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# **Tantalum oxide / carbon nanotubes composite coatings on titanium, and its functionalization with organophosphonic molecular films: a high quality scaffold for hydroxyapatite growth**

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## **Abstract**

Nowadays, titanium is a very commonly used biomaterial for the preparation of orthopedic and dental implants. Its excellent mechanical and biochemical bulk properties are nevertheless counterbalanced by its propensity to long term degradation in physiological conditions and its weak osseointegrative capacities. In this context, surface modifications can significantly hinder titanium weaknesses.

The approach considered in this work relies on the preparation of thin composite coatings based on tantalum oxide and carbon nanotubes by sol-gel process. Tantalum is particularly interesting for its high biocompatibility and bioactivity, as well as its strong resistance to bio-corrosion. Carbon nanotubes are exploited to reinforce the compactness and homogeneity of the coatings, and can act as a favorable factor to strengthen the interaction with bone components by biomimicry. The composite layers are further modified with specific organophosphonic acid molecular films, able to chemically bind the tantalum oxide surface and improve the hydroxyapatite formation process.

The characteristics and the quality of these hybrid inorganic/organic coatings are evaluated by XPS, SEM, TEM, peeling tests, contact angle measurements, and electrochemical characterizations (free potential, polarization curves).

### **Key words**

Titanium-based biomaterials; Tantalum oxide; Carbon nanotubes; Sol-gel co-deposition; Amino-tris-methylene phosphonic acid; Hydroxyapatite.

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# **Tantalum oxide / carbon nanotubes composite coatings on titanium, and its functionalization with organophosphonic molecular films: a high quality scaffold for hydroxyapatite growth**

## **1. Introduction**

Biological and medical orthopedic sciences constantly deal with new issues and problems resulting of the growing ageing of population. Numbers of scientists, practitioners and surgeons daily face problems of osseous structures wearing, breaking and loosening, for which metallic implants are increasingly recommended to ameliorate the longevity and the global quality of life of human beings [1,2]. Elaboration of high-performance biomaterials is thus of crucial importance, as implanted substrates have to present matching mechanical properties as well as high biocompatibility. Bioactivity also constitutes a key factor: orthopedic implants usually have specific intrinsic capacities for inducing and conducting the bone growth process and the osseointegration [1,3,4].

In this context, titanium-based materials constitute a very interesting and useful platform in dental and osseous biomedical applications and devices. Ti and its alloys combine numerous favorable properties which make them almost ideal and widely used biomaterials: low Young modulus and density, high solidity and fatigue strength, complete inertness to human body, reinforced biocompatibility and corrosion resistance due to the spontaneously-formed surface oxide layer [1-8]. However, toxicity of alloying elements (Ni in Nitinol, V in Ti64, ...), long-term degradation under physiological conditions and weak osseointegrative properties remain problematic features [9-14].

A general solving approach should thus consist in retaining volume physical characteristics of Ti substrates while improving and optimizing their surface properties. This can be achieved by the formation of a tantalum coating on the titanium surface. Ta, with its very passivating oxide layer, has proved to be excellently resistant to corrosion and highly biocompatible as well as bioactive, with increased capacities of osseous growth inducement and guidance [15-18]. Its radio-opacity is also particularly appreciated in medical imaging techniques [19]. Nevertheless, the high price of tantalum and its important density restrict its use as a bulk material, so that the deposition of a thin tantalum oxide layer on Ti has to be specifically considered here. Different methodologies can be used to prepare these Ta deposits: electrodeposition [20-22], radio frequency sputtering [23], liquid phase deposition [24], electrostatic spray deposition [25] and sol-gel method [26,27]. This last one has been selected in this work for its practical easiness and its use of non-expensive materials.

Some recent studies also reveal the significant role potentially played by carbon nanotubes (CNTs) in the framework of composite biomaterials. In addition to improve the global mechanical properties of the implant, CNTs are able to specifically interact with osteoblasts and osteoclasts (cellular components of the bone), respectively responsible for bone deposition and dissolution [28-30]. Carbon nanotubes can also promote the bone regeneration process by mimicking the structure of collagen fibers (organic part of the bone matrix) and favoring the formation on the surface of a stronger and tougher hydroxyapatite layer (inorganic part of the bone matrix) [30,31]. Formation of Ta<sub>2</sub>O<sub>5</sub>/CNTs composite coatings on Ti substrates has thus the potential to constitute highly bioactive interfaces that can improve the implant integration ability in the bone [32].

Hydroxyapatite formation is also favored by the presence of appropriate chemical groups on metallic implant surfaces [11,21,27,33-36]. In this way, Ti/Ta<sub>2</sub>O<sub>5</sub>+CNTs hierarchical systems can be further optimized with the grafting of organophosphonic RPO<sub>3</sub>H<sub>2</sub> molecular films on tantalum oxide layers. In particular, utilization of multifunctional phosphonic acids is of particular interest, as some PO<sub>3</sub>H<sub>2</sub> functions can be used as strong anchoring feet with the metallic oxide surface while others, acting as terminal groups, directly favor the HAp growth at the interface with body environment.

This paper reports the formation of tantalum oxide and multiwalled carbon nanotubes composite coatings on titanium substrates via a sol-gel co-deposition process. Those systems are further functionalized with organophosphonic acid molecules. *In vitro* hydroxyapatite growth tests are finally achieved to assess the global impact of the different layers on the system's bioactivity.

## 2. Materials and methods

### 2.1. Chemicals

The following chemicals and solvents are used without further purification for the carbon nanotubes oxidation and sol-gel deposition processes: potassium permanganate (Merck), sulfuric acid (Chem-Lab NV, 95-97%), tantalum butoxide (Aldrich, 98%), hydrochloric acid (Chem-Lab NV, 37%), acetic acid (Aldrich, 99.7%) and absolute ethanol (VWR Prolabo). Surface molecular modification is performed in ultra-pure water (18.2 M $\Omega$  cm) with amino-tris-methylene phosphonic acid, supplied by Thermphos. Simulating body fluid (SBF) is prepared in ultra-pure water with the following salts: sodium chloride (Acros, 99.5%), potassium chloride (Aldrich,  $\geq$ 99.0%), magnesium hexahydrate chloride (Acros, 99%), calcium dihydrate chloride (Merck, 99.5%), sodium hydrogenocarbonate (Acros, 99+%), potassium hydrogenophosphate (Acros, 99+%), sodium sulfate (Janssen Chimica, 99%) and tris(hydroxymethyl)aminomethane (Acros, 99.8%).

