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A two-step approach to surface functionalize Ti dental implants

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***To my family
where life begins
and love never ends.***

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Abstract

For enhancing the osseointegration aptitude of Ti dental implant, titanium oxide (TiO₂) - hydroxyapatite (HA) bi-layered coatings were deposited by means of a two-step process on commercial-grade-IV Ti substrates with different pristine morphologies and topographies (machined, sandblasted, and sandblasted/acid etched). Firstly, dense, compact and crystalline titania was deposited as an inter-layer via Low Pressure Metal-Organic Chemical Vapor Deposition. Then a suitably discontinuous HA upper layer was deposited by means of spray pyrolysis. The thermal treatment at 600°C was assessed as the most suitable, both for material features and for bioactivity.

The novelty of this work is that this synergic two-step approach allows the co-existence of both TiO₂ and HA ceramics on the implant surface, in order to increase the surface bioactivity and improve the short(HA)- and long(TiO₂)-term implant service-life.

The relationship between material processing and functional properties was investigated and evaluated. The electrochemical and tribocorrosive behavior (in artificial saliva) of the functionalized surfaces as well as their metallic ion release (in lactic acid) appeared improved with respect to the uncoated ones. The (nano)hardness of the coated materials was higher than that of bare substrates. In agreement with industrial practice, HA-coating adhesion was qualitatively assessed by tape test. As expected, different results were obtained for different morphologies of substrate surfaces: machined surfaces showed reduced HA retention, while HA coatings better adhered to sandblasted and sandblasted/acid etched substrates. Useful information was obtained by wettability analysis. Freshly prepared surfaces always showed hydrophilicity, and even superhydrophilicity in the case of pure titania coatings. Exposure to air induced gradual wettability decreasing due to atmospheric hydrocarbon contamination. To maintain high-energy surfaces, two industrial-scalable strategies were positively set-up, either storing the samples in distilled water or radiating the contaminated samples by UV.

Acellular *in-vitro* bioactivity was evaluated testing the growth rate of bone-like apatite after immersion in Dulbecco's Phosphate Buffer Saline (DPBS) solution at 37°C. The results showed that a bone-like apatite layer was effectively formed on the TiO₂ – HA functionalized substrates, giving significant results just after 30 minutes incubation in DPBS. Higher HA nucleation rate was, furthermore, detected on the solely TiO₂ coated samples when compared to bare Ti substrates, confirming their improved bioactivity. Finally, according to ISO 10993-5:2009, cell viability confirmed the safety of the samples in terms of cytotoxicity.

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List of principal abbreviations and acronyms

ACP: Amorphous Calcium Phosphate
ALP: Alkaline Phosphatase
AS: Artificial Saliva
ASTM: American Society for Testing and Materials
BIC: Bone-to-Implant Contact
CA: Contact Angle
COD: Crystallography Open Database
COF: Coefficient Of Friction
CPC: Calcium Phosphate Ceramic
cp-Ti: commercial pure Titanium
CVD: Chemical Vapor Deposition
DPBS: Dulbecco's Phosphate Buffered Saline
E: Elastic modulus
 E_{corr} : Corrosion Potential
EDX: Energy Dispersive X-Ray analysis
FT-IR: Fourier Transform Infrared Spectroscopy
H: Hardness
HA: Hydroxyapatite
ICDD: International Centre for Diffraction Data
ICP-AES: Inductively Coupled Plasma Atomic Emission Spectroscopy
ISO: International Standards Organization
 J_0 : Current Density
 J_{pass} : Passive Current Density
LA: Lactic Acid
LP-MOCVD: Low Pressure Metal-Organic CVD
MAUD: Material Analysis Using Diffraction
NRU: Neutral Red Uptake
NC: Negative Control
OCP: Open Circuit Potential
OD: Optical Density
PBS: Phosphate-Buffered Saline
PC: Positive Control
Ra: arithmetical mean roughness value
Rp: maximum profile peak height
Rq: root mean square roughness
Rt: maximum height of the profile
Rv: maximum profile valley depth
Rz: average maximum height of the profile
SBF: Simulated Body Fluid
SCE: Saturated Calomel Electrode
Sdr: surface area excess
SEM: Scanning Electron Microscopy
SLA: Sandblasted Large grit and Acid etched
Ti_m: machined Ti substrate
Ti_m_TiO₂: titania coated machined Ti substrate

Ti_m_TiO₂_HA: titania and hydroxyapatite coated machined Ti substrate

Ti_s: sandblasted Ti substrate

Ti_s_TiO₂: titania coated sandblasted Ti substrate

Ti_s_TiO₂_HA: titania and hydroxyapatite coated sandblasted Ti substrate

Ti_s+a: sandblasted and acid etched Ti substrate

Ti_s+a_TiO₂: titania coated sandblasted and acid etched Ti substrate

Ti_s+a_TiO₂_HA: titania and hydroxyapatite coated sandblasted and acid etched Ti substrate

Ti_TiO₂: titania coated Ti substrate

Ti_TiO₂_HA: titania and hydroxyapatite coated Ti substrate

TTIP: Titanium Tetraisopropoxide

XRD: X-Ray Powder Diffraction

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PREVIEW

I Background

The research topic referred to this Ph.D. project concerns the biomedical sector and, in particular, the dental implants. Dental implants are artificial tooth roots that are placed into the jawbone in order to hold a replacement tooth (the crown). The implant emulates the shape of the root and it is surgically incorporated into the bone and, over time, it becomes a stable base for the crown. Dental implants can replace a single tooth, several teeth, support partial or full dentures [1]. They are made with materials compatible with the human body, mostly titanium [1,2].

Dental implantology is an especially stimulating field because the partial or total edentulous involves numerous functional and aesthetic problems (such as difficulty of mastication and digestion, alteration of the mandibular and body posture, difficulty in verbal expression), with consequences that go beyond the medical state, but also influencing the psychological conditions and social relationships.

In today's society, tooth loss is a widespread problem and, as a result, the use of dental implants is becoming a rather common practice. Statistics on dental implants show that about 69% of the population between 35 and 44 years old has lost at least one permanent tooth because of an accident, gum disease, a failed root canal or tooth decay. Moreover, by age 74, about 26% of adults have lost all of their permanent teeth [2]. These data explain why the number of dental implant procedures has constantly increased worldwide, reaching nowadays about 1 million dental implantations per year [1].

As shown in “Dental Implants and Prosthesis Market” report [3], the growth in life expectancy and the corresponding age-related dental diseases make that the dental implants and prosthesis market is expected to grow from 9.50 USD billion in 2018 to 13.01 USD billion by 2023, at a CAGR (Compound Annual Growth Rate) of 6.5%. Conversely, the high cost of implants and the lack of awareness about the results of the procedures partially restrain the growth of the dental implants market [4].

Even if the reported survival rates of dental implants are generally high (95% for a follow-up period of 10 years) [5], the development of innovative technologies linked to the improvement of the characteristics of dental implants (i.e. type of biomaterials involved, product geometry, chemical and/or physical functionalization of the surface) is currently configured as a sector of particular relevance. The engineering of efficient dental implants, with enhanced osseointegrative performances and increased short- and long-term prospects of success, could absolutely lead to an improvement in the quality of life. For these reasons, implant industries and manufacturers have invested many resources for the development of new products, as well as doctors, scientists, and engineers have deeply researched in this field in the last decades. Nonetheless, this is also expected to greatly be the field of many investigations in the next future [1,6].

The results of a literature search, conducted in the Web of Science database (Web of Science Core Collection), from 1965 to 2018, using the keywords ‘Dental & Implant’, is reported in Figure I. Figure Ia shows the number of publications per year, while Figure Ib presents field of the published documents. It is very clear that before 1995, the topic was in a nascent stage and started to become popular after about 2000, as the number of publications began to increase sharply. The published documents in this examined period cover different scientific fields, being “dentistry oral surgery medicine” the most widespread area.

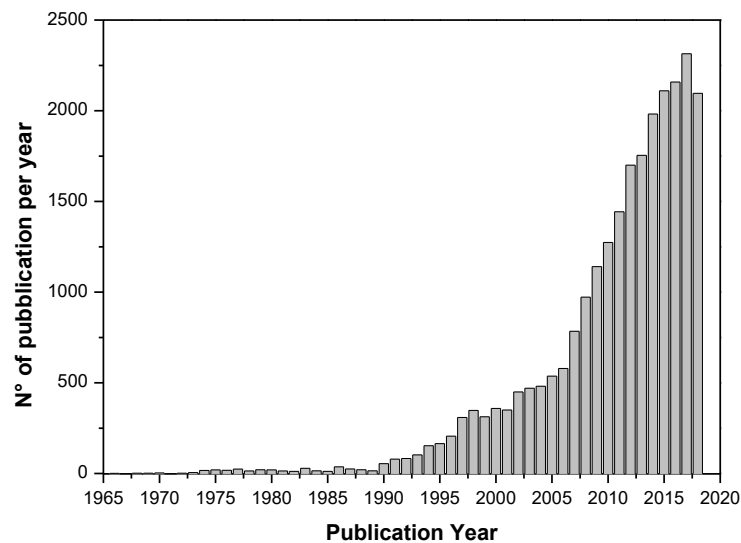
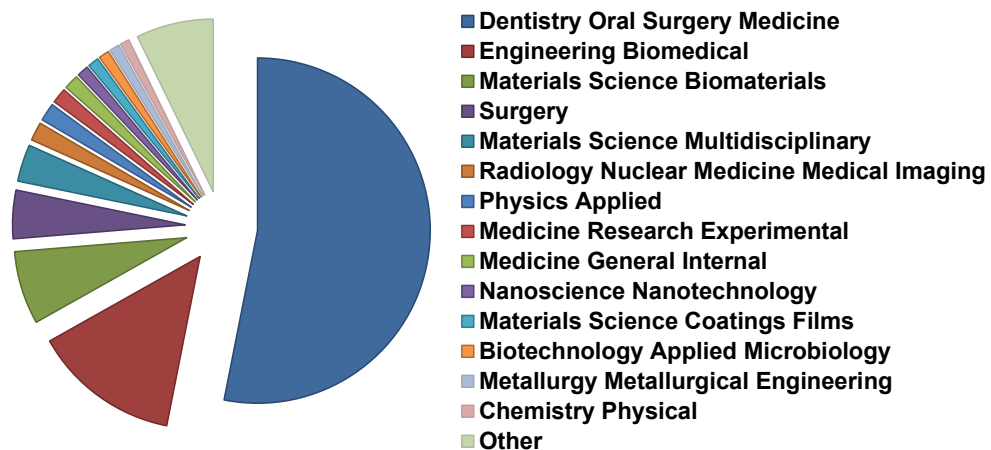
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Figure I Published document per year from 1965 to 2018 (a). Field of the published documents from 1965 to 2018 (b). Source: Web Of Science, from Web of Science Core Collection; Keywords: Dental & Implant. Access date: 17/01/2019.

II Implant based on titanium

Among the various types of implant materials, nowadays about 70÷80% of dental implants are manufactured from biocompatible metals, such as stainless steel, cobalt-chromium alloys, and titanium or Ti alloys [7]. Among them, commercially pure titanium (cp-Ti) and its alloys have been widely used for dental implant applications due to their excellent combination of strength-to-weight ratio, corrosion resistance and biocompatibility [8]. However, due to its limited bioactivity [9,10], the Ti surface has to be functionalized to enhance, accelerate and direct the bone growth. Several methods for the chemical and/or physical implant surface functionalization have been proposed [1,2,11,12]. Surface modification methods can be roughly classified into two main groups: i) procedures targeted to enhance the surface roughness and ii) bio-functional coatings aimed to modify also the

chemical surface composition [1]. Among the first typology blasting with ceramic particles, titanium plasma spraying, etching procedures, and anodization deserve to be mentioned [1,2,12]. Acid etching treatments are often executed subsequently to a blasting phase, in order to remove blasting damaged zones or blasting embedded material, and also to refine the final surface roughness [11].

Beside surface roughness modification, also the chemical composition of the outermost surface layers influences the implant-tissue interactions since the surface chemistry and charge can affect the processes of proteins adsorption and cells attachment, proliferation, and differentiation [1,2,12]. In literature, many procedures for the implant chemical surface modification have been investigated, with the foremost aim to turn a commonly almost bioinert surface into a bioactive one [1,6,12]. Above all, Ti implants have been covered with a layer of bioactive Calcium Phosphate Ceramic (CPC), principally composed of hydroxyapatite (HA, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) [12]. In particular, HA shows chemical and structural similarity to the inorganic component of bone and teeth [13–15], furthermore, among all calcium phosphate ceramics, HA is the most thermodynamically stable under physiological conditions [12,16,17]. As a result of the CPC Ti functionalization, which combines the metal mechanical properties with the biocompatibility and the bioactivity of the ceramics [18], the bone healing and the implant to bone fixation processes are strongly improved [6,12,13,18]. Moreover, the bioactive layer could also minimize the direct metal-body fluid contact (so limiting undesired metallic ion release into the body) [19,20] and improve the roughness of the implant surface, therefore favoring a better mechanical interlocking between bone and implant.

Conversely, CPC coatings on metal implants usually suffer from poor adhesion [19]. Several surface modification techniques have been proposed to improve the coating to implant adhesion, such as the increasing of the titanium roughness (to favor a better mechanical interlocking) or the use of highly energetic CPC deposition techniques [19]. Anyway, these procedures seem not to produce significant results. To ameliorate the adhesion strength between the Ti substrate and the CPC layer, literature has suggested that the insertion of dense and compact ceramic inter-layers reduces the thermal mismatch between metal and the CPC coating and increases the amount of - available anchoring -OH sites [19]. However, it has to be taken into account that the adhesion is also influenced by others factors (such as surface chemical and physical interactions, coating and substrate microstructures, substrate surface roughness, film thickness, and deposition technique [21]) and often the success of the coating adhesion is the result of their synergic contribution.

Among various ceramics, crystalline titania (titanium dioxide, TiO_2) is extensively used as an inter-layer thanks to its well-known biocompatibility, bioactivity and chemical affinity to the already present materials [19,22–26]. This inter-layer has been also proved to increase the corrosion resistance of the metal substrate [22,27]. Additionally, the titania inter-layer may also improve the implant lifetime [25,26]. CPC layers degrade *in-vivo* due to interaction with physiological fluids (coating dissolution and consequent bone-like apatite precipitation) and osteoclast resorption [22,26,28,29]. If the substrate underlying the CPC layer is bioactive and not resorbable (i.e. as TiO_2 is), the bone will have a chance to bond the implant surface also after that the CPC layer is completely dissolved (thus avoiding the complications connected with the dissolution of CPC layer and the resulting loss of anchorage between bone and metallic implant) [22,26]. Crystalline titania (anatase or rutile) has been demonstrated to be bioactive *in-vitro*, therefore it is a suitable and non-degradable surface to bond the bone [25,26].

III Aim of the thesis

The improvement of the bio-functional and mechanical properties of the traditionally used dental implants is a particularly important goal in the medical field. Titanium and its alloys are widely used as starting materials for dental implant purposes. However, despite its excellent biocompatibility, titanium has an intrinsic limit due to reduced bioactivity, a property that is fundamental to trigger a specific biological response. In order to increase osseointegration (and therefore to favor the establishment of an intimate and lasting union between the implant surface and the bone tissue), the titanium surface is often functionalized.

The research activity proposed in the present Ph.D. project concerns with the development of a novel synergic deposition route for the functionalization of Ti substrates, through their coating with suitable ceramic materials. A multi-step process is proposed, with the aim of obtaining a final bi-layered coating made up of a dense, compact and crystalline titania inter-layer (obtained via Low Pressure Metal-Organic Chemical Vapor Deposition technique, LP-MOCVD) and of a homogeneously spread but discontinuous calcium phosphate ceramic (hydroxyapatite) top-layer, with particular crystallinity and morphology (obtained by means of spray pyrolysis technique).

The novelty of this work is based on this synergic two-step approach allowing the co-existence on the implant surface of both titania and calcium phosphate ceramics, with the aim to increase the surface bioactivity and the short(HA)- and long(TiO_2)-term implant service-life. The further goal is obtained thanks to the presence of a homogeneously spread but discontinuous calcium phosphate upper layer that allows to both the ceramic materials to be simultaneously exposed to body fluids and then contribute to the healing process: a bioactive, biocompatible and not resorbable titania inter-layer is available for the long-term integration, whereas a more bioactive and resorbable HA top-layer permits the short-term anchoring. In detail, if a continuous HA layer is present, the new bone rapidly anchors on it, nevertheless, when the HA reabsorption process completely remove it, no more and stable bonds are available between the bone and the implant, thus causing the implant loosening. In the here proposed project, new bone simultaneously starts to anchor on HA (quickly) and on TiO_2 (slowly) and when the HA is completely reabsorbed the implant is still steadily bound.

Figure II summarizes the auspicated osseointegrative behavior of the here presented composite material. At zero time (i.e. just after implantation) synthetic HA islands and TiO_2 are simultaneously present on the Ti surface (Figure IIa). During phase 1, there is rapid osseointegration on the more bioactive HA islands (the white dots simulate the new bone growth), while slow osseointegration on the TiO_2 layer (Figure IIb). In phase 2, the osseointegration on the synthetic HA islands further increases, the HA islands start to be resorbed (due to chemical dissolution and/or osteoclasts activity), while also the osseointegration on TiO_2 layer increases (Figure IIc). Finally, in phase 3, the synthetic HA islands are completely dissolved, but the osseointegration on the TiO_2 layer is well-consolidated (Figure IId).

During all the experiments, considering that besides the chemical composition, the morphology and the topography of the Ti surfaces are significant parameters that affect the rate and quality of the osseointegration process, three types of Ti substrates commonly used in dental implant applications and with different superficial features were coated. Specifically, Ti machined (Ti_m, turned substrate as after mechanical turning processing), Ti sandblasted (Ti_s, machined substrate treated with Al_2O_3 particles) and Ti sandblasted/acid etched (Ti_{se},

s+a, machined substrate treated with Al_2O_3 particles and, subsequently, with an acid bath) were used as substrates.

This Ph.D. project was funded by Mech & Human Srl company (previously 'RT Unipersonale Srl'), Albignasego - Padova, which operates in the field of dental implants. Furthermore, the industrial partner provided the titanium substrates.

During the three years of the Ph.D. School in Industrial Engineering (curricula: Materials Engineering), all the experimental work was performed at the Institute of Condensed Matter Chemistry and Technologies for Energy of the National Research Council (ICMATE-CNR).

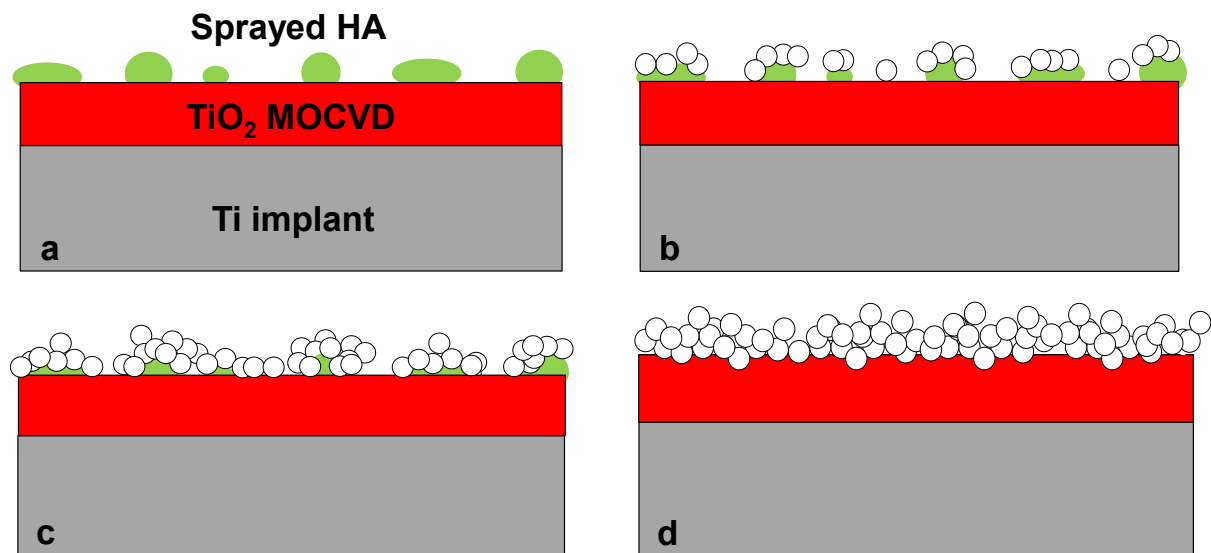


Figure II Schematic representation of the osseointegration process in the two-step functionalized Ti dental implants here obtained.

