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MANUFACTURING AND CHARACTERIZATION OF TiO_2 NANOTUBES ON PURE TITANIUM SURFACES FOR ADVANCED BIOMEDICAL APPLICATIONS

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Scopul lucrării este de a descrie o tehnică de obținere a nanotuburilor de TiO_2 pe suprafața titanului şi de a prezenta parametrii care influențează geometria şi morfologia suprafeței. Lucrarea prezintă posibilele aplicații ale nanotuburilor de TiO_2 în diferite domenii tehnologice şi în special în biomedicină. Creşterea nanotuburilor de TiO_2 s-a realizat prin oxidare anodică în diferite soluții de electrolit. Pentru determinarea parametrilor de suprafață caracteristici, probele au fost analizate prin SEM, studiile despre mecanismul de aderare a osteoblastelor pe suprafața nanotuburilor TiO_2 folosind modelul viscoelastic al interfeței hibride. Combinând procedurile experimentale de creştere a nanotuburilor, analiza SEM şi calculele matematice pentru interfața nanotuburi – osteoblaste s-au stabilit caracteristicile necesare obținerii unor noi materiale pentru aplicații biomedicale.

The purpose of the present work is to describe a technique of producing TiO_2 nanotubes on pure titanium surfaces and to present some parameter affecting their geometry and surface density. Apart from this, the paper describes possible applications of TiO_2 nanotubes to different branches of technology and especially to biomedicine. In the present investigation TiO_2 nanotubes were created on pure titanium surfaces through anodizing method trying different types of electrolytes. The samples were analysed by SEM for defining surface parameters characteristics. Studies about the mechanism of attachment of the osteoblasts on the surface of titanium nanotubes were realized through the viscoelastic hybrid interphase model. Combining experimental procedure of creation of the nanotubes and SEM analysis, as well as the mathematical calculations for the nanotubes-osteoblasts interphase, data were derived about the required characteristics of TiO_2 nanotube surfaces that could be used in order to design a new material for biomedical applications.

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1. Introduction

The prospect of changing the macro-world by working in the nanoworld was first proposed by Richard Feynman back in 1959. But it's only been in the last decade that science and technology has given scientists sufficient mastery to enable them to start working directly in this strange world.

As particle's sizes are reduced to the nanoscale, the ratio of surface area to volume increases dramatically. Since many important chemical reactions – including those involving catalysts – occur at surfaces, it is not too surprising that very small particles are staggeringly reactive. By controlling the manner in which nanometre-scale molecular structures are formed, it is possible to control the fundamental properties of the materials these molecules build: properties such as color, electrical conductivity, melting temperature, hardness, crack-resistance and strength [1].

The synthesis of nanostructured titanium oxide and titanates is often attributed to Kasuga and co-workers in 1998. Since 1998, hundreds of papers have appeared on the synthesis and characterisation of nanostructured titanates and titanium dioxide [2].

Different good mechanical properties of titanium - good resistance at corrosion due to TiO_2 layer at the surface of the metal, good strength - increased researchers' interest for this material and particularly TiO_2 is into their attention due to functional properties as self-cleaning [3], gas sensing [4], solar energy-conversion [5], biomedical applications [6] etc.

Generally, there are three strategies used in the fabrication of TiO_2 nanotubes:

1. Template synthesis
2. Hydrothermal methods and
3. Electrochemical synthesis (anodizing of Ti sheets)

and three generations of nanotubes can be created:

a. Fabricated in HF-based aqueous solutions electrolytes, with limited nanotubes' length [7]
b. Fabricated by adjusting the electrolyte pH, with considerable increase in nanotubes' length [8]
c. Fabricated in almost water-free polar solution (viscous glycerol and ethylene glycol electrolytes) [9]

Among the methods specified the electrochemical anodizing is more used as it is able to build porous titanium oxide films, respectively nanotube films of controllable pore size, good uniformity and conformability over large areas at low cost [10]. Playing with parameters, different research groups obtained TiO_2 nanotube films with several geometry and characteristics of the surface [11,12].

