phase lattice parameters values [17, 18]. Our findings are in accordance with those of R. Montanari *et al.* [19].

255 When the heat treatment temperature increases, a decrease occurs in a_0 as well as c_0 from the α -phase. This is the result of the heat-induced evolution in the chemical composition.

A semi-quantitative evaluation using the SEM-EDX technique was carried out to evaluate this hypothesis. Results are given in Figure 10.

260 In order to preserve this phase at room temperature, the vanadium content observed in the initial β -phase must remain high [20]. When the temperature increases, the β -phase fraction increases and its vanadium content decreases. At 47% of the transformation, the vanadium content is not high enough to form the secondary martensite (α' -phase) [21] or to retain the β -phase. Therefore, α' martensite should form
265 after all heat-treated alloys have been quenched. The chemical differences between the α - and α' -phases are responsible for the double peak appearance observed in the XRD results.

The first attempt to observe the influence of the different martensite fractions on mechanical properties was made using Vickers micro-hardness characterization. As the
270 'soft' β -phase [22] transformed into the martensitic α' -phase, we expected to observe a proportional increase in hardness as the martensite phase fraction increased. The micro-hardness of the different samples is presented in Figure 11.

For the high α' -phase fraction, the expected trend is observed. However, no difference in terms of hardness can be seen between the 47% α' sample and the initial
275 sample. This may be due to the occurrence of a contra-balance effect, by which the hardness of the α' -phase is then decreased by the presence of the α -phase through its lower vanadium content or by a TRIP effect. This point will be discussed later in the manuscript.

Tensile tests were performed to obtain a better understanding of the resulting
280 mechanical properties. Figure 12 presents the resulting tensile curves for all studied alloys. The average value of the principal tensile properties is given in Table 4.

Increasing the percentage of the martensitic α' -phase fraction leads to an increase in the UTS (Ultimate Tensile Strength). For higher α' -phase fractions (77% and 100%) the $YS_{0.2}$ (Yield Strength) also increases significantly. However, a concurrent decrease is
285 observed in the ϵ_u (Uniform Elongation).

The tensile tests agree with previous results for hardness. The 47% α' sample possesses an unexpected mechanical behaviour, displaying an increase in ϵ_u while the $YS_{0.2}$ remains at its initial $\alpha+\beta$ value.

Previous reports suggest the possibility of a TRIP deformation mechanism [9, 23] that could explain the abnormal behaviour of the 47% α' alloy. To verify if a phase transformation occurs during deformation, the work-hardening value has been calculated and is presented in Figure 13.

Improved work hardening is obtained in the 47% α' alloy, however no clear sign of the TRIP effect is observed. Thus, we can conclude that the behaviour of the 47% α' alloy is not due to the TRIP effect.

In agreement with C. Formanoir *et al.*, the abnormal behaviour of the 47% α' alloy and its improved work-hardening could be due to a composite effect [10]. Some multiphase alloys may display unexpected work-hardening property when compared to their equivalent single-phase alloys. The work-hardening improvement can be attributed to the difference in mechanical properties between phases (α - and α' -phase in this case). The consequently unequal distribution of stress and strain leads to the formation of additional dislocations at the interface, thus improving the global work hardening. TEM observations have been carried out on the sample, but due to a low statistic no clear evidence of this increase of dislocation density can be given.

As observed in DP steels, the inhomogeneity of mechanical properties between phases is due to their chemical composition. The work hardening improvement is therefore also related to the amount of martensite in the phase fraction.

The inhomogeneous deformation of the two phases (α - and α' -phase) is shown in Figure 14 via *Jaoul-Crussard* plot analysis. Only three stages can be seen on the 47% α' alloy:

1. Stage I - Homogenous elastic deformation of the α - and α' -phase.
2. Stage II - Inhomogeneous plastically deformation of the α - and α' -phase.
3. Stage III - Homogeneous plastically deformation of the α - and α' -phase.

In contrast to DP steels, the mechanical inhomogeneity of the α - and α' -phase in the studied alloys is much lower. When the percentage of phase fractions turns out to be

dissimilar and martensite becomes the matrix, the work-hardening improvement is lost. Evidence of this is seen in the previous *Jaoul-Crussard* plot (Figure 14).

