UNIVERSITY “POLITEHNICA” OF BUCHAREST
FACULTY OF APPLIED CHEMISTRY AND SCIENCE OF MATERIALS

DOCTORAL THESIS

Theoretical and Experimental Studies on the Manufacturing, Biocompatibility and Modeling of TiO_2 Nanotubes

Abstract

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INTRODUCTION

Nanostructured materials defined as materials which contain clusters, crystallites, molecules or other structural elements comprised between 1-100 nm are nowadays widely studied for biomedical area. The availability of nanomaterials in biomedical branch has two main consequences:

- the progress in nanomaterials research brought to the surface new ways of manufacturing different substances, new techniques of characterization and new modeling techniques
- the specific interactions between biological structures (e.g. enzymes and proteins) and nanostructured materials make possible the development of medical implants and other biostructural elements.

Classical medical implants (stent implants for vessels, grafts or other artificial devices) produce many times, inside the human body, inflammatory reactions (clinical or angiographic restenosis, thrombosis). Replacement of these implants with nanomaterials stops the inflammatory reactions. The interest for nanotechnology is increasing in research in all research fields. In biomedical world nanomaterials are of great importance in new implant design, drug delivery system, and modern imaging techniques.

Between transitional metals, *titanium* is the second most abundant transition metal on Earth and plays a vital role as a material of construction because it has excellent corrosion resistance, high heat transfer efficiency, and superior strength-to-weight ratio. For the same reasons, as well as for its chemical properties and biocompatibility, titanium and its alloys (Ti-6Al-4V) are widely used in replacement of hard tissue, in implant surgeries. Titanium metal is coated with an oxide layer that usually renders it inactive. Once titanium starts to burn in air it forms titanium dioxide, TiO_2 and titanium nitride, TiN. Titanium metal even burns in pure nitrogen to form titanium nitride. When titanium reacts with steam it forms the dioxide, titanium (IV) oxide, TiO_2, and hydrogen, H_2. Dilute aqueous hydrofluoric acid, HF, reacts with titanium to form the complex anion $[TiF_6]^{3-}$ together with hydrogen, H_2. The reactivity of Ti with water and acids make it suitable for surface manufacturing thorough anodizing method.

Therefore, titanium dioxide is a naturally occurring oxide of the element titanium, also referred to as titanium (IV) oxide or Titania; this substance occurs naturally as three mineral compounds known as anatase, brookite, and rutile. The stability and biocompatibility of Ti alloys is ascribed to their ability to form stable and dense oxide mixtures consisting mainly of TiO_2. The native oxide layers on Ti are spontaneously rebuilt in biofluids whenever they are damaged due to an aggressive pH or different mechanical factors.

In spite of its good mechanical and chemical properties, titanium, as any other metal, presents few disadvantages. Related to implant surgery, the main problem of titanium prostheses is the type of interaction that develops between these and the bone tissue. The adhesion tissue - titanium prosthesis is generally too strong and leads to failure of the implant integration into the body. Many times a high amount of medicine and even a subsequent surgery is needed, all these implicating trauma and high costs for the patient. For this reason the manufacturing of titanium and especially titanium surface it is very important, because it can confer to the material better quality as an implant. By exploring different manufacturing methods, there are possibilities to neutralize or minimize titanium disadvantages. The above mentioned chemical properties of titanium are the basis for its surface modification. Under specific conditions, the titanium atoms group and form nanostructures.

Previous studies showed that titanium dioxide nanotubes (TNTs) can represent ideal foils for vascular prostheses. In the latest years titanium dioxide nanotubes produced through electrochemical anodizing are extremely studied as new biomaterials for orthopedic implants, in situ drug delivery systems, in immunoisolation, cellular growth, as biosensors, artificial organs, and tissue engineering.

The doctoral study entitled "**Theoretical and Experimental Studies on the Manufacturing, Biocompatibility and Modeling of TiO_2 Nanotubes**" had the target to improve the quality of a material used for bone implants manufacturing, based on titanium metal.
The main steps in the manufacturing of an implant are:

a) Proper manufacturing (in case of this study, through anodizing method)
b) Biomechanical modeling and testing
c) Biological testing with human cells
d) Sterilizing and packing
e) Integration into the living body through implant surgery

The studies performed having the aim to obtain a material used as a bone implant are interdisciplinary and combine results in the following areas of research:

- ✖ Electrochemistry - Manufacturing of TiO_2 Nanotubes layers on pure titanium surfaces through Electrochemical anodizing.
- ✖ Mechanics – Mechanical modeling for study of the adhesion between TiO_2 Nanotubes layers and human bone cells
- ✖ Biology – Testing the biocompatibility of TNTs layers

The very well aligned nanotubes growing from the titanium oxide layer, enhance and accelerate the hydroxyapatite - $Ca_5(PO_4)_3(OH)$ - deposition and can sustain a drug delivery system.

The original aspect of the present work consists in the effort of correlating the results in the above mentioned scientific areas, having the target to get a quality bone implant material.

The work is divided in two parts:

PART I - Bibliographical study presented in the following chapters:

✖ ***Chapter 1*** A study on titanium and nanostructured titanium
✖ ***Chapter 2*** Methods for Manufacturing TNTs, devices for surface characterization of TNTs
✖ ***Chapter 3*** Applications of TNTs
✖ ***Chapter 4*** Modeling and interphases

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✖***Chapter 8*** Biocompatibility studies; behavior of cells when in contact with titanium surfaces.
✖***Chapter 9*** Materials and methods. Devices.

✖ ***Chapter 10*** General Conclusions

✖ ***Acknowledgements***

✖ ***Bibliography***

In the present abstract the experimental results from chapters 5-10 are briefly presented.

PART II EXPERIMENTAL RESEARCH

Chapter 5

Manufacturing of TiO_2 Nanotubes through Electrochemical Anodizing

Electrochemical anodizing is one of the most convenient methods used for manufacturing nanostructured titanium: nanopowders, nanoparticles or ***titanium dioxide nanotubes (TNTs)***. The two main advantages of this method are the simplicity and the low cost. The very well aligned nanotubes growing from the titanium oxide layer, enhance and accelerate the hydroxyapatite - $Ca_5(PO_4)_3(OH)$ - deposition and can sustain a drug delivery system.

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 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.

5.1 Chemical reactions and energetic processes involved in anodizing. Mechanism of TNTs formation

5.1.1 Chemical reactions

A detailed explanation for the formation mechanism of nanotubes is given. 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:

$$Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^- \tag{5.1}$$

(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:

$$TiO_2 + 6F^- + 4H^+ \rightarrow [TiF]^{2-} + 2H_2O \tag{5.2}$$

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

$$Ti^4 + 6HF \rightarrow [TiF_6]^{2-} \tag{5.3}$$

The competition between electrochemical etching and chemical dissolution determines the structural morphology of nanotubular layer.

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 corrosion speed becomes equal with the dissolution speed for the surface of the superior part of the nanotubes.

5.1.2 Energy processes

Energy processes are of equal importance in the nanotubes' architecture synthesis. 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. 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:

$$\sigma_{er} = \gamma_{11} E B^2 \tag{5.4}$$

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

$$\sigma_{es} = -\frac{1}{2} \varepsilon_o \varepsilon \, B^2 \tag{5.5}$$

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

$$\sigma_{vol} = -\frac{(\partial \upsilon / \upsilon) E (1 - \upsilon)}{(1 + \nu)(1 - 2\nu)} \tag{5.6}$$

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:

$$U_s = \frac{\sigma^2}{2E} \tag{5.7}$$

$$\text{Where } \sigma^2 = \sigma_{er}^2 + \sigma_{es}^2 + \sigma_{vol}^2 \tag{5.8}$$

5.2 Study of synthesis parameters

The manufacturing of a highly ordered TNTs layer is affected by the synthesis parameters:

- Type of electrolyte
- Anoding time
- Electrolysis potential

5.2.1 Type of electrolyte

In the costumized electrochemical cell (see chapter 9) with cathode – graphite electrod and anode – titanium foil were tested a serious of electrolytes – HF solutions of different concentrations or solutions HF - Na_2HPO_4, respectively HF - $(NH4)_2SO_4$. The tested electrolytes are tested in table 5.1.

Table 5.1 Types of electrolytes tested and main parameters applied: voltage and time

	Electrolyte composition	Voltage (V)	Time (min)
a.	HF 0.5wt %	20	120
b.	HF 1wt%	20	120
c.	0.5wt% HF + 5 g/l Na_2HPO_4	20	120
d.	1 M $(NH4)_2SO_4$ + 0.5wt% NH_4F	20	120

For the main experiments two types of electrolytic solutions: a) HF 0.5wt% and b) a glycerol-water mixture (50:50) containing 1wt% HF were used. In table 5.2 the time and voltage combinations as applied to two different electrolytes used in anodizing procedure, are tabulated.

Table 5.2 Main electrolytes and basic parameters applied: voltage and time

	A						**B**					
Electrolyte	**HF (0.5 wt %)**						**HF (1 wt %) in Gly-Water mixture**					
Time	**45(min)**		**2(h)**		**4(h)**		**4(h)**		**8(h)**		**24(h)**	
Voltage	**10**	**20**	**10**	**20**	**10**	**20**	**20**	**25**	**20**	**25**	**20**	**25**

5.2.2 Time of anodizing

The morphology and structure of the titanium oxide nanotubular materials can be studied using a scanning electron microscope (SEM). The images obtained for different anodizing conditions gave information about how parameters influence the structure of the layer.

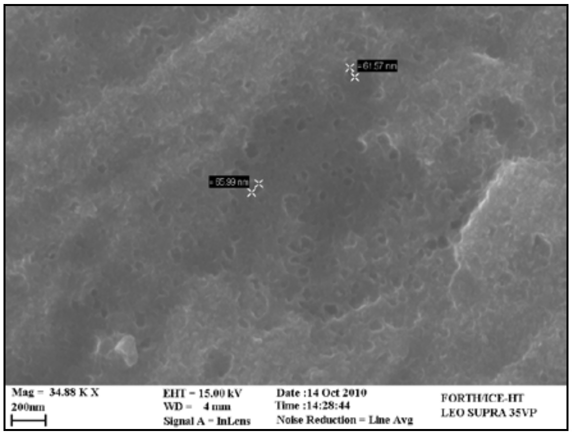


Fig.5.1 SEM of titanium layer anodized in HF 0.5wt%, for 45 min and at 20 V

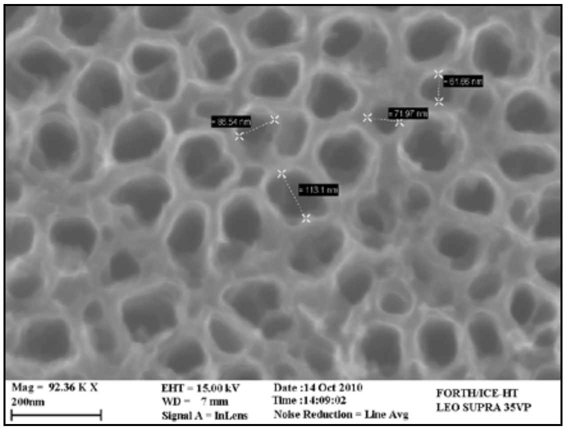


Fig.5.2 SEM of TNTs obtained in HF 0.5wt%, after 2 hrs anodizing at 20 V

It can be observed by comparing Fig.5.1 and Fig.5.2 the importance of the time for performing the experiment. At 45 minutes the titanium oxide layer is partially corroded and the pits which represent the basis for the nanotubes growth appear. After two hours anodizing, the nanotubular architecture is already defined.

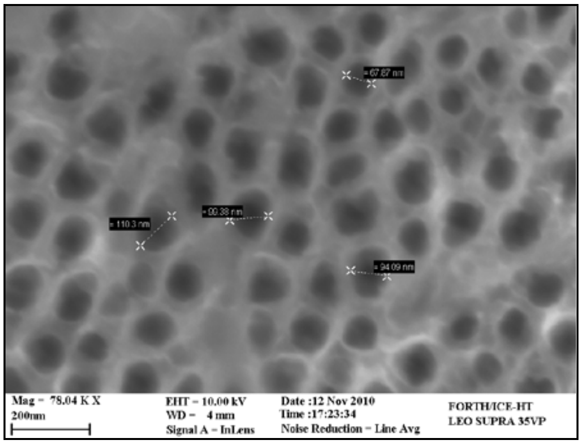


Fig.5.3 SEM of TNTs obtained in HF 0.5wt%, after 4 hrs anodizing at 20 V

In Fig.5.3 the structure of the nanotubes is much more regular due to the increased anodizing time (of four hours).

