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TITANIUM FUNCTIONALIZING AND DERIVATIZING FOR IMPLANTABLE MATERIALS OSSEOINTEGRATION PROPERTIES ENHANCING

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The article focuses on titanium functionalizing and derivatizing reactions for implantable materials osseointegration properties enhancing. Thus, the titanium dioxide was activated to the titanium hydroxide (functionalization), being further immobilized on the titanium surface by ethanolamine covalent reactions and (3-aminopropyl)triethoxysilane (APTS) for the osseointegration membranes reactive coating. The derivatizing was based on ether forming reactions between hydroxyl group from the titanium hydroxide surface and the ethanolamine hydroxyl groups and hydrolyzed APTS respectively. The obtained materials were characterized by scanning electron microscopy, FT-IR infrared spectroscopy, contact angle and X-Ray fluorescence.

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1. Introduction

Bone defects surgical reconstruction is one of the major concerns in biomedical research field. One of the latest large-scale used technics is the animal origin bone addition materials use [1, 2]. The method has advantages in the case of small bone defects especially for spongy bones. In the case of cortical bones or major defects the use of other materials such as titanium and titanium alloys is preferred [3, 4]. These have very good mechanical resistance and an inert chemical character that often prevents further corrosive reactions, secondary products forming in the body or rejection reactions. On the other hand a very big issue in the use of titanium-based implants is the osseointegration process. The oral implantology proves nowadays, by clinical studies and laboratory biochemical methods, the implants surface treatments importance in order to obtain a better biointegration [5].

The dental implants unlike the rest of the implants, supports the biggest mechanical loads per unit area. Thus, their integration must be optimum in a very short time especially in the immediate loading implants case. From the bone integration point of view the latest research unanimously conclude that the implants surface treatments have the biggest potential in the oral implantology [6,7].

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Along with the various methods of increasing the hydrophilicity and of the hydroxyapatite deposition a recent trend is the use of polylactic acid polymer membranes [8] or collagen [9]. For an effective coverage, ideally is to chemically bond the osseointegration membrane to the metal surface, but the chemical inertness of titanium makes it difficult. Literature is relatively limited in presenting functionalization ways of titanium, because of the difficult conditions of this metal chemical processing.

In the case of titanium activation the surface molecular structure modifying method is based on a strongly acidic medium [10] or strong base medium [11] treatment. Basic catalyzed reaction in alcoholic medium offers better efficiency, due to alkoxides intermediaries involved in the synthesis, that present a higher reactivity in acidic medium. Previous studies of APTS immobilization on titanium alloy have been made but with more complicated procedures. Thus the APTS was immobilized on a TiAl6V4 alloy by plasma treatment, the hydroxyl group required for the immobilization being generated during the surface treatment [12]. Other study involves electrochemical attack, annealing stages at 450 or 700 °C and a reaction for 8 hours, in toluene at 70°C [13].

This paper presents new titanium functionalization reactions (activation from titanium dioxide to titanium hydroxide) and derivatization of titanium hydroxide with ethanolamine and (3-aminopropyl)triethoxysilane for subsequent reactive imobilization of collagen membranes for the osseointegration process.

2. Materials and Methods

Square plates samples with the sides of 1cm and thickness of 1mm of CP titanium were used. The elemental analysis was made by Spark Discharge in Argon using a Foundry Master spectrometer the chemical composition being presented in table 1.

Table 1. The average mass concentrations, STDEV and RSD of the main elements of the titanium support

Element	Ti %	Fe %	Al %	V %	C %	Cr %	Sn %	Mn %	Ni %
% mass	99.4	0.154	0.0500	0.038	0.024	0.0180	0.278	0.0160	0.023
STDEV[1]	0.043	0.046	0.0042	0.011	0.014	0.0008	0.040	0.0018	0.0038
RSD[2](%)	0.043	29.68	8.484	28.01	59.64	4.198	14.447	11.100	16.421

1-STDEV-Standard Deviation; 2-RSD-Relative Standard Deviation

2.1. TiO_2 activation

For the functionalization, the titanium plates were washed firstly with hydrochloric acid, nitric acid and deionized water. To increase the content of hydroxyl groups, the titanium was treated with a potassium hydroxide and isopropyl alcohol solution by immersing the 10 plates in 50mL of alcohol in which was dissolved 5g of the hydroxide for 24 hours at 40°C. After the reaction completion the plates were washed and maintained in deionized water to remove the hydroxide traces [11].