320 The low yield strength properties and micro-hardness presented by the 47% α' alloy may be due to the presence of very low and local fractions of the soft martensite α'' -phase or the retained β -phase, although no evidence of this was observed. Indeed, the low fraction of these phases involves using synchrotron diffraction to be possibly measured.

325

The most important mechanical property of any material subjected to repetitive loads is endurance, or fatigue strength. The results of the rotating bending fatigue tests performed on the initial and heat-treated samples are presented in Figure 15.

330 The initial alloy (equiaxed $\alpha+\beta$ of this study) shows higher fatigue resistance than the values reported in previous literature, which range from 423-550MPa for Ti-6Al-4V ELI grade during rotating bending fatigue tests [24, 6]. This could be explained by different factors such as chemical impurities or microstructure size.

335 As observed in this study, all martensite alloys exhibit improved fatigue strength at 10^7 cycles compared to the initial condition. The best fatigue performance is obtained in both 47% and 77% α' alloys, with a 20% increase in the initial fatigue strength, followed closely by the full martensitic alloy.

Results show no difference between alloys in terms of fatigue crack initiation, which first occurs at the surface of the sample where the stress is higher.

340 The improvement in fatigue properties has previously been attributed to a possible transformation-induced plasticity (TRIP) effect [9, 23], where energy dissipation may take place instead of the fatigue damage accumulation. However, none of the martensitic alloys in this work displayed any TRIP behaviour.

345 The fatigue improvement of the 47% α' alloy is mainly due to its improved work-hardening and better accommodation of local dislocations during deformation. The improvement observed for the 77% α' alloy is mainly due to the yield strength increase and its relatively good ductility. The increase in yield strength in our study is reflected by an increase in its high cycle fatigue properties, supporting G. Lutjering's claim that there is a link between yield strength and fatigue life at high number of cycles [22]. A possible effect of the different texture obtained after the heat treatment can also be part of the explanation, as G. Lutjering already mentioned in his work. However, as the

350

phases and a fraction of the phase are different in each of the case presented here, the effect of texture can be determined separately. It is worth mentioning that the other parameters known to modify the fatigue strength (iron composition, inclusions...) are the same in all the samples considered here.

355 From an industrial point of view, the large fraction range of α' -martensite over which optimal fatigue properties can be obtained makes water-quench heat treatment an ideal process for increasing the mechanical properties of Ti-6Al-4V and its ELI grade. It is always difficult to compare values with those found in literature, and this is especially true of titanium alloys, where impurities are so influential. For this reason, we prefer to
360 compare the percentage of improvement rather than provide a precise fatigue value.

The most competitive technique permits the obtaining of a UFG microstructure. The martensitic alloy values of this study were compared to the fatigue strength of the microstructures (obtained from a rotating bending fatigue test) resulting from two different studies by S. Zherebtsov *et. al*, who obtained an improvement of 15% using
365 multiaxial forging [25], and L.R. Saitova *et. al*, who obtained an improvement of 9% using the ECAP process [6].

The water-quench heat-treatment is a more efficient and better technique to improve the mechanical properties of the Ti-6Al-4V ELI alloy: this process is not only remarkably simple in comparison with the complex process required by UFG methods,
370 but also provides improved fatigue strength.

The cytotoxicity of these microstructures must be evaluated prior to their use for biomedical applications. *In vitro* cytotoxicity tests are the primary biocompatibility tests, as they can provide rapid and inexpensive data on biological interactions. The assay is related to the cell's viability and proliferation in the culture, generating a signal
375 that is proportional to the number of living cells [26].

A toxic material is defined as a material that releases a chemical in enough quantities to kill cells or drastic decrease cell proliferation, either directly or indirectly, by inhibiting key metabolic pathways by comparison with cell culture on tissue culture treated plates [27].

380 The Ti-6Al-4V ELI grade has been used as a biomaterial for over a decade and is a leading structural material in the biomedical applications market. However, changes in microstructure and phase composition can affect its toxicity properties.