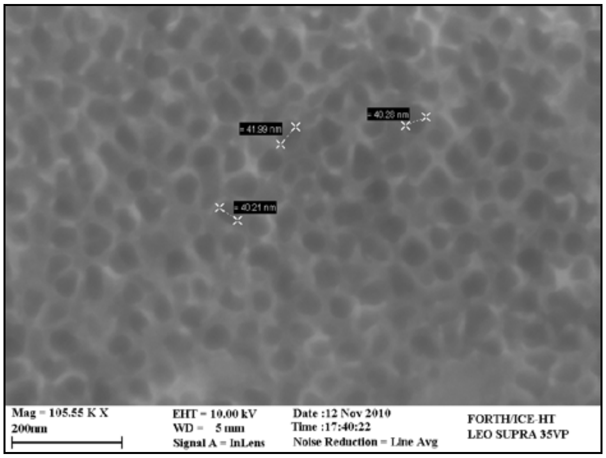


Fig.5.4 SEM of TNTs obtained in HF 0.5wt%, after 45 min anodizing at 10 V

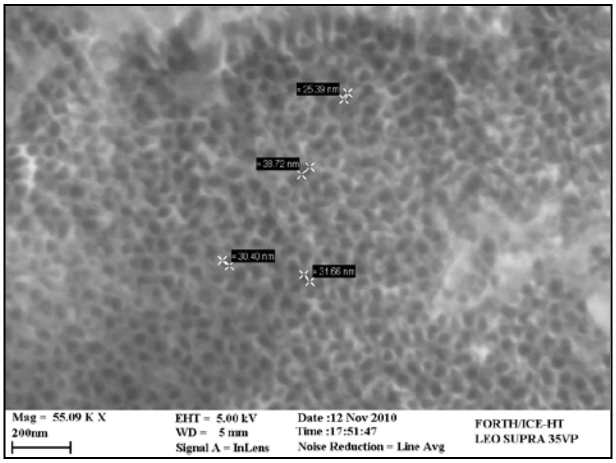


Fig.5.5 SEM of TNTs obtained in HF 0.5wt%, after 4 hrs anodizing at 10 V

5.2.3 Voltage influence

The key factor controlling the tube diameter is the anodizing voltage. Self-organized layers of nanotubes can be defined even at potential as low as 10 V, although their morphology looks better at higher voltage. This can be deduced by comparing Fig.5.3 with Figs. 5.4 and 5.5.

In case of an electrolyte in which is included a natural component as glycerol, the viscosity of the solution increases. Therefore, for activating processes there is a need of higher voltage. The anodizing of titanium in electrolyte containing glycerol did not complete the formation process of nanotubes layer in four hours (Fig.5.6).

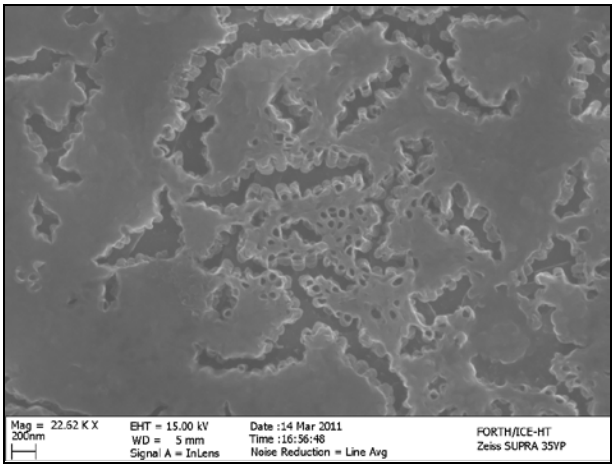


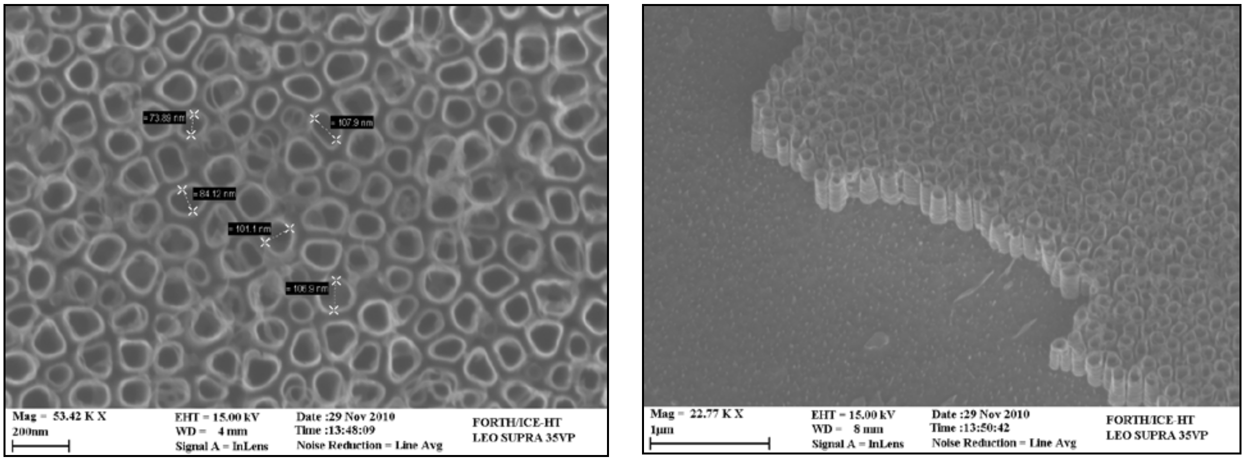
Fig.5.6 SEM of TNTs obtained in Gly containing electrolyte after 4 hrs anodizing

5.2.4 Optimum combination of parameters in anodizing TNTs

The study for finding the optimum combination of parameters responsible of formation of the well organized, well defined and regular nanotubes leaded to the following combination:

- Anodizing electrolyte: Glycerol – HF solution
- Anodizing time: 8 hours
- Anodizing potential: 25 V

Fig.5.7 a and b present SEM images of TNTs obtained in optimum conditions.



a) Panoramic view

b) Lateral view

Fig.5.7 SEM of TNTs obtained in Glycerol **-** containing electrolyte after 8 hrs anodizing at 25V.

5.3 Statistical analysis of SEM images

The structure and density of nanotubes is very important for their application in areas as medicine, aeronautics and environment. All the SEM images were carefully analysed with image analysis software.

The mean diameter and standard deviation of vertically aligned TiO_2 Nanotubes were evaluated from SEM micrographs obtained as a function of the anodizing conditions applied. For each sample, at least five photos were used to calculate the final mean diameter.

In Fig.5.8 it can be seen the statistics of the mean diameter of nanotubes growth on one sample. The nanotube diameter influences the density of nanotubes on the whole layer; this has a great importance when the TNTs layer interacts with a second material and creates an interphase. In Fig.5.9 the mean diameter of several samples were plotted for comparison.

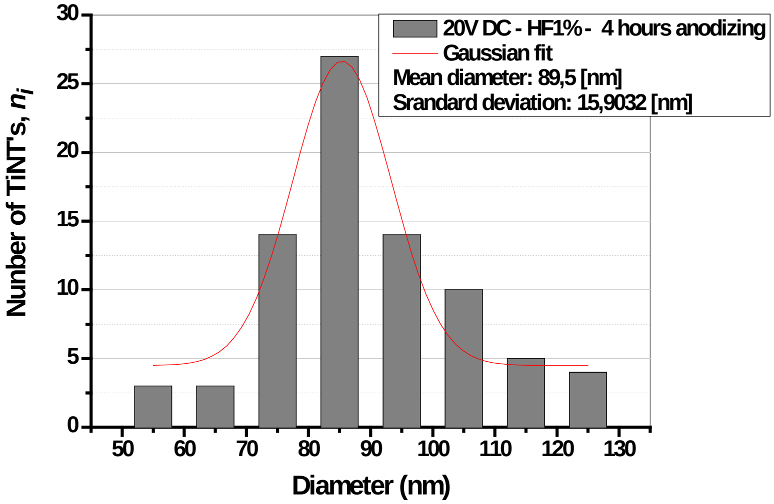


Fig.5.8 Calculation of mean diameter of nanotubes on a sample anodized in HF 1wt % electrolyte

In Fig.5.9 the calculation made for Images 12 and 10 correspond to samples anodized at 10 V, while the calculation for Images 3, 5 and 11 correspond to samples worked at double increased potential, of 20 V.

Image Nr	Electrolyte	Anodizing Time	DC Voltage
3	HF (0.5%)	2h	20V
5	HF (0.5%)	2h	20V
10	HF (0.5%)	4h	10 V
11	HF (0.5%)	4h	20V
12	HF (0.5%)	45 min	10 V
13	HF (0.5%)	2h	10 V
15	HF (0.5%)	2h	20V
21	GLY	4h	20V
22	GLY	8h	25V
24	HF (0.5%)	45 min	20V
26	GLY	24h	25V
40	GLY	4h	25V
41	GLY	8h	20V
42	GLY	24h	20V

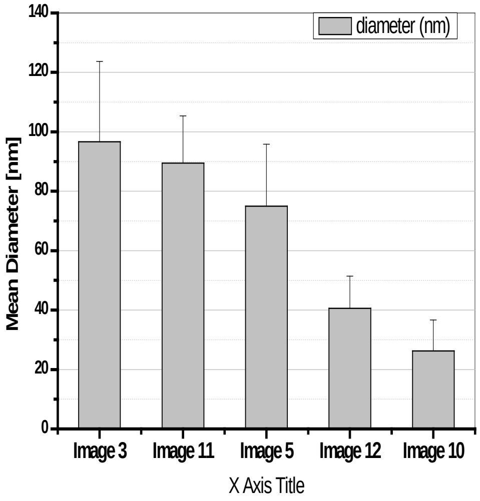


Fig.5.9 The mean diameter of several samples

The up to now presented work aims to underline the method of manufacturing the TNTs, the parameters applied during the electrochemical synthesis and their influence on the structure of the nanotubes, as well as the surface analyses performed to get more information regarding this type of nanostructured titanium.

Chapter 6

Structural, morphological and mechanical analyses of TNTs layers. EDX, PL, XRD, tests and Friction Coefficient

6.1 EDX analysis

Since the first report claiming the hydrothermal synthesis of TNTs many studies have been done on the growth and structure of this type of nanotubes. One-dimensional TNTs, owing to their large length–diameter ratio, have physical, electronic and chemical properties that are different from other forms. Several varieties of techniques, tools and devices can be used to explore the TNTs layers, no matter if it is about layers sustained by the titanium foil or independent layers.
The chemical characterization of the surfaces involved in the experiments can be done through analytical technique, respectively energy dispersive X-ray microscope (EDX connected to SEM). In the image bellow it can be seen the EDX performed on one pure titanium sample, before using it for the anodizing experiment.

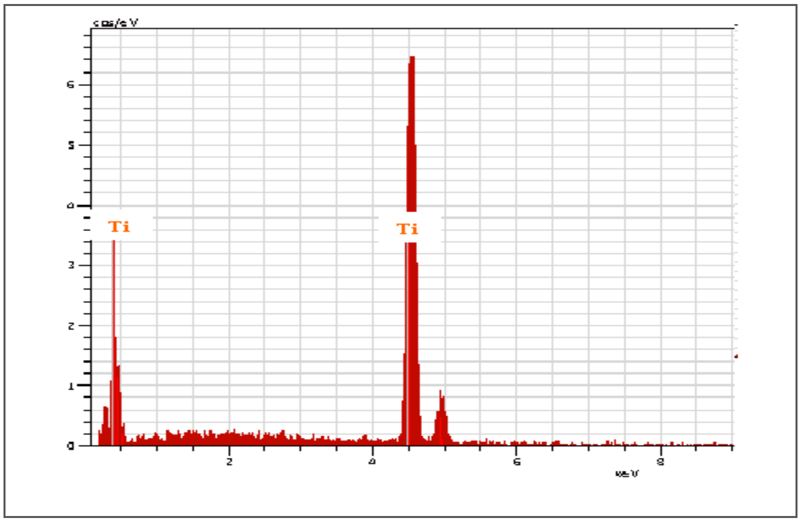


Fig.6.1 EDX- of pure titanium sample

After anodizing and build-up of nanotubes the surface can be considered as a new material, although the matrix in which nanotubes' formation starts it is titanium itself. The processes taking place in the electrochemical cell determine the interaction of the metal with the electrolyte solution; so the final chemical elements present in the layer of nanotubes should be, besides titanium, those that compose the electrolytic solution. Samples anodized in fluorine based electrolyte, containing 0.5 wt % HF in 1000 ml solution were analyzed with EDX device.
As it can be seen in Fig.6.2, after anodizing there are shown:

1) Titanium - the initial sample,
2) Oxide from titanium oxide layer formed under anodizing conditions
3) Fluorine that is component of the electrolyte solution and that has a main role in nanotubes' architecture built up.

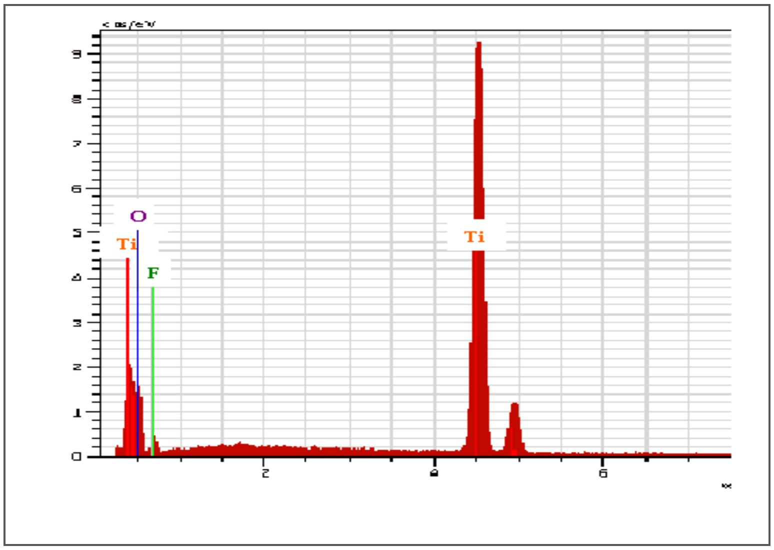


Fig.6.2 EDX – TNTs layer anodized in HF 0.5wt%

For many applications the distribution of chemical elements in the material is basic, as it provides information concerning how the material can be explored and about the type of materials and substances that it could interact with. In case of titanium applied for implants manufacturing, it is preferable to eliminate the content of fluorine because this can be toxic for the human tissue.
As it is known that the fluorine is concentrated in the upper level of the nanotubes, these having structure of rings added one on top of the other (Fig.6.3), a way to eliminate it can be the ultrasonication.