### 2.2. Titanium substrate preparation

Square-shaped (10 mm x 10 mm) 1 mm thick titanium plates are used as substrates (Advent, 99.98%). Coupons are first mechanically polished on a Buehler-Phoenix 4000 instrument using a silicon carbide paper (P320) followed by a 9  $\mu$ m diamond paste (Struers). They are then etched down to 0.02  $\mu$ m using a 4/1 v/v mixture of colloidal silica polishing suspension (Buehler) and hydrogen peroxide (Fluka, 30%). Finally, Ti samples are ultrasonically cleaned through two consecutive steps in absolute ethanol, then in acetone (15 min each), and treated with UV-ozone for 15 min (Jelight 42-220) in

order to remove all physisorbed contaminations prior to modification. A native oxide layer is formed during this process.

### **2.3. Multiwalled carbon nanotubes (MWCNTs) pre-treatment**

0.5 g of MWNT 7000 carbon nanotubes (Nanocyl) are submitted to a specific oxidization treatment (see Table 1 and section 3.1.). The samples are then washed with distilled water until a pH of 5 is obtained. A lyophilisation step finally completes the pre-treatment.

### **2.4. Sol-gel deposition of tantalum oxide / MWCNTs composites (“classical procedure”)**

Sol-gel solution contains 4.0 mL of absolute ethanol, 0.2 mL of acid catalyst (HCl or CH<sub>3</sub>COOH) and 8.0 mg of oxidized MWCNTs. Carbon nanotubes are dispersed ultrasonically during 30 minutes, then 1.0 mL of Ta(OBu)<sub>5</sub> is added dropwise. The solution is kept under stirring for 2 h before use for the modification of mechanically and chemically polished titanium samples. After an immersion step of 10 min in the sol-gel solution, these are further hydrolysed during 10 other min in distilled water and blown dry in a nitrogen stream [32].

### **2.5. Functionalization of tantalum oxide / MWCNTs composites using organophosphonic molecular films**

Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composites elaborated by sol-gel co-deposition are immersed at ambient temperature (25°C) in a 10<sup>-3</sup> M aqueous solution of amino-tris-methylene phosphonic acid N(CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>)<sub>3</sub> (Fig. 1) for 1 h at pH ~ 1. Samples are then washed with ultra-pure water and dried under nitrogen.

## 2.6. Nucleation of hydroxyapatite

The hydroxyapatite formation on functionalized samples is achieved by their immersion in 30 mL of a simulated body fluid (SBF) for 7 days at 37°C. SBF solutions are prepared through the dissolution of NaCl, KCl, MgCl<sub>2</sub>.6H<sub>2</sub>O, CaCl<sub>2</sub>.2H<sub>2</sub>O, NaHCO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>.3H<sub>2</sub>O and Na<sub>2</sub>SO<sub>4</sub> in ultra-pure water, with the following ionic concentrations (in mM): Na<sup>+</sup> 142.0, K<sup>+</sup> 5.0, Mg<sup>2+</sup> 1.5, Ca<sup>2+</sup> 2.5, Cl<sup>-</sup> 148.0, HCO<sub>3</sub><sup>-</sup> 4.2, HPO<sub>4</sub><sup>2-</sup> 1.0, and SO<sub>4</sub><sup>2-</sup> 0.5. They are then buffered at pH 7.25 with tris(hydroxymethyl)aminomethane and 37% hydrochloric acid.

## 2.7. Characterization

### 2.7.1. XPS characterization

X-ray photoelectron spectroscopy (XPS) is used to evaluate the elemental composition of the bare and modified samples. Spectra are collected on a Surface Science SSX-100 spectrometer. The photoelectrons are excited using a monochromatized Al K $\alpha$  radiation as the excitation source, collected at 35° from the surface normal and detected with a hemispherical analyzer. The spot size of the XPS source on the sample is about 600  $\mu$ m, and the analyzer is operated with a pass energy of 20 eV. Pressure is kept below ( $1 \times 10^{-9}$  Torr) during data acquisition, and the binding energies of the obtained peaks is made with reference to the binding energy of the C1s line set at 285.0 eV, characteristic of the aliphatic carbons. Spectra are fitted using a linear combination of Gaussian and Lorentzian profiles, with a Gaussian character let free to vary between 60 and 100%. Peak positions obtained after analysis are found essentially constant ( $\pm 0.3$  eV). The different relative peak areas are finally measured. Sensitivity factor values used for the

different atoms are: C1s 1.00, O1s 2.49, Na1s 0.22, P2p 1.29, Cl2p 2.40, K2p 3.95, Ca2p 4.88, Ti2p 7.11, Ta4p<sub>1/2</sub> 1.86, Ta4p<sub>3/2</sub> 4.66, and Ta4f 9.88.

### **2.7.2. Scanning and transmission electron microscopies imaging**

A scanning electron microscopy (SEM) study is performed on a JEOL microscope 7500F to evaluate the morphological and structural characteristics of the Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings as well as the hydroxyapatite crystals. Carbon nanotubes are also characterized by transmission electron microscopy (TEM) with a Phillips Tecnai 10 instrument.

### **2.7.3. Peeling tests procedure**

Peeling tests for adhesion evaluation of the tantalum coatings are carried out according to the ASTM D3359 protocol. Modified samples are scratched with a metallic comb with spacing streak of 1 mm (Electrometer 1542, ASTM D3359). A scotch tape is then stuck on the peeled surface and removed. SEM pictures of the scatched areas of the coating provide a qualitative assessment of the adhesive nature of the layer.

### **2.7.4. Contact angle goniometry**

Water contact angles are obtained with a Digidrop Contact Angle Meter (GBX Surface Science Technologies). Measurements are carried out with 3  $\mu$ L drops of ultra-pure water under ambient atmospheric conditions. Experimental precision is of  $\pm 3^\circ$ .

### **2.7.5. Electrochemical characterization (free potential, polarization curves)**

Electrochemical studies on bare and modified samples are realized at room temperature on an EG&G Princeton Applied Research Potentiostat/Galvanostat Model VersaStat II in an electrolytic spot-cell (spot  $\varnothing$ : 5.2 mm). The reference and counter electrode consist respectively in a saturated calomel electrode (SCE, +0.246 V vs. SHE) and a

platinum foil, the substrate playing the role of the working electrode. Measurements are achieved in an electrolytic aqueous solution of sodium chloride (0.9%), which is in and out gassed with nitrogen for 15 min prior to any analysis. The free potential  $E_{free}$  is then measured during 30 min, immediately followed by the recording of polarization curves on the basis of a working electrode scanning from -1.0 to +1.0 V/SCE at a 1 mV/s scan rate. Corrosion potential  $E_{cor}$  and corrosion current density  $j_{cor}$  are assessed in this way by the Tafel's extrapolation technique: their values correspond to the intersection of Tafel's cathodic and anodic slopes fitting the corresponding branches on the polarization curves.

