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Optimization of Titanium Surface for Live Cells

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The present article is focused on studying the characteristics of selected titanium alloys before and after heat treatment. The structural characteristics of titanium and titanium alloys, as well as their reactions to heat treatment and different factors, have been described in detail. The specimens were cooled both in water and liquid nitrogen from 900°C and 1000°C for pure titanium and from 1000°C and 1100°C for the Ti-6Al-4V alloy. Also, the article paid attention to the PVD coating process on Ti-6Al-4V alloy. Further, the article deals with line MG63 live bone cells deposited on a titanium base substrate. Proliferation and differentiation are monitored of cells during 7-day in vitro cultivation portraying growth of cells on a biologically selected material.

Keywords: Titanium alloys, Live cells, Mechanical properties, Scanning electron microscopy, Heat treatment

1 Introduction

Titanium and its alloys belong to metallic materials which are considered as most appropriate materials for medical applications. Titanium belongs to a group of allotropic elements which exist in two crystal structures. At room temperature titanium has a hexagonal close-packed (HPC) crystal structure called alpha phase (α). This structure transforms at a temperature of 882°C to a body-centered cubic (BCC) crystal structure called beta phase (β). The mechanical properties of titanium depend on the content of impurities. Among the most deteriorating are nitrogen (N_2), oxygen (O_2), carbon (C) and hydrogen (H_2), which already in small amounts significantly increase the strength of the material but simultaneously are responsible for a marked increase of brittleness. In addition the mechanical properties of titanium are affected by the general magnitude and shape of the grains, i.e. by the method of heat treatment and type of mechanical working [1, 2].

The strength of pure titanium is similar to that of structural steels, however in contrast to them its modulus of elasticity is 50% lower. Hardness in dependence on the chemical purity, is in the range from 100 to 250 HB. One of the main advantages of titanium and titanium alloys are their high resistance to corrosion, comparable with that of corrosion-resistant steels and special corrosion-resistant alloys. In an oxidizing atmosphere and in nitric acid (HNO_3) passivation layers – namely titanium dioxide (TiO_2) – are formed on the surface of titanium which considerably increase its corrosion potential. Due to the above fact titanium is inert in contact with both fresh and salt water. At higher temperatures titanium is resistant to most organic acids, alkaline hydroxide solutions and oxide compounds of chlorine [3, 4].

Due to the inertness, in sense of reacting with current matter in the human body and capability of forming harmless titanium dioxide on its surface, attention is paid in the present paper to the use of pure titanium and a selected titanium alloy in medical applications in the form of various replacement parts for the human body. Biological tests which will serve for comparison of the behavior of other metals versus a live cell were carried out on a selected titanium surface [12, 13, 14, 15].

2 Materials and methods

2.1 Titanium and Titanium alloys

Titanium alloys can be divided into three main groups with respect to phases present at room temperature: α, α/β and β. Each group is specific in dependence on the chemical composition and method of processing. Since Ti is a polymorphous element, the space lattice is usually present in two prevailing structures where atoms of the base material (Ti) and alloying elements form a solid solution thus creating two polymorphous modifications – a high temperature body-centered cubic (BCC) lattice specific to the β - phase and a low-temperature hexagonal close-packed (HCP) lattice specific to the α – phase. The transformation temperature of pure titanium is approx. 882°C. Additions of alloying elements have no effect on this temperature. Diffusion in the α–phase is hundredfold smaller than in the β-phase which is a good base for high resistance to creep [5].

The present trend in research and development of titanium alloys for biomedical applications is directed toward β-type Ti-alloys with a low modulus of elasticity containing absolutely nontoxic elements (e.g. Nb, Ta, Zr etc.) with excellent mechanical properties and workability. These alloys with their behavior are very close to a real bone. β-type Ti-alloys are alloyed with elements expanding the range of existence of the β solid solution, which decreases the β transformation temperature. The higher is the amount of β stabilizers the higher is the resistance to corrosion and hence also the more favorable are the mechanical properties of the alloy for biomedical applications. On the other hand the total mass increases due to the higher density of β stabilizers than due to that of α stabilizers [6].

The decomposition is accompanied by redistribution of atoms and can proceed even at room temperature. The α–phase can then appear in the form of hexagonal α´ or orthorhombic α´´ martensite which is usually present in the form of interconnected needles, stabilizing to a certain extent the given alloy. The recombination can have a diffusion or non-diffusion character depending on whether temperature or deformation stress is concerned. Here an analogy can be found with the metastable γ-phase in austenite of Fe-Ni steels, which is also capable of a martensitic transformation to α´ (α´´). The β –phase can also be obtained from the α/β-Ti-alloy at room temperature after rapid cooling (quenching) from temperatures higher than those of the α↔β transition. Such a titanium alloy is called pseudo-β. The grain in this case is made up of the β –phase surrounded by the α-precipitate along the grain boundaries. Subsequent low-temperature heating of the alloy (ageing) below the β transformation temperature or by annealing of the β –phase leads to a β→α transformation, the α-precipitate coarsens, the structure of the grain is transformed and the alloy is stabilized [7, 9].