Depending on the purpose of which each TNT surface is designed for, scientists try to discover and explore parameters that influence the properties of the nanotubes layer. For the full characterization of TiO_2 nanotubes, the following parameters should be experimentally measured and/or identify:

1. The morphology and structure of the titanium oxide tubular materials
2. The phases of the TiO_2 samples
3. The pore structure of the derived TiO_2 nanotubes
4. The surface area of the samples
5. The pore volume
6. The pore size distribution
7. Spectral data collection in a wide spectral range
8. Changes in weight in relation to change in temperature

In order to make the characterization, different types devices such as AFM, SEM, EDX, TEM, XRD etc., are used.

However, for the determination of the nanotubes length and diameter values several different techniques have been developed and applied. By tailoring the electrochemical conditions high-aspect ratio, self-organized TiO_2 films can be developed during titanium anodizing [13,14,15].

The mechanism of the titanium nanotube formation during anodizing was studied and discussed in many works before and it is finally considered that the main processes that take place within the electrolyte during anodizing was explained [16,17,18]. After elucidating some chemical and electrochemical reactions concerning nanotubes formation researchers started to explore particular properties of the titanium surfaces with purpose to use the material in environment, medical application, aeronautics etc.

In the present work, we produced and studied pure Titanium surface covered with TiO_2 nanotubes with the aim to obtain a material appropriate for prosthesis in biomedical applications.

2. Experimental Procedure

Specimens

The titanium specimens used in the present investigation had dimensions 3cmx2cmx2mm.

The protocol of removing the irregularities and impurities from the pure titanium sample consisted in washing the specimens with ethanol solution, deionized water, acetone and a mixture of acids, after polishing them with sand paper. After all these followed, the samples surface was clean and smooth without any imperfections, which is very important for SEM analysis.

The electrochemical cell

The samples were then introduced into a customized electrochemical cell containing HF 0.5% electrolyte and having as electrodes: graphite as cathode and titanium foil as anode. The voltage applied was in all cases 20 V and two different time periods; 45 min and 2 hours of anodizing were applied.

3. Results and discussions:

3.1. Manufacturing Technique and Formation Mechanism

As it was mentioned before, anodizing electrolysis is the most popular manufacturing TiO_2 nanotubes technique. Generally, for our experiments a home-made electrochemical cell was manufactured and used, consisted of a power supply and two electrodes, one of graphite or platinum as a cathode and a Ti foil as an anode following guidelines given in literature, [18, 19].

The samples require a very efficient cleaning procedure in order to obtain an almost perfect, fine surface. Then, samples were immersed into the electrochemical cell with the electrolyte solution which is mainly based on hydrofluoric solutions or an organic solution. Organic solutions containing glycerol or ethylene glycol in different percentages are more adequate for biomedical applications due to the fact that they decrease the risk of toxicity of the material.

For better control over the morphology and density of the nanotubes, it is important to understand the principles and mechanisms of formation of aligned nanotubes under anodic conditions. The growth of nanotubes by anodizing titanium can be considered as a selective etching. Nanotube growth can be described in terms of a competition between several electrochemical and chemical reactions and energy processes, including: anodic oxide formation, accompanied with chemical dissolution of titanium oxide.

Energy processes

The barrier layer of anodized titanium is characterized by an instability which is the result of the simultaneous action of two or more competing processes. These processes are characterized by respective energies. The first one is related to the surface energy which has a stabilizing effect while the second one is related to the increase in strain energy due to electrostriction and has a destabilizing effect [20]. It is worth to note that by the term surface energy it is meant the work required to increase the surface area of a substance by unit area;

while by the term strain energy is meant the energy stored in the material when stretched.