### 3. Results and discussion

#### 3.1. MWCNTs oxidative treatment

In a general way, this work intends to achieve the deposition on titanium substrates of a thin composite layer made of tantalum oxide ( $\text{Ta}_2\text{O}_5$ ) and multiwalled carbon nanotubes (MWCNTs). To uniformly disperse carbon nanotubes in the tantalum deposit, it seems crucial to manage to solubilize them in the reaction solvent during the sol-gel formation process, i.e. in absolute ethanol. The first strategic step consists thus in a specific functionalization of the MWCNTs in order to favor their homogeneous dispersion in the solution [37-40]. The particular approach envisaged here involves an oxidation process, so that oxygen-based functions like alcohols, ketones and carboxylic acids are generated on the surface of the nanotubes and can facilitate their solubilization in ethanol. On the other hand, such a treatment can also lead to a substantial loss of their mechanical and electrical properties through the destruction of their constitutive double bonds and their shortening. It is thus important to optimize the practical oxidative conditions to allow the presence of a high number of oxidative groups on MWCNTs surface without damaging them too significantly.

Several oxidation treatments have been tested and evaluated by qualitative solubility tests according the following procedure: 1 mg of the differently oxidized MWCNTs are put in presence of 1 ml of absolute ethanol and sonicated, then the mixture is centrifuged during 30 min at 3500 rpm. If a black suspension is obtained, the sample is qualitatively considered as soluble (at least partially). The nature of the oxidative treatment – air oxidation, acidic ( $\text{H}_2\text{SO}_4$ ) and basic (NaOH) solutions of  $\text{KMnO}_4$  and  $\text{K}_2\text{S}_2\text{O}_8$ , “piranha” mixture ( $\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4$ ) –, the applied temperature (from 25 to 500°C)

and the modification time (from 30 min to 24 h) have been respectively modified in order to find the best experimental conditions, leading to a good dispersion of the carbon nanotubes in the solution (Table 1). Out of these experiments, it has appeared that only MWCNTs treated in a 0.1 M  $\text{KMnO}_4/\text{H}_2\text{SO}_4$  mixture at  $60^\circ\text{C}$  during 2 h were soluble in absolute ethanol. Moreover, XPS analyses of the nanotubes have allowed a precise characterization of the nature of the carbons components on the C1s core level (Fig. 2a). The contribution of carbon nanotubes conjugated double bounds is clearly observed at 284.1 eV, along with aliphatic (285.0 eV) and oxidized carbons (C-O, C=O and C(=O)O at 286.3, 288.0 and 288.8 eV, respectively). The satellite “shake-up” peak, characteristic of  $\pi\text{-}\pi^*$  transitions in aromatic structures, is also detected at 290.8 eV. Finally, TEM observations on MWCNTs highlight their global moderate shortening after the oxidation treatment (Fig. 2b): the number of long nanotubes ( $> 2100$  nm) decreases in benefit of the medium tubes (900-2100 nm), while the proportion of oxidized short nanotubes (300-900 nm) increases a bit in comparison with original ones.

### **3.2. Elaboration of $\text{Ta}_2\text{O}_5/\text{MWCNTs}$ composite coatings on Ti substrates by sol-gel co-deposition**

Thanks to its great applied easiness, sol-gel process has been chosen here to realize the co-deposition of  $\text{Ta}_2\text{O}_5$  and MWCNTs on Ti surfaces. This method is based on three main steps (Fig. 3a): adsorption of tantalum butoxide on titanium, hydrolysis of butoxide groups, and drying of the deposit. Many variants are described in the literature, involving different reagents with adjustable conditions (concentrations, acidity, temperature, ...) [26]. The composition of the precursory solution and the experimental parameters have thus to be carefully set up and optimized. In particular, the selected solvent and acid catalyst need to be biocompatible and non- or few toxic in case of

residual incorporation in the resulting layers. In the situation of a combined deposition of tantalum oxide and multiwalled carbon nanotubes, these must be dispersed beforehand in the tantalum butoxide sol in order to trap them into the tantalum oxide matrix during its formation (Fig. 3b).

Our group has already reported the formation of Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings on Ti in a previous paper [32]. One of the main goals of the present study is to optimize the different experimental parameters playing a key role in the sol-gel deposition process. In a first time, the exact composition of the reaction solution is adjusted, in particular with the selection of the best quality acid catalyst. Then hydrolysis and drying steps are optimized in the same perspective. High quality deposits are supposed to be homogeneous, adherent to the substrate, cracks-free and protective against corrosion.

### **3.2.1. Optimization of the reaction solution composition: selection of the acid catalyst**

If sol-gel processes can generally be performed in their absence, acid catalysts are well known to favorably influence the hydrolysis and condensation rates, as well as the structure of the resulting materials. Acids act as protonating species on alkoxide functions and allow the enhancement of the reaction kinetics by producing good leaving alcohol groups [41]. On the basis of previous studies [26,27,32], two acid catalysts have been considered in this work: hydrochloric (HCl) and acetic (CH<sub>3</sub>COOH) acids, with absolute ethanol as solvent. Their impact on deposits features and quality have been assessed and compared by XPS and SEM analyses.

XPS survey spectra (Fig. 4) highlight the existence of a thin Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite layer on the Ti substrate with similar characteristics in both cases. C1s core

level clearly shows the MWCNTs contributions (in a corresponding way to Fig. 2a), and Ta4f peak at 26.9 eV testify for the presence of tantalum under its oxidized Ta<sub>2</sub>O<sub>5</sub> form. The characteristic signal of Ti2p is still observed in both situations around 458 eV, which means that a thin tantalum-based layer has been formed on the titanium surface. Considering that the experimental XPS detection limit goes on the top ~10 nm of the sample surface, and that the underlying Ti substrate is still detected, the Ta coating thickness should indeed be lower than 10 nm. Ta/Ti ratios have been calculated from the Ti2p and Ta4f core levels and are presented in Table 2: in comparison with a pristine Ta<sub>2</sub>O<sub>5</sub> deposit (i.e. without MWCNTs in the sol) prepared with HCl as acid catalyst, composite layers present a significant increasing of Ta quantity on the Ti surface (from 0.2 to 1.0-1.1). This clearly shows and confirms that the MWCNTs addition in the sol-gel solution during the formation of the Ta deposit has a considerable benefic influence. As already highlighted [32], insertion of oxidized nanotubes obviously leads to a consolidated tantalum-based layer on the titanium surface. This can be explained by the presence of hydroxyl groups on the surface of MWCNTs as a result of their oxidation: these functions can indeed act in the same way than water and perform by themselves the hydrolysis of the tantalum butoxide groups, with the resulting formation of a matrix of strong CNTs-O-Ta bonds in the solution. The insertion of oxidized nanotubes in the sol leads thus to a reinforcement of the precursory solution stability, with a resulting reduced material loss during the further hydrolysis step and a global strengthening of the compactness and adherence of the composite layers, in comparison with their pristine counterparts.