IV Outline of the thesis

This thesis is divided into 7 chapters.

Chapter 1 covers the state of art. Biomaterials, dental implants, and osseointegration topics are faced. The biomaterials commonly used for dental implant applications, like metals, polymers, and ceramics are described, enlarging the discussion on Ti and its alloys. Then, the principal physical and chemical Ti surface modification methods are reviewed. Finally, the recent trends to improve the osseointegration of titanium dental implants are discussed.

In Chapter 2 the coatings deposition procedures used in this work are examined. The major chemical vapor deposition and spray pyrolysis features (i.e. principles, configurations, advantages and disadvantages) are presented. Some specific characteristics of the instrumental apparatus and precursors used in this project are briefly presented.

In Chapter 3 the principles of the analytical methods used in this work are shortly described. Structural, morphological and chemical characterization techniques such as X-ray powder diffraction, scanning electron microscopy and profilometry, Fourier transform infrared spectroscopy, energy dispersive X-ray analysis and inductively coupled plasma atomic emission spectroscopy are expounded. Furthermore, potentiodynamic polarization, tribocorrosion, nanoindentation, and contact angle evaluation are discussed.

In Chapter 4 the procedure for the preparation of the sandblasted and acid etched ("s+a") titanium surfaces is delineated. Then the processes used to obtain the final two-step

functionalized materials (MOCVD and spray pyrolysis) are investigated and optimized. Finally, the definition of the post-synthesis thermal treatment temperature is described.

Titania (Ti-TiO_2) and titania/hydroxyapatite ($\text{Ti-TiO}_2\text{-HA}$) coated materials with different superficial features (machined, sandblasted, and sandblasted/acid etched) are widely analyzed and characterized in Chapter 5. Firstly, the functionalized materials are investigated from a morphological, topographical, chemical and structural point of view. After that, the amount of HA deposited is evaluated by means of EDX and weighing experiments. Then, the evolution of the surface wettability with the time is discussed, as well as possible approaches in order to have durable high-energy surfaces. Specifically, the sample storing in water and the UV surface re-activation are taken into account. Functional materials characterizations such as ions release tests, tribocorrosion experiments, potentiodynamic polarization analyses, nanoindentation investigations, and qualitative tape test experiments are discussed. Finally, *in-vitro* acellular bioactivity and human osteoblast-like MG-63 viability are considered.

The main findings of this study are recapitulated in Chapter 6, while the possible future work is reported in Chapter 7. A discussion about the future scale-up of the composite material is also reported in Chapter 7.

Chapter 1

INTRODUCTION

1.1 Biomaterials

Biomaterials have been generally described as natural or manmade (synthetic) materials used to construct artificial organs and rehabilitation devices, or implants to replace natural body tissues. They are organic and/or inorganic biocompatible materials and are used in close or direct contact with the body [12].

Over the years, different definitions have been given to biomaterials [30]. During the II International Consensus Conference of Biomaterials, in Chester (UK, 1991) a biomaterial was defined as “A biomaterial is a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ or function of the body” [31]. It is evident that this definition identifies the biomaterials not in relation to their properties, but to the intended use. Following this definition, for example, polyethylene is not a biomaterial when it is used to produce food containers, but it is a biomaterial when it is used in the hip prosthesis.

In general, biomaterials are used to build medical devices specifically designed to perform certain functions in the body. They can be utilized for various applications in a myriad of areas of the human body. The best-known applications in human are, for example, intraocular lenses, hip, knee, and ankle prosthesis, heart valves, and catheters (Figure 1.1).

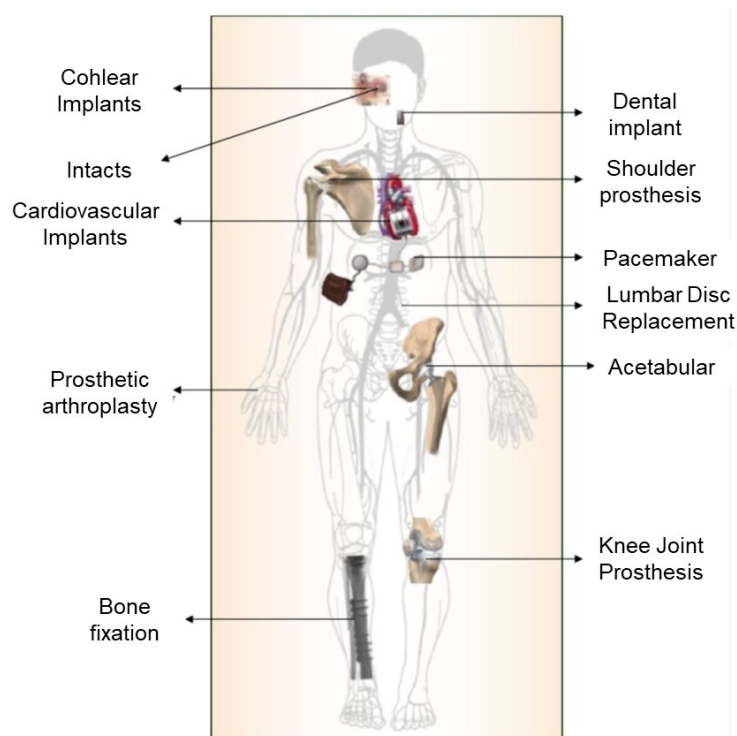


Figure 1.1 Applications of biomaterials in humans [32].

However, the surgical equipment, the materials used in bio-machines, and the devices for controlled drug delivery are also examples of the use of biomaterials. Biomaterials can be used either in permanent implants or prostheses, but also in devices that are in contact with the human body for a limited period (for example, single-use devices employed during surgery). Additionally, some medical devices can be used for any patients who need them, while others must be made for the individual clinical case, greatly increasing the difficulty in the material choice and design.

The field of biomaterials is not new; even if the word 'biomaterial' was established in the mid-twentieth century, biomaterials have been in use from much earlier [33]. Since ancient times, physicians have exploited a wide range of materials to restore natural human functions. Egyptian, Greek, and Roman doctors used vegetable fibers and animal derivate materials to suture the wounds, wood for artificial prostheses and carved seashells and/or stones for dental replacement applications [2].

The use of metal as a biomaterial is much more recent: it can be dated back to the American Civil War (1861-1865) when metal screws were applied to fix fractured or injured limbs. With the beginning of the twentieth century, the medical science began to exploit the new materials put on the market by the industry as biomaterials (i.e. stainless steel, metal alloys, plastics, ceramics, etc.), although not again specifically designed for medical purposes. Consequently, these surgical procedures were generally unsuccessful due to the occurring of infections. For instance, the first artificial hearts contained high flexibility polymer developed for the manufacture of women's socks, while the first material used to produce breast implants was a polymer widely used for the manufacture of mattresses.

Later, with the growth of the demand for new devices designed to replace or integrate the normal functions of the human organism, the research on biomaterials began to take into account the complex mechanisms that regulate the interactions of different materials with biological tissues.

Nowadays, biomaterials are the result of a synergic collaboration between different academic disciplines (chemistry, medicine, pharmacology, biology, and engineering) and the industry.

The design and selection of a biomaterial, to be useful and assure its properties as long as it is required and without rejection, depend on its specific application [34]. In general, biomaterials should meet special requirements [30,33] and the principal characteristics are delineated below.

1) Biocompatibility

Biocompatibility is the only property that is fundamental for all biomaterials. It has been defined as *"the ability of a material to perform with an appropriate host response in a specific application"* [12]. In other words, the devices should not cause adverse reactions in the body and it should well integrate with the surrounding tissue.

The biocompatibility of the medical devices must be acquired with certainty before their commercialization and use. Biocompatibility tests are usually performed following published standards, such as the ISO 10993 [33] series or similar, and measure the level and duration of the adverse biological reaction [33]. The evaluation of the medical device's biological response is a practical approach to determine that the medical device in clinical practice does not introduce unreasonable harm to the patient or user [33].

2) No toxicity

A designed biomaterial should be no toxic, i.e. it should perform its functions without negatively affecting cells, organs, or even the whole organism. Substances that are unintentionally released by the biomaterial into the biologic system generally cause toxicity. Ideally, a biomaterial should not give off anything from its mass, unless it is specifically engineered to do so. In some cases, the toxicity of the designed biomaterials gives an advantage, so certain biomaterials are specifically designed to release the necessary amount of toxic substances (for example a "smart" drug delivery system that targets cancer cells and destroys them) [30,33].

3) Absence of foreign body reaction

“Foreign body reaction” refers to the reactions sequence that generates due to the presence of a foreign body in a living biological system [30]. These reactions differ in intensity and duration, depending upon the anatomical site involved. An understanding of how a foreign object alters the normal inflammatory reaction sequence is an important concern for the biomaterials scientist. A medical device should perform as intended and present no significant harm to the patient or user; however, there will be a chance to develop foreign body reaction, since any material other than the auto-developed bodily material is a foreign material. Hence, the biomaterial should show nil foreign body reaction.

4) Mechanical properties and performance

The biomaterial should have mechanical properties and performances corresponding to that of the organ/tissue in the living system where it will be used.

These requisites can be divided into three categories [30]:

- Mechanical performance. The mechanical features of a biomaterial depend on the function that the device has to perform and the site where it will be employed in the living body. For example, a dialysis membrane must be strong and flexible, but not elastomeric; while an articular cartilage substitute must be soft and elastomeric. A hip prosthesis must be strong and rigid, while a heart valve leaflet must be flexible and tough.
- Mechanical durability. It is the period that the biomaterial has to perform the indented purpose, remaining efficient. Some biomaterials must work for a limited period (a catheter may only have to perform its function for 3 days, a bone plate may carry out its function for 6 months or longer), while others need to work longer (a hip joint must work under heavy loads for more than 10 years).
- Physical properties. The physical properties of the biomaterial may also be carefully considered in order to perform specific tasks. The dialysis membrane must have a defined permeability, while the articular cup of the hip joint must have high lubricity. Clarity and refraction are, instead, necessary for the intraocular lens.

5) Manufacturing

It is linked with the ability to produce biomaterials with the characteristics required for their utilization. Packaging, sterilization, storage, distribution, quality control, and analysis must be faced from biomaterial industries [33]. In particular, the ability of the material to be sterilized is critical, since only through this procedure all the impurities and microorganisms are eliminated, so providing a material that is suitable for implantation [34].

Biomaterials can be classified according to the effects produced on the material itself as **biostable** (biomaterials that, once placed in the human body, do not undergo substantial chemical and/or physical transformations over time) or **biodegradable** (biomaterials that, once placed in the human body, undergo substantial chemical and/or physical transformations that cause them to disappear over time).

Otherwise, depending on the biomaterial/organism interaction, they can be classified as biotoxic, bioinert, bioresorbable and bioactive [35]. **Biotoxic** are the materials that cause adverse reactions by the organism; some alloys based on nickel, cadmium, vanadium belong to this class, as well as some carbon steels, carbides, and methyl methacrylates. The term **bioinert** refers to chemically and physically stable biomaterials, they do not release toxic substances and, once placed in the human body, have minimal interaction with the

surrounding tissue. Examples of these are stainless steel, titanium, alumina, zirconia, and ultra-high molecular weight polyethylene. **Bioresorbable** refers to a material that, after a certain period in contact with the human body, is degraded, solubilized or absorbed. They are of particular interest in clinical applications where it is not recommended the reoperation to remove the implant. Common examples of bioresorbable materials are tricalcium phosphate, polylactic acid, polyglycolic acid, and bioglass. The term **bioactive** concerns to materials that stimulate a specific biological response, favoring a direct biochemical interaction with the biological tissue, which can grow on the surface of the bioactive material itself. The performances of bioactive materials are largely attributable to their capacity to spontaneously produce an apatite layer when in contact with phosphate-containing physiological medium [36]. Examples of these materials are synthetic hydroxyapatite, glass-ceramic, and bioglass.

Based on their chemical nature, biomaterials are either synthetic (metallic, ceramics, polymers and composites) or nature-derived (e.g. plant-derived, tissue-derived) [33].

1.2 Dental implants

Teeth are important tools for the processing of food, but they also deeply influence speech, comfort, facial contour, and esthetics [1]. Loss of natural teeth due to age, injury or disease, is always a disabling event, affecting the quality of life, both for functional constraints and for psychological and social consequences [37].

Dental implantology is the field of dentistry that is concerned with the replacement of missing teeth with synthetic roots anchored to the jawbone. Before the advent of dental implantology, the replacement of missing teeth was made possible by means of fixed prostheses, which were secured to the teeth adjacent to those missing, unavoidably damaging them (dental bridges). When the number and/or the quality of natural teeth were not favorable to the construction of fixed prostheses, the replacement of missing teeth was done with partial or total removable prostheses (dentures). Nowadays, these devices are almost disappearing; using dental implantology it is not more necessary to compromise healthy teeth to replace missing teeth or to apply dentures or otherwise mobile artifacts.

There are four main types of dental implant designs used in clinical dentistry, including a subperiosteal form, blade form, ramus frame, and endosseous form [2]. However, endosseous implants (i.e. implant placed within the alveolar bone) are the most used in dentistry today [2].

A dental endosseous implant (Figure 1.2) is an artificial tooth root, similar in shape to a screw. It is placed into the jawbone and, bonding with natural bone, it becomes a sturdy base for supporting one or more artificial teeth (the crowns). A connector, known as abutment, is placed on top of the dental implant to hold and support the crowns. The crowns are custom-made to match natural teeth and can be cemented or screwed to the abutment [1]. Dental implants can replace a single tooth, several teeth, or support partial or full dentures (Figure 1.3). Endosseous dental implants (also called “root-form” dental implants) can be “screw-type” or “cylinder-shaped” (Figure 1.4). Screw-type is the most used and includes “conical screw dental implants” and “straight (parallel-walled) screw dental implants”. They can be placed in smaller sockets and efficiently transmit biting force to the bone. Cylinder-shape implants have a cylindrical profile, without screw threads and can be placed easily in the jawbone. However, they do not show sufficient primary stability because the surface area is smaller than that of screw-type implants.

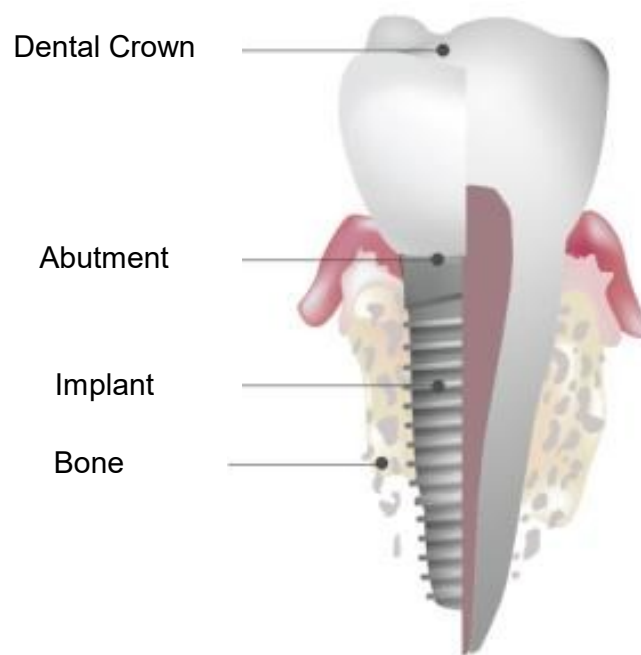


Figure 1.2 Schematic representation of a dental implant [37].



Figure 1.3 A dental implant can support a crown (a), a bridge (b) or a denture (c) [38].

**Cylinder
Dental Implant**



**Straight Screw
Dental Implant**



**Conical Screw
Dental Implant**



Figure 1.4 Types of endosseous dental implants [39].

1.3 Osseointegration

Replacing teeth with a bone-anchored device is not a new concept; to substitute successfully teeth and their supporting structures with artificial teeth has been an aspiration of humankind for centuries. The Mayan civilization has shown some of the earliest examples of dental implants. Archaeologists have found a fragment of a mandible of Mayan origin, dating from about 600 BC, which had three tooth-shaped pieces of shell placed into the sockets of three lower incisor teeth [2]. The basis of modern dental implantology is a biological process, called osseointegration. The most important pioneer of modern implant dentistry was Per-Ingvar Brånemark, a Swedish orthopedic surgeon, from the University of Gothenburg. In the early 1950s, Brånemark affixed a titanium implant chamber to a rabbit's bone to study the activity of blood flow in rabbits. Initially, it was Brånemark's intention to remove later the implant chamber from the rabbit. When he tried to do so, however, he found that the bone tissue surrounding the implant had integrated with the titanium chamber (i.e. it was impossible to remove it from the bone). Although the initial Brånemark purpose was to find out how blood flow aids in healing the bones, he realized that he may have found a way to create dental implants that could be tolerated by the human body for years [40]. Later, in 1965, Brånemark and his associates started clinical trials with titanium dental implants with great success. They proposed a "bone-anchored bridge" to treat edentulous mandibles. They placed the first Ti dental implants into a 34-year-old human patient with missing teeth due to severe chin and jaw deformities. They inserted four Ti fixtures into the patient's mandible and, several months later, they used these fixtures as the foundation for artificial teeth [2]. These dental implants served for more than 40 years, until the end of the patient's life [2]. In 1981, Brånemark and his co-workers coined the term "osseointegration," defined as direct contact between living bone and implant, without intervening fibrous encapsulation [41]. Based on these successes, Brånemark cofounded a company for the development and marketing of dental implants. Brånemark's discovery had a profound impact in dentistry and over 7 million Brånemark System implants have been placed and, currently, hundreds of other companies produce dental implants [2]. Several interconnected key-factors have been recognized as critical for the success of the osseointegration process [2,42]. These involve:

- 1) biocompatibility of the implant material (the compatibility of the material with the bone tissue and its ability to perform a specific function);
- 2) design of the implant (implant width, length, and geometry);
- 3) characteristics of the implant surface (macroscopic and microscopic nature of the implant surface);
- 4) condition of the receiving site (patient health and quality of the implant bed);
- 5) surgical technique;
- 6) loading conditions.

Even if the long-term performances of dental implants have been studied in many works [5,43,44], nowadays it is again difficult to give a uniquely common value to testify their success or nonsuccess. Generally, applied measures for the assessment of the clinical outcome of dental implants are the survival rate and success rate. While implant survival rate only refers to the physical presence of the implant in the mouth (i.e. whether the implant is still physically in the mouth or has been removed), regardless of the occurrence of complications [45], implant success rate requires also the absence of complications over the entire observation period [46,47]. However, survival and success rate are frequently improperly used, so that many times understanding and compare the different studies is not

easy [5]. Furthermore, the large number of existing valuation criteria for implant success as well as the lack of standardization limit the comparability of different studies [43,46,48]. Moraschini et al. [5], for example, have reported an average dental implants survival rates of 94.6% (with variation from 73.4% to 100%) for a follow-up period of 10 years; whereas the success rates vary from 34.9% to 100%, depending on the applied success criteria.