2.2. Ethanolamine functionalization

For the TiOH derivatization with ethanol amine three plates were immersed in 20 mL of isopropyl alcohol in which were dissolved 0.5 g of potassium hydroxide and 2 mL ethanolamine. The reaction was maintained at 70°C for 4 hours. After the reaction completion the plates were washed with deionized water. The chemical reaction equation is shown in Figure 1.

Derivatization with APTS took place under the same conditions as for ethanolamine, but using a volume of 1.5 mL APTS ($6.41x10^{-3}$ moles) and 1 mL of water, the water having the essential role of hydrolyzing the alkoxysilane able to be condensed further (in strongly basic catalysis) on the TiOH surface. This reaction sequence is important in binding the hydroxyl groups from the metal surface, through Si-O-Ti bridges.

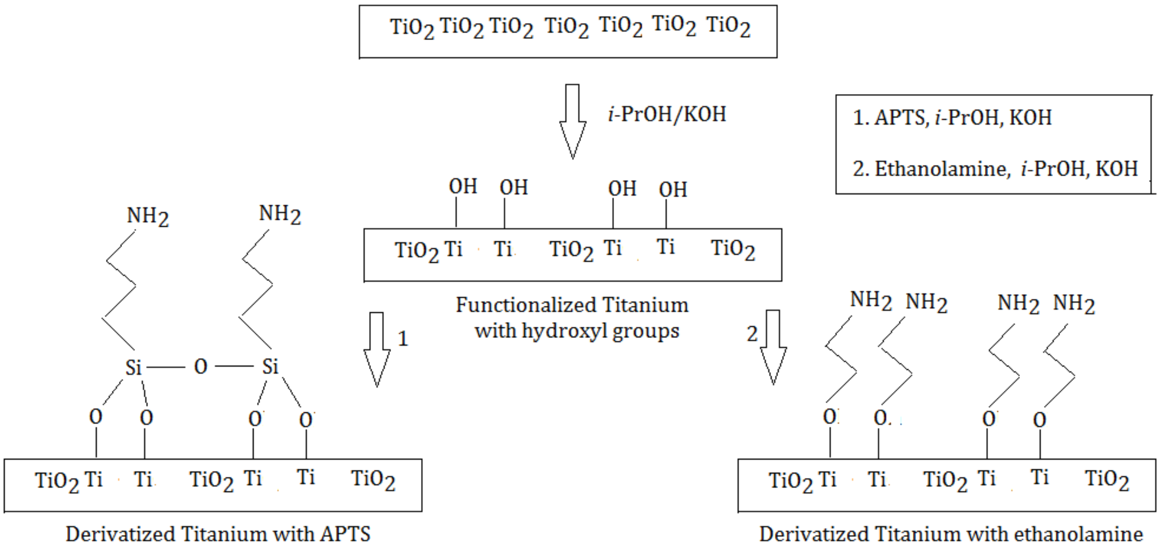


Fig. 1 Idealized reaction schemes for titanium functionalization and derivatization

The obtained materials were analyzed by scanning electron microscopy using a FEI VX 35 microscope equipped with EDAX. FT-IR spectra were recorded on a Jasco FTIR 6300 spectrometer equipped with a device for measuring the specular reflectance at an angle of 30° from Pike Technol. Inc. The spectras were recorded after 30 accumulations at a resolution of 4 cm^{-1} using a Perkin Elmer FT-IR spectrometer and by X-ray fluorescence spectrometry using a PW4025 MiniPal2 (Panalytical) X-ray energy dispersive spectrometer operated at 20 kV and automated amperage. Measurements were recorded for 300 seconds in the He atmosphere. The results were processed using a data analysis software (Origin 8.0 Pro).