SEM characterization of the composite deposits shows important variations in function of the acid catalyst: recorded images reveal homogeneous coatings with HCl against

cracked layers with  $\text{CH}_3\text{COOH}$  (Fig. 5a). Besides those clear morphological differences, the use of different catalysts also modifies the way that MWCNTs are included in the deposit: if the nanotubes are “only” incorporated in the Ta layer with  $\text{CH}_3\text{COOH}$  (red circle on Fig. 5b), they are at the same time included into the Ta coating as well as largely prominent on the surface with HCl. In this case, further TEM analyses are able to determine that a Ta layer is wrapping up the surfacial MWCNTs with a 4.5 nm thickness. Peeling tests have also been performed to check if the deposits really adhere to the titanium surface: this is clearly the case, given that Ta-based coatings are still observed after the samples’ scratching, with conservation of their initial morphological features in respective cases (compact and homogeneous with HCl, cracked and fragile with  $\text{CH}_3\text{COOH}$ ) (Fig. 5c).

On the basis of those results, the use of HCl as acid catalyst rather than  $\text{CH}_3\text{COOH}$  is obviously more conclusive: the obtained deposits are not only of better quality in terms of homogeneity and adhesion, but they also lead to the apparition of Ta-coated MWCNTs on the surface. This particularity can be of significant importance for applied issues, especially with the creation of specific interactions between bone cells (osteoblasts, osteoclasts) and carbon nanotubes present on the surface, which have really the potential to be exploited in the framework of the osseointegration process of a titanium implant.

### **3.2.2. Optimization of hydrolysis and drying steps**

Our previous study has already pointed out the crucial importance of the hydrolysis step of the sol-gel process on the tantalum pristine and composite coatings structure and morphology [32]. In general, water converts butoxide functions on the surface into hydroxyl groups, which allows the formation of strong Ti-O-Ta and Ta-O-Ta bonds

responsible for the strength and adhesion of the Ta layer on Ti (Fig. 3a). When the sample is not dipped in water, the air humidity can hydrolyze some of the butoxide groups, but not in a sufficient way to allow a complete reticulation on the whole sample area. In the situation where oxidized MWCNTs are introduced in the solution (Fig. 3b), the presence of surfacial hydroxyl groups is enough to create by itself a dense and strongly bonded matrix of carbon nanotubes and tantalum oxide composite; in this case, the water post treatment seems to be optional [32]. A relating study is nevertheless considered here in order to check if the achievement of an additional hydrolysis step can have a fundamental impact on the Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings and globally optimize their morphology, compactness and adherence.

On the other hand, the final phase of the procedure, which consists in the drying of the sample, is also essential for the gel solidification process through the matrix restructuration, with the removal of ethanol (solvent) and water. The drying conditions and parameters specifically assist the deposit consolidation and strengthening, and can considerably influence the final nature, structure and morphology of the resulting materials. It appears thus imperative to find a specific and accurate drying procedure for the generation of optimized deposits in terms of homogeneity and adherence.

The practical conditions exploited until now in the so-called “classical procedure” [32] have thus been the subject of an optimization process on the basis of fundamental considerations and experimental observations. The conventional hydrolysis *modus operandi* – a simple dipping of the sample in water – leads to the presence of a residual thin film of tantalum oxide sol-gel in the solution itself. In order to avoid this material loss, we have decided to perform a gradual hydrolysis with a progressive increasing of the water level in the modification pot. Concerning the drying procedure, we have

considered a post treatment at high temperatures (in practical terms, at 300°C during 3 min). In comparison with ambient atmospheric conditions, a heating of the samples can significantly increase the drying and solidification rate of the coating, and reinforce its densification [42].

XPS survey spectrum of an “optimally treated” sample (Fig. 6a) reveals the characteristic contributions of carbon (C1s), oxygen (O1s and Auger O<sub>KLL</sub>) and tantalum (Ta4p, 4d and 4f), while no trace of titanium (Ti2p) is anymore detected here (Table 2). This obviously contrasts with the non-optimized “classical procedure” (section 3.2.1.), for which thinner coatings (< 10 nm) have been characterized (Fig. 6a). According to the corresponding SEM pictures (Fig. 7), the composite layers obtained with optimized conditions also appear to be uncracked and a bit more homogeneous than non-optimized samples (Fig. 5a).

The corrosion resistance of the optimized deposits is also assessed. Free potential versus time and polarization curves of bare and modified Ti are shown on Fig. 8a and b, respectively. Ta-coated substrates, with and without MWCNTs, present higher free potential values than uncoated ones (Table 3):  $E_{free}$  evolves from -232 mV/SCE for bare substrates to -43 mV/SCE for Ti modified with Ta<sub>2</sub>O<sub>5</sub>, and up to +45 mV/SCE for Ta<sub>2</sub>O<sub>5</sub>/MWCNTs films on Ti. This last measurement testifies for a more noble character and a better ability to resist to corrosion for composite layers comparing to pristine ones. Concerning the polarization curves, an anodic stabilization (current decrease on the anodic branch) is highlighted for the two coated samples with respect to bare titanium, from  $1 \times 10^{-6}$  A/cm<sup>2</sup> down to  $1 \times 10^{-7}$ - $10^{-8}$  A/cm<sup>2</sup> (composite and pristine films, respectively). The current plateau on the anodic branch, corresponding to an anodic passivation region (which means that the material becomes less sensitive,

“passive” to oxidation and corrosion), is also more marked for both Ta-modified samples: it starts from around -500 and -250 mV/SCE for pristine and composite coatings, respectively, against +250 mV/SCE for bare Ti (Fig. 8b). Corrosion potentials are listed in Table 3: cathodic protection compartment (displacement of  $E_{cor}$  near more cathodic values) is noticed for the Ta<sub>2</sub>O<sub>5</sub> layer ( $E_{cor}$  varies from -398 mV/SCE for bare Ti to -688 mV/SCE), while anodic inhibition (displacement of  $E_{cor}$  near more anodic values) is observed for the composite coating (increasing of  $E_{cor}$  up to -204 mV/SCE). Corrosion current densities remain in the same order of magnitude, around  $1 \times 10^{-8}$  A/cm<sup>2</sup> (Table 3).