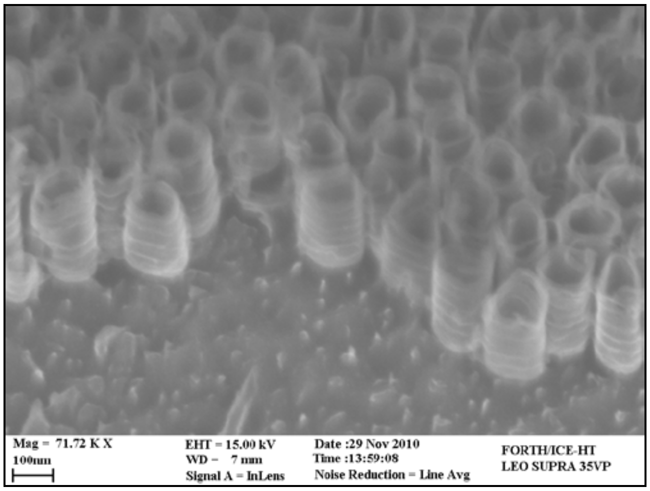


Fig.6.3 SEM - Ring structure of nanotubes

The samples were exposed to ultrasonication. The fluorine existent in the anodized sample was removed in the case of TNTs obtained in HF 0.5wt% electrolyte (Fig. 6.4), but the structure of the nanotubes was affected during the procedure (Fig. 6.5).

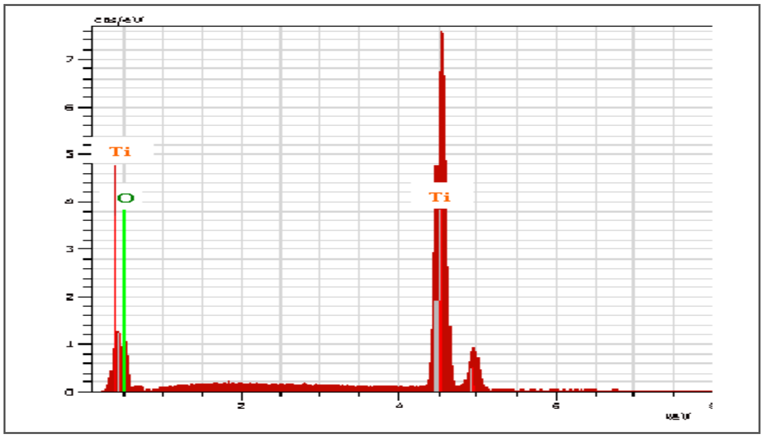


Fig.6.4 EDX – Chemical elements in the anodized layer, after ultrasonication treatment

The ultrasonication method is quite aggressive for the nanotubes and it disorganizes the layer; an important factor when ultrasonication applied is the time. Five minutes of sonication is too much for the sample to keep the initial morphology of the TNTs layer, while 1-2 minutes is enough for the impurities to be removed but not enough for eliminating also the fluorine concentration.

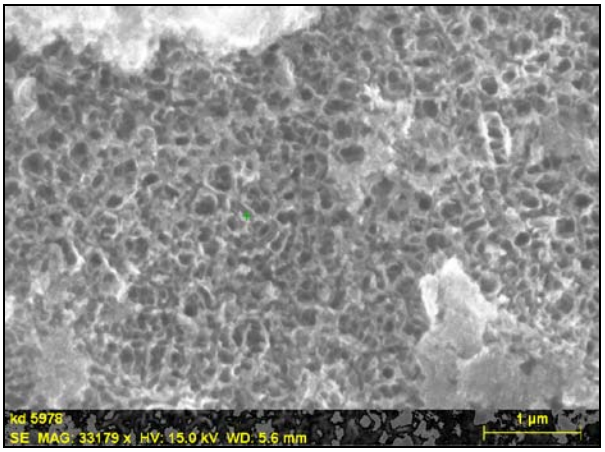


Fig.6.5 SEM – TNTs layer disordered

The structure of the TNTs obtained in glycerol - electrolyte was only in a little percentage affected by the ultrasonication protocol (Fig.6.7), but fluorine was not removed from the surface (Fig.6.6).

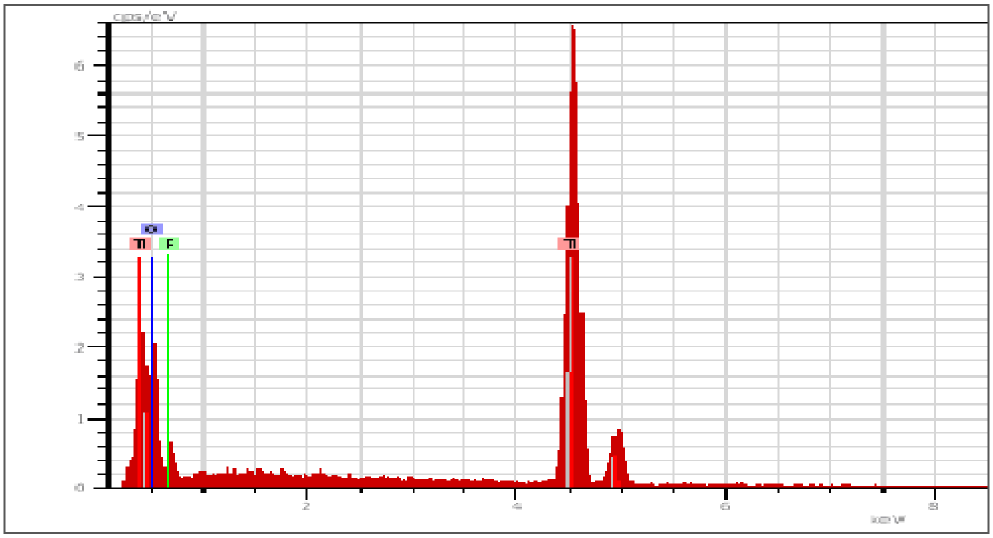


Fig.6.6 EDX – Chemical elements in TNTs layer

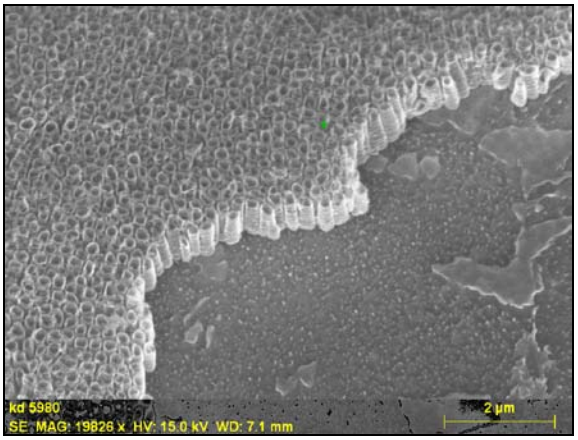


Fig.6.7 SEM – TNTs layer partially removed

The reason for the presence of the fluorine in the layer after five minutes sonication it is still not clear; it might be due to the increased percentage of the hydrofluoric acid (1% wt) in the organic electrolyte or due to a higher penetration of the electrolyte into the layer in short time after the anodizing is ended. It was observed a correlation between the humidity of the samples (when sonicated) and the possible removal of fluorine from its final surface. If samples were sonicated immediately after the anodizing experiment ended, the fluorine content was removed; while the situation was not the same in the case of the samples dried at room temperature for 2-3 days before sonicating, when fluorine remained in the substrate in a high percentage. Although the structure of the individual nanotubes formed in organic electrolyte was not affected during sonication procedure, the architecture of the total entire layer was affected in a little percentage. As it can be seen in Fig.6.7, the layer of nanotubes was partially removed.

6.2 XRD patterns

X-ray crystallography is a method of determining the phase in which a sample finds, as well as the arrangement of atoms within a crystal. XRD tests were performed on samples worked in the same anodizing conditions, respectively: glycerol-HF based electrolyte, 8 hours anodizing and 25 volts potential. The purpose was to determine the phase in which the nanotubes are (crystalline or amorphous) and compare the intensity and arrangements of the picks with standard XRD diffraction of pure titanium.

XRD patterns (Fig.6.8) show that reflections of samples 32 and 33 are similar to metallic Ti, suggesting that TiO_2 nanotubes are in amorphous phase. This is in agreement with the literature were it was several times reported that anodized titania nanotubes are amorphous and transform into crystalline anatase after annealing above 280^{o} C.

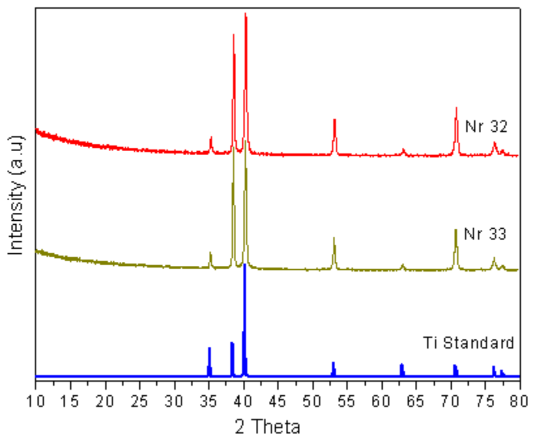


Fig.6.8a XRD for pure titanium and TNTs

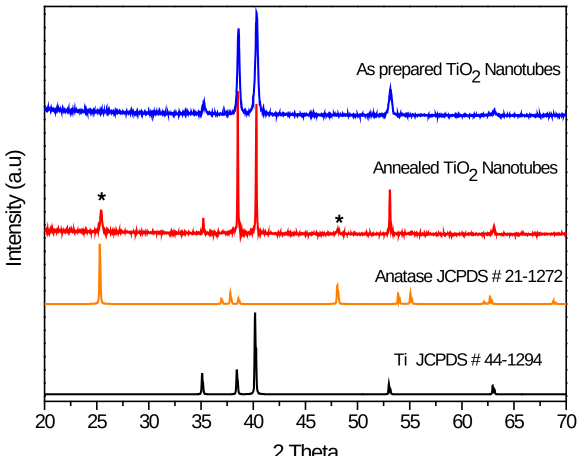


Fig.6.8b XRD for annealed TNTs

6.3 Photoluminiscence

Photoluminescence (PL) is a useful technique that provides information about electronic, optic and photoelectric properties of a material. PL spectra are closely related to the surface stoichiometry and surface states of nanomaterials. It is well known that PL spectra are affected greatly by particle size. The higher the intensity of the peaks in PL spectra, the more uniform interfaces with no trap sites on their surfaces.

Nanotubes were produced on two pure titanium samples; the only parameter changed being the time of anodizing. As it was shown in the SEM images, the sample anodized for longer time has a more uniform surface.

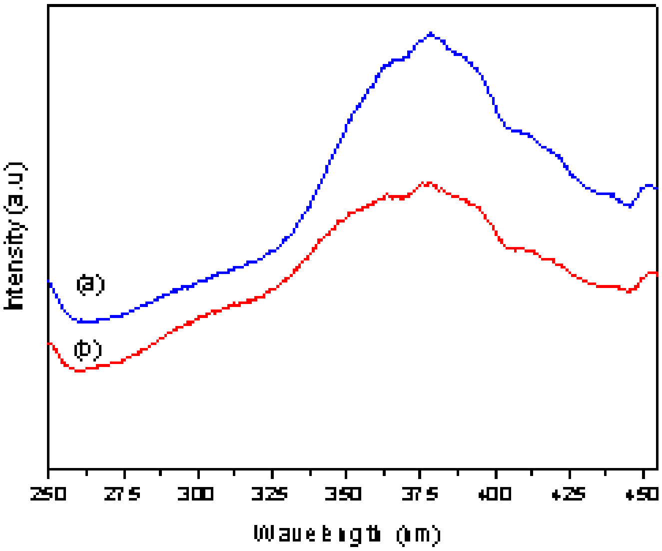


Fig.6.9 PL spectra of TiO_2 nanotubes a) HF, 10 V, 4 hrs
And b) HF, 10 V, 45 min

After several experiments done it was concluded that a higher anodizing time leads to higher order of the nanotubes layer. PL test made to the two samples for comparison (Fig. 6.9) show that the intensity of the pick goes higher in the case of 4 hrs anodizing then in the case of 45 minutes anodizing time.

6.4 Friction coefficient

The static friction coefficient (μ) between two solid surfaces is defined as the ratio of the tangential force (F) required for producing sliding divided by the normal force between the surfaces (N)

$$\mu = F / N \tag{6.1}$$

For a horizontal surface the horizontal force (F) to move a solid resting on a flat surface

$$F = \mu \bullet mass\ of\ solid \bullet g \tag{6.2}$$

If a body rests on an incline plane the body is prevented from sliding down because of the frictional resistance. If the angle of the plane is increased there will be an angle at which the body begins to slide down the plane. This is the angle of repose and the tangent of this angle is the same as the coefficient of friction.