1.3.1 Implant stability and dental implant placement protocol

From the clinical point of view, an indirect indication of the success of the osseointegration process is the measure of dental implant stability. It can be measured at two different stages: primary and secondary [49]. Primary stability is associated with the absence of implant mobility upon insertion and mostly comes from the mechanical interaction of the implant with the surrounding bone. It is also called “mechanical stability” and principally depends on bone quantity and quality, implant geometry and the surgical technique procedure. The primary stability is necessary in order to have successful secondary stability. Secondary stability, also called “biological stability”, involves the healing process around the dental implant and happens through bone regeneration and remodeling phenomena at the implant/bone interface [50]. Primary stability, bone tissue formation, and implant surface features are the main parameters influencing secondary stability. Secondary stability defines the time of functional loading. In general, following the placement of endosseous dental implants, primary stability gradually decreases and biological stability gradually increases [50]. Patient-dependent implant stability and the pattern of implant loading must be carefully considered to have successful implantation [50].

The conventional dental implant placement protocol requires that the procedure be done in two stages [51]. In the first stage (“surgical stage”), the implant installation is performed and the osseointegration process occurs. During this period, the surgical site has to be undisturbed. After that, in the second stage (“prosthetic stage”), the prosthesis is molded, made and finally installed. Only after these stages, the implant is ready to function. According to the conventional dental implant placement protocol, there is a wait time of about 4÷6 months between the surgical stage and the prosthetic stage [49]. However, during this healing period, there is a significant reduction in the patient’s comfort and masticatory function. Consequently, over the years, the dental implant protocol proposed by Brånemark has been upgraded. Currently, thanks to the strong scientific research concerning the modification of the implant shape, superficial chemistry, and morphology with the aim to support and improve cell attachment, growth, and functionality, speedy loading procedures have also been developed and, in certain cases, implant installation and prosthetic activation within 48 hours could be applied [49,51]. Specifically, “immediate implant” loading is defined as loading within 24÷48 hours and “early implant” loading as within 14 days. These types of treatments permit to minimize the patient’s masticator function disability during the healing wait time. Currently, the overall success rate for immediate prosthesis healing of the edentulous patient is similar to that for the traditional two-stage method [51].

1.4 Materials used for dental implants

An ideal dental implant material should be biocompatible and, at the same time, have adequate toughness and strength; furthermore, it should ensure adequate corrosion, wear

and fracture resistance [52]. Lastly, it must be available, that is easy to fabricate and sterilize [1].

Dental implant materials can be classified based on the biological responses that they cause after implantation or according to their chemical composition [35,53]. Following the former classification, they can be classified as bioinert, bioactive or bioresorbable [35]. More often, materials used to manufacture dental implants are classified in function of their chemical composition in three primary groups: metals, ceramics, and polymers (Table 1.1).

Metals	titanium, titanium alloys, stainless steel, cobalt-chromium alloys, gold alloys, tantalum, zirconium, niobium
Ceramics	alumina, hydroxyapatite, beta-tricalcium phosphate, tetracalcium phosphate, fluorapatite, frushite, carbon (vitreous, pyrolytic), carbon-silicon, bioglass, zirconia, zirconia-toughened alumina
Polymers	polymethyl methacrylate, polytetrafluoroethylene, polyethylene, polysulfone, polyurethane, polyether ether ketone

Table 1.1 Classes of material used for the fabrication of endosseous dental implants [35,52,53].

Excellent combination of strength-to-weight ratio and resistance to corrosion in a physiological environment, easy processing, and low cost, and excellent biocompatibility have made titanium the first and the most widespread material used in modern dental implantology [1,8,12]. Furthermore, considering the favorable long-term survival rates reported for titanium (and its alloys) dental implants [52], it is still one of the most utilized materials for current dentistry. However, titanium sensitization has been found in few predisposed subjects [54] and, as a result, alternative materials to titanium have also been researched [55].

Other metals than titanium, such as gold, stainless steel, and cobalt-chromium alloys will not be discussed because they are currently obsolete due to their tendency to adverse tissue reactions and low success rate [52,53]. However, prosthetic components (such as abutment screws, prosthetic screws and various types of other attachments) can still be in gold alloys, stainless steel, cobalt-chromium, and nickel-chromium alloys. However, for these systems, implant failure is frequently due to galvanic processes which are activated when dissimilar metallic surfaces are placed in contact [53].

Since that the dental implants studied in this Ph.D. project are manufactured in titanium, the following part will mainly focus on titanium and its alloys; while only a short introduction on dental implant applications of polymers and ceramics will be carried out.

Polymers are softer and more flexible than ceramics and metals; they present lower mechanical strength, so mechanical fractures during function under high loading forces are more probable. Different polymers (ultrahigh molecular weight polyurethane, polyamide fibers, polymethylmethacrylate resin, polytetrafluoroethylene, and polyurethane) have been used as material for dental implant [53]; nevertheless, the lack of adhesion to living tissues, the inferior mechanical properties, and adverse immunologic responses have caused important limitations [53]. Nowadays, polymers are only used in the fabrication of “shock-absorbing” components, which are generally integrated into the supra-structures supported by the implants screw [53].

For dental implant applications, **ceramic** biomaterials can be classified as bioinert (e.g. zirconia and alumina) or, if they develop a chemical bond with the bone, as bioactive (e.g. hydroxyapatite, bioactive glasses, tricalcium phosphate) [53]. Bioinert ceramics can be used to realize the entire implant, while bioactive ceramic (due to their brittle nature and their solubility in physiological fluid) are generally applied in the form of a coating onto a metallic core to improve osseointegration [53]. The principal goal with bioactive coatings is to achieve strong adhesion between the coating and the metallic core, in order to withstand functional loading and, at the same time, avoid film detaching. Coatings can be dense or porous, depending on the coating method that is employed. Bioactive (ceramic) coating on titanium implant will be discussed later, in the section that analyzes the Ti surface modification methods.

Concerning bioinert ceramic, aluminum oxide (Al_2O_3) was the first ceramic material used for dental implants [55]. Even if it showed good osseointegration, it did not have sufficient mechanical properties for long-term loading [54].

Consequently, subsequently, new ceramic materials, such as zirconia, were introduced. Zirconia (ZrO_2) has a white tooth-like color, which mimics the natural teeth, a very important feature for the aesthetic outcome of the dental implant. Besides, zirconia has good mechanical properties [55], high biocompatibility and low plaque adhesion [55].

At ambient pressure, pure zirconia exists in three crystalline phases depending on temperature: Monoclinic (M), Cubic (C) and Tetragonal (T). The monoclinic phase is stable at room temperatures up to 1170°C , the tetragonal at temperatures of $1170\div 2370^\circ\text{C}$, and the cubic form over 2370°C [56]. The transformation T to M phase upon cooling is accompanied by a substantial increase in volume (about 4.5%), with a consequent material catastrophic failure. Conversely, alloying pure zirconia with stabilizing oxides permits to control the T to M transformation and to preserve the metastable tetragonal structure at room temperature, arresting crack propagation and leading to high toughness.

Yttria (Y_2O_3) is generally used as a stabilizer for maintaining the tetragonal phase of zirconia. Biomedical grade Zirconia usually contains 3 mol. % of yttria: the stabilizing Y^{3+} and the Zr^{4+} cations are randomly distributed over the cationic sites, and the electrical neutrality is achieved by the creation of oxygen vacancies [1]. Y_2O_3 -stabilized tetragonal zirconia (Y-TZP) has high strength and toughness. Dental implant surface modification procedures, such as sandblasting, can trigger a tetragonal to monoclinic transformation. This transformation (accompanied by an increase in volume) induces surface compressive stresses, thereby closing the crack tip and enhancing resistance to further crack propagation. This characteristic is known as “transformation toughening” and increases the fracture strength and fracture toughness of Y-TZP ceramics compared with other dental ceramics [52]. Furthermore, Y-TZP has biological responses that are similar to those induced by titanium. Therefore, it is frequently considered as a potential titanium alternative [55].

1.5 Ti and its alloys

Titanium is one of the most important metals in the industrial sector and, because of Brånemark's studies, it is the most commonly used in dental implantology [1]. This metal, compared to others used since ancient times (such as iron, copper, and bronze), was discovered only relatively recently, in 1791, in a Cornish valley by William Gregor. Titanium is the ninth most common element in the Earth's crust (about 0.6% in weight) and the fourth most abundant metallic element (after aluminum, iron, and magnesium).

The combination of high strength and fracture toughness, low weight, exceptional corrosion resistance, low modulus of elasticity and thermal expansion coefficient, non-magnetic properties, easy to process, high temperatures resistance and biocompatibility, make titanium and its alloy the preferred material for different engineering applications [1].

The first uses of titanium date back to the late 1930s and concern the military sector (tanks, airplanes, and submarines). The studies on its possible applications started in the period between the two world wars, but only after the Second World War titanium began to be used in the aeronautical and aerospace industry. Subsequently, thanks to excellent biocompatibility, titanium and titanium alloys were successfully used in medical surgery for the production of heart valves, bio-immersed devices such as pacemakers, hip joints, pins for fractured bones, hearing aids.

Pure titanium exhibits allotropy. At low temperatures, it occurs as a hexagonal close-packed (HCP) phase, designated as the α -phase. At high temperatures, it transforms into a body-centered cubic crystal (BCC) phase, known as β -phase. The temperature at which titanium switches from one crystal structure to the other is 882°C (β transus temperature) [1]. The β phase is stable from 882°C to the Ti melting point (1660°C). Alloying titanium with different elements allows for an improvement in titanium strength, high-temperature performance, creep resistance, response to aging heat treatments, and formability [1]. Some alloying elements can increase the α to β transformation temperature, while others decrease it. Al, O, N, C tend to stabilize the α phase (increasing the β transus temperature) and are called α -stabilizers; differently, V, Mo, Nb, Ta, Fe, Cr, Fe, W, Si, Co, Mn, H are named β -stabilizers and decrease the β transus temperature. Lastly, elements like Zr and Sn seem not to influence the stability of the α or β phases and only form solid solutions with titanium [1]. Ti alloys can be classified as α type and near α type, ($\alpha+\beta$) type, and β type and near β type.

According to the American Society for Testing and Materials (ASTM 2000), there are six distinct types of titanium used in implant dentistry: four grades of commercially pure titanium (cp-Ti) and two titanium alloys (Table 1.2) [1].

	O (wt. %)	C (wt. %)	Fe (wt. %)	H (wt. %)	N (wt. %)	Al (wt. %)	V (wt. %)	Ti (wt. %)
cp-Ti Grade I	0.18	0.10	0.02	0.015	0.03	-	-	Balance
cp-Ti Grade II	0.25	0.10	0.03	0.015	0.03	-	-	Balance
cp-Ti Grade III	0.35	0.10	0.03	0.015	0.03	-	-	Balance
cp-Ti Grade IV	0.40	0.10	0.05	0.015	0.03	-	-	Balance
Ti-6Al-4V	0.20	0.08	0.30	0.015	0.05	5.50-6.75	3.50-4.50	Balance
Ti-6Al-4V-ELI	0.13	0.08	0.10	0.012	0.05	5.5-6.50	3.50-4.50	Balance

Table 1.2 Grades I-IV of commercially pure titanium and two titanium alloys from ASTM Standard (ASTM 2000).

Grade I, Grade II, Grade III and Grade IV titanium are commercially pure titanium. Grade I-IV cp-Ti contains some trace elements that improve the mechanical properties of titanium [52] (with a slight reduction in osseointegrative performance) and are found in higher amounts from Grade I to Grade IV. Grade IV cp-Ti is the most common used. The two alloys are Ti-

6Al-4V and Ti-6Al-4V-ELI (extra low interstitial alloys). These are ($\alpha+\beta$) type alloys and contain 6% aluminum and 4% vanadium. Heat treatment of these alloys generates fine precipitation, resulting in enhanced yield strength and fatigue properties than pure titanium [1]. Conversely, the long-term performance of Ti-6Al-4V has raised some concerns due to the possible release of aluminum and/or vanadium. The mechanical properties of cp-Ti and of its alloys are reported in Table 1.3 [1].

	Elastic Modulus (MPa)	Yield Strength (MPa)	Tensile Strength (MPa)	Elongation at fracture(%)
cp-Ti Grade I	102	170	240	24
cp-Ti Grade II	102	275	345	20
cp-Ti Grade III	102	380	450	18
cp-Ti Grade IV	104	483	550	15
Ti-6Al-4V	113	860	930	10
Ti-6Al-4V (ELI)	113	795	860	10

Table 1.3 Mechanical properties of cp-Ti Grades I-IV and Ti-6Al-V alloys [1].

1.5.1 Drawback of titanium dental implants

Despite the high success rate of titanium dental implants, three principal drawbacks have been defined associated with the utilization of Ti-based implant materials [1], which could change the primary role of titanium in the next future.

The first problem is the aesthetic issue: the dark grey color of titanium could be evident when the soft mucosa is thin or when it recedes over time. Using white ceramic implants (such as zirconia) could preclude this problem. The second disadvantage is that titanium is not a completely bioinert material; even if this subject is not so far clear, high titanium concentrations have been found in the vicinity of oral implants [1] and titanium sensitization has been found in few predisposed subjects [54]. Finally, the progress in the bioceramic research and manufacture has allowed the production of increasingly advanced materials, strongly addressing the market also toward these new ones [1].

1.6 Titanium surface modification methods

The properties of titanium are of fundamental importance for the success of the dental replacement process. Besides this, also the surface features particularly condition the biological response that the medical device will produce. An essential surface feature of Ti is its capability to naturally form at room temperature a thin ($4\div 6$ nm thick) [57], stable, and amorphous TiO_2 layer under exposure to the atmosphere and/or physiological fluids [58]. However, despite its excellent biocompatibility, native titanium dioxide rarely chemically bonds to bone tissue after implantation [59]. Consequently, Ti is often considered as an inert biomaterial [59]. Furthermore, although this passive layer is widely described as a protective inter-layer, it is too thin and its chemical barrier action is not longtime effective under the action of body fluids. This makes the bulk material to undergo a slow but lasting metal ion

release in the neighboring tissues [60], especially at low pH and in the presence of fluoride (toothpaste for instance) [61]. Metal ion release may activate body defense mechanisms and influence cellular activity, so adverse reactions and even implant rejections may take place [60].

Starting from these considerations, to reduce unfavorable body reactions and, in the meantime, improve dental implants osseointegration, the role of surface regions (morphology, roughness, chemical composition, surface energy, residual stress, presence of impurities, and thickness of Ti oxide film) become essential [62]. Consequently, Ti surface modifications is crucial [1,2,11,12,62]. Table 1.4 shows some different dental implants, with diverse implant surface modifications, that are currently available for the dental implant market.

Two of the most important factors that affect the quality and the speed of the osseointegration process are the chemical and the physical nature of the surface of the implant [2]. A significant number of methods for the chemical and/or physical surface modification of Ti implants have been proposed [1,2,11,12,62], some of which are already used in clinical practice, while others are only on a preclinical level.

Surface	Producer	Type of surface modification
NanoTite™	BIOMET 3i	Calcium phosphate particles deposition by Discrete Crystalline Deposition (DCD™) on OSSEOTITE®
OSSEOTITE®	BIOMET 3i	Dual acid etching
Promote®	CAMLOG	Abrasive-blasting and acid etching
OsseoSpeed™	DENTSPLY Implants	TiO ₂ -blasting and HF acid etching
FRIADENT® plus	DENTSPLY Implants	Grit-blasting and acid etching, followed by a patented neutralizing technique
ITI® TPS	Institut Straumann	Titanium Plasma Spayed (TPS) surface
SLA®	Institut Straumann	Large-grit sandblasting and acid etching
SLActive®	Institut Straumann	Storage in NaCl solution to avoid hydrocarbon contamination of SLA® implants
Sustain®	Lifecore Biomedical Inc	Hydroxyapatite coated surface
TiUnite®	Nobel Biocare	Anodic oxidated surface
Brånemark System®	Nobel Biocare	Turned surface
MTX™	Zimmer Dental	Hydroxyapatite coated surface

Table 1.4 Example of dental implant surfaces available in the market.

Surface modification techniques can be roughly divided into two main groups: procedures targeted to enhance the surface roughness or bio-functional superficial coatings aimed to modify also the chemical surface composition [1].

1.6.1 Surface roughening

Surface roughness is related to the degree of roughness of the dental implant surface and the orientation of the superficial irregularities. The alteration of the implant surface roughness is one of the most commonly applied surface modification techniques to improve the osseointegration process and the bone-to-implant biomechanical fixation. Various methods have been developed in order to induce and control surface roughness [1].

Surface roughness can be divided, depending on the scale of the surface features, into macro-, micro-, and nano-sized. The macro-level is defined for the topographical features in the range of millimeters to tens of microns; this scale is related to implant geometry, with threaded screw and macroporous surface. Many studies [12,37] have shown a positive influence on the osseointegration process of micro-roughness implant: rougher surfaces, with larger exposed surface area, produce enhanced bone anchorage and biomechanical interlocking between implant and bone when compared to smoother ones. However, there is still no standard for the roughness of dental implants [2]. Innumerable investigations show an optimal roughness in the range of $1\div 10\text{ }\mu\text{m}$ [12]; however rougher surfaces may have theoretical clinical drawbacks, such as being more prone to marginal bone resorption (due to peri-implantitis) and/or increased ion release [63]. Conclusively, a moderate roughness of $1\div 2\text{ }\mu\text{m}$ may limit these two parameters [6]. A theoretical approach proposed that the ideal surface should have hemispherical pits about $1.5\text{ }\mu\text{m}$ depth and $4\text{ }\mu\text{m}$ in diameter [6]. Moreover, nano-roughness surfaces have shown a positive effect on the biological response [37], even though the exact mechanism remains again poorly understood. It seems that nano-featured surfaces possess higher surface energy, so influencing the surface wettability and therefore positively influencing the subsequent protein absorption and osteoblasts' adhesion and differentiation processes [37]. Yet, reproducible surface roughness in the nanometer range is again difficult to construct.

Various methods have been developed to alter the titanium surface roughness, so improving the osseointegration. The most common procedures are: titanium plasma spraying, blasting with ceramic particles, etching, and anodization [1].

Titanium plasma spraying (TPS)

It is an additive technique that allows the creation of rougher surfaces by the addition of titanium powders into a plasma torch at high temperatures. Titanium particles are so projected onto the implant surface, where they condense and merge together, forming thick films of about $10\div 40\text{ }\mu\text{m}$, with an average roughness of about $7\text{ }\mu\text{m}$ [1,2]. To obtain uniform plasma sprayed films, the thickness of the coatings must reach $40\div 50\text{ }\mu\text{m}$ [6]. The thickness of the plasma spraying coating depends on many factors such as the particle size and speed, the duration and temperature of the procedure, and the distance from the nozzle tip to the surface [2]. Ti plasma spray coated implants have shown increased mechanical anchorage and fixation to the bone [1]. On the other side, some studies have shown that these coatings are partially unstable: titanium particles have been found in the bone adjacent to the implant site and in the neighboring organs [1].

Grit blasting

Another method to increase the rough of the titanium surface is grit blasting. It is a subtractive mechanical process in which hard particles (hydroxyapatite, alumina, or titania) are projected, through a nozzle at high velocity, onto the implant surface [1,2,12]. The final

implant roughness is of micro-level and depends on particle size and material, time of blasting, pressure, and distance from the particle source to the implant surface. Ideally, the blasting material should be chemically stable, biocompatible and should not hinder the osseointegration process.

Grit blasted implants have shown high levels of osteoblasts adhesion, proliferation and differentiation [2]. Other studies have instead reported that high roughness grit blasted implant increased the mechanical fixation of titanium dental implants to bone [1] (the torque force increased), while only comparable values in new bone apposition have been observed. Contrariwise, blasting particles are often left on the implant surface [2], thus changing the implant surface composition and biocompatibility. Alumina is a common blasting material, yielding a surface roughness that depends on the size of the blasting media [1]. Unfortunately, alumina residues often remain also after the cleaning procedures (alumina is insoluble in acid, so it is particularly hard to remove), thus blasting material is frequently embedded into the implant surface. These residues must be considerably taken into account because they could be released into the surrounding tissues (therefore negatively interfering with the osseointegration process) or create chemical heterogeneity that decreases the titanium corrosion resistance [1].