All said and done, the optimization of hydrolysis and drying procedures is very conclusive. The implementation of a gradual hydrolysis treatment followed by a 300°C heating of the substrates leads to thick and highly compact composite deposits with high electrochemical stability and good protective capacities. In comparison with “classical procedure” conditions (hydrolysis by dipping in water, drying at ambient temperature), modified methodologies allow the preparation of very promising samples with great morphological, structural and adherent characteristics.

### **3.3. Functionalization of Ta<sub>2</sub>O<sub>5</sub>/MWCNTs coatings by organophosphonic acid molecular films**

The second organizational level of the system consists in a molecular film of amino-tris-methylene phosphonic acid, generated on the Ta<sub>2</sub>O<sub>5</sub>-based layer by self-assembling. This molecule is able to graft on the surface via one or two phosphonic functions, leaving the other(s) group(s) available for interfacial reactivity with human environment. In physiological conditions (pH ~ 7.4, 37°C), acid phosphonic entities are

under unprotonated form  $\text{PO}_3^{2-}$ , whence their propensity to complex calcium ions and favor hydroxyapatite nucleation. Moreover, organophosphonic films can also act as effective barriers against corrosive agents; this ability is however not of first interest here considering the already highly protective tantalum oxide surface.

After the immersion of the optimized  $\text{Ti}/\text{Ta}_2\text{O}_5+\text{MWCNTs}$  substrates in an aqueous solution of  $\text{N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  under ambient atmosphere, formed molecular films have been characterized by XPS and contact angle measurements. After practical tests, a pH value of  $\sim 1$  combined with a modification time of 1 h and a  $10^{-3}$  M concentration of  $\text{N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  have been found to be the best experimental conditions for the molecular grafting. XPS survey spectrum (Fig. 6b) points out the presence of carbon, oxygen and tantalum, characteristic elements of the composite deposit. Nitrogen and phosphorus are also detected, with the position of the P2p core level at 134.1 eV testifying for the occurrence of phosphonic functions. A P/Ta ratio of 0.21 is calculated out of P2p and Ta4f core levels, and indicates a substantial covering rate of the molecular film on the surface. Water contact angles values measured for bare Ti,  $\text{Ti}/\text{Ta}_2\text{O}_5+\text{MWCNTs}$  and  $\text{Ti}/\text{Ta}_2\text{O}_5+\text{MWCNTs}/\text{N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  substrates are 40, 31 and  $13^\circ$ , respectively. The observed decreasing is correlated with the reinforcement of the hydrophilic nature of the surface with the successive tantalum-based and organophosphonic coatings. As bone cells attachment is known to be favored with moderate hydrophilic surfaces [43], those contact angle measurements prove that the presence of the organic molecular film on the composite layer is highly appreciated in the perspective of bioactive surfaces for osseointegration.

Grafting of amino-tris-methylene phosphonic acid molecular films is thus confirmed, with a resulting increasing of hydrophilicity. The association between this organic

assembly and the underlying inorganic tantalum layer generates a very interesting scaffold for the hydroxyapatite growth process.

### 3.4. Effect of the inorganic and organic layers on hydroxyapatite growth

Any material designed to be used as bone implant needs to be assessed in terms of osseous growth capacities. Despite its fundamental character, we really wanted to finalize this study with a first stage evaluation of the osseointegrative nature of these hierarchically functionalized titanium substrates. This is achieved by inducing the development of a hydroxyapatite (HAp) layer through their immersion in a simulated body fluid (SBF) solution. Optimized pristine Ta<sub>2</sub>O<sub>5</sub> and composite Ta<sub>2</sub>O<sub>5</sub>/MWCNTs coatings, as well as their N(CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>)<sub>3</sub>-covered counterparts, have been submitted to a 7-days immersion in the previously described SBF at 37°C. HAp films have been analyzed through SEM and XPS measurements.

SEM images of the different systems are shown in Fig. 9. A first important point concerns HAp crystallinity, which is significantly more defined as soon as MWCNTs and/or phosphonic acid molecules are present (Fig. 9b, c, d). With a “simple” Ta<sub>2</sub>O<sub>5</sub> layer (Fig. 9a), HAp is rather deposited under an aggregated form. Another qualitative observation points out an increasing of the crystals density in the following order: (b) Ti/Ta<sub>2</sub>O<sub>5</sub>+MWCNTs < (c) Ti/Ta<sub>2</sub>O<sub>5</sub>/N(CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>)<sub>3</sub> << (d) Ti/Ta<sub>2</sub>O<sub>5</sub>+MWCNTs/N(CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>)<sub>3</sub>. Crystals size and form are of the same range whatever surfacial state is involved (Ø ~ 0.5 µm).

At this point, it is really obvious that the combination of both effects – morphological with carbon nanotubes and chemical with phosphonic acid molecules – leads to the best quality hydroxyapatite crystals in terms of nature, density and crystallinity. MWCNTs

allow a rise of the deposit's roughness and bring supplementary protrusions, acting as nucleation centers for HAp. On the other hand, chemical reactivity supplied by organophosphonic  $\text{PO}_3^{2-}$  functions specifically facilitates the  $\text{Ca}^{2+}$  ions complexation.

XPS study of a “completely-functionalized substrate”  $\text{Ti/Ta}_2\text{O}_5+\text{MWCNTs/N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  has also been pursued. The survey spectrum of a HAp-covered sample (Fig. 6c) displays the characteristic signals peculiar to tantalum-based deposits (Ta, O, C), organophosphonic layers (P, N) and hydroxyapatite film (P, Ca) combined with other constitutive ions of the SBF (K, Na, Cl). Core levels of Ca2p and P2p present binding energy values of 348.0 and 133.7 eV typical of HAp. The experimental Ca/P ratio found here reaches only 0.73, against a theoretical value of 1.6 for HAp ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). Even if the additional phosphorus contribution of organophosphonic molecules is partially responsible for this reduced experimental value of Ca/P, this one remains quite low. An evaluation of its evolution on longer durations (several weeks) should probably lead to higher values and increased apatite growth rates.

#### 4. Conclusions and perspectives

Throughout this study, we intended to ameliorate osseointegrative properties of titanium, a metal commonly used in the framework of bone implants elaboration. The considered approach was based on the preparation of a composite layer made of tantalum oxide and carbon nanotubes on titanium substrates by a sol-gel co-deposition process. The systems were then functionalized with organophosphonic molecular films in order to further induce and favor the growth of a hydroxyapatite layer.