Friction is the force resisting the relative motion of solid surfaces, fluid layers, and/or material elements sliding against each other. When the tangential force F overcomes the frictional force between two surfaces then the surfaces begins to slide relative to each other. In the case of a body resting on a flat surface the body starts to move. The sliding frictional resistance is normally different to the static frictional resistance. The coefficient of sliding friction is expressed using the same formula as the static coefficient and is generally lower than the static coefficient of friction.

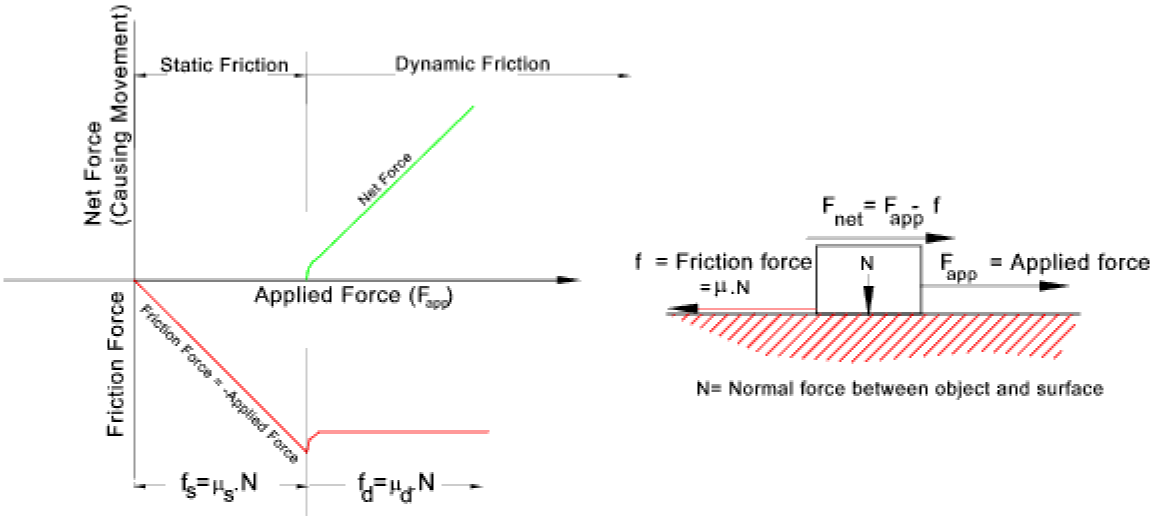


Fig.6.10 Sliding Friction Coefficient scheme

Friction coefficient should not be neglected when it is spoken about implant prosthesis. The friction between implant and tissue is related to the degree of adhesion between the two components, natural and synthetic and in case of improper values they can lead to failure of the implant procedure. The adhesion coefficient is very important in the first hours, day or months after surgery while the friction coefficient is important for long lasting implant - tissue interactions.

For having a general idea on the friction coefficient of titanium in contact with a solid it was chosen the titanium- ice system. For comparison there were made tests to the following materials in contact:

a) Pure commercial titanium and ice
b) TiO_2 nanotubes and ice
c) Fe and ice

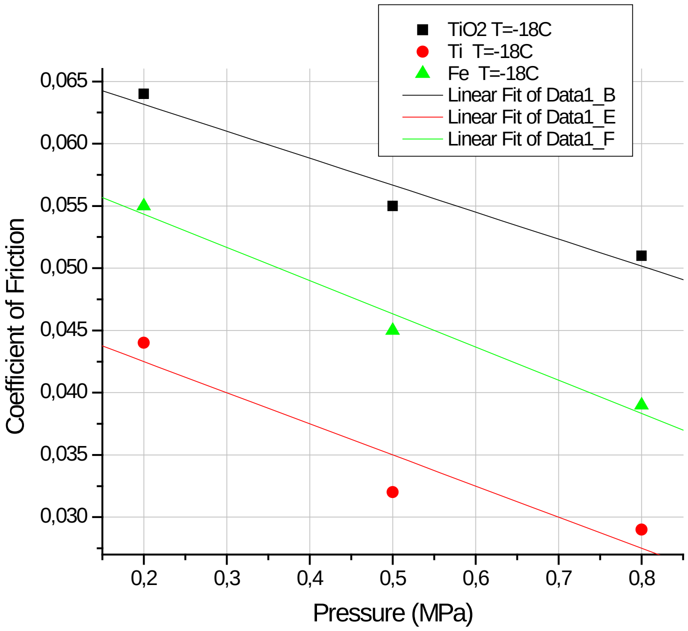


Fig.6.11 Variation of the Friction coefficient for TNTs on ice and Steel on ice systems at -18°C for different levels of applied pressure

Based on Physical and Mechanical considerations, and taking into account all experimental results already presented, the following analytical relation was found connecting the coefficient of friction of TiO_2 nanotubes on ice with applied normal pressure and temperature.

$$\mu = \mu_0 C_f \left(1 - P\right) \exp\left[-2\mu_0 C_{fT} \left(1 + 2P\right) T\right] \qquad (6.3)$$

Where μ is the friction coefficient, P the applied normal pressure in (MPa), T the temperature in (^{0}C), C_f the friction Compliance for dry friction in (MPa^{-1}) which is equal to one, C_{fT} the friction Compliance per degree Celsius for dry friction in ($MPa^{-1}\ {}^0C^{-1}$) which is equal to unity and μ_0 the static coefficient of friction at T=0^{0}C and for applied normal pressure P tending to zero; which for the present combination of materials equals to $\mu_0 = 0.02373$. For the application of the model, the experimental value of μ_0 is the only one needed for the prediction. Also all parameters included in the above equation have a clear physical meaning. A comparison between experimental values and theoretical predictions was made.

Table 6.1 Coefficient of friction for the different materials at different temperatures

Pressure (MPa)	TiO_2 T= -18°C	TiO_2 T= -10°C	TiO_2 T= -5°C	Ti T= -18°C	Fe T= -18°C
0,2	0,064	0,04	0,018	0,044	0,055
0,5	0,055	0,027	0,012	0,032	0,045
0,8	0,051	0,017	0,01	0,029	0,039

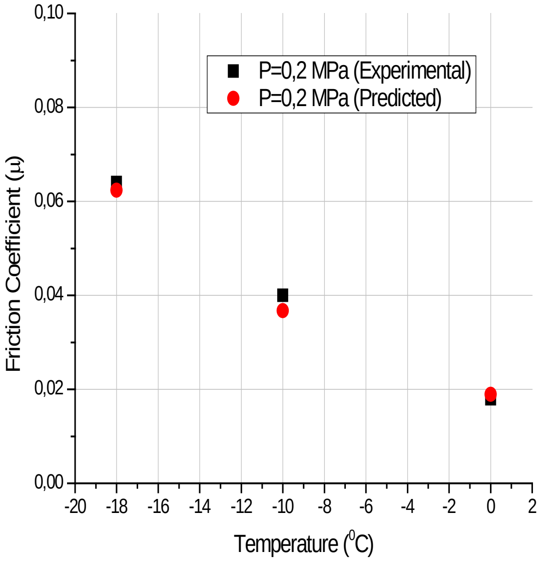


Fig.6.12 Variation of the friction coefficient with temperature. Comparison between experimental and predicted values

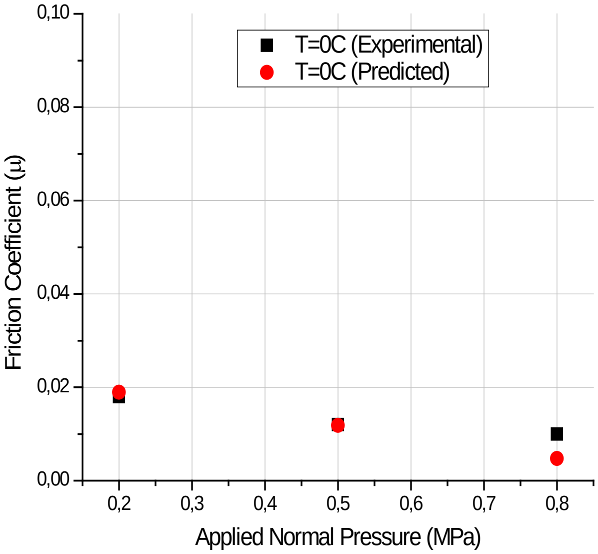


Fig.6.13 Variation of the friction coefficient with applied stress. Comparison between experimental and predicted values

Chapter 7

A new Concept for Living – Nonliving Interphasial Layers Modeling

According to the IUPAC Compendium of Chemical Terminology, an interfacial layer is defined as the inhomogeneous space region intermediate between two bulk phases in contact, and where properties are significantly different from, but related to, the properties of the bulk phases. Examples of such properties are: compositions, molecular density, orientation or conformation, charge density, pressure tensor, electron density, etc. The interfacial properties vary in the direction normal to the surface. Complex profiles of interfacial properties occur in the multi-component systems with coexisting sorption or depletion of one or several components. This interfacial region may also be regarded as a distinct, though not autonomous phase and be called the interphase.

A first preliminary definition for the interfacial area had been given in 1978 by Papanicolaou et.al and it was the following: "*Around an inclusion embedded in a matrix a rather complex situation develops consisting of areas of imperfect bonding, where mechanical stresses are abundantly developed due to shrinkage and high stress gradients or even stress singularities due to the geometry of the inclusion are apparent and finally voids, microcracks, and other similar discontinuities may be created*. In this case the composite may be considered as consisting of three phases, that is the two actual phases and a third one, which may also arise during thermal treatment of the material, because of component interaction. This extra phase is what we call "*interphase*" and it is obviously inhomogeneous.

Next, a novel approach of the interphase concept introduced according which *the interphase volume fraction represents the percentage of the bulk matrix surrounding the inclusions in which a specific matrix property is strongly affected by the existence of the reinforcement, while the interphase thickness represents the maximum radial distance from the inclusion boundary at which this property varies.* This means that both the interphase volume fraction and the interphase thickness are not simple geometrical/structural concepts but they are property-dependent, that is, their values depend on the property considered at the time. An interphase considering all these is the so-called *"hybrid interphase".*

Interphasial phenomena are studied mainly through modeling. The RVE is a technique used in the modelling of materials. According to this technique, the material is considered to be constructed by the repetition of infinite number of representative volume elements. Each one of them has the same percentages of phases with the whole composite, while individual phases are retaining their geometrical characteristics.

The representative volume element (RVE) for an FRC (Fiber Reinforced Composite) is constituted from three material phases, i.e. the fiber, the interphase and the matrix. The radius of the fiber is denoted r_f, and that of the matrix r_m. The interphase extends between the other two phases and has inner and outer radii denoted r_f and r_i, respectively. The extent of the interphase and, correspondingly, its outer radius, r_i, is controlled by the volume fractions of the constituent phases in the FRC. The thickness of the interphase is very small compared with the fiber radius and depends on the fiber volume fraction. The fiber and matrix materials are assumed to be homogeneous and isotropic.

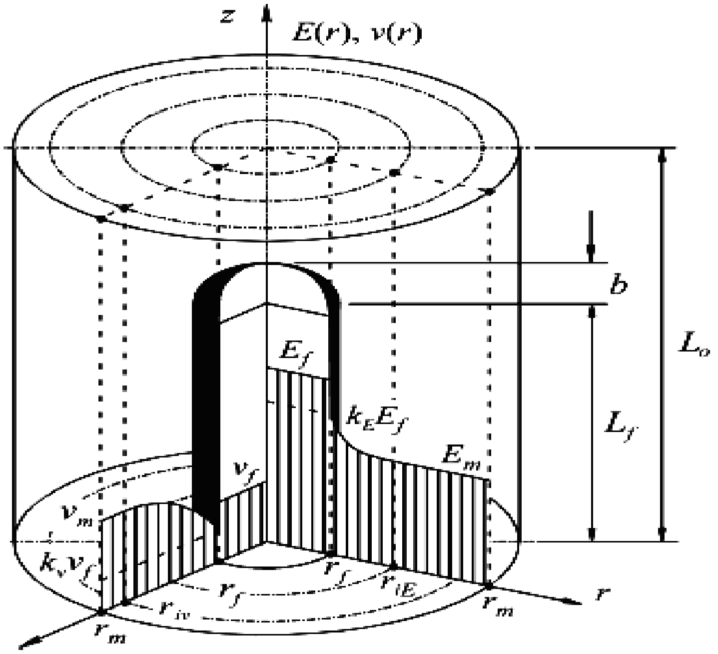


Fig.7.1 RVE along with the hybrid interphase concept

The experimental study of interphases is time consuming and a number of devices are needed for accurate measurement of the degree of adhesion between phases. This experimentation becomes more complicated in the case of biological tests, where living osteoblast cells are involved. Modeling is useful for predicting living-not living interphasial interactions since the living tissue reacts as a homogeneous layer, independently of each cell's behavior. In this case modeling can be applied and can replace many costly and time consuming procedures, as long as tissue' main properties are considered.

7.1 Viscoelastic Hybrid Interphase Model

Interphasial phenomena at the close vicinity of the TNTs were analytically investigated by means of the Viscoelastic Hybrid Interphase Model.