An alternative to alumina is titanium dioxide (TiO_2), which determines a moderate roughness (i.e. 25 μm titania particles produce 1÷2 μm roughness), but it has no toxicity problems. An experimental study of Ivanoff et al. [64] has shown significant improvement for bone-to-implant contact (BIC) for TiO_2 blasted implants in comparison with machined ones. Other studies [1] on titania blasted titanium implants have shown high success rates up to 10 years after implantation.

To realize at the same time clean and textured Ti surfaces, biocompatible, osseointegrative and resorbable calcium phosphates (hydroxyapatite and/or β -tricalcium phosphate) have also been used as blasting materials. These surfaces showed higher BIC when compared to untreated machined surfaces, while comparable BIC values were found when contrasted with the common alumina or titania blasted surfaces (even if lower final roughness was obtained with calcium phosphates particles) [12].

Etching

The acid etching procedure is a subtractive chemical treatment used to clean the surface and, together, modify the roughness. It mainly consists of the erosion of metal surfaces by acid attack, producing isotropic surfaces with micro pits, with sizes ranging from 0.5 to 2 μm [2]. Acid solutions containing hydrofluoric, nitric, hydrochloric and sulfuric acids in different concentrations are commonly used. The result of the chemical attack depends on acid composition and concentration, process duration and temperature [2]. These methods, performed on machined titanium surfaces, have shown increased cell adhesion and bone formation, thus enhancing the osseointegration [11]. Notwithstanding, acid etching procedures might reduce the mechanical properties of titanium due to the incorporation of hydrogen (hydrogen embrittlement), both dissolved in the matrix and forming hydrides [65]. The type of acid, its concentration, the temperature, and the duration of the acid attack procedure are factors that influence the degree of hydrogen incorporated. Lin et al. [65] proposed that higher temperatures and shorter etching times are ideal to get a homogeneous surface and decrease hydrogen embrittlement.

A common variation of the technique is the Dual Acid Etching (DAE), where the Ti surfaces are chemically treated with acids in sequence or with their combination [11]. Examples of the acid mixture used are $\text{HCl}/\text{H}_2\text{SO}_4$ or HF/HNO_3 . For example, micro-rough Ti surfaces have

been produced after immersion for several minutes of titanium implants in a mixture of HCl and H₂SO₄, heated above 100°C (dual acid etching). It has been shown that this type of surface promotes rapid osseointegration [1].

Acid etching could be used alone or combined with other treatments. Often, acid etching treatments are carried out after a blasting phase, in order to remove blasting damaged zones or blasting embedded material, and also to refine the final surface roughness. After blasting, the surface reactivity is different from that of untreated titanium, so various modifications in final roughness can be achieved. SLA surfaces (sandblasted large grit and acid etched) are blasted with large-grit sand particles and then acid etched, so surface topographies of different scales are produced (macro roughness and micro pits) [1,11]. SLA implants have shown superior quality in several *in-vitro* and *in-vivo* investigations [1].

Micro-rough titanium dental implants have also been produced by treating titanium implants with fluoride solutions [6]. Titanium is very reactive to fluoride ions, forming soluble TiF₄ species. This chemical process produced a micro-roughness surface, but also the fluoride incorporation in the surface, enhancing osteoblast differentiation in comparison with untreated Ti samples [6]. Furthermore, fluoridated rough implants have shown a significantly higher torque removal than that of the control implants [6].

The chemical treatment of Ti implants can also be performed with alkaline solutions (such as NaOH or other alkaline concentrated aqueous solution) [66]. The high pH existing during the alkaline attacks induces before the formation of -OH groups on the native TiO₂ superficial layer and then the formation of titanate salts. These salts form and dissolve so that the surface is progressively covered by a hydrogel. It is reported [12] that amorphous sodium titanate hydrogel, obtained by exposing pure titanium into alkaline solutions and subsequent heat treatment, would also induce apatite formation when soaked in simulated body fluid. Usually, this process is carried out at high temperatures (more than 100°C) and a sub-micrometric roughness is produced.

Anodization

Anodization is another usual Ti surface modification technique. It consists of an electrochemical process where the implant is the anode electrode and it is immersed in an electrolytic solution with strong acids (often H₂SO₄, H₃PO₄, HNO₃, HF). High potential (100 V) is applied to the electrical circuit [1]. This process thickens the titanium native oxide layer, which grows from the usual 5÷10 nm to more than 1000 nm [6]. The anodization process depends on various parameters such as current density, concentration, and composition of acids, and electrolyte temperature [6]. When strong acids are used in an electrolyte solution, the oxide layer will be dissolved along current convection lines and thickened in other regions. The dissolution of the oxide layer along the current convection lines creates micro- or nano-pores on the native titanium surface [6].

Anodization changes both the microstructure and the crystallinity of the titanium oxide layer [6]; anodized surfaces have shown higher clinical success rates [6] and better implant to bone fixation in comparison with turned titanium surfaces of similar shapes [6].

1.6.2 Surface coatings

Besides the modification of the surface roughness, also the chemical composition of the outermost surface layers influences the implant-tissue interactions since the surface chemistry and charge can affect proteins adsorption and cell attachment, proliferation, and

differentiation [1,2,12]. In literature, many procedures for the implant chemical surface modification have been investigated, with the foremost aim to turn a commonly bioinert surface into a bioactive one [1,6,12]. The most used approach to modify the chemistry of an implant surface is the deposition of bioactive coatings [1,2,12,67]. Coatings can be dense or porous, depending on the coating process; furthermore, these treatments might also improve the implant surface roughness, allowing a parallel enhancement in terms of biomechanical performances [12].

Above all, metal implants have been coated with layers of bioactive calcium phosphate ceramics, mainly composed of hydroxyapatite [12], thanks to its similarity to the inorganic component of bone and teeth [13–15]. Calcium phosphate is the common name of a family of minerals containing calcium cations (Ca^{2+}) and orthophosphate (PO_4^{3-}), metaphosphate (PO_3^-) or pyrophosphate ($\text{P}_2\text{O}_7^{4-}$) anions, and sometimes hydrogen (H^+) or hydroxide (OH^-) ions [14]. Calcium phosphate is the major inorganic constituent of bone (~ 60 wt. % of bone), and the main constituent of tooth enamel (~ 90 wt. %). If the Ca/P atomic ratio is between 1.5 and 1.67, the calcium phosphates are called apatites (e.g. hydroxyapatite or fluorapatite) [14]. It is reported that, following implantation, calcium and phosphate ions are released from the coating, therefore increasing the saturation of body fluids with respect to these ions and so inducing the precipitation onto the implant surface of a apatite layer [6], which might also contain endogenous proteins and serve as a matrix for osteogenic cell attachment and growth [6].

Thanks to the combination of the metal mechanical properties with the biological properties of calcium phosphates, the bone healing process, the implant to bone fixation and, therefore, the implant success rates are strongly improved with calcium phosphate coatings compared with uncoated implants [6,12].

Different methods, as following described, have been developed for depositing calcium phosphate coating on titanium implants [12,67].

Hydroxyapatite plasma spray coatings

As well as for the TPS procedure described above, hydroxyapatite particles are injected into a plasma torch at high temperature and projected on to the surface of the titanium, where they condense and fuse together, forming a film. Plasma spraying is the most commonly used technique for depositing calcium phosphates coatings, and it is the only chemical modification technique actually used in clinical practice. Plasma spraying treatment also induces an increased surface roughness, therefore hydroxyapatite coatings can combine enhanced biomechanical fixation with biochemical effects [12].

However, HA plasma spraying coating shows some disadvantages. First, to achieve complete coverage of the substrate material, the sprayed depositions must have a minimum thickness of about 50 μm . This results in relatively poor bonding strength between the plasma sprayed apatite coating and the substrate material [12]. To improve the mechanical retention of the coating, the implant surface is often pre-roughened. For HA plasma spraying coated implants, possible inflammatory reactions due to the failure of the implant-coating interface (although the coating is well-attached to the bone tissue) with consequent coating delamination and particles release have been reported [12]. Furthermore, due to the high energy involved during the plasma spraying procedure, uncontrollable changes in the composition and crystallinity of the initial calcium phosphate particles can also occur; and several calcium phosphate phases (such as tricalcium phosphates, tetracalcium phosphate, calcium oxide, and amorphous calcium phosphate) could be formed [6]. It follows that plasma-sprayed HA coatings are usually composed of large crystalline HA particles

embedded into a highly soluble amorphous calcium phosphate phase. The discrepancy in dissolution rate between the various CPC phases can further induce coating delamination, particle release and thus the clinical failure of the implants [6]. Lastly, the plasma-spraying technique is not very effective for coating tiny dental implants with a complex shape.

For all these reasons, the clinical use of plasma-sprayed HA coated dental implants is limited. To overcome these limitations several alternative coating procedures have been investigated. However, apart from the specific advantages or disadvantages of each procedure, detailed clinical experiments in order to extend the knowledge on the bone responses of these coated implant surfaces are again missing, so future studies are strongly recommended before their introduction into the everyday life.

Other techniques

Sol-gel

Sol-gel technique permits the deposition of porous and low density [12] bioactive coatings, with high chemical homogeneity and fine grain structure at low crystallization temperature. Calcium phosphate coatings are prepared by dipping the substrate in calcium (usually nitrate salts) and phosphorus gels [12]. Yet, the industrial use of sol-gel coating on dental implants is limited due to poor coating to substrate adhesion. To improve the adhesion strength, CPC coatings are frequently sintered at high temperatures and, depending upon the sintering temperatures, different calcium phosphates can be obtained [12].

Physical vapor deposition

Physical vapor deposition methods, such as ion plating and magnetron sputtering [12], have been used to deposit thin calcium phosphate layers onto titanium implants. Normally, the as-prepared coatings are amorphous, consequently, post-synthesis thermal treatments [12] are necessary; the main component of the annealed films is hydroxyapatite. However, the practical use of these procedures is restricted due to the expensive synthesis requirements (ultra-high vacuum systems and subsequent sintering steps at high temperatures) [12].

Electrophoretic deposition

It is a process based on the migration of precursor particles suspended in a colloidal solution towards the substrate surfaces. The deposition takes place in an electrochemical cell in which one of the electrodes is the substrate to be coated. When the potential is applied, the suspended coating particles are polarized and pulled toward the substrate (electrophoresis) to form the coating. Yet, at this point, the film is a powder agglomeration and must undergo additional thermal treatment to secure the bonds among the particles and between the coating and the substrate. Electrophoretic deposition enables uniform coatings, even on complex shape substrates, at high deposition rates [12]. Furthermore, it requires no expensive apparatus. Nevertheless, as a consequence of the post-sintering high temperatures, it is difficult to produce dense and crack-free coatings, and the adhesive strength of such layers is very low [12].

Ion implantation

Ion implantation is a clean, versatile, highly controllable, and reproducible technique to induce a superficial modification of a target material and, in the meantime, preserving its bulk structure and characteristics. It is performed at low-temperature and high-vacuum conditions. During an ion implantation process, previously selected ions at high velocities are accelerated into a solid surface, thereby changing its physical, chemical, and/or electrical

properties. The ions penetrate and remain in the material surface due to their high kinetic energy. Differently from other coating techniques, the implanted zone forms an integrated part of the material, thus avoiding the risk of material delamination and loss. Mechanical, chemical, electrochemical and biological benefits have been found in ion-implanted surfaces [1]. Different species (like Au, N, C, CO, and Ne) have been implanted in Ti–6Al–4V dental implants [1]. It has been reported [1] that CO ion implantation supported the osseointegration of the Ti–6Al–4V dental implants, thanks to a positive modification of the native oxide layer.

Cathodic deposition

It is an electrochemical method where hydroxyapatite is formed from an electrolyte solution containing calcium and phosphate ions. This process can be carried out under mild experimental conditions (i.e. relatively low temperature), so unwanted phase changes could be avoided [68]. It shows high controllability and conformability, so allowing the covering of complex shape substrate. It is possible to vary the calcium phosphate coating properties by regulating the pH, the current density and the agitation of the electrolyte solution. Post deposition thermal treatments are carried out to crystallize the desired crystalline phase [12]. Deposition can be carried out in acidic calcium phosphate solutions or in simulated body fluids [12]. Cathodic deposited nanocrystalline calcium phosphate coatings have shown good biological activity [12].

1.7 Recent achievements

Current trends to improve the short- and long-term osseointegration of titanium dental implants are: i) the modifications of titanium surface roughness at the nanoscale level for promoting protein adsorption and cell adhesion; ii) biomimetic calcium phosphate coatings for enhancing bone growth and iii) the incorporation of biological drugs for accelerating the bone healing process [1,6,12].

Surface roughness at the nanoscale level

As underlined, surface chemical composition and roughness of implant play a major role in the osseointegration process that follows implantation [1,2,12]. Most of the available implant surfaces have random surface topography, with structures ranging from nanometers to millimeters, thus implant/biological tissue interaction occurs at both length scales.

The interest of surface modifications at nanoscale level (in the range of 1÷100 nm in dimension) is that the conventional properties of a bulk materials are very different from those of a nanomaterial (e.g. increased number of atoms at the surface, presence of surface grain boundaries, enhanced surface energy and surface area, electron delocalization, etc.). Nanoscale surface modifications influence the chemical reactivity of the biomedical material and thus affect its interactions with the host tissue. Nanoscale surface changes can modify the wetting properties, lead to different protein adsorption, or influence the bone mineralization process. However, up to now, there are no standardized surfaces with repetitive topography at the nano-sized level, so the exact role of nano- and micro-metric features on the biological response is poorly understood [6,12]; as a consequence, the nanoscale level roughness modifications of the implant surface is an approach of current interest. Recent data support the role of nano-topography into the osseointegration process and suggest that the critical steps during the implant to bone fixation process can be facilitated by the nanoscale modification of the implant surface [1]. Lately, various

approaches have been developed and implemented in order to create nano-rough surfaces that can directly influence the biological performance of oral implants [1,6,69]. Most of these methodologies have used processing methods from the electronic industry such as lithography and surface laser-pitting [6]. Also, other physical and/or chemical approaches have also been taken into account [1], but their major limitation is that they frequently are random processes, so it is hard to control the uniformity and distribution of the surface modification.

Biomimetic coatings

The biomimetic coating technique is inspired by the natural process of biomineralization and results in more bioactive implants. It has been developed to try to overcome the drawbacks of the above-described coating procedures. This method involves the heterogeneous nucleation and growth of calcium phosphate apatite crystals (bone-like apatite) on the surface of the implant from Simulated Body Fluid (SBF), at physiological temperature and pH (37°C and 7.4, respectively) [12].

Generally, the process consists of an initial pre-treatment step of the Ti surface (alkali, acid or heat treatment) before the soaking phase in the SBF. After that, the implant is soaked in buffered SBF solution; typically the implants are soaked for about three weeks [70].

Taking into account the specific example of titanium and related alloys, Ti is initially treated with an aqueous solution of NaOH, at 60°C for 24 h, and then heated at 600°C [12]. The alkaline and heat treatment procedures result in a thin amorphous sodium titanate layer on the Ti surface. During SBF soaking, the sodium titanate layer allows the formation of Ti–OH groups on the Ti surface. After that, the Ti–OH groups interact with the local calcium ions from the SBF solution to form a layer of amorphous calcium titanate. As the calcium ions accumulate, the positively charged surface reacts with negatively charged phosphate ions, forming an amorphous calcium phosphate layer (ACP). In the final step, the ACP is converted into bone-like apatite [70].

The major problem with biomimetic coating using SBF is the slow kinetics of coating deposition. Consequently, in order to accelerate the process, several approaches have been proposed [12]. Using the above described cathodic electrochemical deposition technique, the biomimetic coating production is performed in an electrochemical cell, with a simulated body fluid solution and at neutral pH [6,12]. Different titanium surface modification treatments have been carried out before the soaking in SBF to form a bioactive surface on metal-based implants [12]. Other strategies have been developed to improve the kinetic of the biomimetic deposition process; these use higher strength SBF solutions (high concentrations of calcium and phosphate ions in an increasing pH solution) [12].

The osseointegration of titanium implants coated with biomimetic calcium phosphate has been investigated in preclinical studies; however, up to now, their osseointegration route has not again been compared with other surface treatments in preclinical models [1].

Biochemical treatments

To directly influence, control and guide the events that happen at the implant-bone interface, the surface of titanium dental implants could also be coated with bone-stimulating agents. As a result, bioactive surfaces are produced enriching the implant surface with specific biomolecules (i.e. growth factors and adhesive proteins), normally present on the cell membranes and/or extra-cellular matrix [66].

Growth factors are naturally occurring substances, important for regulating a variety of cellular processes. They are capable of stimulating cellular growth, proliferation, and

differentiation. Members of the transforming growth factor (TGF- β) superfamily, and in particular bone morphogenetic proteins (BMPs), TGF- β 1, platelet-derived growth factor (PDGF), and insulin-like growth factors (IGF-1 and 2) are some of the most promising candidates as bone formation inductors for dental implants [1]. However, due to their low chemical stability and solubility in the biological environment, and their high cost, the application of an entire protein is often difficult. An alternative approach, named “peptide-mimicry” [66], consists in the identification of stable, soluble and biologically active protein sequences (bioactive peptide), belonging to longer proteins, and then chemically synthesize them, at relatively low cost [66]. Lately, Arginine-Glycine-Aspartate (RGD) peptide and many other sequences have been studied [1,66]. RGD sequence (able to promote cell adhesion via integrin) is the minimal cell recognizable sequence in many adhesive proteins, therefore it is one of the most investigated bioactive peptide [66].

Concerning metals, three techniques can be considered to functionalize the metal surface:

- ✓ Physico-chemical absorption of the active peptide on the surface. It is the simplest approach: the metal is immersed in the bioactive peptide containing solution. With this approach, it is not possible to determine the right amount of bioactive peptide (which is of critical importance in controlling the interaction with biological tissue); furthermore, it is not feasible to ascertain the molecule distribution and, in some case, peptides are found away from the incorporation site.
- ✓ Covalent bond (direct or through spacer) of the active peptide on the surface. Peptides are covalently bound to the metal surface, exploiting the functionality already present into the surface. Usually reactive -OH groups are used, whose quantity can be eventually increased with different strategies (i.e. gold coating). This technique does not allow good control of the quantity of adsorbed peptides.
- ✓ Peptide inclusion into entrapment material. The active molecule is incorporated in an entrapment material, which acts as a coating material. Entrapment material can be simply impregnated or covalently bonded with the peptide. Using this approach, there is satisfactory control of the density of the peptide. Polylactic and polyglycolic acids and hydrogels are examples of biocompatible and bioresorbable entrapment material [66].

The surface of implants could also be loaded with molecules controlling the bone remodeling process. In this context, the incorporation of bone antiresorptive drugs, such as bisphosphonates, might be very relevant in clinical cases where the amount of bone is insufficient [6].

Chapter 2
COATINGS
DEPOSITION
PROCEDURES

2.1 Preface

By means of deposition processes, it is possible to achieve coated materials, which have a new surface with mechanical, electrical, thermal, optical, corrosive, aesthetic, etc. features different from those of the pristine substrates on which the coating was carried out. Based on the nature of the process, the methods employed for film deposition can be divided into two groups, i.e. chemical and physical methods (Figure 2.1).