In a first time, a methodological optimization of the sol-gel experimental parameters has been performed through the functionalization of MWCNTs and their dispersion in the sol-gel precursory solution, the selection of the best quality acid catalyst as well as hydrolysis and drying conditions. Obtained deposits were highly homogeneous, cracks-free and resistant to corrosion. Afterwards, functionalization of those layers with phosphonic acid molecules has permitted to introduce reactive  $\text{PO}_3^{2-}$  functions on the surface in the perspective of osseous integration. Finally, *in vitro* tests of hydroxyapatite growing in a simulated body fluid have proved that the hierarchical modifications considerably reinforced the global bioactivity of the systems.

In terms of perspectives, osseointegrative properties assessment can be completed by testing the proliferation and adhesion capacities of osteoblasts on those surfaces. Other methodologies can also be considered for the preparation of the tantalum-based layers on titanium and its alloys: in particular, electrochemical techniques such as electrodeposition and electrophoretic deposition have clearly the potential to prepare good quality pristine and composite coatings with a high level of control and precision [44].

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## Figures caption

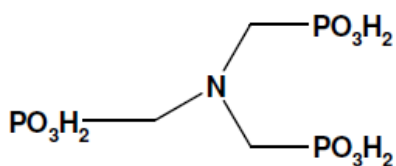


Fig. 1: Molecular structure of amino-tris-methylene phosphonic acid.

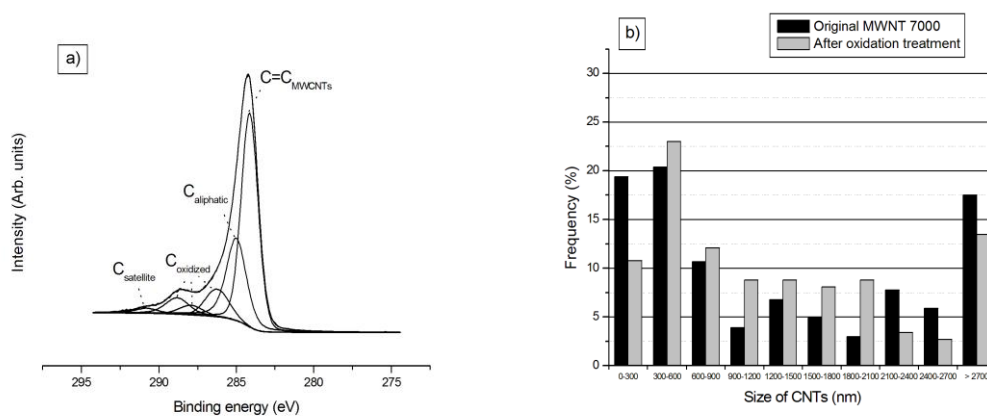


Fig. 2: (a) XPS core level of C1s of MWCNTs oxidized by a 0.1 M  $\text{KMnO}_4/\text{H}_2\text{SO}_4$  solution at 60°C during 2 h. (b) Comparison of the length distribution for MWNT 7000 carbon nanotubes before and after the oxidation treatment. Frequency values are obtained with a reproducibility of ~ 5 %.

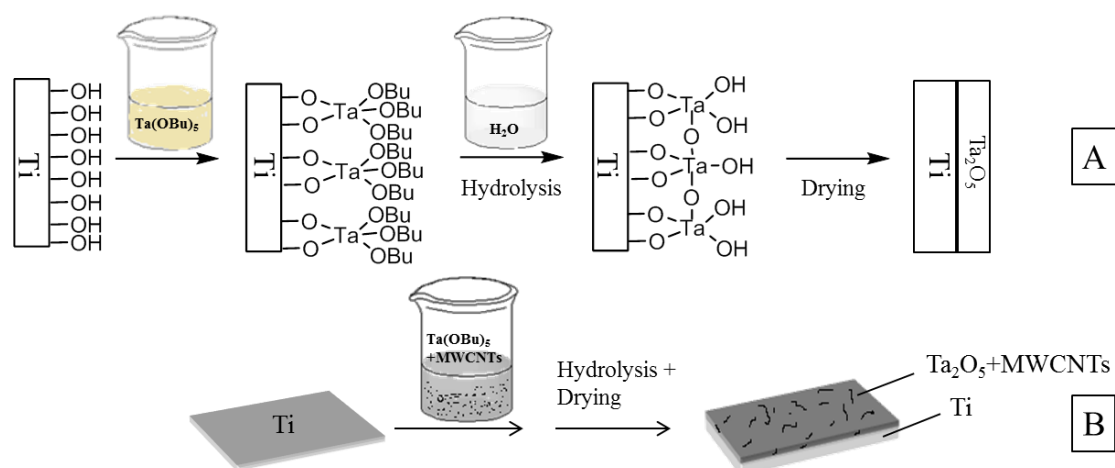


Fig. 3: Principle of the sol-gel process: (a) reactions occurring in the sol-gel deposition of  $\text{Ta}_2\text{O}_5$  on Ti, (b) sol-gel co-deposition of  $\text{Ta}_2\text{O}_5$  and MWCNTs on Ti.

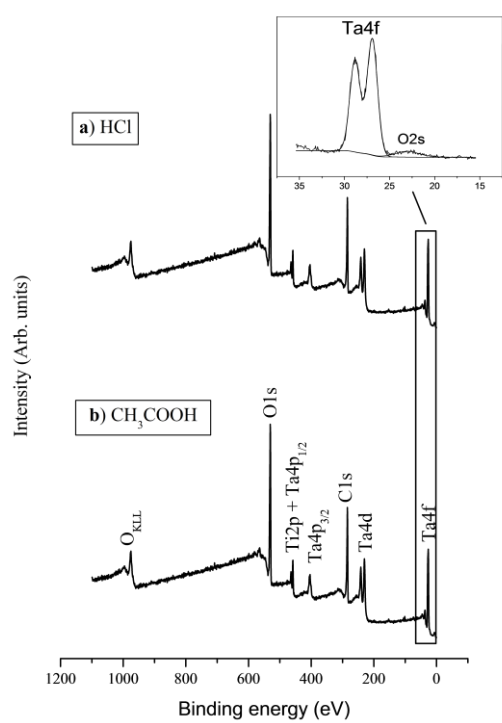


Fig. 4: XPS survey spectra of  $\text{Ta}_2\text{O}_5/\text{MWCNTs}$  composite coatings generated on Ti substrates by sol-gel co-deposition, with (a) HCl and (b)  $\text{CH}_3\text{COOH}$  as acid catalyst. Enlarged region: core level of Ta4f (similar in both cases).