Even if the cytotoxicity could depend on other aspects of the microstructure (surface energy, crystallographic texture, grain boundary density, phase boundary density, surface topography...) in our case, we feel that the other parameters than composition are of second order. Indeed, most of the features of the microstructure will not change drastically between the samples. Surface topology is erased by polishing whereas surface energies should not change in that range of composition. In this study, cytotoxicity was evaluated in one of the various studied microstructures. Important factors as: surface energy [37,41,42], topography [32-40] or crystallographic texture [29-31] are not analysed in this study, even if they could influence the cytotoxicity results. The main objective of the experiment is to verify that the good cytotoxicity of the reference material is not degraded by the heat treatment. Thus, the results presented here are mainly comparative ones. It shows that the optimized microstructure in terms of mechanical properties does not exhibit a deteriorated biocompatibility considering that all the others important parameters cited before did not change.

The cytotoxicity experiment was done through 'direct assays': cells were directly placed on the material inside a culture plate before being incubated with a culture medium. The 47% α' alloy, sample showing one of the best fatigue properties, was tested with human MG-63 osteoblast-like cells and fibroblasts at various intervals (3, 6 and 10 days). Results of the cell proliferation evolution over time for both assays are plotted in Figure 16.

The MG-63 osteoblast-like cell cytotoxicity assays show that the new microstructure has a very limited effect on cell proliferation, with a main value which decreases but remains within the standard deviation of the comparison data. The presence of the 47% α' alloy in the fibroblasts assays seems to decrease the proliferation rate further, although cell proliferation does not cease and increases with time, albeit at a slower rate. It must be noted that fibroblast proliferation started also at day 3 and still increases with time. The heightened sensitivity to the presence of the 47% α' alloy for the fibroblasts may be explained by one of the following hypotheses:

1. Fibroblasts cells are primary cells and consequently more 'fragile' than the MG-63 osteoblast-like cells after several passages [28].
2. Fibroblasts cells needs longer time to adapt to the metal surface.

415 These results clearly show that, on the alloy considered here with this method, no
cytotoxicity can be observed due to the presence of the α' -phase. We can conclude that
the Ti-6Al-4V ELI grade, with the presence of a high fraction α' -martensite phase, is
not cytotoxic. However, it is certain that further studies, such as *in vivo* assays, must be
carried out to assess the safety of the heat treatments considered in this study.

420 4. Conclusions

This paper reports the use of a water-quench rapid heat treatment to optimize the
microstructure and increase the mechanical properties of the Ti-6Al-4V ELI alloy
currently used in biomedical applications.

425 The partial or total transformation of the Ti-6Al-4V ELI microstructure into a
martensitic α' -phase improves the high cycle (10^7 cycles) fatigue strength when
compared to the initial $\alpha+\beta$ equiaxed microstructure (Ti-6Al-4V ELI standard
microstructure for biomedical applications).

430 Tensile test results reveal an unexpected behaviour of the 47% α' alloy. An increase
in ductility is observed, whilst the same yield strength value as the initial condition is
maintained. This phenomenon may possibly be related to a 'composite effect' resulting
from a difference in the chemical compositions of the α and α' phases.

435 The optimum fatigue performance is located within in a wide phase fraction range
ranging from 47% to 77% α' -phase, making the water-quench heat treatment an
economic and efficient technique. The martensitic alloys remain cytocompatibility with
the initial $\alpha+\beta$ microstructure, which is currently used in biomedical applications.

The results presented show that martensitic dual phase ($\alpha+\beta$) Ti-6Al-4V ELI alloys
can be proposed as a new standard in biomedical applications. Indeed, heat treatment
improves the static and fatigue properties of these microstructures without modifying
the cytotoxicity of the alloy.

440 References

- [1] Bothe, R. T. (1940). Reaction of bone to multiple metallic implants. Surg
Gynecol Obstet, 71, 598-602.