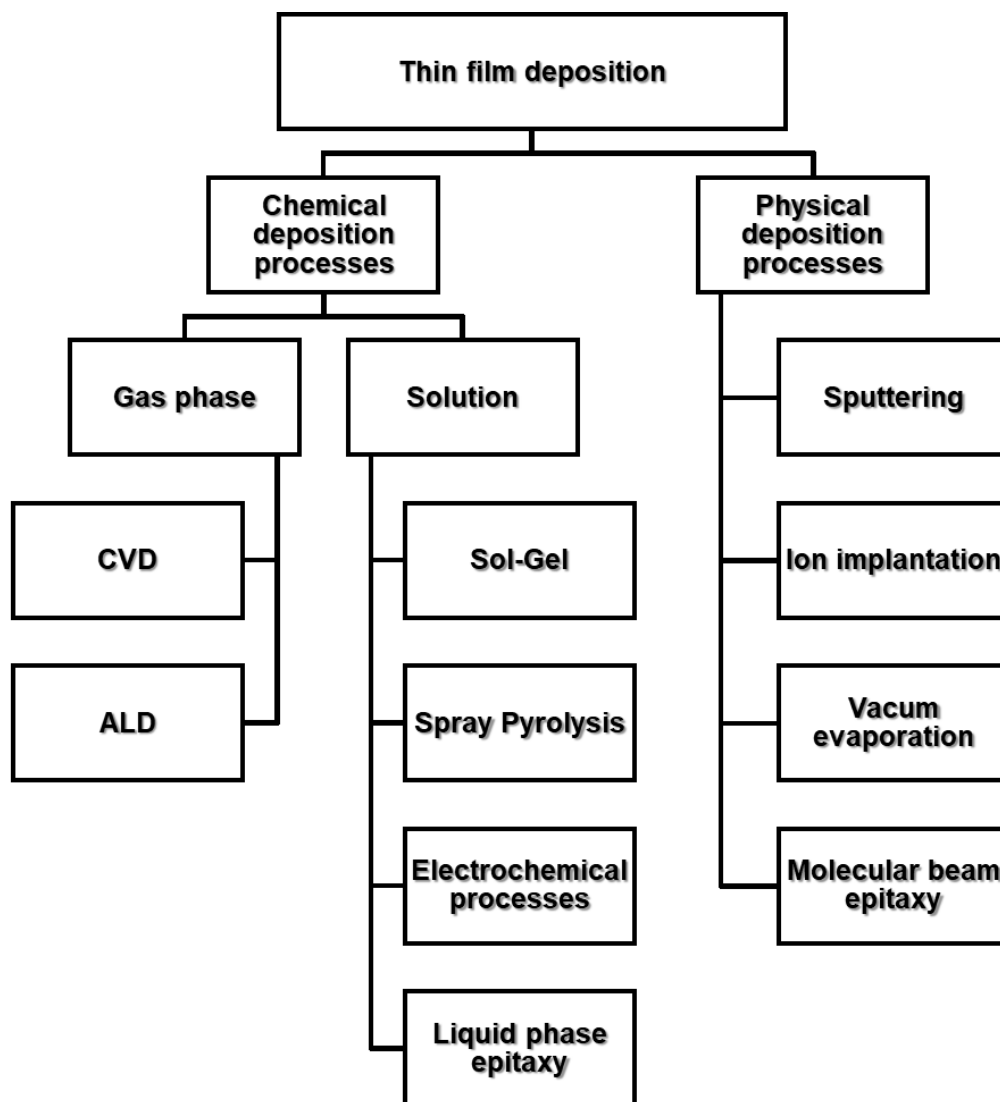


Figure 2.1 Principal chemical and physical thin film deposition processes.

Many of these deposition methods have been used to deposit titanium dioxide (TiO_2) and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) coatings [1,12,71]. Each deposition technique has its advantages and drawbacks, and produces films with various properties and characteristics, with different growth rates, costs, and efficiencies. In this Ph.D. thesis, TiO_2 thin films on titanium substrates were obtained by means of a Chemical Vapor Deposition technique (CVD), while the homogeneously spread and discontinuous calcium phosphate ceramic (mainly composed of hydroxyapatite) top-layers were deposited by means of a chemical deposition process from solution (spray pyrolysis).

2.2 Chemical vapor deposition technique

2.2.1 Introduction

Chemical vapor deposition is one of the mainly used deposition techniques for the production of innovative materials. The majority of its applications concerns the deposition of thin films, but it is also used in the production of ultra-pure materials both in the massive form (bulk) and as powders [72].

In its simplest form, the technique involves the vaporization of a solid or liquid precursor, which is then transported by an inert gas into the deposition chamber, where there is the substrate to be coated. The chemical reaction of vapor phase precursors (activated by simple heating or by other ways that will be mentioned later) takes place and allows the formation of stable solid products. The deposition involves homogeneous gas phase reactions, which occur in the gas phase, and/or heterogeneous chemical reactions, which occur near the vicinity of a heated surface, leading to the formation of powders or films, respectively. Gaseous reaction by-products and unreacted precursors are evacuated by the carrier gas flow.

One of the most important features of the CVD technique is its versatility. This method is one of the main processing approaches used to deposit amorphous, single-crystalline and, polycrystalline coatings for a wide range of applications [73]. Examples include [73]:

- semiconductors (Si, Ge, III–V, II–VI) for microelectronics, optoelectronics, energy conversion devices (solar cells);
- dielectrics (SiO_2 , AlN, Si_3N_4 , etc.) for microelectronics;
- metallic films (W, Pt, Mo, Al, Cu, etc.) for electronics;
- refractory ceramic materials (e.g. TiB_2 , SiC, B_4C , BN, TiN, Al_2O_3 , ZrO_2 , MoSi_2 , diamond, etc.) used for hard coatings, protection against wear, corrosion, oxidation, chemical reaction, thermal shock, neutron adsorption or as diffusion barriers;
- ceramic fibers (e.g. SiC and C) and ceramic matrix composites (e.g. SiC/SiC, SiC/C);
- ferroelectrics (e.g. PbTiO_3 , PbZrTiO_3);
- superconductors (e.g. $\text{YBa}_2\text{Cu}_3\text{O}_7$);
- perovskite materials (e.g. $\text{La}(\text{Sr})\text{MnO}_3$) for solid oxide fuel cell applications.

As well as there are a large number of materials that can be deposited using this technique, there are many variants of the CVD process. CVD methods can be categorized based on the experimental conditions, the activation mechanism and the nature of precursors. The more common acronyms used are defined below.

With respect to the activation mechanism, thermal CVD refers to processes where the reactions are promoted by heat, while Photo-Assisted CVD (PA-CVD) or Plasma-Enhanced CVD (PE-CVD) describe processes where high-frequency radiations or plasma activate the chemical reactions, respectively. A major advantage of PE-CVD, with respect to thermal CVD, is that deposition can occur at low temperatures, even close to room temperature, which allows temperature-sensitive substrates to be used. In Laser CVD method the use of laser sources can provide localized surface heating (i.e. without heating the whole bulk substrate) for selected area depositions.

Regarding the operative pressure, Low Pressure CVD (LP-CVD) processes are carried out at reduced pressure, while Atmospheric Pressure CVD (AP-CVD) refers to depositions carried out at atmospheric pressure. Concerning the nature of the precursors, Metal-Organic Chemical Vapor Deposition (MOCVD) and Organometallic Chemical Vapor Deposition

(OMCVD) are specific types of CVD that utilize metal-organic and organometallic precursors, respectively. Metal-Organic Vapor Phase Epitaxy (MOVPE) and Organometallic Vapor Phase Epitaxy (OMVPE) are CVD processes that produce single crystal (i.e. epitaxial) films (rather than polycrystalline or amorphous) on single crystal substrates. Atomic Layer Deposition (ALD) refers instead to processes in which gaseous precursors are introduced sequentially to the substrate surface and the reactor is purged with an inert gas between the precursor pulses, to deposit high-quality thin films with thicknesses down to a fraction of a monolayer.

In addition to the high versatility already mentioned above, the CVD technique has numerous advantages. The first, which is one of its strength points, is the possibility to deposit thin uniform layers over the entire surface of an object, regardless of its shape or size, and including the insides and underside parts. Therefore, this technique can also be used to uniformly coat complex-shaped components. Such distinctive feature outweighs the PVD process. The second advantage is the capability of producing highly pure materials. Furthermore, the CVD method produces uniform films with good reproducibility and adhesion at reasonably high deposition rates. This method does not require high vacuum conditions (as in the PVD process) and allows also to deposit at relatively low deposition temperatures (e.g. MOCVD), so permitting reasonable processing cost. Furthermore, it is also possible to modify the coating crystal structure, morphology, and orientation by controlling the CVD process parameters. Besides, CVD process can be easily scaled-up to an industrial level.

CVD disadvantages mainly concern with the nature and properties of the precursor. Ideally, the precursors need to be volatile at temperatures near room temperature. This is non-trivial for numerous elements in the periodic table, although the use of metal-organic precursors has eased this situation [72]. Furthermore, most of the suitable precursors are toxic, corrosive, flammable and/or explosive as well as very expensive. For example, many organometallic precursors used in OMCVD, such as $\text{Ni}(\text{CO})_4$, are toxic and can be a source of carbon contamination of the film due to their incomplete decomposition. Precursors, such as B_2H_6 , are explosive, while others are corrosive (like SiCl_4). Also, some precursors produce perilous reaction by-products such as CO , H_2 or HF , therefore it is necessary to adopt particular precautions. Beside the nature of the precursor, the temperature generally used (ranging from 200°C to 2000°C) greatly restricts the range of usable substrates. In addition to the substrate thermal stability, it is important that the deposited layer and the substrate have compatible thermal expansion coefficients to avoid dangerous stresses, which can be generated by the cooling process, and involve mechanical instability. Finally, with this technique, it is often difficult to deposit multi-component materials with well-controlled stoichiometry using multi-source precursors because different precursors have different vaporization rates. Furthermore, different precursors can have diverse thermal stability temperatures. However, these limitations can be overcome using single-source precursors.

2.2.2 CVD variants

As briefly describe before, there are many variants of the CVD process, each of which is characterized by a specific process and/or by a particular reactor. The choice of one method compared to another is mainly determined by:

- the type of the substrate;
- the chemical nature of the precursor;
- the desired morphology, thickness, and uniformity of the coating;

- the process costs.

Thermally activated CVD (thermal CVD) is a conventional CVD process in which the chemical reactions are initiated by thermal energy. Hot-wall (Figure 2.2a) or cold-wall (Figure 2.2b), horizontal or vertical reactors can be employed. The thermal energy can be supplied as RF heating, infrared radiation or resistive heating. Most of the thermal CVD techniques use hot-wall reactors, in which the entire deposition chamber is contained inside a furnace.

Hot-wall reactors (Figure 2.2a) can be very large, therefore permitting the simultaneous deposition on numerous substrates (consequently they are often used for industrial applications), meanwhile maintaining uniform temperatures and thicknesses of the deposited layer. A three-zone resistively heated furnace is commonly used to facilitate the temperature control of the outer zones, so letting a uniform temperature profile. Hot-wall reactors suffer from depletion of gaseous reactants as the distance from the tube inlet increases. As a result, the substrates often have to be positioned at 45 degrees with respect to the gaseous reactants flow [73]. However, despite their simplicity, the presence of the entire deposition chamber in the furnace involves some disadvantages: first, the energy spent to heat the whole apparatus is much larger than that which is necessary to heat only the substrates. Furthermore, the deposition could take place also on the walls of the reactor or in the gas phase (homogeneous nucleation), with consequent waste of precursor, need of frequent cleaning of the reactor and powders formation. Finally, in this configuration, the substrate can remain for a long time at high temperatures and this could involve its thermal deterioration.

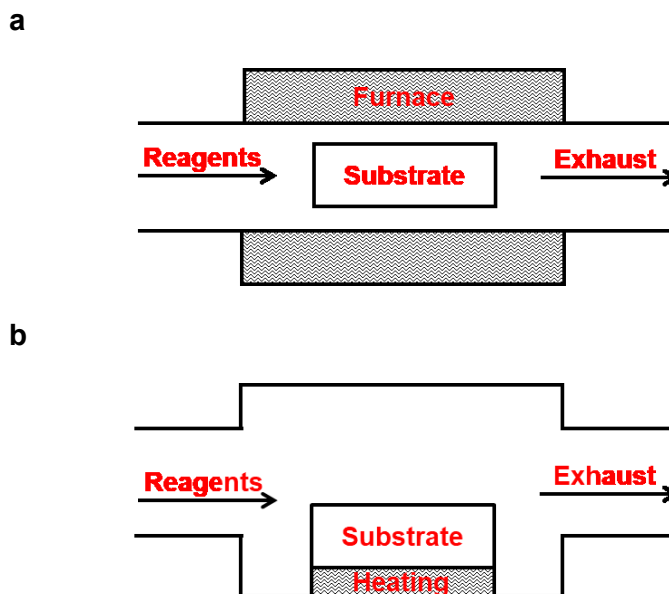


Figure 2.2 Horizontal hot-wall (a) and horizontal cold-wall (b) CVD reactors.

A large number of CVD techniques use cold-wall reactors (Figure 2.2b), in which only the substrate is heated, by irradiation, high-frequency induction or Joule effect. With these apparatuses, the depositions can be carried out at pressures ranging from tens of Pa up to atmospheric pressure. By means of cold-wall reactors, the problems of the hot-wall reactors are solved: the walls of the chamber are colder, therefore they get less dirty and less precursor is wasted, and the energy used is lower. The main disadvantages are: (i) the non-uniformity of the substrate temperature, which can lead to films with non-uniform

thicknesses, (ii) difficulties in the coating of complex shaped substrates due to problems in their heating.

Thermally activated CVD processes can be further subdivided according to the pressure range in which the deposition process occurs in Atmospheric Pressure CVD and Low Pressure CVD (operating from about 100 Pa to 1 Pa). In this context, it should be underlined that gas transport phenomena, which play a crucial role in the dynamics of the CVD process, strongly depends both on the type of reaction chamber and on the working pressure.

AP-CVD technique uses a high concentration of precursors, which can cause secondary reactions with the formation of particles in the vapor phase [74]. This event is undesirable for various reasons: first, it involves relatively high consumption of precursor, which is particularly disadvantageous due to the high cost of some reagents; secondly, the formed powder can deposit on the surface of the substrate, strongly compromising the quality of the deposited film. Additionally, at atmospheric pressure, the reagents must be diluted with inert gases in order to prevent gas phase reactions, while this is not necessary at low pressures.

At low pressures (< 100 Pa), the kinetic of the precursor reaction becomes the key-point of the process, while the gas mass transfer is less critical than that at atmospheric pressure. Reduced pressure conditions limit the side reactions in the gas phase; therefore, more uniform films are obtained, with better coverage and quality, and free from defects and impurities [75]. Moreover, at low pressure, the gaseous reagents have faster diffusion towards the substrate surface (the diffusion rate, in fact, is inversely proportional to the total pressure), therefore improving the growth rate.

Even if thermal CVD is the most extensively used procedure, as it was previously described, in some cases thermal CVD can be disadvantageous. One way of reducing the growth temperature is to use plasma-assisted or plasma-enhanced CVD technique. During the PE-CVD process, which operates at low pressures (from tenths to tens of Pa) and at low temperature (even close to room ambient temperature), an ionized gas (the plasma) is used to dissociate the precursor vapors into highly reactive molecules. These species activate the reactions and lead to the film formation on the surface of the substrate. This permits high deposition rates while keeping the temperature relatively low. Non-isothermal plasmas (glow discharge) are typically used in order to further reduce the deposition temperature: in these systems, the temperature of the electrons is much higher than that of the gas, and the inelastic collisions of the electrons with the precursor molecules form chemically active species. The main advantage of PE-CVD, over other CVD methods, is that operating at low temperatures it is possible to expand the range of available substrates to be coated, thus including those that suffer from thermal instability. Besides, the effects due to the different film-substrate thermal expansion are considerably reduced. On the other hand, the main disadvantage regards the poor quality of the deposited layer: there are often impurities (due to incomplete desorption of by-products and unreacted precursors) and damages of the growing film and/or substrate caused by the plasma. Finally, the film features strongly depend on several process parameters such as RF power and frequency, gas pressure, reagent flow rate, reactor geometry, etc.

Another method to supply energy during a CVD process is to use high-energy photons; photon-assisted and laser-assisted CVD are other categories of CVD processes. In these cases, light from either a laser or a high energy lamp is used to promote the film deposition. The photons can either be incident on the surface or only interact with the gas phase molecules. In both cases, the light can influence the chemical reaction either through simple heating of the gas and/or substrate surface or by electronic/molecular/substrate excitation.

Precisely, the photo-assisted CVD process involves the interaction of the light radiation with the precursor molecules, either in the gas phase or on the growth surface; UV radiation is commonly used for inorganic precursors. Photo-assisted CVD allows low deposition temperatures, thus minimizing dopant diffusion, defects, interlayer diffusion and thermal stress created with high-temperature processes. Conversely, the principal disadvantage is the low deposition rate, which partially limits its possibility of use. On the other hand, laser-assisted CVD is particularly advantageous to realize spatially resolved deposition, where selected area deposition or patterned films can be achieved by scanning a focused laser. Furthermore, the use of pulsed laser sources can provide localized surface heating, without heating the bulk substrate.

Metal-organic chemical vapor deposition is another CVD variant, which has been classified according to the nature of the precursor used (i.e. metal-organic precursors). Compounds containing metal atoms bonded to organic ligand are known as metal organics. In the strictest sense, an organometallic compound contains a direct metal-carbon bond (e.g. metal alkyls, metal carbonyls), while metal-organic precursors contain metal-O/N/P/S-R bonds, where R is the organic group (e.g. metal-alkoxides, metal- β -diketonates, metal alkylamides, etc). MOCVD can be used to deposit a wide range of materials in the form of amorphous, polycrystalline and epitaxial films. The MOCVD reactions occur mainly in a temperature range from 300°C to 800°C [76], and at pressures ranging from 100 Pa to atmospheric pressure. It is often advantageous to use very bulky ligands in the precursor, as this can increase its vapor pressure and render it less air/moisture sensitive [77]. With respect to metal halides (WF_6 , $TiCl_4$) or hydrides (SiH_4 , AsH_3), metal-organic precursors have lower decomposition or pyrolysis temperatures, allowing that MOCVD process can be performed at an inferior deposition temperature. Furthermore, inorganic precursors are often toxic, corrosive, and explosive or produce hazardous by-products. Metal-organic and organometallic compounds are usually more volatile than most of the inorganic precursors. However, metal-organic precursors tend to be very expensive compared to halides or hydrides and, in some cases, they are not commercially available. Therefore, they need to be synthesized specifically for certain applications.

In addition to the CVD variants described above, there are other variants less common but with good potentiality.

Hot filament CVD technique uses a filament (W or Ta) heated at high temperatures (typically 1500-2000°C) and pressure of 10^3 - 10^4 Pa to produce precursor dissociations into highly reactive radicals. Diamond films and carbon nano-horns have been synthesized using hot filament chemical vapor deposition. Although this is a simple and low-cost technique for the deposition of diamond films, the hot filament has a rather short lifetime because, unavoidably at high temperatures, it undergoes carburization and embrittlement (in a hydrocarbon-rich atmosphere). Moreover, the filament is prone to evaporate at elevated temperatures and contaminate the films, too.

In the combustion CVD process, the chemical precursors are initially dissolved in an organic combustible solvent. The resultant solution is atomized into submicron droplets, which are subsequently carried into a flame using an oxidizing gas. In the flame, the droplets undergo combustion and pyrolysis in order to deposit a thin film onto a substrate. Different and low cost inorganic materials, as well as complex and multilayer films, can be deposited on metallic, ceramic and polymeric substrates, with excellent control of stoichiometry.

Chemical vapor infiltration (CVI) is another variant of the CVD process, that works at low temperature and pressure, which is used to manufacture fiber-reinforced composite ceramic materials and in the densification of solids. During the CVI process, the gaseous precursors

initially have to diffuse and infiltrate through the porous structures. Then, the gaseous reactants undergo decomposition and chemical reactions to deposit the material.