2.2 Selected Ti-6Al-4V alloy

This alloy is the most widespread and most frequently used titanium alloy for structural purposes and more than 50 grades of titanium alloys are derived from it. Ti-6Al-4V with its high strength, low mass and high corrosion resistance is widely used in many fields of industry. It is applied in aerospace and automobile industry, in chemical and food production, in crude-oil and natural gas production, in the production of sporting outfit, etc. It is widely used in medicine – above all in surgery [3, 10].

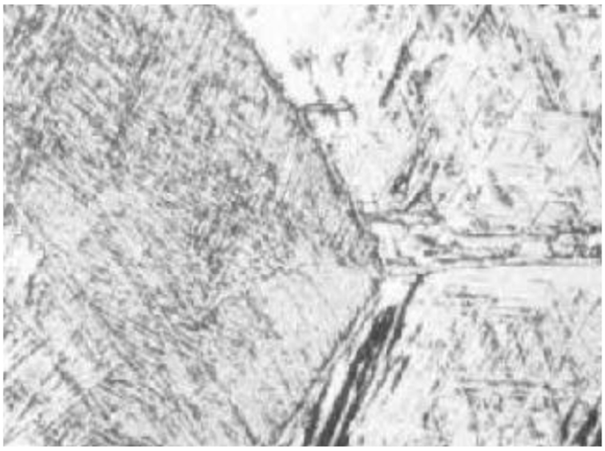
The Ti-6Al-4V alloy contains 6% aluminium which stabilizes the α- phase and 4% vanadium which stabilizes the β –phase. Due to the presence of these alloying elements both these phases are present in the alloy also at room temperature – α/β-alloy. In addition to the main alloying elements it also contains traces of iron and interstitial elements (hydrogen, oxygen, nitrogen, carbon). In most cases the designation of this titanium alloy for commercial purposes complies with the ASTM (US) standard. The name of this alloy is Titan Grade 5 and/or Titan Grade 23. The difference between the Ti-6Al-4V and Ti-6Al-4V ELI alloys is in the decrease of the oxygen content from 0.2% to 0.13% respectively and decrease of the carbon content from 0.40% to 0.25% respectively. This gives the Ti-6Al-4V ELI alloy higher ductility associated with lower strength. Owing to the presence of alloying elements, the physical properties of the Ti-6Al-4V alloy slightly differ from those of pure titanium. Aluminium in the alloy, compared with pure titanium, is responsible for a minor decrease of the melting temperature to 1630°C and vanadium increases the temperature of structural transformation from 882°C to 975°C. In contrast to pure titanium the thermal conductivity of the alloy is one third of that of pure titanium (λ=6.7 Wm-1K-1), the value of the specific electrical resistivity is fourfold higher (ϱ = 1.76.10-6 Ω·m), and is affected by the presence of aluminium which is a better conductor of electricity. Due to the presence of alloying elements, the mechanical properties of the alloy are radically different. From the viewpoint of mechanical properties the alloy has considerably higher values of the ultimate tensile strength (UTS), yield point in tension and hardness, which in effect decreases the ductility of the alloy.

The modulus of elasticity depends on the magnitude of interatomic forces which correspond with the spacing between atoms in the space lattice. Hence β stabilizers, supporting the existence of a HCP lattice with greater interatomic spacing, decrease the value of the modulus of elasticity. In addition to excellent corrosion resistant properties the Ti-6Al-4V alloy has good biocompatibility, which in some cases can be reduced due to a moderate reaction with the living tissue. This reaction is caused by the presence of aluminium and vanadium assuming harmlessness to the human organism. The appropriateness of the alloy for medical purposes is confirmed by the capability to form a coating of titanium dioxide (TiO_2) on the surface of the body replacement and in body fluids. After damage caused to the coating it can renew itself immediately or after a short while [1, 11].

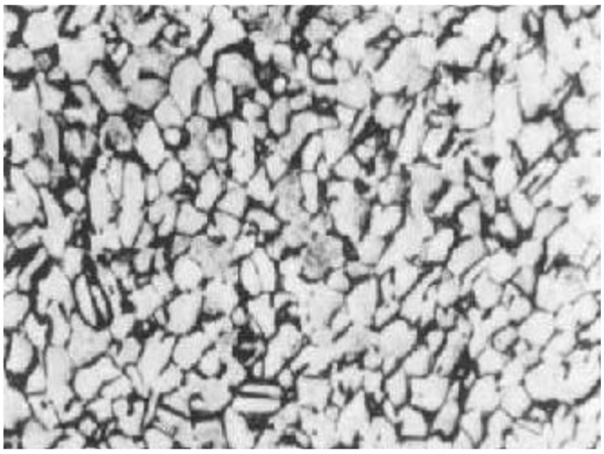
Owing to the fact that formability is a capability of the material to experience plastic deformation without failure, this technology is used with the Ti-6Al-4V alloy. The alloy is usually formed at temperatures in the range 950 to 980°C, not excluding cold working. During cold working a distinct texture is established which gives rise to an anisotropy of properties similarly as in corrosion resistant steel.