- 445 [2] Leventhal, G. S. (1951). Titanium, a metal for surgery. *J Bone Joint Surg Am*, 33(2), 473-474.
- [3] Beder, O. E., & Eade, G. (1956). An investigation of tissue tolerance to titanium metal implants in dogs. *Surgery*, 39(3), 470-473.
- 450 [4] A. Standard, F136-02a: Standard specification for wrought titanium-6 aluminum-4 vanadium ELi (extra low interstitial) alloy for surgical implant applications (unpublished). West Conshohocken, PA: ASTM International; 2002, doi: 10.1520/f0136-02a, West Conshohocken, PA: ASTM International.
- 455 [5] Saitova, L. R., Höppel, H. W., Göken, M., Semenova, I. P., & Valiev, R. Z. (2009). Cyclic deformation behavior and fatigue lives of ultrafine-grained Ti-6Al-4V ELI alloy for medical use. *International Journal of Fatigue*, 31(2), 322-331.
- [6] Saitova, L. R., Höppel, H. W., Göken, M., Semenova, I. P., Raab, G. I., & Valiev, R. Z. (2009). Fatigue behavior of ultrafine-grained Ti-6Al-4V 'ELI' alloy for medical applications. *Materials Science and Engineering: A*, 503(1-2), 145-147.
- 460 [7] Zharebtsov, S., Kudryavtsev, E., Kostjuchenko, S., Malysheva, S., & Salishchev, G. (2012). Strength and ductility-related properties of ultrafine grained two-phase titanium alloy produced by warm multiaxial forging. *Materials Science and Engineering: A*, 536, 190-196.
- 465 [8] Sherman, R. G., & Kessler, H. D. (1956). Investigation of the heat treatability of the 6% aluminum-4% vanadium titanium based alloy. *Trans ASM*, 48, 657-676.
- [9] Imam, M. A., & Gilmore, C. M. (1983). Fatigue and microstructural properties of quenched Ti-6Al-4V. *Metallurgical Transactions A*, 14(1), 233-240.
- 470 [10] de Formanoir, C., Brulard, A., Vivès, S., Martin, G., Prima, F., Michotte, S., ... & Godet, S. (2017). A strategy to improve the work-hardening behavior of Ti-6Al-4V parts produced by additive manufacturing. *Materials Research Letters*, 5(3), 201-208.

- 475 [11] Matsumoto, H., Yoneda, H., Fabregue, D., Maire, E., Chiba, A., & Gejima, F. (2011). Mechanical behaviors of Ti–V–(Al, Sn) alloys with α' martensite microstructure. *Journal of Alloys and Compounds*, 509(6), 2684-2692.
- [12] Matsumoto, H., Yoneda, H., Sato, K., Kurosu, S., Maire, E., Fabregue, D., ... & Chiba, A. (2011). Room-temperature ductility of Ti–6Al–4V alloy with α' martensite microstructure. *Materials Science and Engineering: A*, 528(3), 1512-1520.
- 480 [13] Kahveci, A. I., & Welsch, G. E. (1986). Effect of oxygen on the hardness and alpha/beta phase ratio of Ti-6Al-4V alloy. *Scripta metallurgica*, 20(9), 1287-1290.
- [14] Welsch, G., Boyer, R., & Collings, E. W. (Eds.). (1993). *Materials properties handbook: titanium alloys*. ASM international.
- 485 [15] Elmer, J. W., Palmer, T. A., Babu, S. S., & Specht, E. D. (2005). Low temperature relaxation of residual stress in Ti–6Al–4V. *Scripta materialia*, 52(10), 1051-1056.
- [16] E. A. Brandes, G. Brook, *Smithells materials reference book* (1992).
- 490 [17] Grabovetskaya, G. P., Melnikova, E. N., Kolobov, Y. R., Chernov, I. P., Naidenkin, E. V., Nikitenkov, N. N., & Mishin, I. P. (2006). Evolution of the structural and phase states of a Ti-6Al-4V alloy in forming submicrocrystalline structure with the use of temporary hydrogenation. *Russian Physics Journal*, 49(4), 442-447.
- 495 [18] Ivanov, M. B., Manokhin, S. S., Kolobov, Y. R., & Nechayenko, D. A. (2010). Phase composition and microstructure of Ti-6Al-4V alloy after hydrogen-plastic working.
- [19] Montanari, R., Costanza, G., Tata, M. E., & Testani, C. (2008). Lattice expansion of Ti–6Al–4V by nitrogen and oxygen absorption. *Materials characterization*, 59(3), 334-337.
- 500 [20] E. K. Molchanova, *Phase diagrams of titanium alloys*, IPST, 1965.