The degradation of the elastic modulus within the hybrid interphase region is given by:

$$E_i(r,t) = E_m(t) + \left(k_E E_f - E_m(t)\right)\exp\left\{-\frac{k_E}{1-k_E}\frac{E_l}{E_t}\frac{r-r_f}{r_f}\right\} \qquad r_f \le r \le r_{iE} \tag{7.1}$$

Where E_f is the nanotubes modulus;
E_i is the interphase modulus;
$E_m(t)$ is the matrix time dependent modulus;
E_l and E_t are the macroscopic elasticity moduli of the composite along the longitudinal and the transverse direction, respectively;

Nanotube-matrix adhesion efficiency coefficient is:

$$k_E = \frac{E_i\left(r = r_f^+\right)}{E_f} \tag{7.2}$$

When subjected to a constant low level mechanical load, the matrix modulus, E_m, is the inverse to the creep compliance, C(t), and can be either determined experimentally or predicted by a theoretical model. The Maxwell model, which was applied within the framework of the present study, considers:

$$E_m(t) = \left(C(t)\right)^{-1} = \left(\frac{1}{E} + \frac{1}{\eta}t\right)^{-1} \tag{7.3}$$

Where E represents the matrix' instantaneous elasticity modulus while parameter η represents the matrix coefficient of viscosity.

7.1.1 Constituent phases in case of TNTs layers and human tissue Modeling

Titanium implants are known as the most long lasting prostheses, sometimes keeping their good mechanical properties until 20 years after the implant surgery. The real problem of integration of the titanium implant into the body doesn't occur as time passes, but in the first months after the surgery. Many titanium implants surgeries failed because of the difficulty created by adhesion between the tissue and the implant in the first month after the intervention; the adhesion is too strong and restricts the proliferation of the cells. Prediction of the adhesion implant – tissue through modeling could be a key to partially solve this problem. For the model we have applied, there were considered many biochemical and biomechanical influent factors and data.

The parameters were chosen corresponding to the experimental protocols used in parallel in the biology laboratory. There were considered the titanium foils covered on an area of 1 cm^2 with perpendicular nanotubes. Mineral Essential Medium containing roughly 25 000 cells per ml was evenly spread on a surface of 1 cm^2. The device used for the measurement of the population of cells per unit volume was a *hemocytometer*.

Our assumption is that the cells in the Mineral Essential Medium create an interphase; the two components of the interphase create an inhomogeneous liquid on the surface of nanotubes during culture period. Between the two surfaces, i.e. that of the titanium and the osteoblasts layer there are clearly considerable differences in properties. The purpose of this work was to analyse on what extent the two phases interact to each other in order to create the interphase.

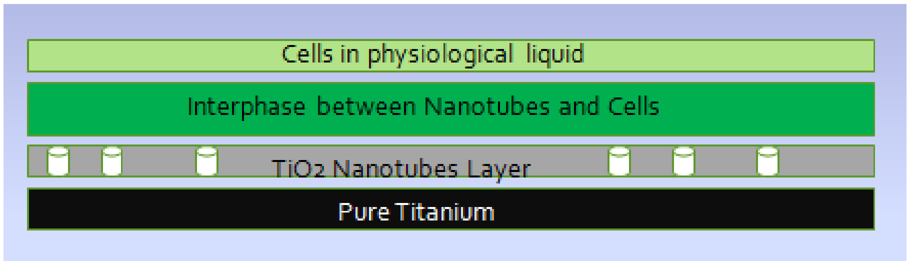


Fig.7.2 Simplified scheme of the interphase created

The values of the elastic and viscous properties of the constituent phases as well as their geometrical features are given in Table 7.1. However, the concept of the *hybrid interphase* was applied for the two phase system containing the linear elastic reinforcement (TiO_2 nanotube) and the homogeneous linearly viscoelastic medium, also referred to as matrix, immediately surrounding each nanotube.

Table 7.1 Properties of the constituent phases

	TiO_2 Nanotube	Osteoblast	Mineral Essential Medium
Viscosity (Pa s)	-	0.03	0.0015
Modulus of Elasticity (Pa)	$20 * 10^9$	5000	-
Mean Diameter (m)	$40 * 10^{-9}$	$25 * 10^{-6}$	-

The matrix was assumed to have properties equivalent to these of the solution of Mineral Essential Medium with spherical osteobast cells embedded in it at a ratio of 25000 per ml of solvent. The properties of the elastic and viscous component of the Maxwell model, for the matrix material are given in Table 7.2.

Table 7.2 Properties of reinforcement and equivalent matrix material

Volume fraction of Osteoblast in the MEM (%)	Matrix viscosity η (Pa s)	Matrix elastic modulus E (Pa)	Reinforcement's elastic modulus E (Pa)
0.02	0.00151	1.022	$20\ 10^9$

For the calculation of the matrix properties, the assumption of the rule of mixtures was made. A number of 25 000 spherical osteoblast cells per ml of solution, with a mean diameter of 25 μm, correspond to a volume fraction of 0.02%.

Interphase properties and the extension of the interphase are affected by the quality of adhesion between the two phases. As we approach the conditions of perfect adhesion the interphase thickness tends to zero. In Fig.7.3 it can be seen that for perfect adhesion (k=0.999) between one nanotube and Mineral Essential Medium containing cells, the interphase thickness practically tends to zero.

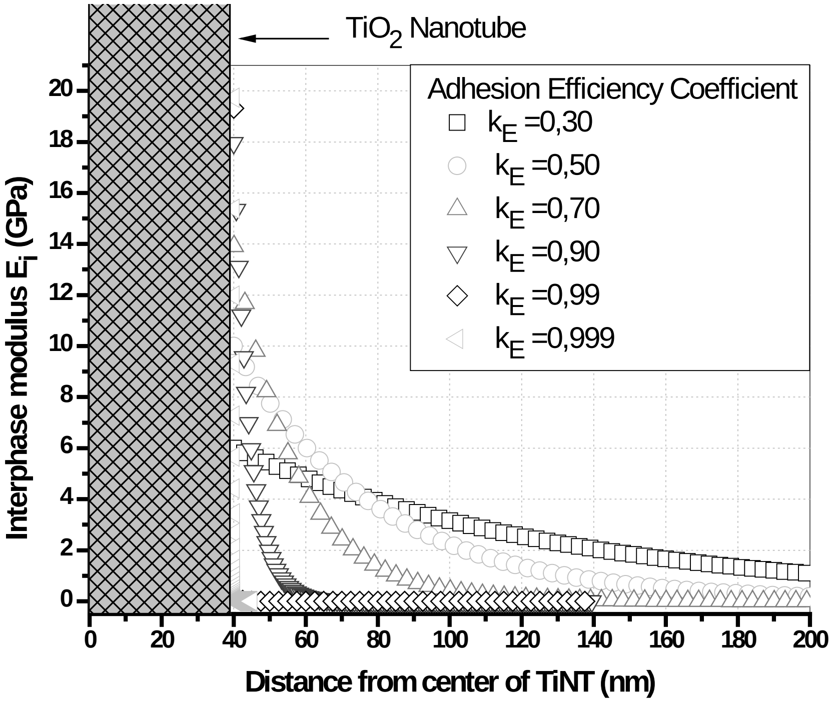


Fig.7.3 One nanotube in contact with Mineral Essential Medium

Osteoblasts properties are highly dependent on the substrate on which they grow. When they are spread on a biomaterial, osteoblasts try to imitate it and to adapt to its surface, taking some of its properties. This happens due to the proteins implicated in osteoblasts adhesion on a layer; it is valuable for osteoblasts – titanium adhesion as well.

As radial distance from the nanotube center increases, the interphase modulus tends to attain a constant value. Depending on the degree of adhesion, the osteoblasts close to the nanotube surface try to imitate and to adapt to the properties of titanium until a limit. In any case, the interaction cell material is accentuated as time passes and the adhesion can be perfect (Fig.7.4), fact which can determine the death of the cells. The too strong, almost perfect adhesion is not recommended because it keeps the cells stacked on the substrate.

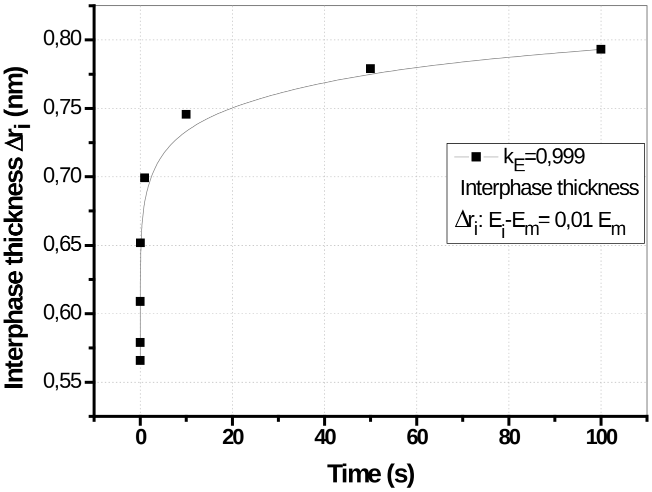


Fig.7.4 Interphase thickness as a function of time for perfect adhesion (k_E=0.999)

In order to achieve a strong fixation of the metal to the tissue while at the same time to have a good environment for the development of the osteoblast cells, extreme values corresponding to both, perfect adhesion or zero adhesion, should be excluded. For perfect adhesion the interphase thickness tends to zero and the properties variation when going from the inclusion surface to the bulk matrix are changing in an abrupt way following a step function variation. Also, in case of two adjacent nanotubes imbedded into the same matrix, corresponding interphases are interacting leading to the total variation of the properties of the matrix material (Fig.7.5).
The adhesion of the osteoblasts to the titanium surface was several times reported as being very strong. When TNTs are present the surface on which osteoblasts are spread is rougher. Therefore, the degree of adhesion is higher. To this phenomenon it is added the pressure of the tissue. While movements take place, also an important factor is the friction coefficient. All these factors shall be calculated before realizing the new implant design.

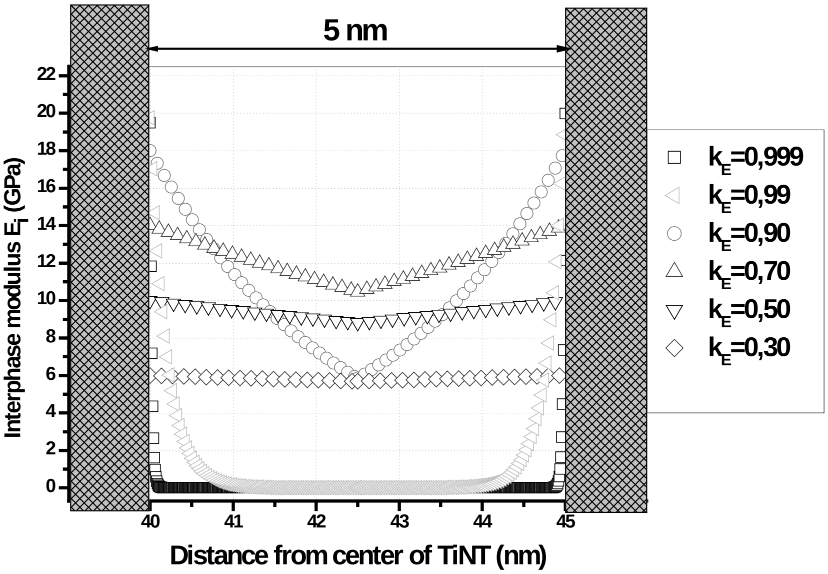


Fig.7.5 Interphase Modulus variation within the interphasial area between two adjacent TiO_2 Nanotubes for various values of adhesion efficiency coefficient k_E

7.2 Interphase between TNTs and a secondary layer

Nowadays there are in fashion the coatings manufactured on the implant materials. Coatings can be biosynthetic or synthetic. Such examples are hydroxyapatite, polymers and lately CNTs. In the frame of this work it was initialized a study of interphases created by TNTs layers in contact with one of the mentioned coatings, having the purpose to create a better environment for cells' development.

7.2.1 TNTs and hydroxyapatite

Synthetic hydroxyapatite (HA) is a very good activator in tissue rebuilding processes because it imitates in high extent the natural HA, component of the human bone.
For synthesis of HAP coating on TNTs, the samples were left for one week in Simulated Body Fluid (Table 7.3), at 37^o C.

Table 7.3 Reagents for preparation of SBF (pH 7.25, for 1000 ml solution)

Reagent	**Amount**
1. $NaCl$	7.996 g
2. $NaHCO_3$	0.350 g
3. KCl	0.224 g
4. $K_2HPO_4 \cdot 3H_2O$	0.228 g
5. $MgCl_2 \cdot 6H_2O$	0.305 g/ l
6. 1 kmol/ m^3 HCl	87.28 mL of 35.4% diluted to 1000 mL
7. $CaCl_2$	0.278 g
8. Na_2SO_4	0.071 g
9. $(CH_2OH)_3CNH_2$	6.057 g
10. 1 kmol/ m^3 HCl	Appropriate amount for adjusting pH

The SEM images indicated the deposition of HA particles (Fig.7.6).

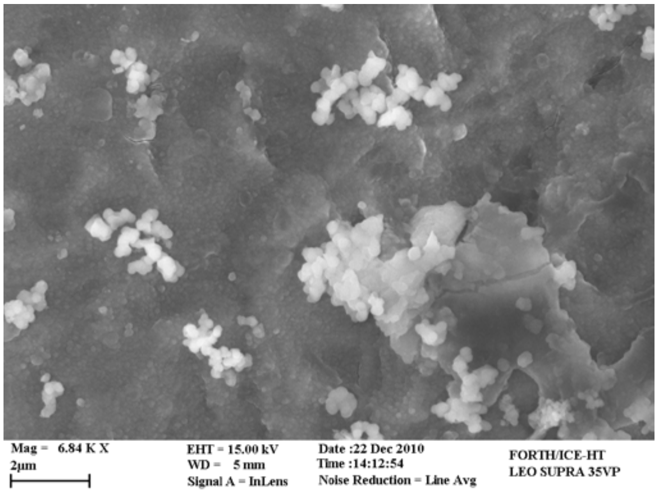


Fig.7.6 Synthetic HA particles on the TNTs layer

7.2.2 A Hybrid Nanocomposite reinforced with both TNTs and CNTs

An aqueous solution with a low concentration of multiwalled carbonanotubes (MWCNTs) was prepared through ultrasonication method. Afterwards the solution was uniformly injected on the TNTs layer surface and left for drying through evaporation of the solution for one day on a glass, under the sun, at high temperature.