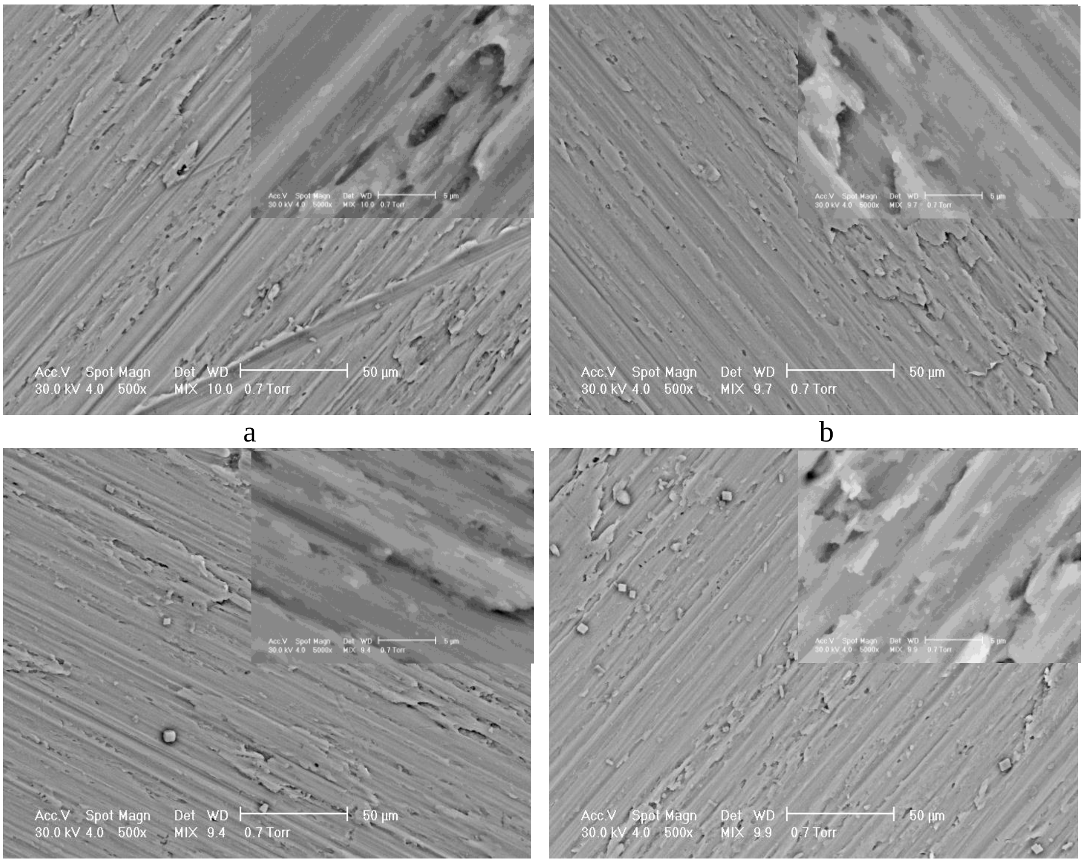
Contact angle measurements were performed at room temperature using a Contact Angle Tensiometer CAM 200, KSV Instruments system type. Measurements were made using the static system on 1cm x1cm specimens (6 µL drop, ±2°, CA error ± 2°).

3. Results and discussions

The main novelty of the present investigation is the presentation for both the functionalization method and the derivatization of titanium, a metal that is substantially chemically inert. The functionalized, as a concept in organic chemistry means to introduce a functional group in a specific chemical species or material. Derivatization referres to the subsequent reaction between the introduced functional group and another functional group of another compound to be added in order to imobilize it to introduce the original material a specific property or as additional spacing arm for a subsequent derivatization. In this case, the role of ethanolamine and APTS is to allow subsequent immobilization of the collagen as a osseointegration membrane by free amino groups. Unlike conventional layering, that does not involve any chemical interaction between the metal and polymer (rather than physical process of adhesion), the molecular interaction between the metal and polymer implantable material should provide additional stability and strength during the bone mounting.