- [21] Bagariatskii, I. A., Nosova, G. I., & Tagunova, T. V. (1958, September). Factors in the formation of metastable phases in titanium-base alloys. In Soviet Physics Doklady (Vol. 3, p. 1014).
- 505 [22] Lütjering, G. E. R. D. (1998). Influence of processing on microstructure and mechanical properties of (α + β) titanium alloys. Materials Science and Engineering: A, 243(1-2), 32-45.
- [23] Kennedy, J. R. (1983). Fatigue behavior of solution-treated and quenched Ti-6Al-4V. Materials Science and Engineering, 57(2), 197-204.
- 510 [24] Niinomi, M. (2007). Fatigue characteristics of metallic biomaterials. International Journal of Fatigue, 29(6), 992-1000.
- [25] Zharebtsov, S., Salishchev, G., Galeyev, R., & Maekawa, K. (2005). Mechanical properties of Ti-6Al-4V titanium alloy with submicrocrystalline structure produced by severe plastic deformation. Materials transactions, 46(9), 2020-2025.
- 515 [26] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 65(1-2), 55-63.
- [27] Ratner, B. D., Hoffman, A. S., Schoen, F. J., & Lemons, J. E. (2004). Biomaterials science: an introduction to materials in medicine. Elsevier.
- 520 [28] Cristofalo, V. J., Allen, R. G., Pignolo, R. J., Martin, B. G., & Beck, J. C. (1998). Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proceedings of the National Academy of Sciences, 95(18), 10614-10619.
- 525 [29] S. Faghihi et al., "The significance of crystallographic texture of titanium alloy substrates on pre-osteoblast responses," Biomaterials, vol. 27, pp. 3532-3539, 2006.
- [30] S. Faghihi, H. Vali, and M. Tabrizian, "Effects of crystal size and orientation of substrates on cell adhesion: Implication for medical implants," Int. J. Mod. Phys. B, vol. 22, no. 18-19, pp. 3069-3081, 2008.

- 530 [31] S. Faghihi, M. R. Bateni, F. Azari, J. A. Szpunar, H. Vali, and M. Tabrizian,
"The Role of Crystallographic Texture of Ti-6Al-4V Alloy on Cell
Attachment and Proliferation," *Mater. Sci. Forum*, vol. 495–497, pp. 705–710,
2005.
- [32] C. H. Lohmann et al., "Surface roughness modulates the response of MG63
535 osteoblast-like cells to 1,25-(OH)₂D₃ through regulation of phospholipase A₂
activity and activation of protein kinase A," *J. Biomed. Mater. Res.*, vol. 47,
no. 2, pp. 139–151, Nov. 1999.
- [33] R. A. Gittens et al., "The effects of combined micron-/submicron-scale surface
roughness and nanoscale features on cell proliferation and differentiation,"
540 *Biomaterials*, vol. 32, no. 13, pp. 3395–3403, 2011.
- [34] R. A. Gittens et al., "A review on the wettability of dental implant surfaces II:
Biological and clinical aspects," *Acta Biomaterialia*, vol. 10, no. 7, 2014.
- [35] A. Iost, D. Najjar, K. Anselme, and M. Biggerelle, "Relationships between the
surface morphology and a physical response in the biomaterial field," in
545 *Thermec'2003, Pts 1-5*, vol. 426–4, T. Chandra, J. M. Torralba, and T. Sakai,
Eds. Zurich-Uetikon: Trans Tech Publications Ltd, 2003, pp. 3031–3036.
- [36] K. Anselme and M. Biggerelle, "Modelling approach in cell/material
interactions studies," *Biomaterials*, vol. 27, no. 8, pp. 1187–1199, 2006.
- [37] G. Zhao, A. L. Raines, M. Wieland, Z. Schwartz, and B. D. Boyan,
550 "Requirement for both micron- and submicron scale structure for synergistic
responses of osteoblasts to substrate surface energy and topography,"
Biomaterials, vol. 28, no. 18, pp. 2821–2829, 2007.
- [38] K. M. Hotchkiss, G. B. Reddy, S. L. Hyzy, Z. Schwartz, B. D. Boyan, and R.
Olivares-Navarrete, "Titanium surface characteristics, including topography
555 and wettability, alter macrophage activation," *Acta Biomater.*, vol. 31, 2016.
- [39] R. A. Gittens et al., "The roles of titanium surface micro/nanotopography and
wettability on the differential response of human osteoblast lineage cells,"
Acta Biomater., vol. 9, no. 4, 2013.