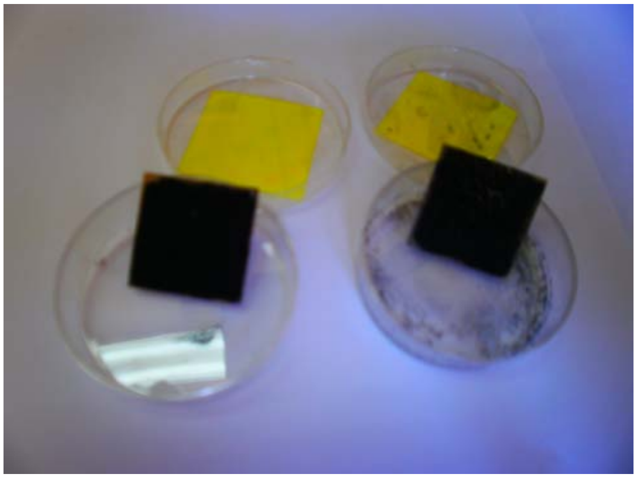


Fig.7.7 Titanium covered by layer of TNTs and reinforced with CNTs

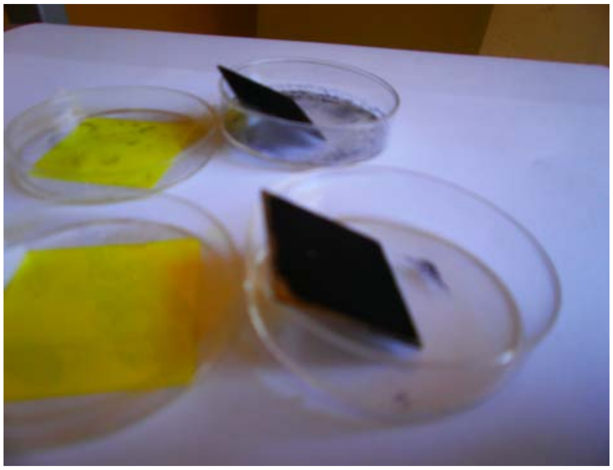


Fig.7.8 Titanium covered by layer of TNTs and reinforced with CNTs, lateral view

7.2.3 TNTs and polymer

Deposition of a very thin polymer/ resin film on the TNTs it is recommended as a simple solution which can assure a better adaptation of the cells to this layer. In biology laboratories the biocompatible polymer is most efficient material used for the proliferation of the cell cultures.

For this study, a thin polymer film (acrylic resin) resistant to aggressive environmental conditions, was sprayed on the TNTs layer and left for drying at room temperature.

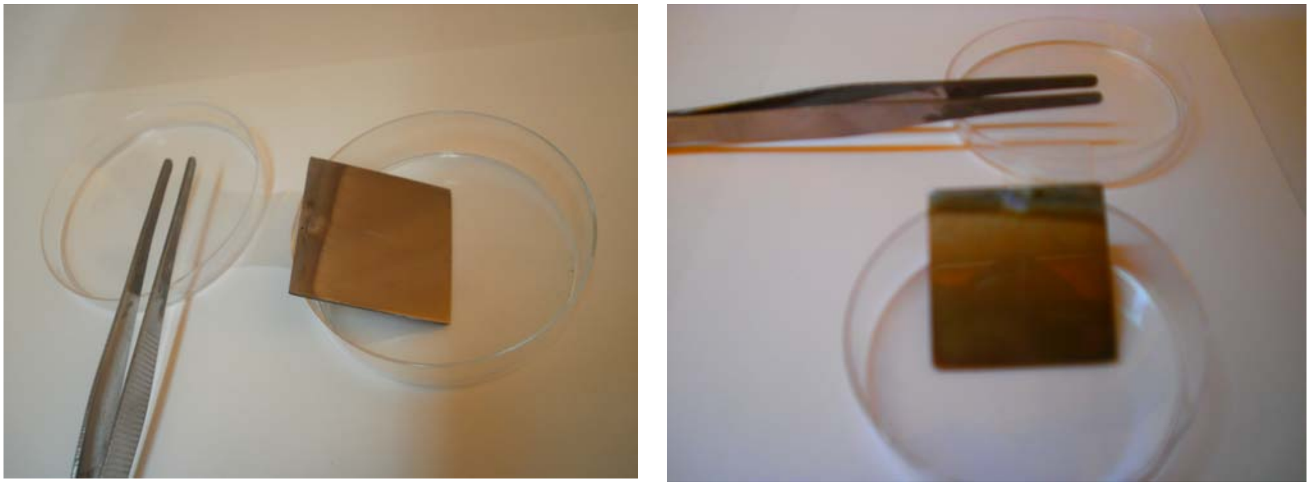


Fig.7.9 TNTs protected by a thin polymer layer

Chapter 8

Behavior of Human Bone cells on Titanium surfaces

Osteoblasts are the bone cells; this type of cells has to adhere to the implant in order to develop, until they can form the connective-tissue which together with the implant it is capable to replace the damaged part of the bone. The rapidly stabilization of the implant by creating a fast anchorage between the prosthesis and the surrounding bone tissue represent a very important aspect to be considered in any biocompatibility study. Recent studies have shown that alkaline phosphatase (orthophosphoric-monoester phosphohydrolase) specific activity is enhanced on rough pure titanium or titanium alloy surfaces.

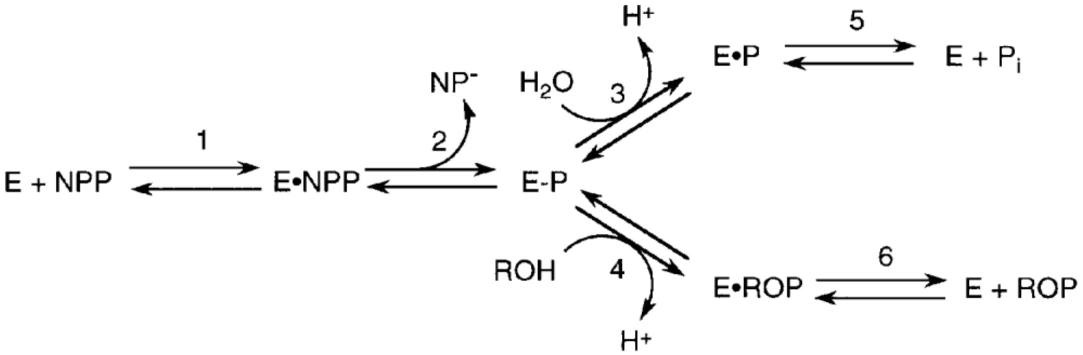


Fig.8.1 Reaction mechanism of human placental alkaline phosphatase

Cells grown on rougher surfaces exhibited increased production of collagen, prostaglandin E_2 (7-[3-hydroxy-2-(3-hydroxyoct-1-enyl)- 5-oxo-cyclopentyl] hept-5-enoic acid), and transforming growth factor β.

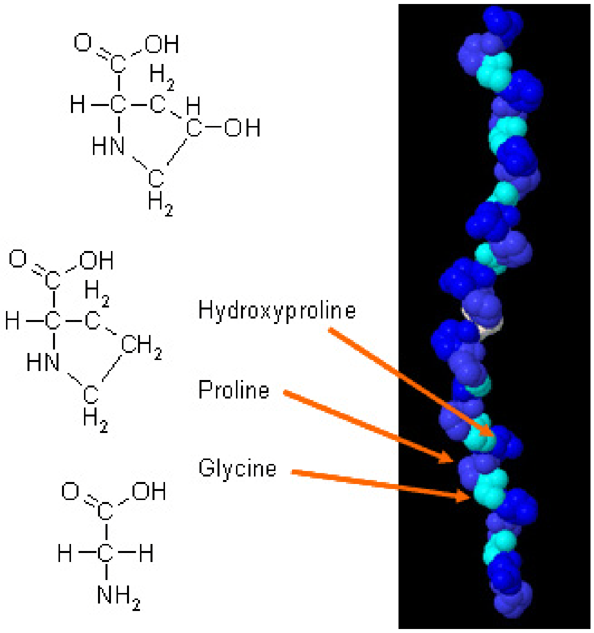


Fig.8.2 Collagen's triple helix and its structural formula

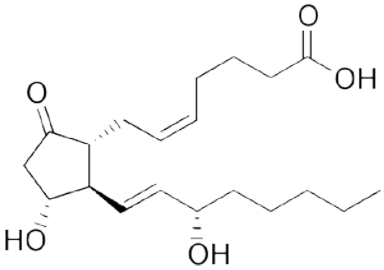


Fig.8.3 Prostaglandin E_2

Human bone marrow cells which were obtained by aspiration from the femoral diaphysis of patients undergoing total hip replacement surgery were used in this study. The patient had an age of between 40 until 60 years old and from each donor, a single-cell suspension was prepared by repeatedly aspirating the cells successively through 19 and 21-gauge needles.

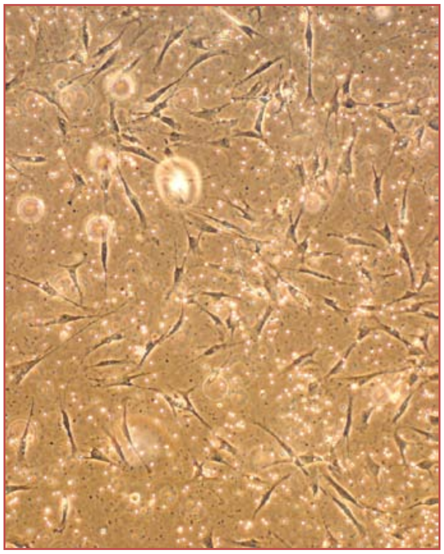


Fig.8.4 Cell culture

Cell attachment on the substrate was evaluated by analyzing the morphology of the cells attached after the incubation period of one week. The cells were seeded onto the materials and incubated. Afterwards, the specimens were washed with PBS to eliminate the unattached cells. Tissue culture polystyrene, pure titanium foils and CNTs were used as a control surface.

Tissue culture polystyrene is well known as one of the best materials for conserving cultures of cells. In the photo bellow (Fig.8.5) it can be seen how the cell expands. This is how cells should look like when they are well develop and when the material they adhere to is highly biocompatible.

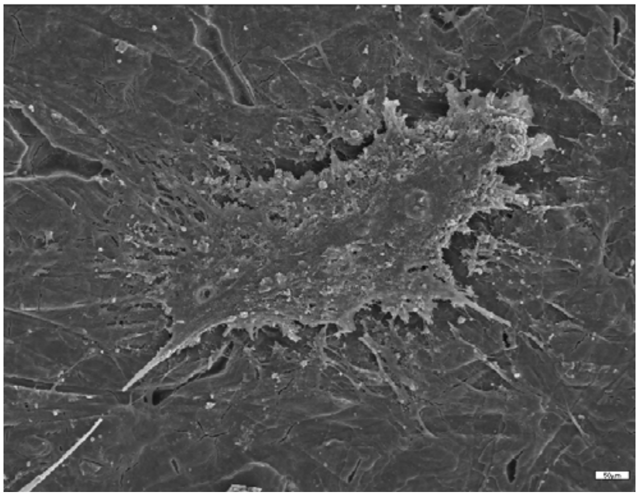


Fig.8.5 SEM image of one cell on polymer after one week incubation time

Fig.8.6 SEM image of one cell on pure titanium after one week incubation time

In Fig.8.6 it can be seen that the cell is less expanded. The titanium surface restricts partially the freedom of the cell.

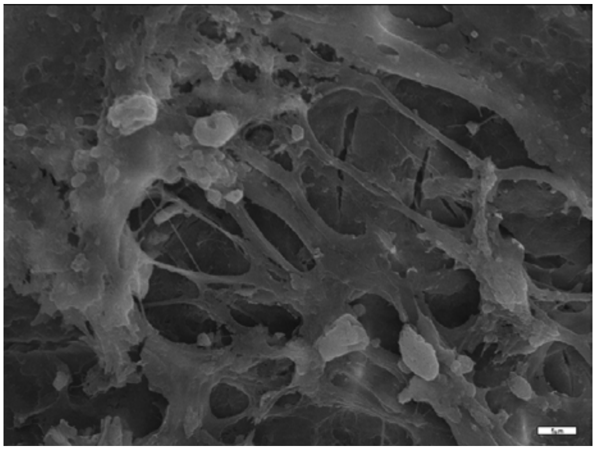


Fig.8.7 Many cells on polymer after one week incubation time

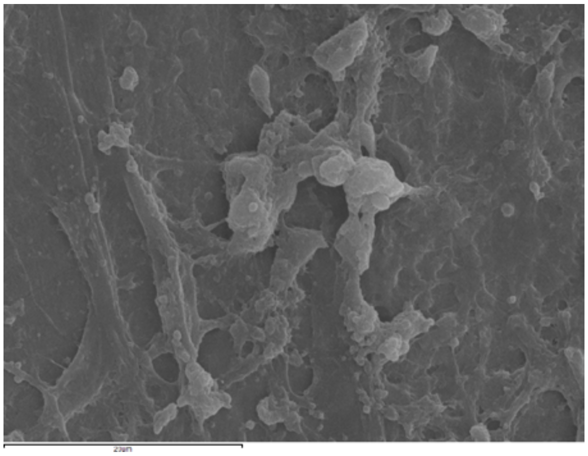


Fig.8.8 Many cells on pure titanium after one week incubation time

When the cells develop properly they start to overlap and step by step they initialize the formation of the new layer of tissue. When freedom of cells is limited this cannot happen, therefore it is affected not only the proliferation of individual cells but also the synthesis of the connecting tissue between implant and natural tissue of the body.