2.3 Structural Properties of the Ti-6Al-4V Alloy

By forming the Ti-6Al-4V alloy above the temperature of structural transformation, i.e. in the region of the presence of the β –phase and subsequent violent cooling in water a lamellar structure is formed comprising α´-martensite with residues of the β –phase. By quenching below the temperature of structural transformation a similar lamellar structure is created with the only difference that initiating α –phase appears in the structure. At lower quenching temperatures a morphology is formed where the lamellar structure merges into an acicular one. This structure is formed predominantly by the initiating α –phase and an unstable β –phase (Fig.1a).



(a) Acicular α –phase (with the initiating α –phase) and residual β –phase



(b) Equiaxial α –phase with intergranular β –phase

Fig. 1 *Selected structures after various modes of heat treatment*

By cooling in air the structure of the alloy heated above the temperature of structural transformation consists of an acicular α –phase with β –phase residues. By heating below the temperature of structural transformation the structure consists of an acicular α –phase (with the initiating α –phase) and residual β –phase. Slower cooling in an electrically heated furnace is also known. In this case, after cooling from a temperature higher than the temperature of structural transformation, the structure formed comprises a lamelliform α –phase and β –phase. After cooling from a lower temperature the structure formed comprises an equiaxial α –phase with an intergranular β –phase (Fig.1b).

2.4 Factors affecting Heat Treatment

Heat treatment was selected on the basis of literary knowledge of obtaining the β –phase which is more suited to interaction with live cells.

The most significant factors which affect the selection of the process of heat treatment of Ti-alloys are [1]:

- Low thermal conductivity of titanium and its alloys. This conditions the limitation of heating and cooling rate (at considerable variations of the temperature and stress in different sections) interrelated with stress-strain conditions.
- High chemical activity of Ti and its alloys (saturation of the surface with gases) which is related with subsequent different methods of processing (blasting, etching, vacuum annealing, application of protective coating). Most appropriate is the use of a furnace with a neutral atmosphere, induction or contact heating furnace or resistance furnace.
- Existence of a polymorphous α/β transformation. The temperature of a complete α/β→β transformation in most industrial Ti-alloys is within the range 850-1020°C and depends on the amount of alloying components and additions. In the β region titanium is softer. An optimum structure with high exploitation properties is created during deformation in the α/β region and also by combining β and α/β deformation (creation of an optimum fine-grained structure).
- Limited potential plastic deformation of Ti-alloys during cold working. Creation of intensive strain hardening and large resilient recoil (spring back, due to the enhanced value of the ratio of the yield point to the modulus of elasticity) leads to susceptibility to failure. Heating to 200-300°C considerably decreases the resistance to deformation (by 40-50%). In order to improve rolling and forging of semi-products, temperatures are selected in the range between 550 and 700°C.

2.5 Structural changes during Heat Treatment

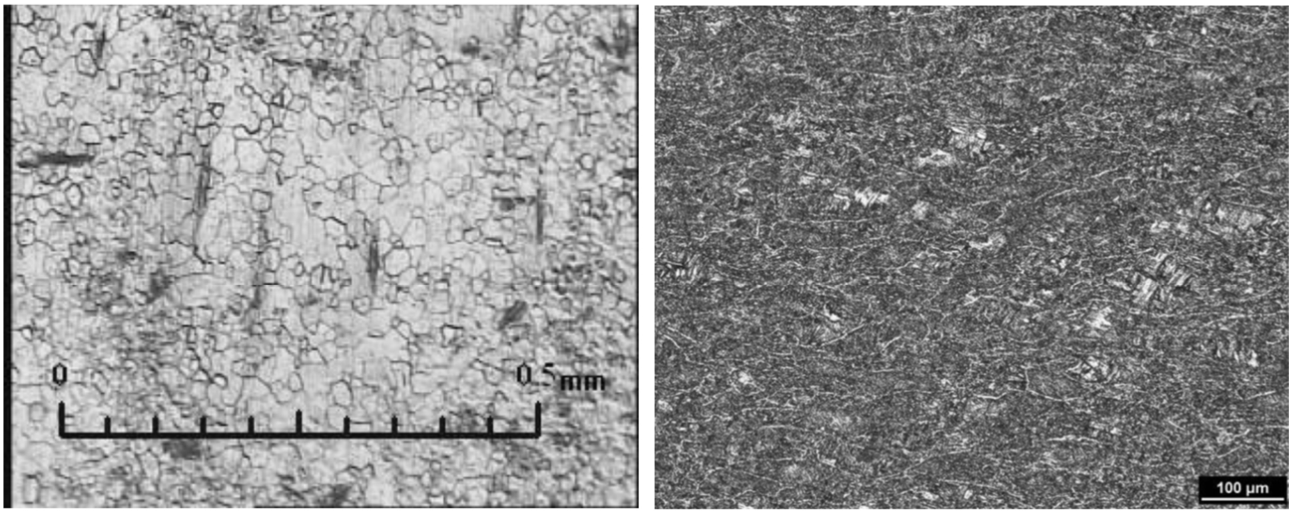
In our work, we performed an experiment with the heat treatment of titanium and the mentioned above Ti-6Al-4V titanium alloy. The heat treatment process was selected to produce a final structure made up predominantly of the β – phase. Due to the fact that the material will be used in biomedical applications, heat treatment of structural elements cannot be performed. Selection of an optimum dwelling temperature and subsequent cooling is related with the alloying elements and the preceding applied production technology. Titanium and the titanium alloy were heated at various temperatures and cooled at various cooling rates with a constant dwelling time.