Moreover, electrostriction is the strain response of a dielectric material proportional to the square of an applied electric field. This effect is related to the strain – dependence of the dielectric function. However, this strain produces an electrostriction stress which is given by the relation:

$$\sigma_{er} = \gamma_{11} E B^2 \tag{1}$$

where: σ_{er} is the electrostriction stress, E is the Young's modulus, γ_{11} the electrostriction coefficient in the direction of the field and B is the electric field.

Under the condition that the barrier layer of anodized titanium is non-ferroelectric, a negligible dilatational strain due to electrostriction is developed. Next, an electrostatic compressive stress is developed which is expressed as

$$\sigma_{es} = -\frac{1}{2}\varepsilon_o \varepsilon B^2 \tag{2}$$

where: σ_{es}, is the electrostatic stress which is compressive, ε_o the permittivity of free space and ε is the relative dielectric constant of barrier layer.

Finally, the formation of oxide layer results in a volume expansion, which in turn develops a compressive stress and can be expressed as

$$\sigma_{vol} = -\frac{(\partial\upsilon/\upsilon)E(1-\upsilon)}{(1+\nu)(1-2\nu)} \tag{3}$$

where: $(\partial\upsilon/\upsilon)$ is the volumetric strain, E the Young's modulus of elasticity and ν, the Poisson's ratio.

In a molecule, strain energy is released when the constituent atoms are allowed to rearrange themselves in a chemical reaction or a change of chemical conformation in a way that: angle strain, torsional strain, ring strain and/or steric strain, allylic strain, and pentane interference are reduced. The external work done on an elastic member in causing it to distort from its unstressed state is transformed into strain energy which is a form of potential energy. The strain energy in the form of elastic deformation is mostly recoverable in the form of mechanical work. Finally, we define the strain energy density of a solid as the work done per unit volume to deform a material from a stress free reference state to a loaded state.

The above stresses contribute to the total strain energy density which can be expressed as:

$$U_s = \frac{\sigma^2}{2E}, \text{ where } \sigma^2 = \sigma_{er}^2 + \sigma_{es}^2 + \sigma_{vol}^2 \tag{4}$$

Formation of nanotubes

Preliminary results of our research have shown that by following the anodizing method described above and using as basic material pure titanium specimens introduced into the customized electrochemical cell, titanium dioxide nanotubes have been produced and this is shown in the SEM photo below, (Fig.1).

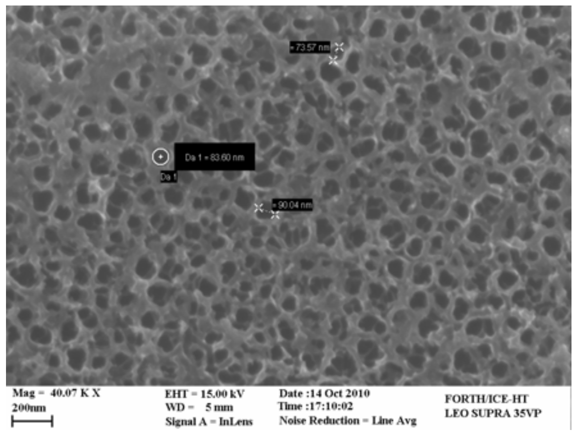

Fig.5. SEM image of titanium nanotubes created in HF 0.5%

A detailed explanation for the formation mechanism of nanotubes is given in the sequence of the present paper.

- Chemical reactions

Nanoporous structure is formed by two processes. Consequently, the two reactions are described as following:

(1) Electrochemical etching, when the initial oxide layer forms at the surface of the titanium as a result of the following anodic reactions:

$$Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$$

and:

(2) Chemical dissolution, of the titanium oxide as soluble fluoride complexes.