Alloy surface hydroxy groups derivatization has been a particular challenge in this case, since this group is reactive with both the hydroxyl group and the amino group of ethanolamine and the hydrolysed APTS. In fact, regardless of the reaction conditions, a mixture of the two compounds is obtained, but the working parameters may determine the reaction that will predominate. For an increased reactivity of the amino group and to drive the reaction between the hydroxyl and amino groups, the catalyst must be weakly basic, at 80-100^{o}C. The use of strong base catalyst, the reaction results in the formation of ether type derivatives, the amino group exhibiting a low reactivity [11]. In the same time, strong basic catalyze causes EO hydrolysis of APTS, as an intermediate step of the reaction.

Scanning electron microscopy (Fig. 2) revealed no significant changes between the four types of materials, no visible changes being noticed in the EDAX analysis. The channels presented in the metallographic microscopic analysis resulted from the titanium processing, differences between the synthesized materials being unnoticed. The lack of changes in the morphology does not exclude modifications at the molecular level, these being revealed as a first method of analysis by FT-IR and further directly confirmed by fluorescence analyses, and respectively indirectly by contact angle analyses.



a b

c d

Fig. 2 Scanning electron microscopy images an Ti a), TiOH b), APTS derivatized TiOH c) and ethanolamine derivatized TiOH d).

The titanium dioxide presence at the samples surface is evidenced by the FTIR spectrum characteristic bands presence, as it can be seen in the Figure 3.

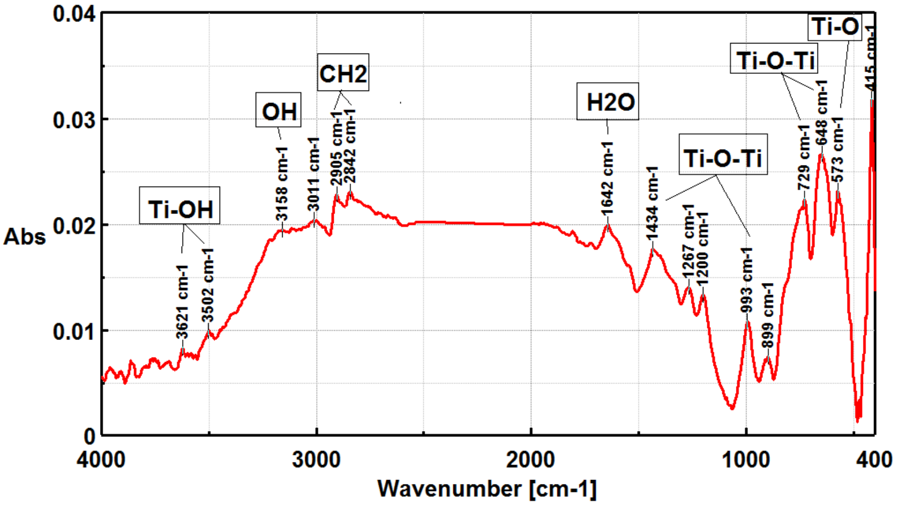


Fig. 3 Alcalyne medium treated titanium plates FTIR spectrum

TiOH spectrum reveals the bands at 3621 and 3602 cm^{-1}, of medium intensity, specific to the non-associated by hydrogen bonds hydroxyl groups while at 3158 cm^{-1} a broad band is found due to the hydrogen bond associations of these groups. The intensity of the two bands gives us information on the group's existence, but also shows that they do not have a very wide distribution on the material surface. This conclusion can be drawn by studying the intensity of the bands located at 2842, 2905 cm^{-1} respectively corresponding to the symmetric and asymmetric stretching vibration of CH$_2$ groups remaining from the isopropyl alcohol. Certainty on the reaction completion comes also from the 648, 729, 993 or 1434 cm^{-1} bands that are specific to the Ti-O-Ti bonds type, created by condensation in strongly basic catalyst from Ti-OH groups associations [15].

Titanium plates with APTS immobilized on the surface FT-IR spectrum reveals also the band at 1602 cm^{-1} that corresponds to the plane deforming vibrations of the primary amino group, a medium intensity band, which demonstrates the existence of large amounts of free NH$_2$ groups, as can be seen from Fig. 4.