2.6 Structural Characteristics after Heat Treatment

The initial microstructure of pure Ti consists of α grains with various sizes (Fig.2a) and a very small amount of the β –phase. This structure is characterized by a large number of grains with slip lines. By electrolytic treatment of the microstructure of pure titanium the presence can be revealed of hydrogen which is monitored and classified during the input control (greenish, transparent and irregular formations).

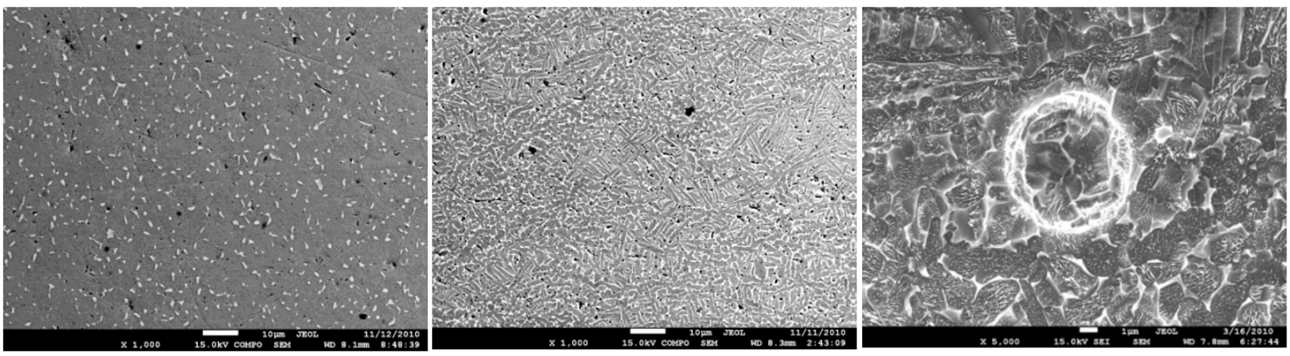
The structure of commercially obtained Ti-6Al-4V alloy is characterized by numerous pores and heterogeneity of grain sizes (Fig.2b). The grain boundaries are formed by the α –phase which creates a base of lamellae and spheres interconnected with the β matrix. By measurement and evaluation of grain sizes it was shown that for the selected pure titanium and the titanium alloy the tendency to change the grain size is as follows: the grain size of α-Ti is 10 μm, the grain size of the α/β Ti-6Al-4V alloy is 20 μm, the grain size of the α/β Ti-6Al-4V alloy after quenching is 300 μm.

Microstructures of the initial Ti-6Al-4V alloy materials were studied in detail at higher magnification with an electron microscope and are shown in Fig.3. The Ti-6Al-4V alloy (Fig.3a) is characterized by a microstructure without distinctive grain boundaries with a localized β –phase (light particles). The heterogeneity of the structure of the Ti-6Al-4V alloy will be related with the more complicated character of the distribution and type of the alloying elements (Fig.3b) and the presence of pores (Fig.3c).



a) b)

Fig. 2 *Initial structures of pure titanium (a) and Ti-6Al-4V titanium alloy (b)*



a) Ti-6Al-4V ELI b) Ti-6Al-4V c) Ti-6Al-4V Pore

Fig. 3 *Microstructure of the initial Ti-6Al-4V alloy material*

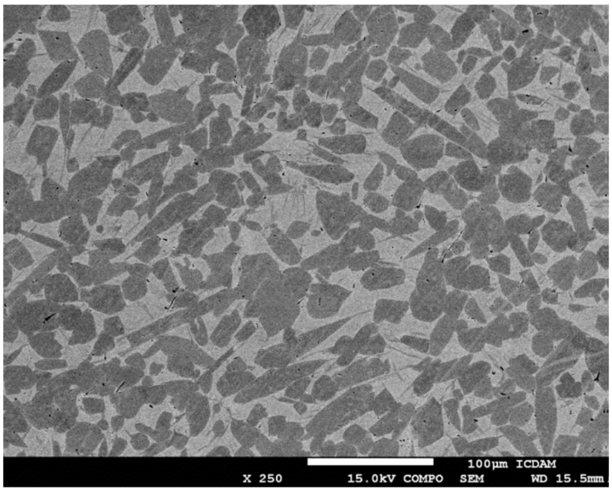


Fig. 4 *Effect of temperature gradient on the final microstructure*

Regions rich in Al are located mainly at α grain boundaries and the grain boundaries themselves are uniformly made up of the β matrix. The β matrix in the vicinity of the primary α grains is richer in vanadium (which was confirmed by chemical analysis).