The hydrogen fluoride (HF) is predominant in acidic fluoride solution. In the presence of acidic fluoride solution, the oxide layer dissolves locally and nanotubes are created from small pits that are formed in the oxide layer. These pits are created from the following reactions between TiO_2 and HF:

a) $TiO_2 + 6F^- + 4H^+ \rightarrow [TiF_6]^{2-} + 2H_2O$

Continued with the direct complexation of Ti^{4+} ions migrating through the film:

$$\text{b)} \quad Ti^{4} + 6HF^{-} \rightarrow [TiF_6]^{2-}$$

The competition between electrochemical etching and chemical dissolution determines the structural morphology of nanotubular layer [2, 16, 20, 21].

As mentioned before, based on the model of the local dissolution, nanotubes form from little pits resulted as a consequence of these dissolutions. Behind the formation of the pits is the chemical dissolution as well as the electrochemical corrosion, thinning the barrier layer and increasing the intensity of the electrical field which is followed by the growing of the pores. The chemical dissolution removes the superior part of the column of the pores, which represents the metallic part un-anodized in between the pores, available for the electrochemical corrosion and the chemical dissolution.

The channels formed in this region separate the pores one from the other forming the nanotubes. The lenght of the nanotubes increases until the electrochemical corossion speed becomes equal with the dissolution speed for the surface of the superior part of the nanotubes.

3.2. Parameters affecting the geometry and surface density of nanotubes

In the present study, some of the most important parameters in nanotubes formation will be described.

a) Type of Electrolyte

The widely common used electrolyte from the beginning of TiO_2 nanotubes elaboration was the hydrofluoric acid solution at different concentrations. The type of the electrolyte used in the experiment influences the nanotubes structure due to the existence of different concentrations of ions. In biomedical applications it is better the electrolyte to contain mainly organic components as glycerol or ethylene glycol. These organic solutions make easier the adherence of the human tissue to the surface of the prosthesis.

In Table 1 a list of some electrolytes used for nanotubes elaboration is tabulated.

Table 1

Electrolytes used for nanotubes elaboration and properties of nanotubes created in these electrolytes

	Electrolyte composition	**Voltage**	**Time (minutes)**	**Pores diameter (nm)**	**Length of nanotubes (μm)**
1.	HF 0.5 %	20 V	120	100	0.2-0.5
2.	HF 1%	20 V	120	90-110	0.5-5
3.	0.5% HF + 5 g/l Na_2HPO_4	20 V	120	100	2.4
4.	1 M $(NH4)_2SO_4$ + 0.5% NH_4F	20 V	120	110	1.8
5	H_2O–glycerol (50:50 vol. %), 0.27 %^{3} NH_4F	20 V	120	60-120	0.15–3
6	0.5 wt% NH_4F in CH_3COOH	20 V	120	20	0.1–0.5

The anodizing method on titanium performed in two kinds of electrolytes a mixture of fluoride salts as inorganic solution, and a hybrid inorganic + organic components as glycerol and acetic acid leads to the formation of different self-organized oxide nanotubes.

In Fig. 2 SEM images of nanostrucures obtained using anodizing for 2 hours at 20 V in an aqueous mixture of fluoride are illustrated.

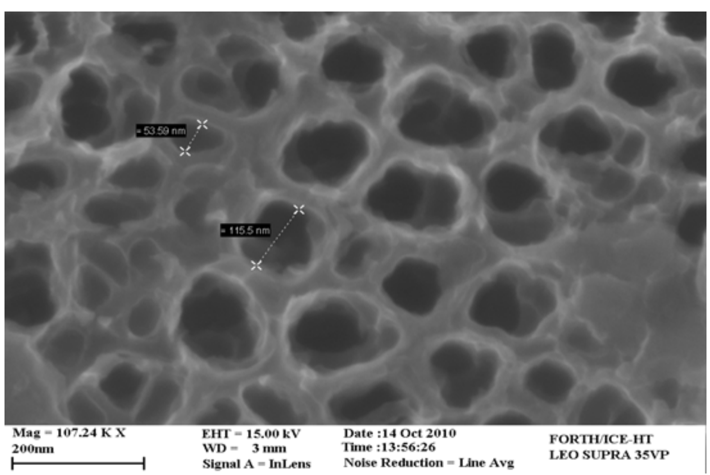

Fig. 2. SEM image of TiO_2 nanotube obtained in a HF 0.5 % aqueous solution

Fig. 3 shows SEM images corresponding to oxides structures obtained on Ti6Al7Nb alloy in a hybrid organic + inorganic electrolyte (50% glycerol + 50% H_2O + NH_4F 0.27 wt% mixture).