Also, the band at 955 cm^{-1} specific to the Si -O-Ti bonds, demonstrates the APTS immobilization through hydroxyl groups generated by APTS hydrolysis. The band at 1166cm^{-1}, specific to Si-O-Si [16], shows the binding between several APTS molecules via ether bridges, giving extra stability to the material and to the immobilization process.

The presence of the sharp bands at 3797, 3722 cm^{-1} is characteristic to the free silanol groups generated from organosilane compounds and found at the samples surface, while the broadband centered at 3297cm^{-1} can be attributed to OH and NH$_2$ groups involved in hydrogen bonds. The symmetric and asymmetric stretching vibrations characteristic bands of the methylene groups from the APTS, located at 2911, 2844 cm^{-1}, respectively can also be found in the spectrum.

In addition, one can find the bonds corresponding to the C-N stretching vibration located at 1287 cm^{-1} and the one corresponding to the amino group deformation vibration overlapped with that of Ti-O-Ti bond at 719 cm^{-1}. The existence of titanium dioxide layer on the titanium plates surface subjected to functionalization is evidenced by the presence of 671 cm^{-1} bands in the spectrum due to the Ti-O-Ti bond, while at 575 cm^{-1} a very extended band that can be attributed to the Ti-O bond is found [17].

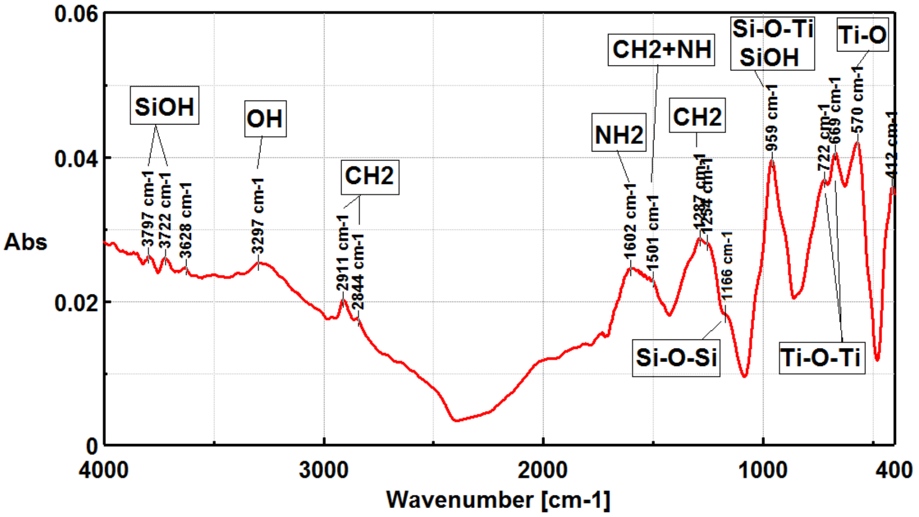


Fig. 4 Titanium plates with APTS surface imobized FTIR spectrum

The FTIR spectra of the samples obtained by monoethanolamine surface modifying reveals the amino group specific bands, namely the stretching vibration from 3483 cm^{-1} and from 3175 cm^{-1} due to the hydrogen bonds involvement. Asymmetric and symmetric stretching vibrations of methylene groups can be found in the spectrum at 2908, 2837 cm^{-1}, respectively, while at 1400 cm^{-1} the methylene group deformation vibration is found.