Hypothetically it can be claimed that the grain at a higher temperature is composed of an α/β matrix and the superfluous quantity of Al and V (from the supersaturated α-Ti solid solution) migrates during variation of the temperature gradient into the α boundary between the grains. This proceeds either during crystallization or during heat treatment (e.g. annealing). This process can initiate, during decomposition of the β matrix, so-called diffusion flow composed of an α/β matrix. This assumption is in agreement with the thermodynamic analysis of Ti-6Al-4V alloys according to Rault´s law [1], where the Ti-V system has a higher activity than the Ti-Al and Ti-V systems (the latter having the lowest activity). Our assumptions are based on the condition that the melting temperature of aluminum is lowest of all elements present in the alloy. This indicates that during cooling of a Ti-alloy from the range of α/β temperatures (below the temperature of the β transition), aluminum can still exist in a more mobile fluid state. Hence structural formations rich in Al (forming prevailingly the boundaries of primary α grains) can cool the forming grains. Thus a temperature gradient develops between the boundary and the grain (Fig.4).

Chemical analysis was performed on a scanned area of 50 mm² with a JEOL JSM-7600F scanning electron microscope with an energy dispersive X-ray spectrometer (EDS) (Oxford Instruments, UK).

2.7 Plan of Heat Treatment Experiment

Heat treatment of the studied materials was carried out in a laboratory LH furnace (LAC Comp.) at the laboratories of the Department of Materials Engineering of the Czech Technical University in Prague. Specimens of pure titanium were cooled from 900°C and 1000°C, specimens of the titanium alloy were cooled from 1000°C and 1100°C. Two different cooling media were used – water and liquid nitrogen. The temperatures selected for both pure titanium and the titanium alloy were slightly above the temperature of structural transformation and in the β –phase region. Before quenching in liquid nitrogen the specimens were first cooled for 1 sec. in water and then finally cooled in liquid nitrogen. Conditions of the heat treatment of individual specimens are in Table 1. The dwelling temperatures were adjusted by the operating mode of the furnace.

Tab. 1 *Heat treatment of selected materials*

Material	Heating [h]	Dwell[°C - h]	Cooling – dwell in medium [min]	
			H_2O (+ 20 °C)	N_2 (- 183 °C)
Ti	2	900 – 1.5	20	-
Ti	2	900 – 1.5	1/60	120
Ti	2.5	1000 – 1.5	20	-
Ti	2.5	1000 – 1.5	1/60	120
Ti-6Al-4V	2.5	1000 – 1.5	20	-
Ti-6Al-4V	2.5	1000 – 1.5	1/60	120
Ti-6Al-4V	4	1100 – 1.5	20	-
Ti-6Al-4V	4	1100 – 1.5	1/60	120

After heat treatment, the microstructure which reflected the actual process affected by the temperature gradient, was evaluated by metallography. Chemical analysis of elements in the obtained structures was carried out for all elements in the Ti-6Al-4V alloy (Fig.5). Ti, Al and V are distributed uniformly in the original structure. After heat treatment elements participating in the formation of new phases are redistributed. This is evidenced by the obtained structure rich in the β –phase. Hence it must be considered that also the morphology of the grains are affected by heat treatment.