A rather different variation of CVD is atomic layer deposition (ALD) and the dedicated version atomic layer epitaxy (ALE). In this modification of CVD, gaseous precursors (two, at least) are introduced alternately to the reaction chamber, where they reach on the substrate surface. A general ALD process consists of four steps (see Figure 2.3). The first step is the injection of the first precursor, part of it adsorbs to the available sites on the substrate surface by chemisorption. Then (second step) the excess of the first-step precursor and gaseous by-products are purged out from the reactor with inert gas, leaving only a monolayer of the precursors on the substrate surface. After that (third step), the second precursor is introduced into the reactor. It can react with the first precursor adsorbed on the substrate surface in a self-limiting manner to produce a monolayer of film and some gaseous by-products. Finally, the fourth step removes any excess of precursor molecules and by-products.

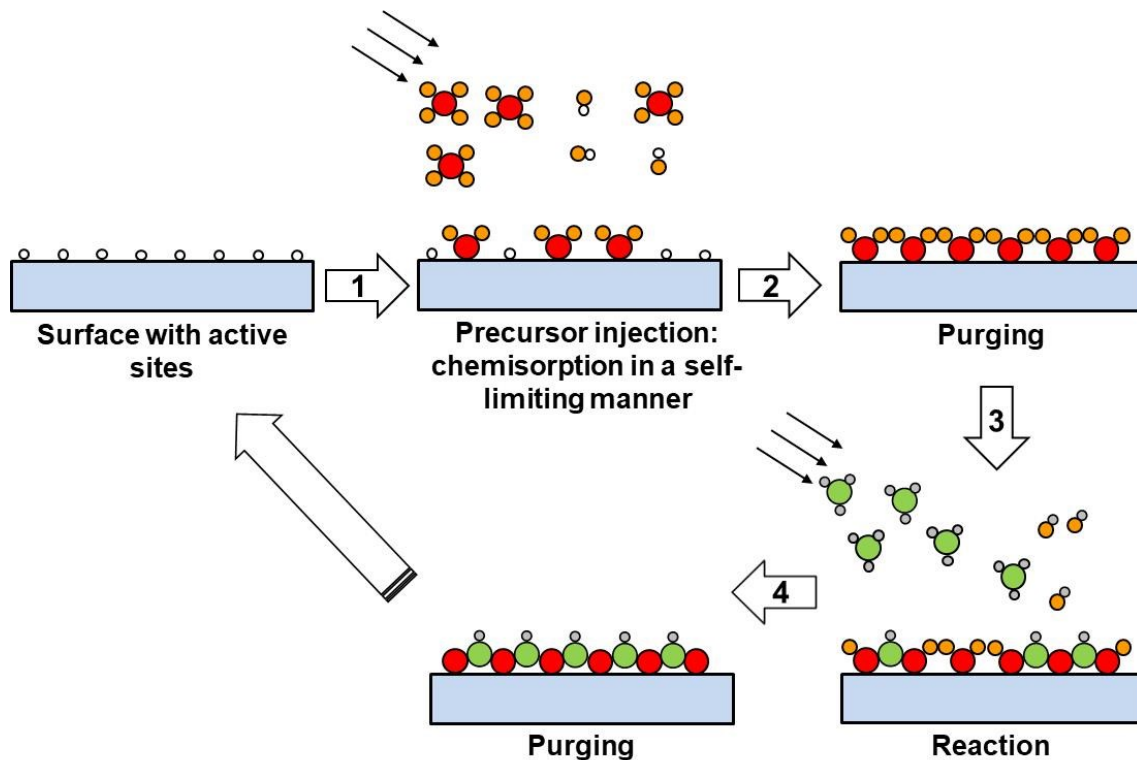


Figure 2.3 Schematic of a 4-step ALD system, with separate introductions of the two precursors and with the purging of the chamber between the precursors filling steps.

With these mechanisms, the film is formed by a full or partial monolayer of atoms per cycle: as a result, the thickness can be controlled simply by defining the number of cycles (at the angstrom level). This technique can grow homogeneous, high quality, dense and pinhole-free films on large surface area substrates with high conformality. Furthermore, it has good reproducibility and scaling-up ability, and low process temperature. While ALD has many promising features, it suffers from slow deposition rates and waste of precursor, and it requires vacuum systems, leading to high production costs.

2.2.3 CVD process

CVD process occurs through chemical reactions that include pyrolysis, oxidation, reduction, hydrolysis or a combination of these [73], and may also be catalyzed by the substrate. The type of chemical reaction often determines the operating temperature range required. Besides the reaction chemistry, CVD processes involve other disciplines such as thermodynamics, fluid dynamics, and deposition kinetics. In this section, a general overview of these aspects is given, but a more detailed discussion about the modeling of CVD processes is given elsewhere [73,77].

Thermodynamics of CVD

CVD is a non-equilibrium process. Thermodynamic calculations of the CVD process are useful to provide basic information about the achievability of the procedure (i.e. if the process is feasible or not), but they do not state how long the process will take. Thermodynamic can also provide guidelines in order to select the process conditions based on the equilibrium analysis of the phases and the amount of the species present in the CVD system. However, a more precise and accurate analysis would require also the consideration of the chemical kinetics and mass transport phenomena.

According to the thermodynamic approach, the desired CVD reaction will take place if the free energy change during the reaction, G_r , is negative.

$$G_r = G_{f(\text{products})} - G_{f(\text{reactants})} < 0 \quad (2.1)$$

Where $G_{f(\text{products})}$ is the products free energy and $G_{f(\text{reactants})}$ is the reagents free energy. G_r depends on the nature of products and reactants, as well as the gas phase composition, pressure, and temperature. The selection of the precursors based on this analysis constitutes an important topic in order to achieve the optimal and favorable growth mechanism. Complicated methods are commonly used for the calculation of G_r [76,78].

Fluid dynamics and deposition kinetics

The knowledge of fluid dynamics and deposition kinetics represents a key issue to control the composition, structure, and homogeneity of desired coatings and to design and build new reactor geometries for novel CVD processes and deposition strategies.

The analysis of a CVD process from a fluid dynamical point of view clearly represents an extremely complex problem, because many gas-phase and surface reactions are involved. In general, the process and the reactor design are targeted to obtain a laminar flow, which leads to the null velocity at the substrate surface. A significant feature is the presence of a hot layer of gas immediately above the substrate surface, called “boundary layer”, in which a composition and thermal gradient is generated. In this region, the flow velocity decreases rapidly to zero and mass transport only occurs by diffusion. The thickness of this layer is proportional to $\sqrt{(x/R_e)}$, where x is the distance from the inlet in flow direction and R_e represents the Reynolds number (it is a dimensionless parameter that characterizes the flow of a fluid) [76].

Generally, the CVD process involves different key-steps and Figure 2.4 summarizes the different stages that are assumed to follow each other [77]:

- 1) Evaporation of the reagents;

- 2) Transport of the reagents to the reaction chamber. This is a critical step since, to ensure composition uniformity of the deposited material, the precursor flow in proximity to the growth surface must be as constant and as uniform as possible;
- 3) **A.** Diffusion of reactants across the boundary layer and their adsorption to the substrate surface;
B. Homogeneous gas-phase reactions resulting in powdery products and gaseous by-products. This is an undesirable aspect because it induces the formation of powders, which compromise the film quality and cause the detrimental consumption of precursor;
- 4) Surface diffusion to growth sites;
- 5) Nucleation and surface chemical reactions leading to film formation (heterogeneous reaction);
- 6) Desorption of gaseous by-products and diffusion across boundary layer;
- 7) Removal of gaseous by-products from the reaction chamber.

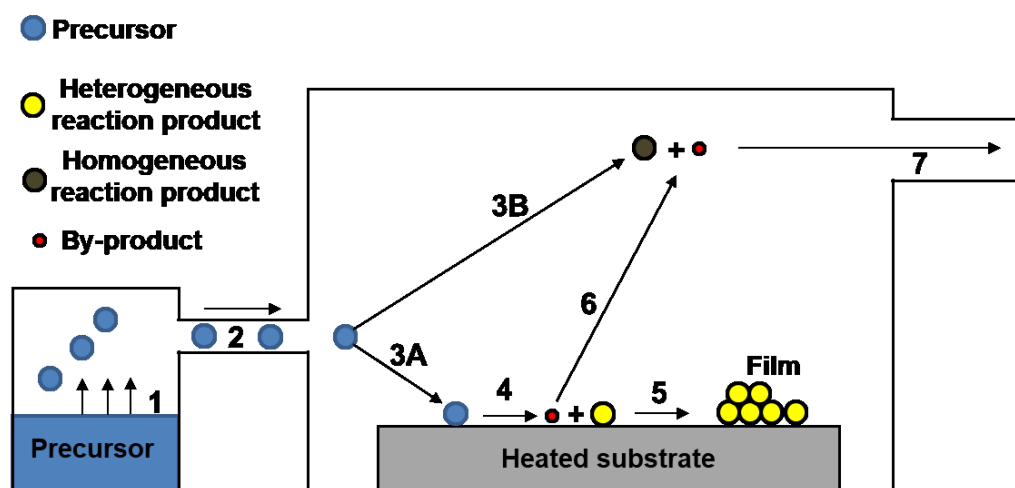


Figure 2.4 Schematic of the key-steps in a CVD process. Adapted from [77].

For the deposition of dense films, the process conditions are tailored in order to favor the heterogeneous reaction. Whereas, a combination of heterogeneous and homogenous gas phase reaction is preferred for the deposition of porous coatings.

As previously declared, in the conventional thermal CVD process, the film growth rate is determined by several parameters, the primary ones being:

- the temperature of the substrate,
- the operating pressure,
- the composition and the chemistry of the gas phase.

The temperature at which the coating is deposited is critical as it controls both the thermodynamics and the kinetics of the coating process. The dependence of film growth rate on substrate temperature is illustrated in Figure 2.5, where a plot of the log of growth rate vs. reciprocal temperature is reported. In the graph of Figure 2.5, three regions are evident.

At lower growth temperatures, the growth rate is controlled by the kinetics of the chemical reactions occurring either in the gas-phase or on the substrate surface. This region is the region of “kinetic growth control” and the film growth rate increases exponentially with substrate temperature, according to the Arrhenius equation (equation 2.2) [73]:

$$\text{Growth rate} \propto e^{-\frac{E_A}{RT}} \quad (2.2)$$

where E_A is the reaction activation energy, R is the gas constant and T is the temperature. In this situation, uniform film thickness can be achieved by minimizing temperature variations over the substrate surface. This region should be employed in hot-wall CVD reactors (thanks to the huge uniformity of the temperature, it is possible to obtain coatings with constant thickness).

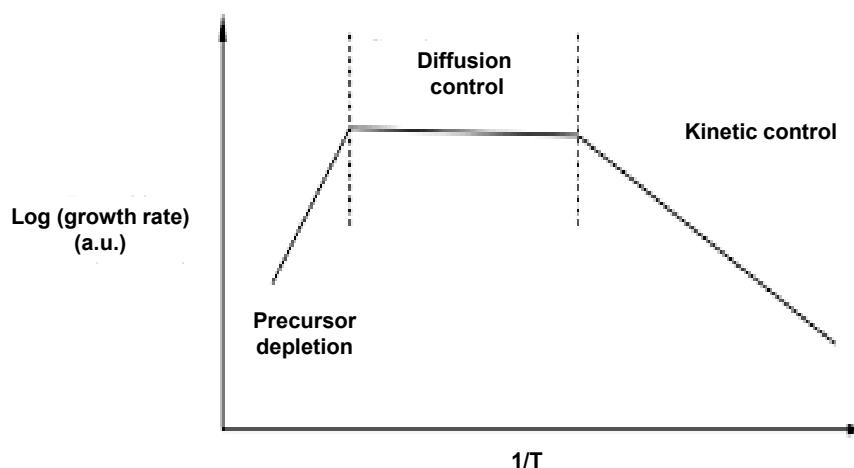


Figure 2.5. Dependence of film growth rate from the substrate temperature.

As the temperature increases, the growth rate becomes nearly independent from the temperature and it is controlled by the mass transport of the reagents, through the boundary layer, to the substrates. This second region is the “mass transport or diffusion controlled growth”. It should be employed in cold-wall CVD reactors, where only the substrate is heated and slight differences in temperature do not induce important alteration of the coating thickness.

Finally, at even higher temperatures, the growth rate tends to decrease, due to the precursor desorption from the substrate and/or depletion of reagents on the reactor walls due to parasitic gas phase reactions. Gas-phase reactions become increasingly important with increasing temperature and higher partial pressures of the reactants.

In addition to the temperature, the pressure of the CVD reactor is another crucial parameter, which determines the relative importance of each regime since the gas diffusion is inversely related to its pressure. From atmospheric pressure to intermediate pressures (~ 1000 Pa), gas phase reactions are important and a significant boundary layer is present. Kinetics and mass transport can both play a significant role in the deposition. As the pressure falls, gas phase reactions tend to become less important and, particularly at pressures below 100 Pa, the film growth is often controlled by surface reactions. At very low pressures (e.g. < 0.01 Pa) mass transport is absent and the layer growth is mostly controlled by the gas and substrate temperature and by the desorption of precursor from the substrate surface [77].

2.2.4 CVD Precursors

The choice of the CVD precursor is of fundamental importance for the control of the characteristics of the deposited film. The design and the synthesis of suitable precursors are

essential for the success of the vapor phase chemical deposition process. Early examples of CVD films were limited principally due to the lack of commercially available precursors. CVD depositions can be conducted using either single-source precursor (which contains all necessary components for the film growth) or multiple source precursors. Single-source precursors enable high control over film stoichiometry and generally decompose at lower temperatures; their use moreover allows a simplification of the precursor delivery system apparatus. Nevertheless, single-source precursors are higher molecular weight molecules (i.e. with low volatility) than multiple source ones, and the films are typically deposited with low crystallinity content. Differently, dual-source precursors typically employ commercially available species and generally result in low film impurities; however, inconsistencies between vaporization rates and deposition temperatures of various precursors typically result in nonstoichiometric films.

The selection of a specific precursor depends, in first, from the material to be deposited. However, an “ideal” CVD precursor should have certain fundamental characteristics.

- ✓ Adequate volatility. This characteristic is required because the precursor is transferred to the substrate in the gaseous phase. The vapor pressure of the precursor must be appreciable in the selected operating conditions. With precursors that occur naturally in a gaseous state (e.g. silane, diborane, ammonia, etc.) volatility is not a problem. For liquid or solid precursors, volatility can be a problem and, often, it has to be enhanced. The use of liquid precursors allows very efficient control of the precursor flow rate and guarantees its constancy over time, thanks to a constant evaporation surface. This does not happen generally with solid precursors, because the sublimation rate of a solid depends on the solid surface and the size of its grains (consequently it can change during the process). The chemical composition, as well as the molecular structure of the precursor, are decisive for its volatility. Generally, in order to improve the volatility, it should be chosen precursors with the lowest molecular weight compatibly with the need for a high steric hindrance and with ligands that produce minimal intermolecular interactions (that might lead to dimer, oligomer or polymer formation).
- ✓ Thermal stability during storage, vaporization and vapor transport into the reaction chamber. The precursor should be sufficiently stable for long-term storage under ambient conditions (i.e. unaffected by air or moisture), and it should not undergo any appreciable decomposition at the evaporation temperatures necessary to achieve adequate gas phase transport. Furthermore, for safety reasons, it is also essential to ensure that the rapid thermal decomposition of a precursor does not occur by misuse or by accident, particularly when the precursor is manufactured and used in large quantities. The precursor thermal stability is a fundamental prerequisite for the detailed knowledge of the experimental conditions and their reproducibility. If this condition is not satisfied, it is possible to have unwanted precursor losses and different precursor decomposition paths, causing heterogeneity at different process levels.
- ✓ A sufficiently large temperature “window” between evaporation and decomposition temperature.
- ✓ High chemical purity or easiness of purification. The precursor purity strongly influences the film properties because unintentional impurities can seriously degrade the final material features. Nevertheless, unintentional dopants are generally introduced during the synthesis processes and, therefore, precursor purification should be considered [77].

- ✓ Clean decomposition without the incorporation of impurities in the coating. The decomposition of the precursor on the substrate must be clean and well defined, without any secondary reactions; furthermore, it should produce by-products that can be rapidly removed from the reactor, in order to prevent parasitic reactions with consequent contamination of the growing film. Film contamination is a common problem when metal-organic precursors are used and, consequently, often carbon is incorporated in the film. The level of carbon incorporation is strongly dependent on the molecular structure of the precursor. Carbon can be present as carbonate (in the presence of oxygen), carbide (in inert or reducing conditions) or as a ligand group not dissociated from the metal during precursor decomposition. In this regard, strong metal-ligands bonds should be avoided (even if with metal-organic species this is not simple). The problem can be solved by using highly labile ligands, which form complexes that are thermodynamically but not kinematically stable. In this way, the ligand is easily dissociated during the precursor decomposition. Furthermore, the metal chemical nature also plays an important role: the more the metal is noble (at the bottom and right part of the periodic table), the more the metal-ligand bond is labile. To summarize, the nature of the ligand and the type of metal-ligand bonds determinate and control the decomposition pathway. The conditions in which the problem of carbon contamination is minimized occur in a strongly oxidizing environment (presence of a sufficient O_2 partial pressure), in a strongly reducing atmosphere (presence of a sufficient H_2 partial pressure) or with the use of co-reagents that favor clean decomposition paths (carbon dioxide or water, for example, react with carbon to form carbon monoxide and hydrogen).
- ✓ Decomposition at a temperature below the melting temperature and phase transformation of the substrate.
- ✓ Good compatibility with co-precursors during the growth of complex materials.
- ✓ Readily manufactured in high yield and at low cost.
- ✓ Non-hazardous or with a low hazard risk (both for the precursor and for the by-products). It should have low toxicity, explosivity, and inflammability for the safety of handling and disposing of the unreacted precursor.
- ✓ Low environmental impact.

Actually, precursors rarely meet all the criteria.

2.2.5 The precursor used

Titanium tetraisopropoxide has been chosen in this study as the precursor to carry out the growth of TiO_2 thin films. It decomposes at a relatively low temperature and does not need additional oxygen atoms to produce stoichiometric TiO_2 by thermal decomposition.

Titanium tetraisopropoxide (Figure 2.6), TTIP, has the formula $Ti(OC_3H_7)_4$. Table 2.1 summarizes some of its physical properties. It is a liquid and colorless precursor (it becomes yellow if kept for some time at light) and must be kept in a controlled atmosphere, as it is sensitive to moisture (the moisture in the atmosphere induces its decomposition that will propagate throughout the entire precursor volume once started). Consequently, its handling has always been performed in a dry-box, i.e. in an inert atmosphere of nitrogen and practically in the absence of O_2 and H_2O .

TTIP can be easily found on the market (in our case it was purchased by Sigma-Aldrich), at a relatively low price (500 mL costs about 50 euros), and does not present particular storage

difficulties. It is irritating if inhaled or in contact with skin and eyes, thus caution is required in handling it.

It was found that during a CVD process TTIP decomposes at $T \leq 400^{\circ}\text{C}$ through the formation of acetone, isopropanol, and propene as by-products [79]

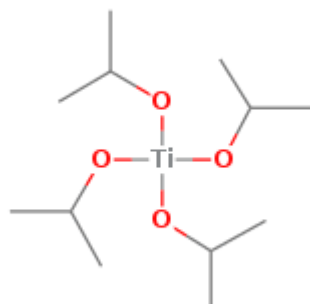


Figure 2.6 2D chemical structure of TTIP.

Physical Properties	TTIP
Molecular weight	284.26 u.m.a
Density	0.955 g/cm ³
Boiling point	232°C
Melting point	16-20°C
Vapor pressure at 25°C	20.71 Pa
Appearance	Colorless to light yellow liquid
Refractive index	1.46
Solubility in water	Decomposes

Table 2.1 Physical properties of TTIP.

2.2.6 Properties of CVD films

The main purpose of any deposited CVD film is to confer to the underlying 'raw' material specific superficial properties (such as mechanical, electrical, magnetic, optical or chemical, or even a combination of some of them). Independently from the type of the CVD layer, there are some peculiarities common to all the films deposited by CVD, listed below.