- 560 [40] B. D. Boyan et al., "Osteoblast-Mediated Mineral Deposition in Culture is
Dependent on Surface Microtopography," *Calcif. Tissue Int.*, vol. 71, pp. 519–
529, 2002.
- [41] D. J. Irvine, K.-A. Hue, A. M. Mayes, and L. G. Griffith, "Simulations of Cell-
Surface Integrin Binding to Nanoscale-Clustered Adhesion Ligands."
- 565 [42] J. Luo and Y.-M. Chiang, "Existence and stability of nanometer-thick
disordered films on oxide surfaces," *Acta Mater.*, vol. 48, no. 18, pp. 4501–
4515, 2000.

570

Table 1: Chemical composition of the Ti-6Al-4V ELI alloy used in the present research [Wt. %]

Ti	Al	V	C	Fe	N	O	H
Bal.	6.01	3.93	0.015	0.18	0.005	0.12	0.0025

575

Table 2: SEM (BSE) and Dilatometry phase fraction calculation of the resulting α' -martensite.

Heat treatment temperature [°C]	α' fraction [%]	
	SEM (BSE)	Dilatometry
923	47	66
948	77	87
1030	100	100

Table 3: Lattice parameters of α -phase and α' -phase for the initial and heat-treated alloys.

α' fraction [%]	α		α'	
	a_0 [nm]	c_0 [nm]	a_0 [nm]	c_0 [nm]
Initial	0.29336	0.46804		
47	0.29217	0.46657	0.29140	0.46357
77	0.29194	0.46648	0.29134	0.46489
100			0.29142	0.46576

Table 4: Principal tensile properties from initial and heat-treated samples.

α' fraction [%]	YS _{0.2} [MPa]		UTS		ϵ_u [%]		YS _{0.2} / ϵ_u Ratio	A _r [%]
	SE_x		SE_x		SE_x			
Initial	872	2	1076	9	8.1	0.3	108	30
47	875	9	1140	1	10.3	0.8	85	68
77	923	2	1131	6	5.7	0.5	100	37
100	960	16	1122	6	4	0.4	204	30

580

Figure 1: a) EBSD quality map of the initial microstructure. b) EBSD map with β -phase in yellow.

585

Figure 2: Sample design for dilatometry testing (dimensions in mm.).

590

Figure 3: Sample design for tensile testing (dimensions in mm.).

595

Figure 4: Sample design for rotating bending fatigue testing (dimensions in mm.).

Figure 5: Dilatometry curve of the initial Ti-6AL-4V ELI alloy.

Figure 6: SEM (BSE) observation. a) Initial $\alpha + \beta$ microstructure (β in bright). b) and c) 923°C, 948°C WQ-HT dual $\alpha + \alpha'$ microstructure respective (martensite in bright). d) 1030°C WQ-SHT full α' microstructure.

605

Figure 7: β -phase transformation during heating calculated from the dilatometry test.

610

Figure 8: Grain size of β -phase as a function of holding temperature.

Figure 9: XRD diffractograms of the initial and different heat-treated samples.

620 **Figure 10:** EDX-SEM composition of the main alloying elements in each phase. a) α -phase. b) β/α' -phase.

Figure 11: Vickers micro-hardness results for initial and different martensitic alloys.

625

Figure 12: Tensile test curves for the initial and different martensitic alloys.

630 **Figure 13:** Work hardening rate curves of tensile testing for initial and different martensitic alloys.

Figure 14: Jaoul-Crussard plot analysis of the heat-treated samples.

Figure 15: 10⁷ cycles fatigue life for the initial and different martensitic alloys.

Figure 1: Cytotoxicity assays on the 47% α' alloy and culture treated polystyrene: LEFT) MG-63 osteoblast-like cells. RIGHT) Fibroblasts. (mean _ standard deviation).