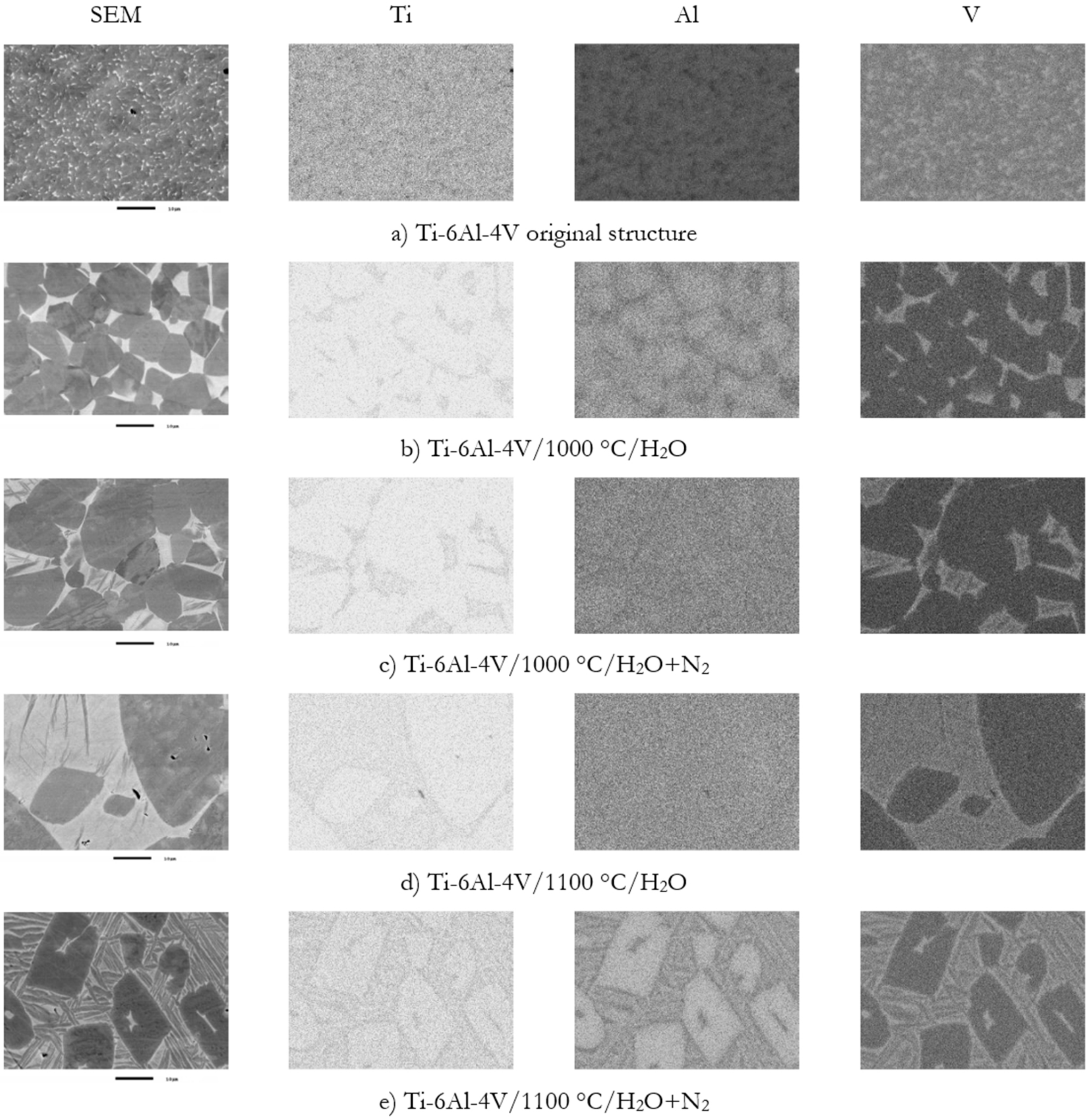


Fig. 5 *Distribution of elements in the structure of Ti-6Al-4V alloy after various heat treatment processes (the scale is 10 μm)*

Heat treatment of pure titanium and a titanium alloy stabilizes the structure which in further applications should not change. In order to achieve better interaction between the metal and the living tissue and to achieve better mechanical properties of products used in biomedical applications our investigations were focused also on titanium specimens coated with TiN.

2.8 PVD on Titanium

The concept of the coating procedure is based on commercially available pure titanium on which the procedure was tested. The material of concern was inserted into a living organism and consequently more detailed attention was paid to it. The concept is based on interaction between the metal and the living tissue, where on certain occasions mutual diffusion can occur. The PVD method applying the TiN coating was selected from a number of coating methods. It is known that TiN creates a hard layer resistant to wear and to nitrogen and owing to its presence in the living organism can serve as a bridge between the metal and the tissue. The coated sites (Fig.6a) were further studied with respect to adhesion, distribution of elements, coating thickness and character of the subcoating layer. The study showed that titanium was uniformly distributed in the base material (Fig.6b) and identified an increased concentration of nitrogen in the coating (Fig.6c) which corresponds to the character of the coating.

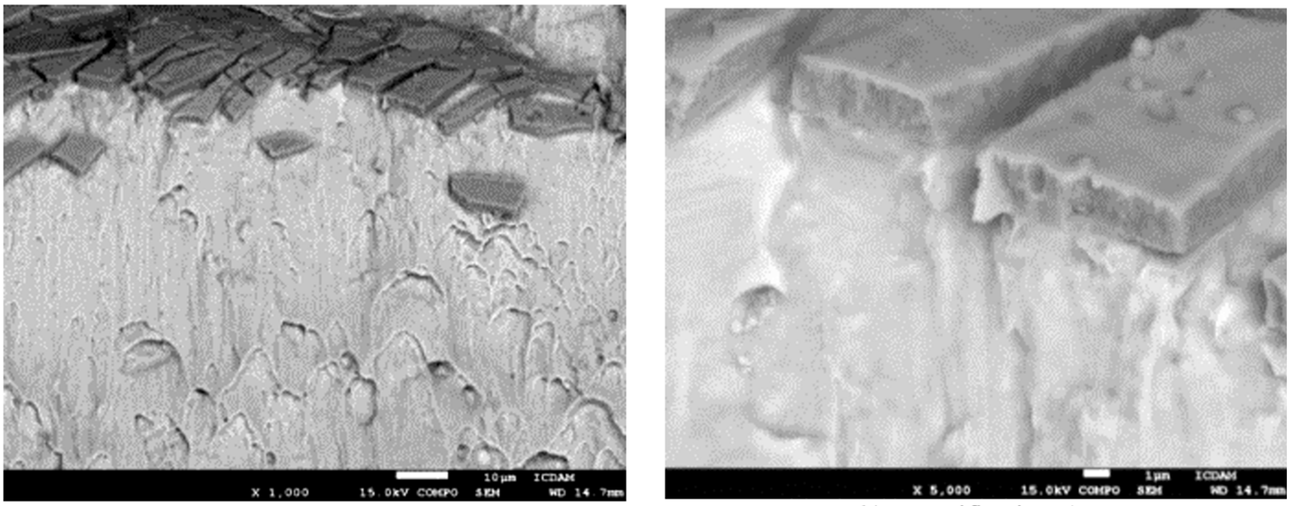
a) b) c)

Fig. 6 *Mapping of pure titanium with a TiN coating (magnification identical for all pictures); a) selected area of measurement; b) distribution of titanium (Ti); c) distribution of nitrogen (N)*

Of greatest interest was the distribution of nitrogen in pure titanium with a TiN coating which owing to the diameter of the atom will be active during diffusion. Our selection took into consideration the subsequent deposition of live cells for which it is assumed that they will respond to the chemical composition of the surface.