On the samples containing layers of TNTs there were quite big differences from one area to another of the sample. There were detected many traumatized cells, not expanded and stacked on the surface of the material (Fig.8.9). Thus, in some parts of the same sample the cells expanded better as it can be seen in Fig.8.10.

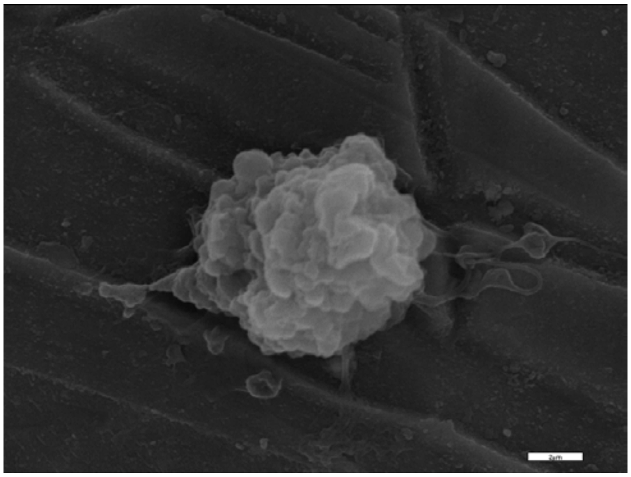


Fig.8.9 Cell expanded on TNTs,
after one week incubation time

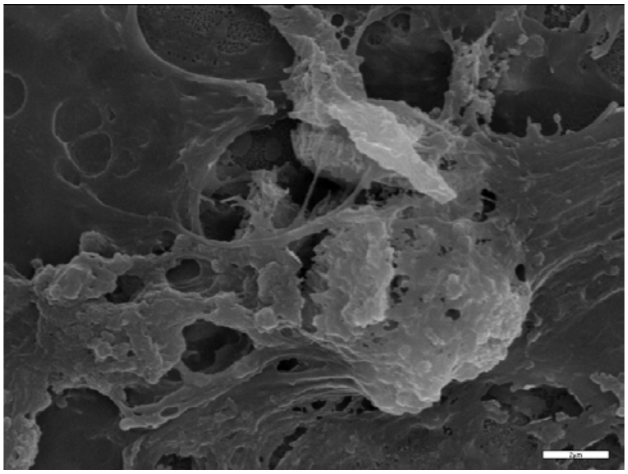


Fig.8.10 Cells expanded on TNTs,
after one week incubation time

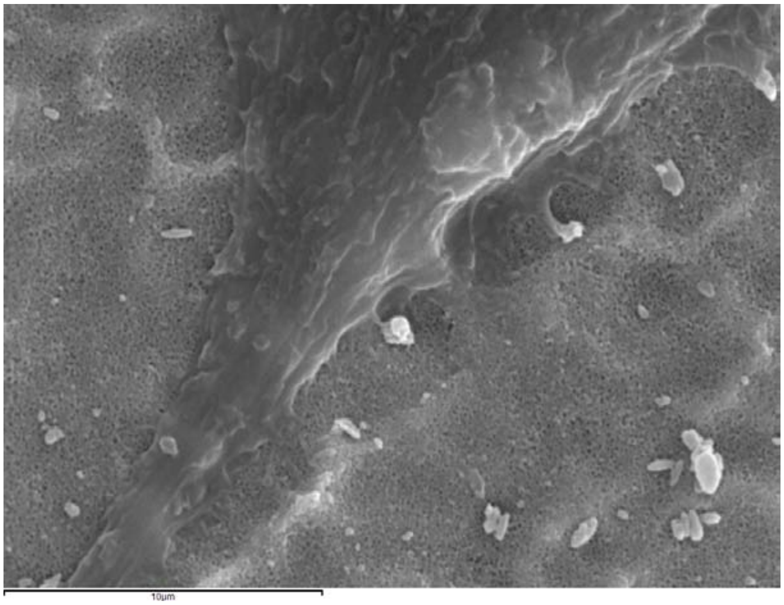


Fig.8.11 “Filopodia” of a cell on the TNTs layer after one week incubation time

It was concluded that the CNTs layers create a better environment than the TNTs, these being several times tested with human cells. In Fig.8.12 it can be observed the proper good morphology of the cells spread between the CNTs.

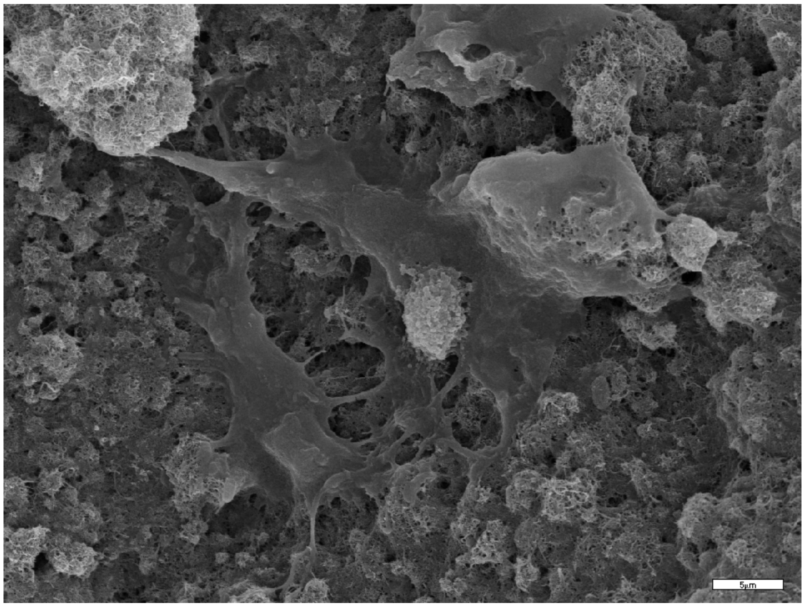


Fig.8.12 Cells on CNTs after one week incubation time
after one week incubation time

In the efforts of improving the quality of adhesion between cells and TNTs it was deposited HA on the nanostructured titanium. On the samples including HA nanoparticles the behavior of cells was different, these being in a higher number in colonies and exposing a proper morphology, compared to that of the cells spread on simple TNTs layer (Fig.8.13 a and b).

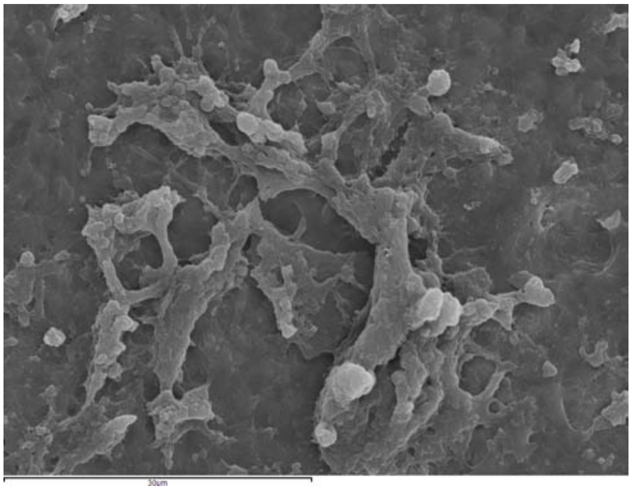


Fig.8.13 a) Cells on TNTs- HA layer
after one week incubation time

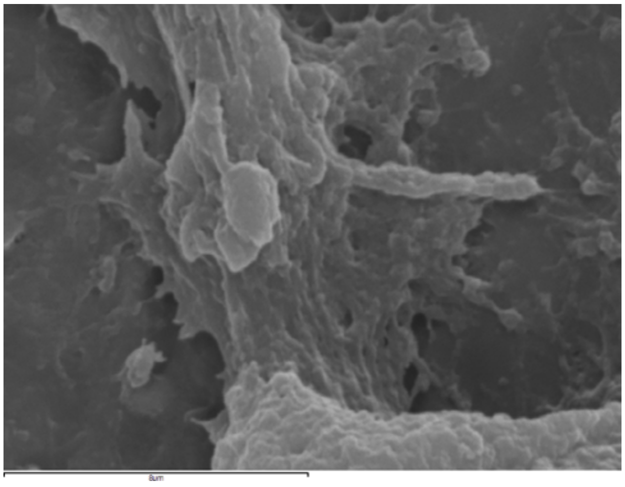


Fig.8.13 b) Cells on TNTs- HA layer
after one week incubation time

Chapter 10

General Conclusions

Titanium remains one of the performing hard tissue replacing implant materials, mainly due to its good mechanical properties; also, titanium is a promising material due to the possibilities to manufacture its surface, procedure that can transform it in a high biocompatible material. Through nanotechnology we can nanostructure titanium under different shapes.

The titanium dioxide layer naturally occurring at the surface of titanium when this comes in contact with water molecules or air it gives to the material antimicrobial and catalytic properties. Under specific conditions the titanium atoms group to form the titanium dioxide nanotubes (TNTs).

In the present PhD the anodizing method for manufacturing TNTs is presented. There were performed experiments for surface analysis of the TNTs layers; interphasial phenomena for inert TNTs layer and osteoblast cells were studies through the Viscoelastic Interphase Model and through real biological experiments. The following devices and techniques were used: Electrochemical anodizing, SEM, EDX connected to SEM, XRD, PL, IR, Image Tool, Nikon Microscopy, Interphase Modelling, Human Cells culture seeding, to bring together the following research areas:

- ✓ Chemistry and electrochemistry
- ✓ Mechanics and physics and
- ✓ Biology Sciences

It was concluded that:

- The very good combination of parameters to get well organized single wall TNTs through anodizing method is: HF 1% in glycerol - water (50:50) electrolyte solution, 8 hours anodizing and 25 V potential
- Depending on the titanium positioning in the electrochemical cell, single or multiwall TNTs layers can be elaborated
- The SEM, EDX, PL and XRD devices can offer a lot of information with respect to the structure and chemical composition of the TNTs layers
- Mechanical branch it is very important for aspects related to material - tissue adhesion and the Viscoelastic Hybrid Interphase Model (Papanicolaou Model) can predict the behavior of the osteoblasts (bone cells) in contact with titanium implant
- The biological experiments show improper morphology of the osteoblasts on TNTs layer because of their strong adhesion to this material; the environment of the cells can be improved through deposition of HA or other intermediate layers that can attenuate the strong adhesion of the tissue to the implant
- The comparative study between modeling and biological results can be the basis for creating a predictive system for any tissue-implant interaction; this can exclude failure of the implant surgery, it can reduce the medical costs and trauma of patients

Some of these results were published in the following categories of written and oral presented works:

Distinctions:

1 **Personal invitation for a plenary lecture of 25min**, Title of Presentation: "*Parameters Affecting the Structure and Geometry of TiO2 Nanotubes Produced for Advanced Biomedical Application*", on the 5th Conference for Biomaterials of the Hellenic Society for Biomaterials (HSB) together with the Scientific Societies of Knee, Hip and Reconstructive Knee, Hip Surgery of the Hellenic Society for Surgical Orthepaedics and Traumatology (EEXOT), **Thessaloniki**, 26-28 Nov., 2010.

2. **Personal invitation for a plenary lecture of 30min**, *"TiO_2 Nanotubes Produced for Advanced Biomedical and Technological Applications"*, **University Lulea-Sweden**, invited lecture of 30 min duration, Thursday 8th of December, 2010.

3. **Personal invitation for** teaching a seminar to the *"Master of Civil and Mechanical Engineering, at the University ABDELHAMID IBN BADIS DE MOSTAGANEM*, Faculty of Pure and Engineering Sciences", on Nanotechnology, **Algeria**, 1 of May – 6 of May 2011.

4. **Personal invitation for invited lecture of 45min**, *"Manufacturing - Characterization and Applications of TiO_2 Nanotubes for Advanced Biomedical Applications"*, Invited Lecture, The Hellenic Society of Biomaterials, **KAT – General Hospital of Attica**, Athens, Sat. 28 of May 2011.

5. **Personal invitation** for presenting the research done up-to now to explore the possibility of international research cooperation on TiO_2 nanotubes manufacturing and applications, **University of Latvia, Institute of Polymer Mechanics,** 19-21 May, 2011.

Invited lectures

1. **D.V. Portan and G.C.Papanicolaou**, " *Parameters Affecting the Structure and Geometry of TiO_2 Nanotubes Produced for Advanced Biomedical Application*", 5th Conference for Biomaterials of the Hellenic Society for Biomaterials (HSB) together with the Scientific Societies of Knee, Hip and Reconstructive Knee, Hip Surgery of the Hellenic Society for Surgical Orthepaedics and Traumatology (EEXOT), **Thessaloniki**, 26-28 Nov., 2010.