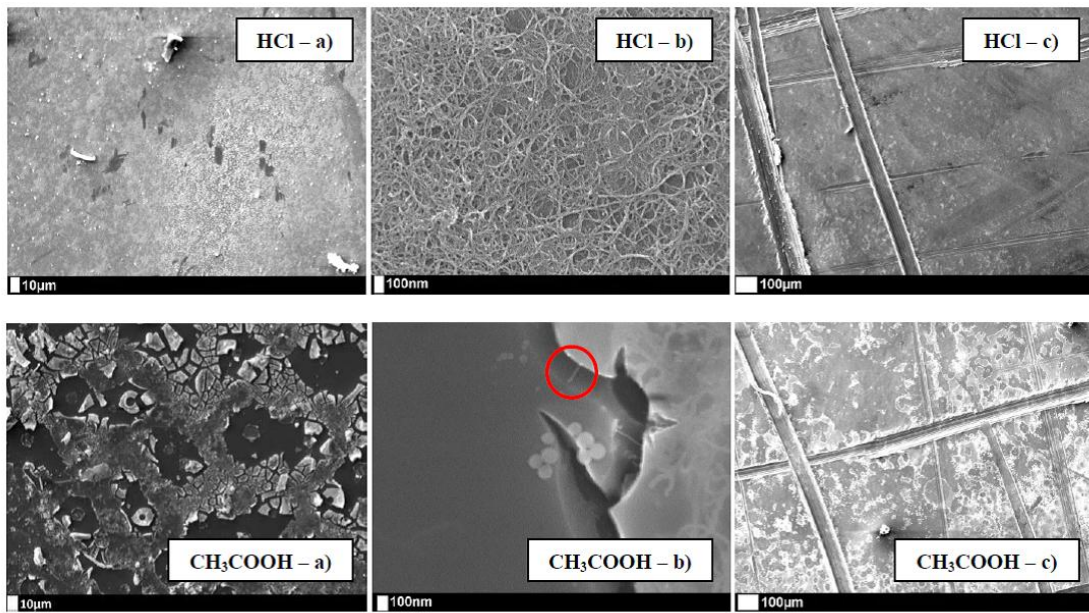


Fig. 5: SEM pictures of Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings generated on Ti substrates by sol-gel co-deposition, with HCl (up) and CH<sub>3</sub>COOH (down) as acid catalyst: (a) after deposition, large scale; (b) after deposition, restricted scale; (c) after peeling test.

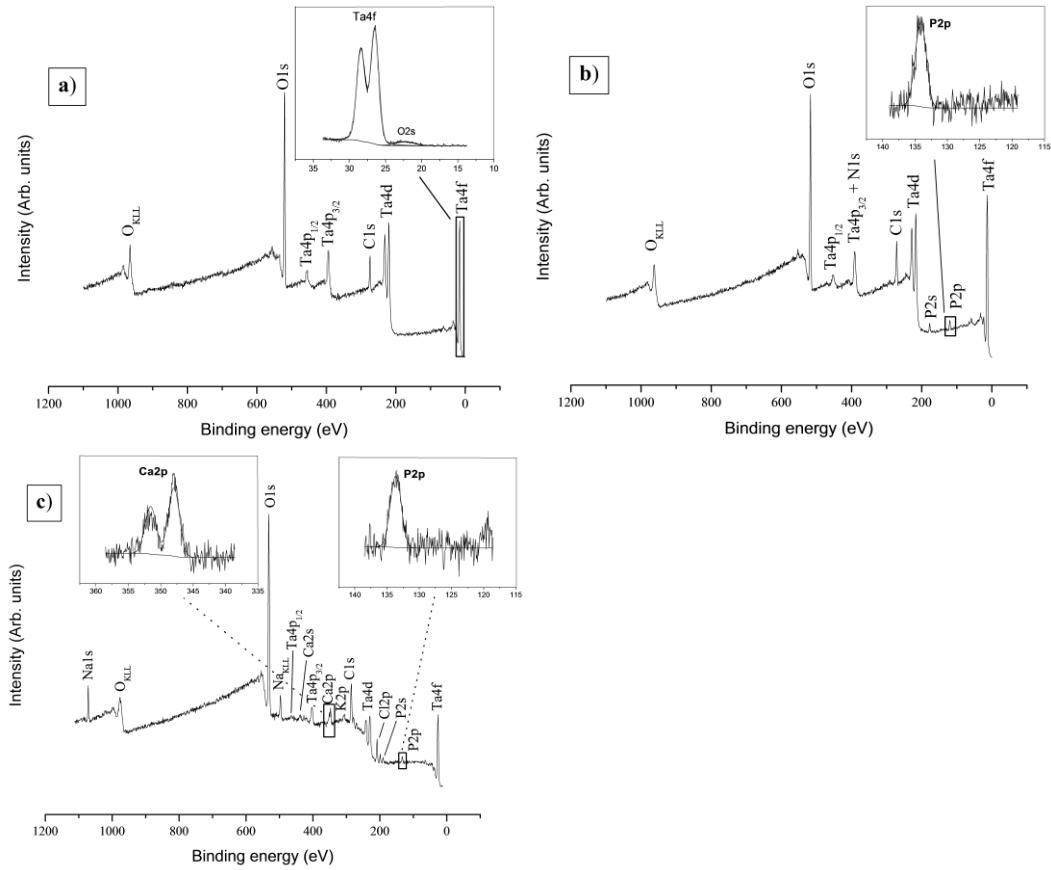


Fig. 6: XPS survey spectra of Ti substrates covered by (a) Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings generated by sol-gel co-deposition in optimized conditions (HCl as acid catalyst, gradual hydrolysis followed by drying at 300°C during 3 min), (b) amino-tris-methylene phosphonic acid molecular films grafted on optimized Ta<sub>2</sub>O<sub>5</sub>/MWCNTs composite coatings, and (c) hydroxyapatite layer formed after 7 days on optimized Ta<sub>2</sub>O<sub>5</sub>/MWCNTs/N(CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>)<sub>3</sub> coatings.