It can be claimed that the thickness of the coating (approx. 4 µm) is uniform and is not subject to changes which could be related with the initial roughness of the titanium base.

A study in more detail was carried out on the fracture surface morphology with a JEOL JSM-7600F scanning electron microscope. The fracture surface shows that the coating adheres well to the base material. A part of the damaged coating tracing separated parts on the fracture surface is shown in detail. The measured hardness (approx. value) of the coating was higher than that of the base material and was documented by the character of the damaged layer (Fig.7a and 7.b).



a) Magnification 1000x b) Magnification 5000x

Fig. 7 *Fracture surface of pure titanium with a TiN coating*

The experiments indicated that coating of pure titanium and/or a titanium alloy must be subject to more extensive attention in further investigations.

2.9 Cell cultivation on pure titanium with tin coating

Cell cultivation was carried out on the prepared samples of pure titanium coated with TiN. The cell cultures were studied at the Physiological Institute of the Academy of Sciences of the Czech Republic in Prague. The titanium specimens were sterilized at a temperature of 160°C for 2 hours and after cooling they were deposited onto a well cell culture plate for cell cultivation. One titanium specimen with the coated side facing upwards was placed in each well on the plate. Polystyrene platelets serving as a reference material for subsequent evaluation of cell proliferation and overall behavior of the cells were located in all unoccupied wells.

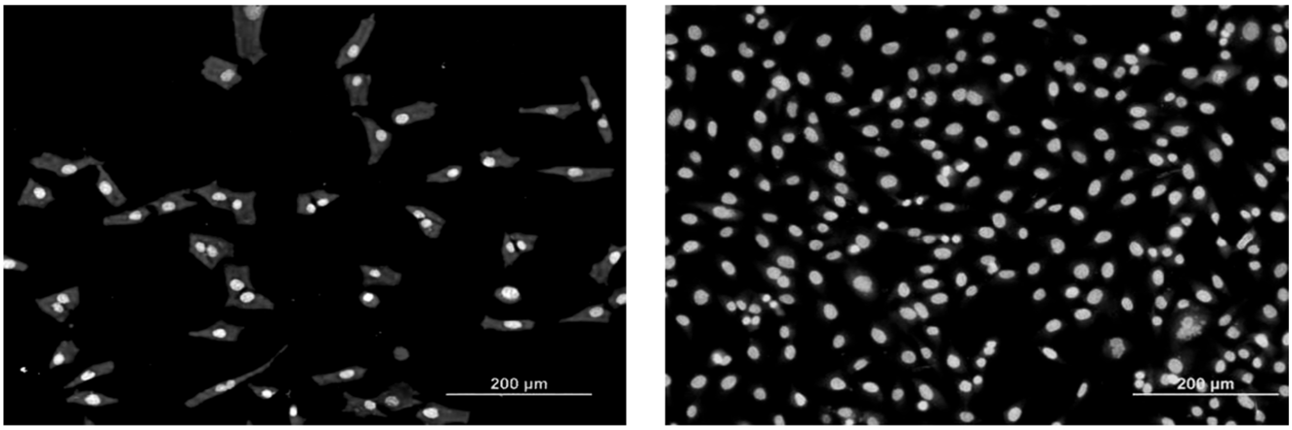
Human line MG63 bone cells from the European Collection of Cell Cultures (Salisbury, UK) were subsequently placed on the titanium specimens. The cultivated cells were immersed in a suspension of DMEM cultivating medium (Dulbecco´s Modified Eagle Medium), 10% fetal bovine serum, gentamicin and vitamins. Fetal bovine serum contains a great number of growth factors and helps the cells to reproduce, gentamicin is an antibiotic preventing bacterial infection of the culture. 3 ml of the medium containing approx. 21 000 cells were placed in each well.

The cells were cultivated in an incubator for a period of 1, 3 and 7 days at a temperature of 37°C in a moistened atmosphere of air with 5% CO_2 in order to be able to perform an easy to survey evaluation. A set of 3 specimens of coated titanium and 1 polystyrene platelet – 3x(3Ti+1PS) – were prepared for each monitored cultivation period.