2. **G.C.Papanicolaou and D.V. Portan**, *"TiO_2 Nanotubes Produced for Advanced Biomedical and Technological Applications"*, **University Lulea-Sweden**, invited lecture of 30 min duration, Thursday 8th of December, 2010.

3. **K.P. Papaefthymiou, E.D. Drakopoulos, A.F. Koutsomitopoulou,**
D.V. Portan, S.P. Zaoutsos, G.C. Papanicolaou, "*Dynamic Mechanical Behavior of Epoxy Matrix-MWCNT Nanocomposites"*, Invited lecture, **DFC-11 & SI-5** Conference, **Queens' College,Cambridge**, 13-15 April 2011.

4. **G.C.Papanicolaou and Diana Portan,** *"Manufacturing - Characterization and Applications of TiO_2 Nanotubes for Advanced Biomedical Applications"*, Invited Lecture, The Hellenic Society of Biomaterials, **KAT – General Hospital of Attica**, Athens, Sat. 28 of May 2011.

5. **G.C.Papanicolaou and Diana Portan,** *"Synthesis Characterization and Biomedical Applications of TiO_2 Nanotubes"*, Invited Lecture, **The 8th International Conference on Nanosciences & Nanotechnologies – NN11,** Workshop 3: Nanomedicine, Thessaloniki, 12-15 July 2011.

Papers in Conference Proceedings

1. **Demetrescu, I., Ionita, D., Pirvu, C., Portan, D., Manole, C., Miculescu, F., Cimpan, A.,** *"Aspects of correlation between structures, properties and bioapplications of TIO2 nanotubes".* 2009 3rd ICTON Mediterranean Winter Conference, ICTON-MW 2009, art. no. 5385642

2. **I Demetrescu, D. Ionita, M. Prodana, A Mazare, D Portan, "***Aspects of biointeractive and bioactive nature of TiO_2 micro and nanostructure".*Oral presentation, **Duracosys 2010**, http://www.mech.upatras.gr/~dur2010/, 9th International Conference on Durability of Composite Systems, Patras, 12-15 September 2010.

3. **G.C.Papanicolaou, I.Demetrescu, D.Portan, K.P. Papaefthymiou,** *"Interphase Modeling of human osteoblasts spreaded on pure titanium surface covered with TiO2 nanotubes"*, oral presentation, **Duracosys 2010**, http://www.mech.upatras.gr/~dur2010/ , 9th International Conference on Durability of Composite Systems, Patras, 12-15 September 2010

4. **K.P. Papaefthymiou,, E.D.Drakopoulos, A.Koutsomitopoulou, D.V.Portan, S.P.Zaoutsos, G.C.Papanicolaou, "***Effect of Dispersion of MWCNT's on the Dynamic Mechanical Behavior of Epoxy Matrix Nanocomposites"*, oral presentation, **THERMA 2010,** http://www.mech.upatras.gr/~therma09/, 4TH National Conference on Thermal Analysis, 23-24 October, 2010, Patras, Greece.

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6. **George C. Papanicolaou, Diana Portan,** *"TiO_2 Nanotubes Produced for Advanced Biomedical and Technological Applications"*, **University Lulea-Sweden**, invited lecture of 30 min duration, Thursday 8th of December, 2010.

7. **Diana Portan, N. Bouropoulos, G.C. Papanicolaou**, *"Manufacturing and characterization of TiO2 nanotubes for solar cell and biomedical applications"*, Oral presentation, **DFC-11 & SI-5** Conference, **Queens' College,Cambridge**, 13-15 April 2011.

8. **K.P. Papaefthymiou, E.D. Drakopoulos, A.F. Koutsomitopoulou, D.V. Portan, S.P. Zaoutsos, G.C. Papanicolaou, "***Dynamic Mechanical Behavior of Epoxy Matrix-MWCNT Nanocomposites"*, Invited lecture, **DFC-11 & SI-5** Conference, **Queens' College,Cambridge**, 13-15 April 2011.

9. **G.C.Papanicolaou and Diana Portan,** *"Manufacturing - Characterization and Applications of TiO_2 Nanotubes for Advanced Biomedical Applications"*, Invited Lecture, The Hellenic Society of Biomaterials, **KAT – General Hospital of Attica**, Athens, Sat. 28 of May 2011.

10. **G.C.Papanicolaou and Diana Portan,** *"Synthesis Characterization and Biomedical Applications of TiO_2 Nanotubes"*, Invited Lecture, **The 8th International Conference on Nanosciences & Nanotechnologies – NN11,** Workshop 3: Nanomedicine, Thessaloniki, 12-15 July 2011.

11. **G.C.Papanicolaou, D.V.Portan, K.Papaefthymiou,** *"Elastic and Viscoelastic Interphase Modeling Applied to Nanocomposites"*, **ICSAAM 2011**, September 7-10, 2011, Sinaia, Romania.

12. **D.V.Portana, K.Papaefthymiou, E.Arvanita, G.C.Papanicolaou**, *"Statistical and Interphasial Investigation of TiO_2 Nanotubes Geometry Produced Under Different Anodizing Conditions"*, **ICSAAM 2011**, September 7-10, 2011, Sinaia, Romania.

13. **D. V. Portan, G. C. Papanicolaou, N. Bouropoulos, A. Kroustalli**, *"Surface Modification of TiO_2 Nanotubes Manufactured by means of Electrochemical Anodizing Method"*, **ICSAAM 2011**, September 7-10, 2011, Sinaia, Romania.

Papers in International Journals

1. **Portan D, Ionita D, Demetrescu I. "***Monitoring TiO_2 nanotubes elaboration condition, a way for obtaining various characteristics of nanostructures"*. **Key Engineering Materials** 2009; 415:9-12.

2. **Demetrescu I, Ionita D, Pirvu C, Portan D. "***Present and future trends in TiO_2 nanotubes elaboration, characterization and potential applications"*. **Molecular Crystals and Liquid Crystals** 2010; 521: 195-203.

3. **G.C. Papanicolaou, I. Demetrescu, D. V. Portan, K.P. Papaefthymiou, "***Interphase modeling of human osteoblasts spread on pure titanium surface covered with TiO_2 nanotubes",* **Composites Interfaces**, Volume 18, Number 1, pp. 23-35(13) (2011).

4. **Diana Portan, Kostas Papaefthymiou, Ioana Demetrescu, Cristian Pirvu, George Papanicolaou,** *"Manufacturing and characterization of TiO_2 nanotubes on Pure Titanium surfaces for advanced biomedical applications",* **U.P.B. Sci. Bull., Series B, Vol. 73, Iss. 2, 2011** ISSN 1454-2331, **(2011).**

5. **Daniela Ionita, Anca Mazare, Diana Portan, and Ioana Demetrescu,** *"Aspects Relating to Stability of Modified Passive Stratum on TiO2 Nanostructure",* **Met. Mater. Int.**, Vol. 17, No. 2 (2011), pp. 321~327, doi: 10.1007/s12540-011-0421-8 Published 26 April 2011.

6. **G.C. Papanicolaou, E.D. Drakopoulos, N.K. Anifantis, K.P. Papaefthymiou, D. V. Portan**, *"Experimental - Analytical and numerical investigation of interphasial stress and stress and strain fields in MWCNT polymer composites "***,** Accepted to **Journal of Applied Polymer Science,** 2011.

7. **G. C. Papanicolaou, A.G. Xepapadaki, E.D. Drakopoulos, K.P. Papaefthymiou, D. V. Portan, "***Interphasial viscoelastic behaviour of CNT reinforced nanocomposites studied by means of the concept of the hybrid viscoelastic interphase"*, accepted to **Journal of Applied Polymer Science,** 2011.

8. **G.C. Papanicolaou, K.P. Papaefthymiou, A.F. Koutsomitopoulou,D.V. Portan, S.P. Zaoutsos,** *"Effect of Dispersion of MWCNTs on the Dynamic Mechanical Behavior of Epoxy Matrix Nanocomposites"*, accepted to **Journal of Materials Science,** May 2011.

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Selective Bibliography:

[1] *Dawei Gong, Craig A. Grimes, and Oomman K. Varghese,* “Titanium oxide nanotube arrays prepared by anodic oxidation”, in RAPID COMMUNICATIONS, **vol. 16**, no. 12, Dec 2001, pp. 3331-3334.

[2] *M.C. Kuo, S.K. Yen,* “The process of electrochemical deposited hydroxyapatite coatings on biomedical titanium at room temperature”, in Materials Science and Engineering, Part C, **vol.20** , 2002, pp.153–160.

[3] *K.S. RajaT, M. Misra, K. Paramguru,* “Deposition of calcium phosphate coating on nanotubular anodized titanium”, in Materials Letters, **vol. 59,** 2005, pp. 2137–2141.

[4] *K.S. Raja , M. Misra, K. Paramguru,* “Formation of self-ordered nano-tubular structure of anodic oxide layer on Titanium”, in Electrochimica Acta **vol. 51,** April 2005, pp. 154–165.

[5] *Seunghan Oh, Chiara Daraio, Li-Han Chen, Thomas R. Pisanic, Rita R. Fin˜ ones, Sungho Jin,* “Significantly accelerated osteoblast cell growth on aligned TiO2 nanotubes”, in Journal of Biomedical Materials Research, Part A, DOI 10.1002/jbm.a, December 2005, pp. 97-103.

[6] *Logothetidis S,* “Nanotechnology in Medicine: The Medicine of Tomorrow and Nanomedicine”, in HIPPOKRATIA, **vol.10**, no. 1, 2006, pp. 7-21.

[7] *G.C. Papanicolaou , D.J. Bakos, Th.V. Kosmidou,* “Effect of the interface stiffness and skin–core adhesion efficiency on the interfacial stress distribution of sandwich structures”, in Composites: Part A, **vol. 38** , 2007, pp. 1099–1106.

[8] *J.M. Macak, H. Tsuchiya, A. Ghicov, K. Yasuda, R. Hahn, S. Bauer, P. Schmuki,* “TiO_2 nanotubes: Self-organized electrochemical formation, properties and applications”, in Current Opinion in Solid State and Materials Science, **vol. 11,** August 2007, pp. 3-18.

[9] *ZHU Zhi-ping, 2, HUANG Ke-long, ZHOU Yi,* “Preparation and characterization of new photocatalyst combined MWCNTs with TiO2 nanotubes”, in Trans. Nonferrous Met.Soc. China, **vol. 17,** Sept. 2007, pp.1117-1121.

[10] *Seong-Je Choa,, Kyu-Shik Mun, Dae-Jin Yang, Hun-Park, Yong-Joon Park, Jong-Oh Kimd and Won-Youl Choi* “Titanium oxide nanotubes anodized in aqueous and non-aqueous electrolytes”, in Journal of Ceramic Processing Research, **vol.9**, no.5, 2008, pp. 449-451.

[11] *LÉNA BRUNET, DELINA Y. LYON, ERNEST M. HOTZE, PEDRO J.J. ALVAREZ, AND MARK R. WIESNER,* “Comparative Photoactivity and Antibacterial Properties of C60 Fullerenes and Titanium Dioxide Nanoparticles”, in Environ. Sci.Technol.**, vol. 43**, March 2009, pp.4355–4360.

[12] *Clarisse CH Ng, Susan Liao, Casey K Chan,* “Drug delivery and titanium dioxide nanotubes”, in Nanomedicine, **vol. 4**, no.4, 2009, pp. 381-383.

[13] *Sergey V. Dorozhkin,* “Nanodimensional and Nanocrystalline Apatites and Other Calcium Orthophosphates in Biomedical Engineering, Biology and Medicine”, in Materials, **vol. 2**, Nov. 2009, pp. 1975-2045.

[14] *Karla S. Brammer a, Seunghan Oh d, Christine J. Cobb a, Lars M. Bjursten b, Henri van der Heyde c, Sungho Jin,* “Improved bone-forming functionality on diameter-controlled TiO_2 nanotube surface”, Acta Biomaterialia, **vol. 5**, 2009, pp. 3215–3223.

[15] *Xianhui Meng, Tae-Young Lee, Huiyu Chen, Dong-Wook Shin, Kee-Won Kwon, Sang Jik Kwon, and Ji-Beom Yoo,* “Fabrication of Free Standing Anodic Titanium Oxide Membranes with Clean Surface Using Recycling Process”, in Journal of Nanoscience and Nanotechnology, **vol. 10**, no. 7, 2010, pp. 74259–4265.

[16] *Tohru Sekino,* “Synthesis and Applications of Titanium Oxide Nanotubes”, in Inorganic and Metallic Nanotubular Materials, Topics in Applied Physics, **vol. 117**, 2010, DOI 10.1007/978-3-642-03622-4 2, pp. 17–32.

[17] *Dmitry V.Bavykin and Frank C.Walsh,* “Titanate and Titania Nanotubes Synthesis, Properties and Applications”, Ed. RSC Nanoscience & Nanotechnology, Cambridge, UK, 2010.

[18] *Tolou Shokuhfar,* “STRUCTURAL AND SURFACE PROPERTY CHARACTERIZATION OF TITANIUM DIOXIDE NANOTUBES FOR ORTHOPEDIC IMPLANTS”, Doctoral Thesis 2010, Michigan Technological University;

[19] *G. C. Papanicolaou, I. Demetrescu, D. V. Portan and K. P. Papaefthymiou,* “Interphase Modelling of Human Osteoblasts Spread on Pure Titanium Surface Covered with TiO2 Nanotubes”, in Composite Interfaces, **vol. 18** 2011, pp. 23–35.