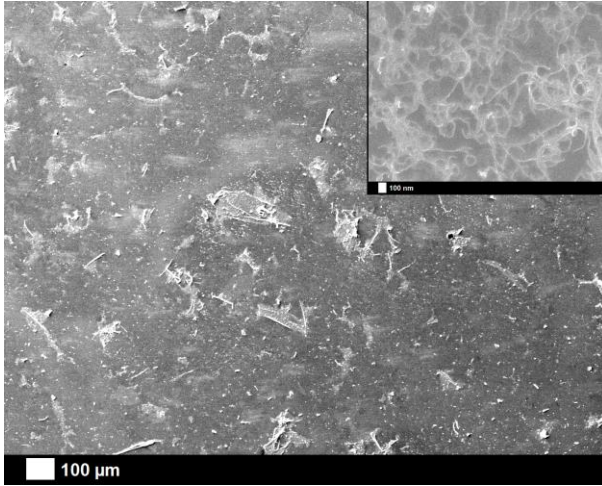


Fig. 7: SEM pictures of  $Ta_2O_5/MWCNTs$  composite coatings generated on Ti substrates by sol-gel co-deposition, in optimized conditions: HCl as acid catalyst, gradual hydrolysis followed by drying at  $300^\circ C$  during 3 min.

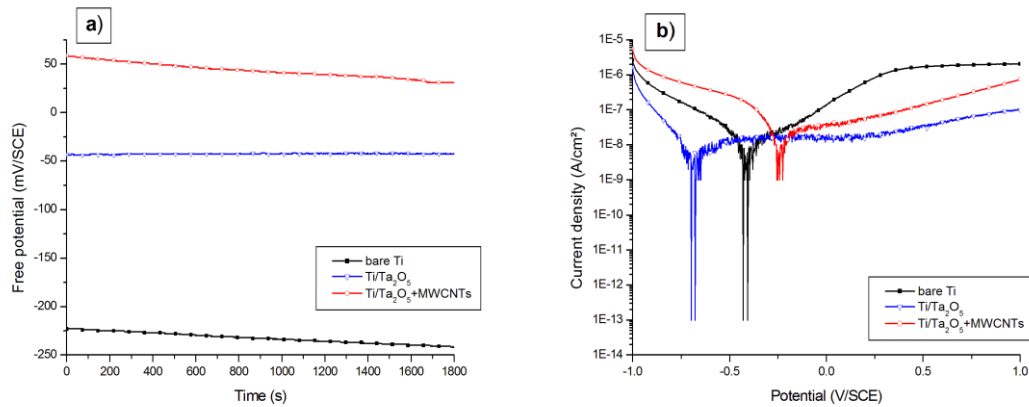


Fig. 8: electrochemical characterizations of *bare Ti* and Ti modified with pristine  $Ta_2O_5$  ( $Ti/Ta_2O_5$ ) and  $Ta_2O_5/MWCNTs$  composite ( $Ti/Ta_2O_5+MWCNTs$ ) coatings (both prepared according optimized conditions). (a) Free potential measurements in NaCl 0.9%; (b) polarization curves in NaCl 0.9%, recorded at a scan rate of 1 mV/s.

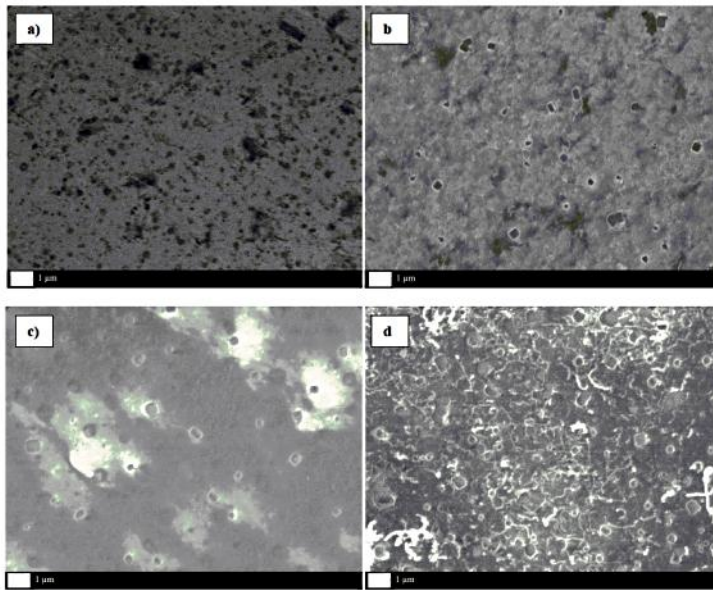


Fig. 9: SEM pictures of hydroxyapatite deposited on Ti substrates covered by optimized (a) pristine  $\text{Ta}_2\text{O}_5$ , (b) composite  $\text{Ta}_2\text{O}_5/\text{MWCNTs}$ , (c)  $\text{Ta}_2\text{O}_5 + \text{N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  and (d) composite  $\text{Ta}_2\text{O}_5/\text{MWCNTs} + \text{N}(\text{CH}_2\text{PO}_3\text{H}_2)_3$  coatings.

## Tables

Oxidative conditions	Temperature (°C)	Duration (min)
O <sub>2</sub>	400, 500	30, 120, 240, 480
<u>0.1 M KMnO<sub>4</sub>/H<sub>2</sub>SO<sub>4</sub></u>	<u>60</u>	60, <u>120</u>
0.2 M KMnO <sub>4</sub> /NaOH	40, reflux	40, 120
6.5 M H <sub>2</sub> O <sub>2</sub> /H <sub>2</sub> SO <sub>4</sub>	25, 60	60, 120, 240
0.2 M K <sub>2</sub> S <sub>2</sub> O <sub>8</sub> /H <sub>2</sub> SO <sub>4</sub>	25, 60, reflux	24 h

Table 1: experimental parameters of the different tested oxidation methods of MWCNTs. Underlined: leads to a soluble dispersion in absolute ethanol.

Deposit	Ta/Ti
Pristine Ta <sub>2</sub> O <sub>5</sub>	0.18
<b>Composite Ta<sub>2</sub>O<sub>5</sub>/MWCNTs</b>	
<i>Acid catalyst optimization</i>	
HCl	1.09
CH <sub>3</sub> COOH	1.00
<i>Hydrolysis and heating optimization</i>	
Gradual hydrolysis + 3 min drying at 300°C	Ti undetected

Table 2: XPS Ta/Ti ratios for tantalum-based layers prepared on Ti substrates by sol-gel deposition.

	E <sub>free</sub> (mV/ECS)	E <sub>cor</sub> (mV/ECS)	j <sub>cor</sub> (10 <sup>-8</sup> A/cm <sup>2</sup> )
Bare Ti	- 232	- 398	1.1
Ti/Ta <sub>2</sub> O <sub>5</sub>	- 43	- 688	0.57
Ti/Ta <sub>2</sub> O <sub>5</sub> +MWCNTs	+ 45	- 204	2.1

Table 3: free potential, corrosion potential and corrosion current density of bare and modified Ti substrates.