3 Results and discussion

3.1 Viability of Cells

The first set of 3 titanium specimens and 1 PS platelet for comparison were analyzed with respect to the number of cells and their condition. The specimens were rinsed in a phosphate buffer stabilizing pH (7.2-7.4), then fixed with 70% deeply subcooled ethanol and stained with a combination of two dyes. The first dye with a brand name Hoechst # 33258 stained the cell nucleus, the second dye Texas Red C2 – Maleimide stained the cell membranes and cytoplasm. The fluorescent dyes were applied for 2 hours at room temperature. The number of cells and their shape on the surface of the material were evaluated from the photographs made by an inverted microscope Olympus IX51 equipped with a digital camera DP70 (Olympus, Japan, lens 10x). The number of cells per cm^2 was subsequently determined from the number of cells on the photograph. A selected photograph of the cells cultivated on pure titanium with a TiN coating after 1 day of cultivation is in Fig.8a. From the depicted cells it is apparent that they are captured on the surfaces of the specimens and the number of cells on coated titanium is higher than that on the polystyrene check-up specimen.



a) b)

Fig. 8 *Distribution of cells after the 1st (a) and 3rd (b) day of cultivation*

The second set of specimens - (3Ti+1PS) – was investigated after 3 days of cultivation. Valuation was carried out identically to that performed after the 1st day of cultivation. Owing to an increased number of cells only the Hoechst # 33258 dye staining the cell nuclei was applied. The dye staining the cell membranes could not be applied since individual cells on the photograph were indistinguishable. The selected photograph is in Fig.8b on which substantial cell growth is quite apparent.

Specimens after seven days of cultivation were evaluated with an automatic Vi-CELL XR analyzer. In this case the optical counting of cell nuclei could not be applied due to the high number of cells which were stacked in more layers and consequently individual cell nuclei could not be distinguished. Before measurement the cells had to be removed from the surface of the specimen and separated one from another without damage. A Trypsin-EDTA Solution (Sigma, USA, Cat.No.T4174), in fact a digestive enzyme, was used for this purpose. Consequently the slightly digested cells disengage themselves from the surface and also one from another. After an extended exposure to Trypsin the cells could be completely destroyed and therefore after separation they are rinsed out with a phosphate buffer (for 10 minutes) which neutralizes the effect of the Trypsin. The cells disengaged from the surface were put in a test tube and inserted into an analyzer which automatically selected the necessary amount of cellular solution and automatically specified the number of cells. The viability of the cells was evaluated from the photographs according to the form of the distribution of the cell membranes giving evidence of how well they are doing. Those cells are at their best which have a "stellar" shape, however most frequently they were elongated which was observed also in specimens investigated by us in coated titanium and the reference polystyrene platelet. The most inappropriate form of the distribution of membranes is a circular one since in this case the cell is either dying or preparing to differentiate. At the very beginning of cell differentiation it is impossible to determine whether the cell is differentiating or dying. In an advanced phase two explicitly separated nuclei are formed in the cell and the cell is viable.

3.2 Results of Cell Cultivation

The cultivated specimens were evaluated statistically and simultaneously the average value ($\overline{x}$) of the number of cells per cm² for individual periods of cultivation (Table 2) and the statistical deviation represented by the mean error of the average (SEM) were determined.

Tab. 2 *Average number of cells per cm²*

Number of cultivation days	Ti (TiN) $\bar{x} \pm SEM$	PS $\bar{x} \pm SEM$
1	7331 ±679	3725 ±244
3	39750 ±2688	21138 ±1118
7	412327 ±17093	286133 ±8596

Values of the average number of cells ($\overline{x}$) for individual periods of cultivation are plotted in the diagram for cultivation periods (Fig.9). This diagram shows an apparent slow increase of cell growth during the first day and a gradual increase of their number with time following a geometric series.

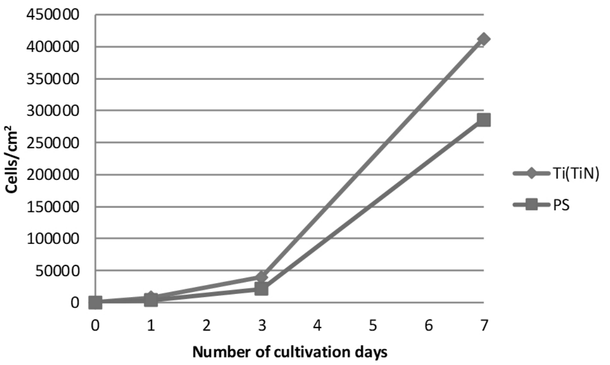


Fig. 9 *Number of cells during the entire range of biological tests*

4 Conclusions

From literature is known that roughness has not only a significant effect on the process of coating but also on the final surface geometry which consequently affects adherence of the cells.

Heat treatment stabilizes the structure which should be invariable during subsequent processes. Coating generally serves as a barrier to the surrounding environment, which would otherwise be in direct contact with the substrate. Concepts of coating methods take into account the ratio between the hardness of the coating and the substrate. It is assumed that the thickness and quality of the coating will be selected assuring that the surface layers will not be disrupted. Selection of the chemical composition of the coating must be based on its assumed function. Sine this paper is devoted to the interaction between the metallic material and the live cell, the coating must be biocompatible (bioinert) and at the same time must act as a barrier to elements diffusing from the basic structure into the living organism.

The applied live cells tested not only their proliferation but also their morphologic distribution affecting their adherence to the surface of the body replacement part.

In the tests the deposited cells were elongated, which shape is considered to be optimum and was probably affected by the character of the surface of the coated substrate.

On the basis of the biological tests performed and investigation of the surfaces, statistical verification can be recommended of a large number of specimens with identical quality and a single type of live cell. In our next investigations and studies attention will be paid to quantified roughness of the surface which should be optimal for the adherence of viable cells.

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