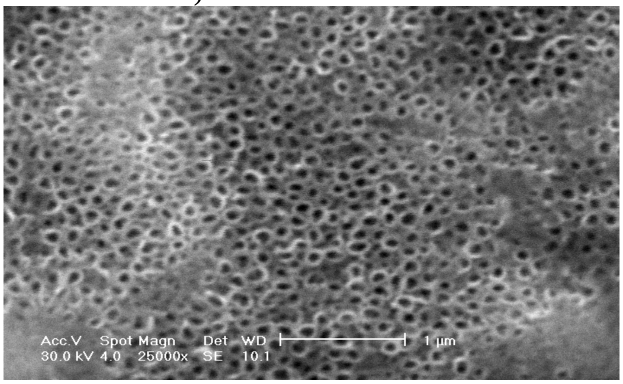

Fig. 3. SEM image of TiO_2 nanotube obtained in NH_4F and HF 0.5 % in 50% H_2O si 50% glycerol solution.

b) Anodization time

The time for anodizing it is also a very important parameter. In literature references can be found for anodizing time ranging from few minutes till 10 or even more hours. When the anodizing time is not enough, the pits start to form but finally they are not well defined and do not determine the formation of the nanotubes. Depending on the design of the electrochemical cell, one can have control on the time given for the anodizing process.

Most of the time, organic electrolytes need more than two hours for the electrochemical processes to be completed. During the building of the nanotubes the processes mentioned in the previous paragraph as the electrochemical etching, the chemical dissolution and the surface energy processes follow an order that can be changed due to the environmental conditions for short periods of time. If it's not given to the global system the needed time in order to the processes take place till to confer a maximum stability and length of the nanotubes, the surfaces remain covered only by some unclear pores that indicate the beginning of the architectural structure of the nanotube.

The evolution of the current curve during the formation of the nanotubes decreases at the beginning, then increases exponentialy and finally a stady-state value is achieved. These is revealed in most of the studies concerning the elcetrochemical course of the anodizing. The time needed for the steady-state to be reached reached depends on the concentration of fluorine in the electrolyte.

The evolution of the current is important in order to understand if the time period applied given is enough for the different electrolytes for the good developing of all the processes implicated in the electrolyses.

It can be observed that the potentiostatic anodizing time applied greatly affects the length and structure of nanotubes created. More precisely, the increase in length is proportional to the increase of the anodizing time. Also the anodizing time has effect on the value of the density of the nanotubes on the titanium surface.

Nanotubes shown in Figures 4 and 5 were created under the same conditions, i.e. HF 0.5% electrolyte, voltage 20 V and room temperature, except of the time of the potentiostatic anodizing which for the sample shown in Fig. 4 was 45 minutes and that shown in Fig. 5 was two hours, respectively.

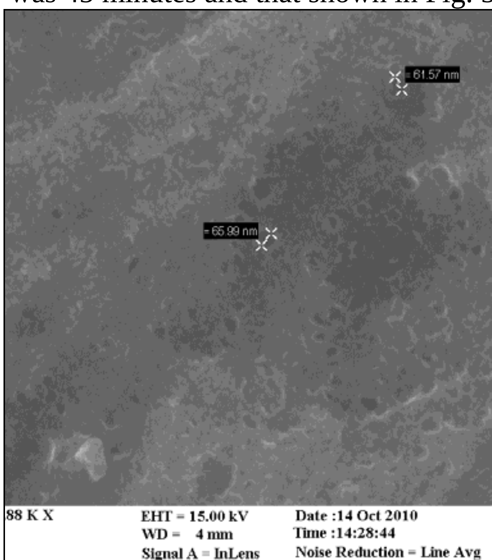

Fig. 4. TiO_2 nanotubes created when 45min potentiostatic anodizing time was applied