- **Thickness.** The thickness of the CVD film can vary within rather large limits: from a single layer of atoms up to a few hundred micrometers. Generally, the layer thickness is measured after deposition by optical techniques (interferometry and ellipsometry), microscopy, and mechanical or optical profilometer. The simple weighing of a sample before and after the deposition process can be a very accurate method in some circumstances (i.e. if the deposited layer is uniform and homogeneous, the film density is known, and the coated surface is wide to eliminate the influence of the edges).

- **Adhesion.** To achieve good adhesion, it is generally recognized that it is important to clean the substrate before deposition. Furthermore, improved adhesion can be achieved inducing the interdiffusion of substrate-coating elements (chemically induced adhesion). This new transitional layer has intermediate characteristics between the substrate and the coating, thus reducing the tensions caused by the mismatch of the crystallographic parameters, by different coefficients of thermal expansion or by microstructural defects. Adhesion can also be enhanced by improving the roughness of the surface of the substrate in order to have a better film-to-substrate interlocking (mechanical induced adhesion) or via post-synthesis thermal treatments. Homogeneous gas phase reactions can cause the formation of weakly adhered powdery deposits; while the depletion of gaseous precursors can cause differences in gas composition and therefore in coating thicknesses with stress concentration. The measurement of adhesion is generally made by scratch tests or (less sophisticated) qualitative tape tests.
- **Purity and composition.** The composition of a film strongly influences its properties, therefore small traces of impurities are often sufficient to cause significant changes in the film characteristics (this is, for example, the case of deposits used for microelectronic applications). These impurities may be present either in the gas flow or somewhere in the reactor: in such cases, once the problem is diagnosed, the remedies can be found easily. Differently, impurities deriving by diffusion from the substrate can contaminate the growing film (above all at high temperatures) and are more difficult to control. Layer purity and composition can be measured by Auger Electron Spectroscopy (AES), X-ray Photoelectron Spectroscopy (XPS), Rutherford Back Scattering (RBS), Energy Dispersive X-ray analyses (EDX) and Secondary Ion Mass Spectrometry (SIMS).

2.2.7 The microstructure of the deposited films

During a CVD process, thin film growth takes place inside the reactor exposing the substrate to the precursor vapors. The growth model and the relative film microstructure are determined, beyond the nature of the deposited material, by the precursor's diffusion at the substrate surface and by the nucleation processes. These processes are, in turn, influenced by the substrate temperature, pressure and gas phase composition.

The three basic models for thin film growth [80] are shown in Figure 2.7. In the Volmer-Weber or 3D island growth model (Figure 2.7a), small clusters nucleate on the substrate surface and grow in three-dimensional islands. This generally takes place when the interactions between the atoms/molecules of the film are stronger than those between the film and the substrate. This is often the growth model when film and substrate are composed of dissimilar materials, and polycrystalline thin films with rough surfaces are usually obtained. The opposite features of 3D island growth are displayed in the Frank-van der Merwe or layer-by-layer growth model (Figure 2.7b). It occurs when the atoms/molecules of the film are attracted more intensively to those of the substrate than to each other: first, a monolayer is formed and then the deposition of the second layer begins. This growth mode usually happens when the substrate and film are similar materials or some particular dissimilar materials. The intermediate mechanism is the Stranski-Krastanov growth (Figure 2.7c) which combines the features of the layer-by-layer growth and island growth. Thin films firstly grow two-dimensionally in layer-by-layer mode and then grow three-dimensionally in island mode.

The transition from a two-dimensional growth model to a three-dimensional one is not yet clear; it occurs at a critical layer thickness which is highly dependent on the chemical and physical properties (such as surface energies and lattice parameters) of the substrate and film. This sort of growth is quite common and has been observed for several metal-metal and metal-semiconductor systems.

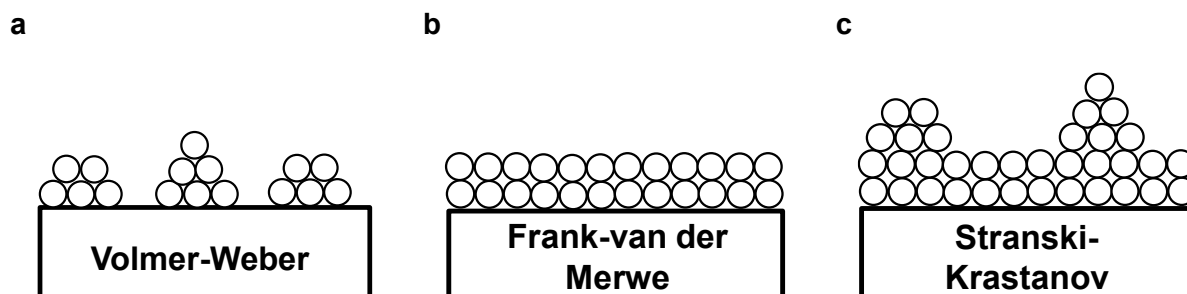


Figure 2.7 Three basic models proposed for the growth of thin films [80].

By varying temperature, pressure and gas composition, epitaxial, polycrystalline or amorphous films can be obtained via CVD. Epitaxial growth, i.e. the growth of a crystalline layer on a crystalline substrate of the same material (homoepitaxy) or different material (heteroepitaxy) [78], typically occurs at high temperatures and with low growth rates. In these conditions, the surface diffusion is fast with respect to the arrival of new reagents; so favoring the formation of a layer that replicates the underlying substrate. In order to facilitate such growth, it is important to have substrate surfaces free from contaminants and defects. The growth of epitaxial layers leads to high-quality materials and it is vastly used in the semiconductor industry. At the other extreme, i.e. at very high growth rates and low substrate temperatures, the surface diffusion is slow with respect to the speed at which the precursor reagents arrive at the substrate. Thus, a layer without a crystalline structure is often obtained, i.e. an amorphous film. However, if the amorphous film stays in a hot reactor for a long time, crystallization could also happen. At intermediate temperatures and growth rates, nucleation occurs at many different surface sites, leading to the growth of islands that will coalesce and form a polycrystalline layer. The control of polycrystalline growth is important in determining the properties of CVD films. Polycrystalline growth is facilitated by polycrystalline substrates where nucleation occurs at many different surface sites.

Basically, the structure of CVD materials is classified according to three different morphologies (Figure 2.8) [76]: columnar grains, which are capped by a dome-like top (Figure 2.8a), columnar grains with a more faceted and angular top (Figure 2.8b), and randomly orientated fine equiaxed grains (Figure 2.8c). Often, the film could include two or more types of these structures: this usually happens in thick deposits, in which a uniform structure is more difficult to obtain. Metallic deposits tend to have (a) or (b) microstructures, while most of the ceramic obtained via CVD (such as SiO_2 , Al_2O_3 , Si_3N_4) and dielectric materials tend to have (c) microstructure.

It is possible to control the film structure by the proper manipulation of the CVD deposition parameters (temperature, pressure, precursor supersaturation, and type of reaction). At low pressure, as well as at low temperatures and high supersaturation, the deposited structures tend to be fine-grained. The equiaxed structure is desirable for high mechanical strength and fracture toughness properties. At higher temperatures, films tend to be columnar as the result of the uninterrupted growth of the grains toward the source of the reagents. Columnar

structures are usually undesirable because of structural, chemical, and electrical anisotropy, and the rapid diffusion of impurities along the grain boundaries. It is possible to reduce columnar deposits by mechanical or chemical treatments at regular intervals to permit at the growing surface to re-nucleate [76].

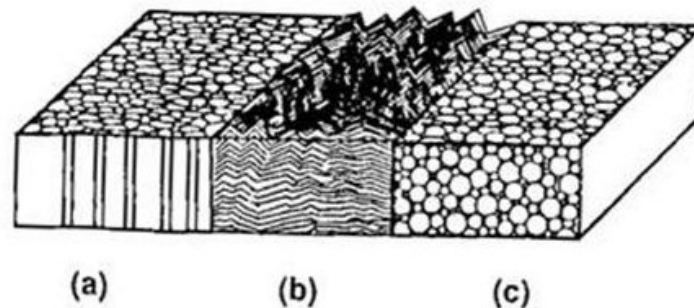


Figure 2.8 Schematic of structures obtained by CVD: (a) columnar grains with domed tops, (b) faceted columnar grains, (c) randomly orientated fine equiaxed grains[76].

2.2.8 Description of the MOCVD apparatus

Every CVD reactor can be tailored to produce specific coating materials or to cover substrates with particular geometry; furthermore, different apparatuses have been developed for R&D or commercial production purposes. In general, irrespective of all variations, all thermal CVD reactors have some common features:

- a) a precursor supply and gas handling system to control the input of gases or vapors to the reaction zone;
- b) a reaction zone;
- c) an exhaust system, which may include a vacuum pump for low-pressure operation and to remove waste products, and a waste treatment facility with any necessary waste monitoring devices.

The precursor supply system has to generate precursor vapors, while the gas handling system has to deliver the precursors vapor into the reactor. As a function of the type of the starting precursor material (i.e. solid, liquid or gas), different designs are possible. Solid precursors must be sublimated. Liquid precursors are kept in containers, commonly called “bubbler”, made of stainless steel, quartz or pyrex glass. Many precursors have very low vapor pressures at RT: thus bubblers are commonly held in temperature-controlled baths. When the precursor container is heated, also the inlet pipes that connect the bubbler to the reactor must be heated to avoid the precursor condensation. As a general rule, these pipes must be kept at about $10\div 20^{\circ}\text{C}$ above the bubbler temperature. Normally, the pipes are heated simply wrapping heating tapes around the lines. Precursors are transported, after vaporization, into the reactor by means of a carrier gas flow (commonly inert gases, such as Ar or N_2). These gases are often further purified to remove any oxygen, moisture or other contaminants picked up in the gas transfer lines before entering the reactor. A mass flow controller, upstream of the bubbler, controls the rate of precursor transport.

Another method (in addition to the temperature-controlled baths) to vaporize liquid precursors is the flash evaporation, where the liquid precursor is initially metered (with a syringe pump or peristaltic pump) and then instantaneously evaporated. Differently, in liquid

injection, the precursor is firstly dissolved in an inert solvent (ether or hydrocarbon). Subsequently, during the deposition process, the precursor solution (generally kept RT) is delivered at a controlled rate and quantity into a heated evaporator and then transported by a carrier gas into the reactor zone.

The second zone of a CVD apparatus is the reactor. It is used to heat the substrate to the deposition temperature and it mainly consists of a reaction chamber, a substrate holder and a heating system with a temperature controller. As described in 2.2.2 section ("CVD variants"), in a thermal CVD system, the reactor can be either hot-wall or cold-wall, horizontal or vertical.

The exhaust system serves to remove unreacted precursors and by-products from the reactor chamber and provides the required reduced pressure. The common elements in a laboratory-scale reactor are one or more particle filters, a pressure sensor to control the deposition pressure, a liquid nitrogen trap and a vacuum pump. CVD reactants and by-products are often corrosive, toxic, hygroscopic, inflammable and readily oxidizing. Furthermore, abrasion problems could also arise due to solid particles that form in the homogeneous gas phase reactions. There are several methods of effluent treatment. Unreacted precursors and corrosive by-products (such as HCl) are trapped using a liquid nitrogen trap, so preventing that these gases, entering the pump and damage it. Inflammable gases (such as H_2) or unreacted precursors could be burned off or made to chemically react to form stable species. Unreacted expensive precursors (e.g. BCl_3) could also be collected at the outlet of the apparatus and recycled. It is essential that this part of the reactor is efficient to render the substances harmless; often toxic gas monitors are required to be installed. Finally, low-pressure CVD systems necessitate a vacuum system (i.e. a corrosion-resistant rotary pump), very simpler as compared to those required in the PVD process.

2.2.9 MOCVD equipment here used

The MOCVD apparatus used during this Ph.D. project is shown in Figure 2.9. It is a low-pressure horizontal hot-wall reactor. It is a multiple purpose system and consists essentially of three parts: i) the precursor supply and gas handling system, ii) the reactor and iii) the exhausting system.

In the precursor supply and gas handling system the liquid precursors are evaporated and the gas flows are regulated. All the inlet pipes are equipped with mass flow meters connected to a mass-flow controller. Inlet pipes and connections carry the carrier gases and the precursor vapors to the entrance of the reactor. The here used reactor has four lines (Figure 2.10). Line 1 is used for the precursor and its carrier gas flow. Line 2 is employed for the dilution gas, such as N_2 or Ar. Line 3 serves for the O_2 flow. It is connected to a tank placed outside of the laboratory and allows the oxide film deposition. A separate O_2 line is necessary to ensure that the oxygen is not exposed to the precursor vapor before the reactor. Finally, Line 4 allows the use of a second precursor and its carrier flow.

Needle valves are placed upstream and downstream of each flow meter and the U-shaped pyrex tubes (bubbler) containing the precursor. Line 1 and Line 4 are also equipped with by-pass valves in order to be able to install or remove the bubbler, even while the system is operating. Heating baths (water or oil) are used to thermostat the precursors contained in the bubbler; stirred heating baths are normally preferred thanks to better heat exchange and reduced heating time. The pipes and the connections, especially those immediately after the bubblers, are wrapped by heating tapes to prevent the precursor condensation.

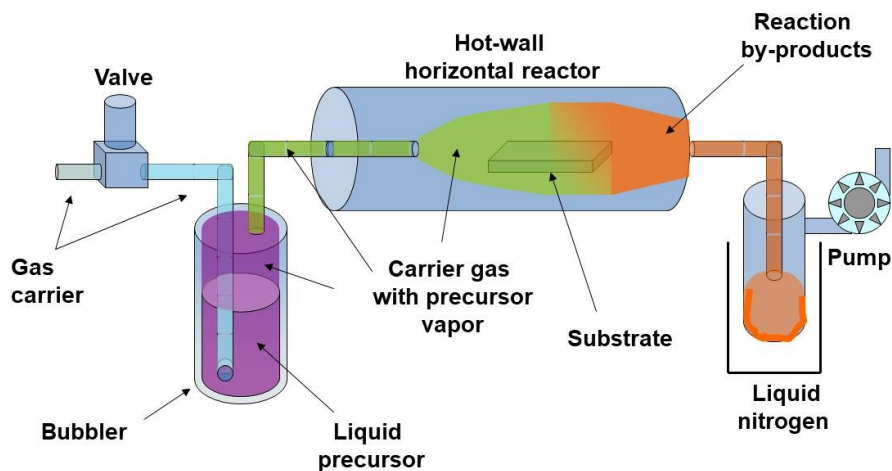
a**b**

Figure 2.9 Photo (a) and schematic representation (b) of the MOCVD apparatus used.

In this Ph.D. project, titanium tetraisopropoxide was used as the precursor for the growth of TiO_2 thin films. It does not need additional oxygen atoms to produce stoichiometric TiO_2 by thermal decomposition. Therefore, in this work, for the deposition of titania film only Line 1 and Line 2 were employed.

The reactor is equipped with a glass tube (resistant to 550°C) or a quartz tube (resistant to 1300°C), about 60 cm long and with a diameter of 18 inches. The deposition chamber is kept under vacuum by two Viton O-rings compressed between two steel flanges and the reactor tube. The substrates and the reactor are heated by an electric oven, equipped with a thermostatic system that allows the setting and control of the deposition temperature. A thermocouple is also located inside the furnace tube through which it is possible to check the actual substrate temperature, as well as the temperature fluctuations as the substrate position changes.

Downstream of the reactor there is the exhausting system. It consists of one output line, along which the following devices are positioned: i) a Baratron pressure gauge (it measures the pressure inside the reactor and it is able to provide accurate pressure measurements in the range $1 \div 10^4$ Pa); ii) a liquid nitrogen cooled trap (it prevents that exhausted products are

sucked into the pump or are released into the environment); iii) a Pirani pressure gauge (it measures the pressure downstream of the vacuum pump, pressure range: $10^{-1} \div 10^5$ Pa); and iv) a rotative pump (pumping rate 12 L/min) to keep the entire system under vacuum.

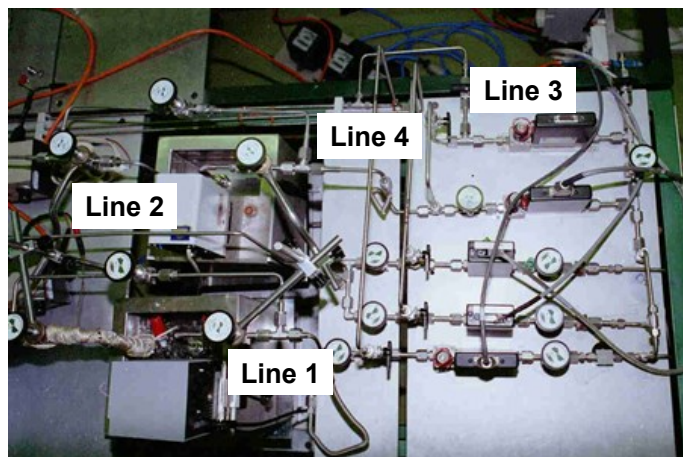


Figure 2.10 MOCVD reactor inlet lines.

2.3 Spray pyrolysis technique

2.3.1 Introduction

Spray pyrolysis, as shown in Figure 2.1, consists of spraying, through an atomizer, a precursor solution on a heated surface. Droplets hit the substrate surface and react to form the film. The morphology and the quality of the deposited films strongly depend on the substrate temperature.

Films via spray pyrolysis have been deposited on a variety of substrates (such as glass, ceramic or metal) and various devices such as solar cells, sensors, anti-reflection materials, and solid oxide fuel cells have been obtained [81]. Since the pioneering work of Chamberlin et al. [82] for the deposition of CdS and CdSe films, during the last decades researchers as well as industries have taken into account this method, as it is one of the most suitable processing techniques to get a large variety of materials such as thin or thick, dense or porous films, and powders. Nowadays it is widely used to synthesize a variety of metal oxides as well as binary and ternary chalcogenides. One of the most important features of the spray pyrolysis is that, differently from many other film deposition techniques, it is a simple and relatively cost-effective process, especially regarding the equipment cost. Typical spray pyrolysis apparatus consists of an atomizer, a precursor solution, a substrate heater, a temperature controller, an air compressor or other sources of carrier gas, and a rotameter (Figure 2.11). Different types of atomizers can be used, the most common are air blasts, ultrasonic atomizers, and electrostatic atomizers [81].

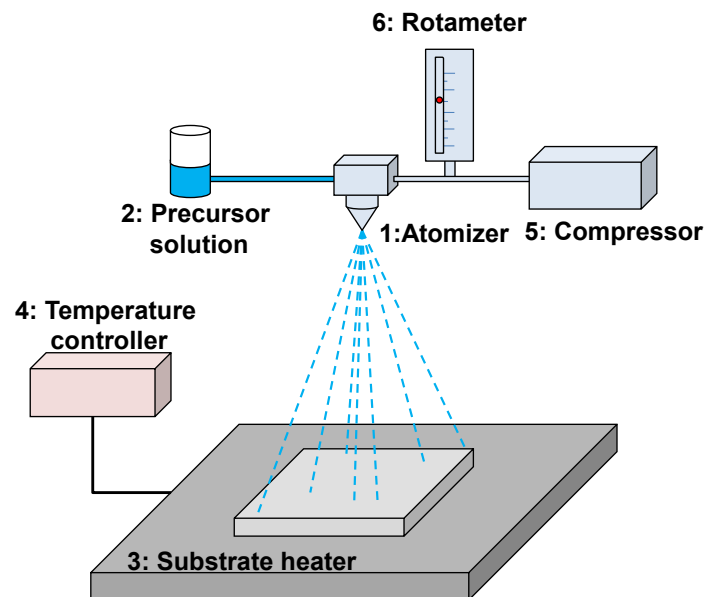


Figure 2.11 Representation of spray pyrolysis equipment: 1) atomizer, 2) precursor solution, 3) substrate heater, 4) temperature controller, 5) compressor, 6) rotameter.