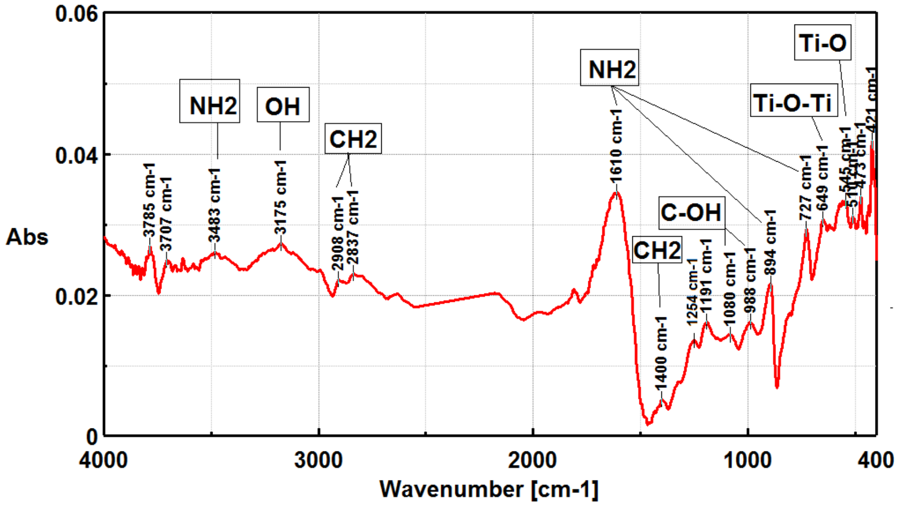


Fig. 5 The FTIR spectra of the Ti samples with monoethanolamine on the surface

In the ethanolamine functionalized material spectrum, the band found at 1610 cm^{-1} is attributed to the amino group. Its much higher intensity as compared to the bands of 3175 cm^{-1}, specific to the hydroxyl group, suggests the bonding also with the ethanolamine by hydroxyl group an not by the amino group.

One can also find in the spectrum: the primary amine group located as a doublet at 894 or 727 cm^{-1} deformation vibration, C-OH bond located at 1080, 988 cm^{-1} stretching vibration and the bands corresponding to the presence of Ti-O and Ti-O-Ti located at 649 or 545 cm^{-1}.

The fluorescence radiation, specific to each chemical element is directly proportional to the concentration of that element in the material under investigation [18].

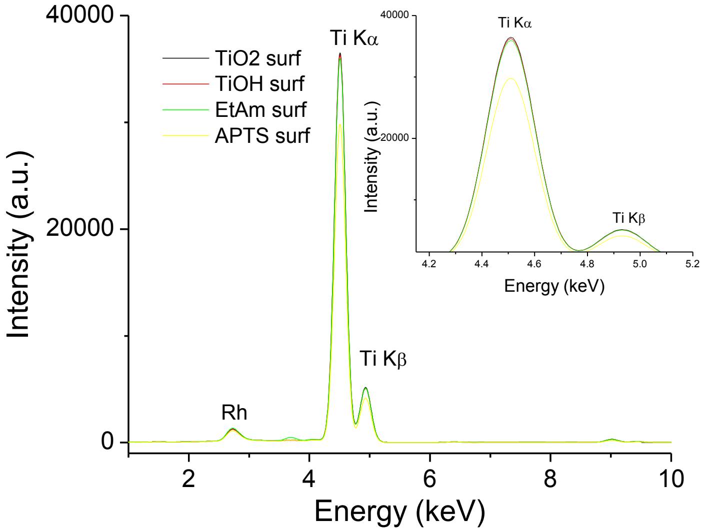


Fig. 6. EDXRF spectra of the witness sample ant of the three functionalized and derivatized samples. Inset - Titanium characteristic lines (Kα and Kβ)

In our case, the titanium coating can be analyzed by X-ray fluorescence, by the titanium characteristic fluorescence radiation intensity variation in the sample spectrum (Figure 6, inset presents the two characteristic lines of titanium - Kα and Kβ). Decreased intensity of titanium Kα line (fig. 7) can be correlated with the appearance of the support material surface coating (titanium plate) that attenuates the titanium characteristic fluorescence radiation. This decrease is directly proportional with the coating thickness (ie molecular weight of coating material).