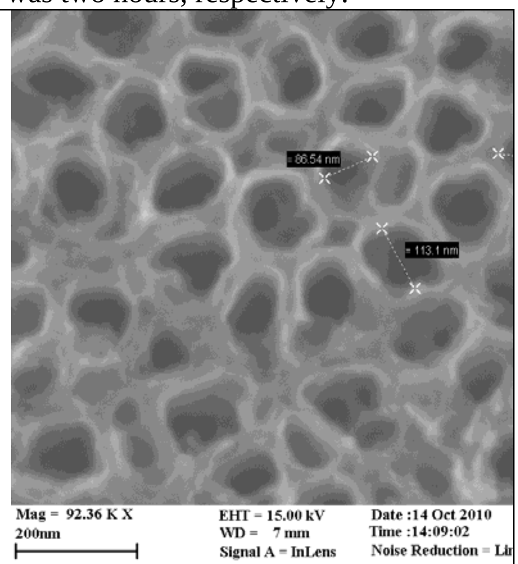

Fig. 5. TiO_2 nanotubes created when 2 hrs potentiostatic anodizing time was applied

In these images one can observe the difference in structure and surface density of nanotubes. More precisely where the anodizing time applied was 45 min, no nanotubes were created but only few nanopores, which are the initial stage of nanotubes creation, exist. In contrary, in Fig.5 which corresponds to 2 hrs anodizing time, a dense population of nanotubes can be observed to exist on specimen’s surface. These observations lead us to the conclusion that anodizing time in combination with other parameters plays an important role in creating nanotubes as well as in their respective geometry and surface density.

3.3. Effect of surface density of nanotubes on the modification of properties of liquids in contact with nanotubes

Implant failure due to a lack or loss of the osseointegration is a big problem for both doctors and patients. To optimize osseointegration, implant prostheses have traditionally undergone surface modifications in terms of surface chemistry or surface topography, and micron rough surfaces have been shown to have greater-bone implant contact and bone- implant bond strength compared with smooth surfaces.

The human tissue is formed of different types of cells, each group having a specific behavior when it comes in contact with a synthetic or semi-natural material [21, 22, 23].

The adhesion factor of the bone cells on the prostheses is extremely important for the development of the tissue especially in the first moments after the surgery. The cells are alive only in their physiological environment. Osteoblasts survive if the adhesion on the prosthetic material is not too low because in this case the tissue does not adhere to the surface of the prostheses but neither too strong because osteoblasts in such a case do not have the freedom to develop. For making a study of adhesion from mechanical point of view we considered the cells in the physiological solution spread on the titanium surface.

This study was done to avoid damage of the tissue and to create a good biocompatibility of the prostheses. We needed for this purpose to predict with tests and models the consequences of the implantation surgery. For the tests and models all biological, biomedical and mechanical parameters consulted from literature were used [24, 25]. It is worth to mention that one of the main characteristics of osteoblast cells is that as they are adapted on the titanium surface, their mechanical and physical properties close to the surface are changing following an exponential rule of variation.

In such a way, the combination of cells dispersed into the physiological liquid and the whole system lying on the titanium material can be considered as a composite system having three phases. Namely, the matrix (cells dispersed into the physiological liquid), reinforcement (titanium nanotubes) and the interphase, lying in the space between the two main phases. In this case one can consider a interphasial phenomenon which is known to govern the global behavior of the two main phase systems. The study of the interphasial phenomena in the present investigation can provide substantial information concerning the degree of biocompatibility between titanium prostheses and human tissue. Also we can deduce more information about the wanted structure of the titanium surfaces for different types of prostheses. The stiffness variation into the interphasial area close to nanotubes was analytically modeled by means of the hybrid interphase model.