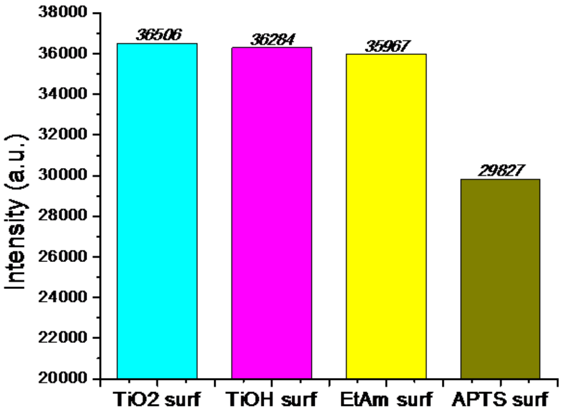


Fig. 7 Titanium characteristic fluorescence radiation intensity variation (Kα line)

Thus, at the appearance of hydroxyl groups on the support material surface a decrease in the titanium fluorescence radiation intensity of approx. 0.61% is observed, while at the derivatization of titanium hydroxide with ethanolamine and amino propyl triethoxy silane, the decrease is more significant (approx. 1.48% and approx. 18.30% respectively). The X-ray fluorescence, by monitoring the decrease in titanium specific fluorescence radiation intensity, directly confirms the changes made.

Analyzes of surfaces by contact angle before and after chemical modification with different grafting agents, sustains a drastic polarity change after these processes (Figure 8).

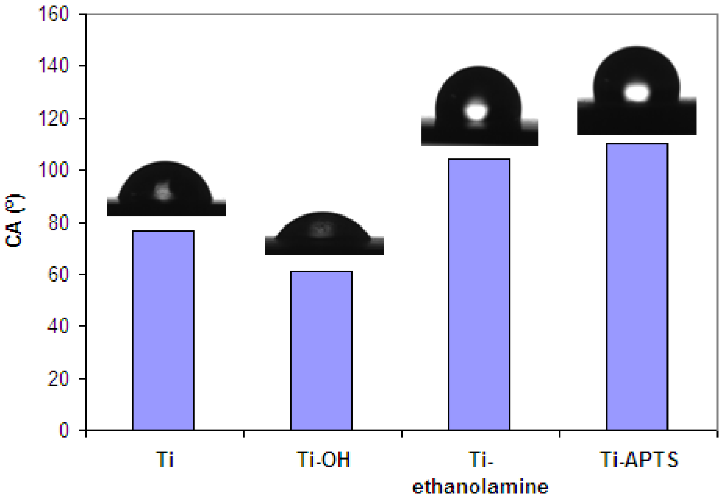


Fig. 8 Water contact angle evolution as consequence of chemical modifying nature.

Hydroxyl groups functionalized has as result the decrease of the water contact angle with arround 30%. This phenomenon is particularly important both in terms of surfaces use for subsequent graft of functional biocompatible membranes and in terms of using it as a potential implant by itself. Hydroxyl units increase can bring benefits both to the physical relationship of biological fluids adsorption involved in biointegration processes and also from the chemically point of view related to the condensing centers establishment necessary to the hydroxyapatite so as to the collagen fibers. Chemical binding of ethanolamine units is supported by a large increase in the surface hydrophobicity at 104° (Figure 8). The phenomenon is even more pronounced when treating with APTS. The coupling agent efficiently occupies by condensation the initially generated hydroxyl groups on the surface, exposing the amino groups outward.

The data obtained by water contact angle analysis along with FTIR and X-ray fluorescence analyses sustain the proposed reaction mechanisms and create the conditions for a better chemical integration of biological molecules.

4. Conclusions

The article presented a new method of titanium functionalization and derivatization in order to improve its chemical properties for reactive membrane coating binding in order to improve the osseointegration process of the titanium based implantable materials. The method consists of the activation of titanium from titanium dioxide to the titanium hydroxide in base catalyst, followed by ethanolamine or APTS covalent attachment via the hydroxyl groups with ether links forming.

The synthesis was demonstrated by several direct and indirect analysis methods. FTIR spectra confirmed the presence at the metallic plate surface of the functionalization compounds or the free primary amine functional groups when using as the functionalizing agents of monoethanolamine and APTS. It was shown the existence on the titanium plate's surface of free Ti-OH groups after the treatment in alkaline medium. The interactions between the support and functionalization reagents are made mainly through hydroxyl groups (generated by the alkoxysilane hydrolysis or monoethanolamine existing) and Ti-OH from the oxide layer surface.

The surface properties modification has been demonstrated directly by contact angle analysis. The most pronounced hydrophilic character was evident after treatment in strong alkaline medium.

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