The Hybrid interphase model

In the present work, interphasial phenomena at the close vicinity of the TiO_2 nanotubes were analytically investigated by means of the *hybrid interphase model*. According to this model, the interphase thickness is not a simple geometrical concept, but instead, it depends on the property considered at the time. As shown in previous publications, the degradation of the elastic modulus within the hybrid interphase region is given by:

$$E_i(r,t) = E_m(t) + \left(k_E E_f - E_m(t)\right)\exp\left\{-\frac{k_E}{1-k_E}\frac{E_l}{E_t}\frac{r-r_f}{r_f}\right\},\quad r_f \le r \le r_{iE} \qquad (5)$$

where E_f is the fiber's (nanotube) modulus;

E_i is the interphase modulus;

$E_m(t)$ is the matrix time depended modulus;

E_l & E_t are the macroscopic elasticity moduli of the composite along the longitudinal and the transverse direction, respectively;

and

k_E is the nanotube-matrix adhesion efficiency coefficient.

The adhesion efficiency coefficient represents the concentration of micro or, in our case, nano-defects on the TNT-cell interphase. This means that there exist regions where cells are improperly jointed to the nanotube surface which, as a consequence leads to an increase in the interphase thickness. Analytical results indicated a strong impact of the degree of adhesion upon the interphasial properties in general, and more precisely upon the extent of interphase. This can be observed in some changed values of different parameters of the osteoblasts, like it would be the value of the Elasticity Modulus.

Under specific circumstances, the interphases formed at the contours of neighboring nanotubes can overlap to each other. This, results to a transformed matrix (physiological liquid with cells) with properties distinct from the ones initially considered. Under these conditions, osteoblasts show different physical and mechanical properties comparing to the respective ones before the contact with the prostheses,

Interphase overlapping

Requisite condition for interphase overlapping to exist is that the interphase thickness to be higher than the half distance between the nanotubes. The two determinant parameters that govern this behavior are, of course, the thickness and geometry of the nanotubes array and also the thickness of interphase, which is a strong function of the degree of adhesion (and also time).

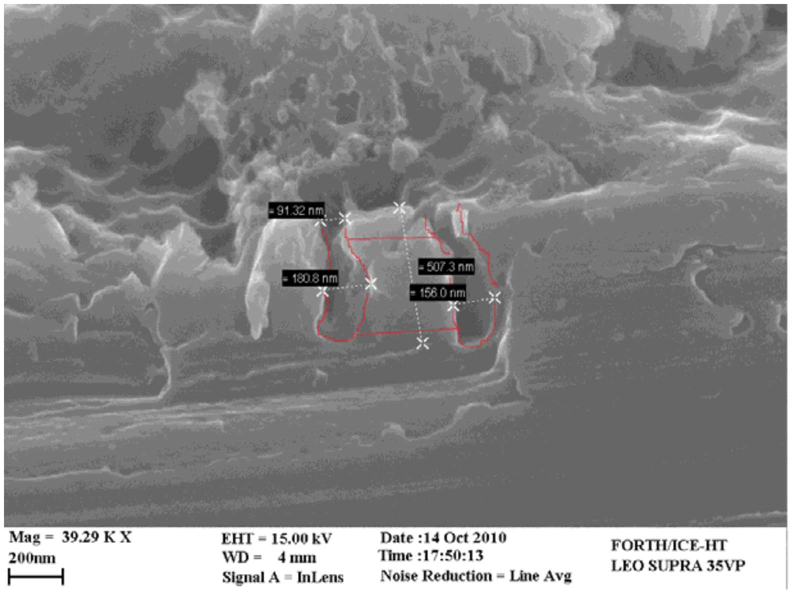

Fig.6. SEM analysis of nanotube layer scratched from pure titanium surface

Fig.6 represents a SEM photomicrograph where neighboring TiO_2 nanotubes of about 507 nm length are shown. As the nanotubes layer was scratched, the distance between the nanotubes is not valuable for a layer that is removed through special protocol. The image was realized with the purpose of studying the nanotubes length at a specific moment of synthesizing.

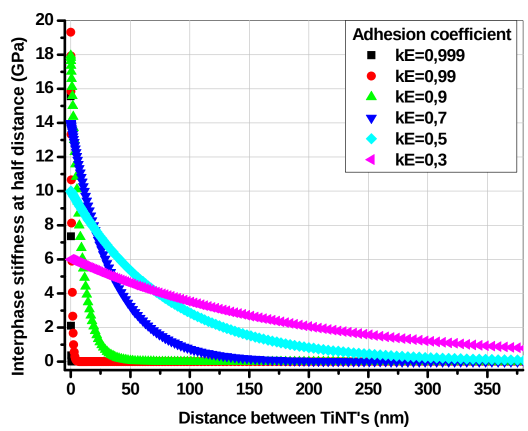

Fig.7.Interphase modulus at the midpoint between two adjacent nanotubes as a function of the respective distance between them for various values of the degree of adhesion

Thus, in an effort to investigate the possibility of overlapping, the interphase modulus at the midpoint between two nanotubes was parametrically calculated as a function of the distance between the nanotubes for the various degrees of adhesion. In the case of perfect adhesion the cells although they are in perfect contact with the substrate, they cannot move through the physiological liquid in order to have a correct evolution in development.

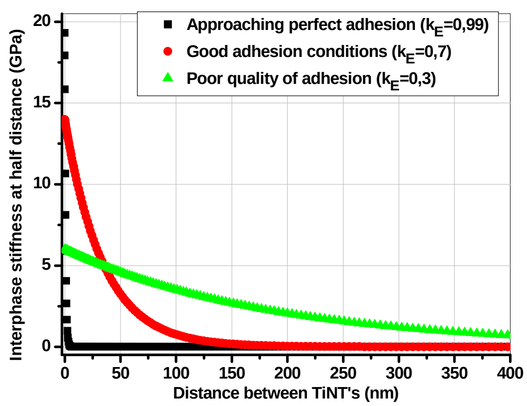

Fig. 8. Interphase modulus at the midpoint between two adjacent nanotubes as a function of the respective distance between them for almost ideal, good and poor adhesion conditions

As it can be observed in Figs.7-8, as the quality of adhesion degrades, the possibility of interphase overlapping increases even for a sparse array of nanotubes. More precisely, for good adhesion conditions, k_E=0.70, which is thought to correspond to good biocompatibility, there is an overlapping of the interphases even for distance between neighboring nanotubes lying within the range of 100-150 nm. As a result, a large fraction of the matrix lying in the area between nanotubes transforms into a material with higher stiffness, and more resistant to deformation. On the contrary, as ideal adhesion conditions are approached interphase thickness tends to zero and, thus, the nanotube distance in order to have overlapping is within the range of some nanometers.

4. Conclusions

o The present work describes techniques and respective electrochemichal mechanisms of producing TiO_2 nanotubes on pure titanium surfaces and presents some parameters that affect their geometry and surface density.

o The pure titanium surface covered with TiO_2 nanotubes can have several applications to different branches of technology and especially to biomedicine.

o TiO_2 nanotubes were created on pure titanium surfaces through anodizing method trying different types of electrolytes.

o Studies about the mechanism of attachment of the osteoblasts on the surface of titanium nanotubes were realized through the viscoelastic hybrid interphase model and in parallel in vitro with osteoblast cells in order to both experimentally observe and theoretically predict the adaptation of the human tissue on a new designed prosthesis material.

o Combining experimental procedure of creation of the nanotubes, as well as the mathematical calculations for the interphase nanotubes-osteoblasts data are given about the required characteristics of TiO_2 nanotube surfaces for making the environment adequate for a proper developing of the cells.

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