Besides the method simplicity and low cost, the main advantages of spray pyrolysis over other techniques are summarized below.

- It is an easy way to prepare films of any chemical composition by simply controlling the reagents used in the precursor solution. It is possible to achieve doped films with practically any element in any proportion, simply adding it into the spray solution.

- It leads to relatively uniform and high-quality coatings. High purity films can be obtained depending only on the purity of the precursors' solutions.
- It does not require high-quality targets and/or substrates. There are ideally no restrictions to substrate materials (if compatible).
- Large-area or complex-geometry substrates can be coated.
- It does not necessitate of high vacuums; therefore, the process can be easily scaled up for industrial applications.
- No high temperatures are required during processing.
- Films with composition gradients or multilayers can be obtained by changing the composition of the precursor solutions during the process.
- The process is reproducible and available for mass production.
- The deposition rate and the film thickness can be easily controlled by changing the process parameters.
- Inexpensive precursors such as acetates, nitrates, and chlorides can be used.

Nevertheless, spray pyrolysis has some disadvantages. First, different variables influence the final products, so all at the same time must be considered. Furthermore, difficulties in the precise growth temperature determination and problems due to that, after long processing times, the spray nozzle may ruin partially, limit this method. Anyway, spray pyrolysis stimulating advantages make this method attractive and competitive compared to many other techniques used for materials synthesis.

2.3.2 Influence of Deposition Parameters on Thin Film Properties

The spray pyrolysis process involves a thermally activated chemical reaction between liquid or vapor precursors. It consists of spraying a targeted salt solution (generally aqueous) on a heated substrate. The precursor solution must contain the atoms of the desired coating. The sprayed droplets impact against the substrate surface, spread into disk-shaped structures and undergo thermal decomposition or pyrolysis to form the film, while the other volatile by-products and the solvent escape in the vapor phase. The film is usually composed by means of their overlapping. Different conditions mainly affect the drop impact characteristics against a solid surface: the fluid precursor properties, the kinematic parameters (drop velocity and diameter), and the substrate surface characteristics (surface energy, morphology, and temperature) [83]. Among them, the main process parameters that influence the structure and properties of the deposited films are the substrate temperature and the composition of the precursor solution [81]. However, the nature of the substrate, the gas flow rate, the deposition time, the solution amount and the nozzle to substrate distance are other important parameters that affect the spray pyrolysis process [84]. The type of substrate could also influence the film nucleation and growth, as well as the substrate porosity and roughness condition the film morphology. The film thickness depends (beyond the concentration of the precursor solution and, of course, the substrate temperature) on the distance between the spray nozzle and substrate, the quantity of the precursor solution sprayed and the time of deposit.

2.3.2.1 Influence of Temperature

Spray pyrolysis involves many processes occurring either simultaneously or consecutively. The most important steps are the atomization of the precursor solution, the transport of the

aerosol toward the substrate surface, the evaporation of the solvent, the droplet impact with consecutive spreading, and the drying and decomposition of the precursor [81]. The substrate temperature, being involved in all mentioned processes except that in the aerosol generation, is the key parameter in determining the final film morphology and properties. The film morphology changes by increasing the substrate temperature: the higher the substrate temperature, the rougher and more porous are the films. If the process temperature is too low, the films are cracked. In the middle, dense and smoother films can be obtained. The deposition temperature also influences crystallinity, texture and other properties of the deposited films [81]. Therefore, the properties of the coatings can be defined and controlled by modifying the deposition temperature.

2.3.2.2 Influence of Precursor Solution

The precursor solution strongly affects the properties of the deposited films. Specifically, the nature of the solvent, type of salt, concentration of salt, and the presence of additives influence the physical and chemical properties of the precursor solution. Thus, film features can be changed by varying the composition of the precursor solution. For example, highly volatile solvents could induce the formation of cracked films because the film dries completely before the precursor pyrolysis.

2.3.3 Models for Film Deposition by Spray Pyrolysis

As it is extensively described in the literature [85,86], many processes occur either sequentially or simultaneously during a spray pyrolysis process. Understanding and modeling these processes will help to improve film quality. Thin film deposition by means of spray pyrolysis can be divided into three main steps: the atomization of the precursor solution, the transportation of the resultant aerosol and the decomposition of the precursor on the substrate.

2.3.3.1 Atomization of Precursor Solution

Atomization is defined as the production of droplets and their dispersion into a gas [87]. It is the first step in the spray pyrolysis deposition process. Normally used atomizers in spray pyrolysis techniques are air blast, ultrasonic and electrostatic. It is essential to know which atomizer is more appropriate for a targeted application and how the precursor properties and the working conditions influence the atomization process. Typically, many drops are deflected or reflected from the substrate surface, thus decreasing the deposition efficiency. Airblast, ultrasonic and electrostatic atomizers differ in droplet size, rate of atomization and droplet velocity. The size of the generated droplets (D : droplet diameter) is not related to any fluid property of the precursor solution and depends solely on the fluid charge density level (ρ_e) [88,89]:

$$D^2 = \left(\frac{-\alpha}{\beta} \right) \frac{3\varepsilon_0}{q\rho_e} \quad (2.3)$$

where ϵ_0 is the vacuum permittivity ($8.854 \times 10^{-12} \text{ C}^2/\text{m}^2\text{N}$), q is the fundamental charge ($1.602 \times 10^{-19} \text{ C}$), and $(-\alpha/\beta)$ is a constant value equal to $1.0 \times 10^{-17} \text{ J}$. Therefore, all fluids will spray the same if charged to the same level.

The mass of a spherical droplet depends instead on its density [88]:

$$m = \frac{4\pi}{3} \rho_q r^3 \quad (2.4)$$

where r is the droplet radius and ρ_q is the droplet density.

The velocity of the droplet, when it leaves the atomizer, is important because it determines the droplet heating rate and the residence time of the droplet during the spray pyrolysis process. Table 2.2 summarizes the properties of droplets for the different atomizers commonly used for spray pyrolysis deposition.

Atomizer	Droplet diameter (μm)	Droplet velocity (m/s)
Pressure	5 ÷ 50	5 ÷ 20
Ultrasonic	1 ÷ 100	0.2 ÷ 0.4
Electrostatic	5 ÷ 70	1 ÷ 4

Table 2.2 Characteristics of atomizers commonly used for spray pyrolysis [90].

Airblast atomizer (pressure atomizer) uses high-speed compressed air (or other gases) in order to generate an aerosol from the precursor solution. The liquid precursor is introduced into the airflow and the gas flow kinetic energy is used to transform the liquid precursor in fine droplets. The mean droplet diameter decreases by increasing the gas pressure [88]. As it is shown in the work of Perednis [91], all droplets sprayed from an air blast atomizer are contained within a 70° spray cone angle, while half of them are within a narrower 12° angle. Nevertheless, such atomizers generally do not allow the obtainment of reproducible micrometer or sub-micrometer size droplets and to finely control the droplets' size distribution.

Differently, ultrasonic atomizers use ultrasonic frequencies to produce fine liquid precursor atomization, with much lower velocity compared to that of the air blast atomizer; by means of this type of atomization, the size and distribution of the droplets can be precisely controlled. In electrostatic atomizers the atomization of the precursor solution is obtained by exposing the liquid precursor to a strong electric field; in such way fine (sub-micrometer), self-dispersive (no agglomerating), highly wettable, and adhesive droplets are obtained.

2.3.3.2 Aerosol Transport

The atomization of the precursor solution forms an aerosol. It is defined as a suspension of solid or liquid particles in a gas [87]. In the aerosol, the droplets are transported and, eventually, evaporated. To make more dense and thick films, it is important that, during transportation, as many droplets as possible strike the substrate and spread, without forming powders or particles before reaching the substrate surface.

When the droplets move toward the heated substrates, they can undergo physical and chemical changes [88] (Figure 2.12), and solvent evaporation can take place. The evaporation of the solvent yields a droplet size decrease and a concentration gradient. Furthermore, when the precursor concentration exceeds the solubility limit, it can precipitate on the surface of the drop. This situation will result in a crust and subsequently in porous hollow particles. This effect is not desired in film formation due to the increased roughness of the film [81]. Numerical simulations models indicated that solid particles can be formed when the reactor temperature is low or irregularly distributed, when the precursor solution is concentrated and when the carrier gas flow rate is low [81].

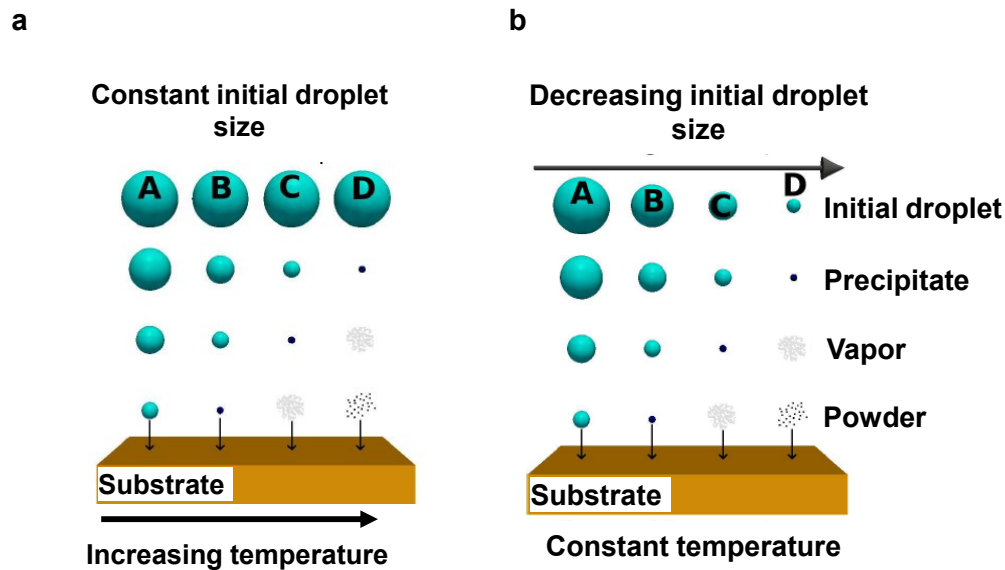


Figure 2.12 Droplet changes during the aerosol transportation, when the substrate temperature (a) or the initial dropped size (b) is altered. Four different processes are possible when the droplets move towards the substrate (A-D) [88].

Four different forces simultaneously act on the droplets moving toward the substrate and, thus, influence their path. These forces are i) gravitational, ii) electrical, iii) thermophoretic, and iv) Stokes force. Gravitational force pulls the droplet down according to the mass of the traveling droplet (i.e. small droplets are subjected to small gravitational force). The electrical force exists on spray pyrolysis apparatus where additional electrical sources are used to influence the droplet's trajectory (i.e. ultrasonic and electric spray deposition), while air blast atomizers do not have this additional contribution and the droplet transport is only driven by the gravitational force and the initial velocity of the droplets. Stokes force is caused by the friction between the droplets and air molecules and depends on the particle velocity and size (i.e. large and quick droplets have the largest retarding force). Finally, thermophoretic force causes a reduction of the droplets' velocity when they approach hot substrates. It depends on the thermal gradient in the transport atmosphere and, when the droplet is more than several mm ($\sim 5\div 7$ mm) away from the substrate, it does not affect the droplet movement. In non-electrostatic spray process, thermophoretic forces could keep most droplets away from the substrate surface [88].

2.3.3.3 Decomposition of Precursor

Many processes can occur when a droplet of precursor moves and hits the substrate surface (Figure 2.12), such as the evaporation of the residual solvent, the spreading of the droplet, and the salt decomposition. There are many models to describe the decomposition of the precursor salt and most of the authors proposed that only the CVD-like process gives high-quality films by spray pyrolysis [81,86].

According to Perednis et al. [81], the following processes occur on the substrate surface when the temperature is increased (Figure 2.12a). At low temperatures, the drops spread on the substrate surface and then decompose (A). At higher temperature (B), the solvent in the droplets initially completely evaporates, thus dried precipitate is formed. Then, the precipitate hits the substrate surface and its decomposition occurs. At even higher temperatures (C) again the solvent firstly evaporates and dried precipitate is obtained. As the precipitate reaches the immediate vicinity of the substrate, it melts and vaporizes (without decomposition). The precursor vapors diffuse into the substrate and undergo a heterogeneous CVD process. At the highest temperatures (D) the precursor vapors react before reaching the substrate surface (homogeneous nucleation) and, consequently, solid particles are formed. In the processes A and D rough and non-adherent films are obtained, while adherent films are achieved at intermediate temperatures (process C) via CVD-like procedure. However, processes type A or B allow the formation of high-quality adherent films too.

It must be underlined that the process C can rarely occur in most spray pyrolysis depositions, because often the spray pyrolysis deposition temperature is too low in order to vaporize the precursor and/or the precursor decomposes without melting and vaporization.

2.3.4 Description of the spray pyrolysis apparatus

The used spray pyrolysis apparatus is shown in Figure 2.13. This system was specifically projected, assembled and optimized at ICMATE-CNR laboratory to obtain homogeneously spread but discontinuous calcium phosphate ceramic coatings on planar titanium substrates with different pristine morphologies and topographies.

It essentially consists of:

1) The atomizer: a Paasche VL airbrush.

An airbrush atomizer works by passing a stream of carrier gas (N_2 in our case) through a tube that first narrows and then expands in cross-sectional area (Venturi effect). Thus a local reduction in the gas pressure is created that allows the solution to be pulled (suction) from a connected tank at normal atmospheric pressure. Then the high velocity of the carrier gas atomizes the precursor solution into very tiny droplets.

The here employed spray pyrolysis apparatus uses a Paasche VL airbrush (Figure 2.14), with internal mixing (i.e. the precursor atomization takes place inside the airbrush body) and double action. Differently from a single-action airbrush, where it is only possible to regulate the airflow (pushing down the trigger button), with a dual-action airbrush, it is possible to regulate the airflow and, at the same time, the precursor solution flow. Paasche VL can work both double and single action (a roller in front of the trigger allows it to be used as a single action system for simpler control). The airbrush body is machined brass, polished and chrome-plated, while the needle is in stainless steel. It can be used with both water-based or solvent-based mediums. It can achieve spray patterns

from fine detail to broader coverage. The carrier flow is supplied by an N₂ tank equipped with a pressure controller.

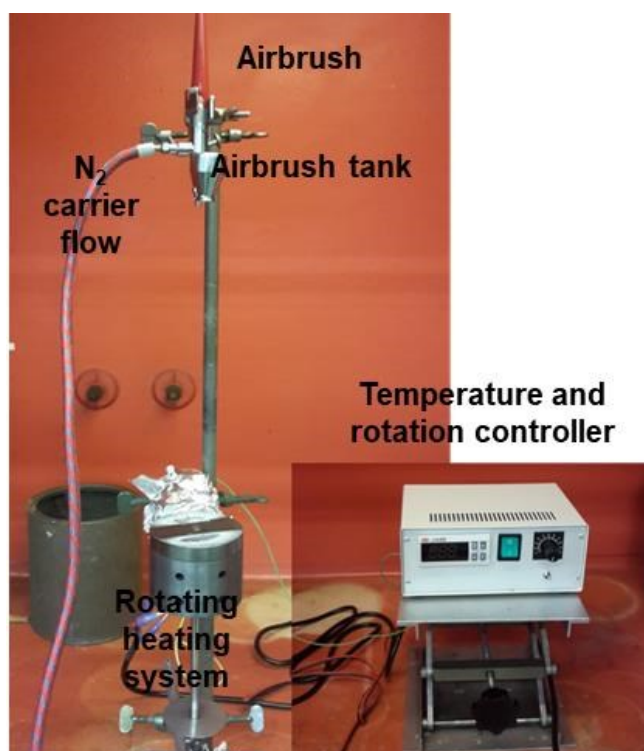


Figure 2.13 Photo of the spray pyrolysis apparatus used in this work.



Figure 2.14 Paasche VL airbrush.

- 2) The rotating heating system and the temperature/rotation controller. The rotating heating system is a bronze plate in which four resistors and a thermocouple are inserted. It can reach temperatures up to about 370°C. A motor connected to it also allows its rotation. The temperature/rotation controller is a Carel ir33 Universale.

2.3.5 The precursor solution used

Before the film deposition processes by spray pyrolysis, a solution of the required material is needed to be prepared. In this work, calcium nitrate tetrahydrate $\geq 99.0\%$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and ammonium monohydrogen phosphate 100.0% , $(\text{NH}_4)_2\text{HPO}_4$, were purchased from

Sigma-Aldrich and J.T. Baker, respectively; and used as reagents for the spray pyrolysis process.

Table 2.3 summaries some of their physical properties. Both can easily be found on the market at relatively low cost (500 g costs less than 70 euros), and do not present particular storage difficulties.

Calcium nitrate tetrahydrate irritates skin, eyes and respiratory tract, and it is harmful if swallowed or inhaled; while ammonium monohydrogen phosphate is irritating to the eyes. Therefore, carefulness is required in handling them.

Physical Properties	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	$(\text{NH}_4)_2\text{HPO}_4$
Molecular weight	236.15 u.m.a	132.06 u.m.a
Density	1.896 g/cm ³	1.619 g/cm ³
Boiling point	132°C	-
Melting point	42.7°C	155°C
Solubility in water	1290 g/L (20°C)	368 g/L (20°C)
Appearance	White hygroscopic solid	White powder

Table 2.3 Calcium nitrate tetrahydrate and ammonium monohydrogen phosphate physical properties.

Chapter 3

ANALYTICAL METHODS

3.1 Structural characterization: X-ray diffraction

X-ray diffraction is a versatile and non-destructive technique to analyze material properties of powders, solid samples or even liquid samples. X-ray radiation is a well-adapted tool to observe atomic details since inter-atomic dimensions are about $1\text{--}2\text{ \AA}$ and X-ray wavelength is in the range of 0.1 to $1000\text{ \AA}$. Nevertheless, X-ray diffraction as a means of establishing the crystal properties of material was not used until the early part of the 20th century. It was in 1912 when Max von Laue suggested that when X-rays pass through a crystal they are diffracted and that there is a relationship between their wavelengths and the separation of planes in a crystal lattice.

According to the Bragg model, in the crystal lattice, there are several families of planes, each of which may be interested in the "reflection" of the incident waves. However, given the nature of the incident wave and the periodicity characteristics of the crystal lattice, reflection is only limited to some special angular relationships between the incidence direction and the plane in question. The conditions for which constructive interference is generated (Figure 3.1) are such that the difference in the optical path between the incident ray and diffracted ray is equal to a whole number of wavelengths (Bragg law):

$$2 d_{hkl} \sin\theta = n \lambda \quad (3.1)$$

where n is the order of reflection, d_{hkl} is the inter-planar spacing of the planes which makes an angle θ with incident beam, λ is the wavelength of X-rays and 2θ is the angle by which the Bragg reflection deviates from the incident beam.

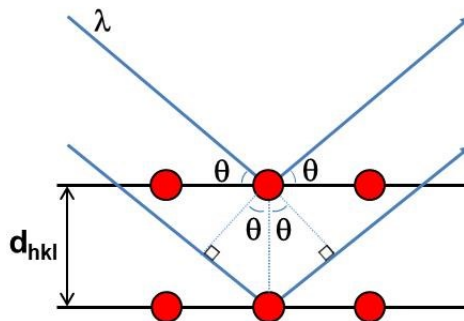


Figure 3.1 Bragg model of lattice planes.

By means of X-ray diffraction, it is possible to:

- carry out quantitative and qualitative phase analyses of pure substances or mixture;
- analyze the microstructure of the material (crystallite size, preferred orientation effects, and residual stresses);
- analyze the influence of temperature and/or other non-ambient variables, such as humidity or applied pressure.

In single-crystal X-ray diffraction, a single crystal is rotated around various axes in order to find a large number of different reflections. The crystal structure is determined from the angular position and the intensities of these reflections.

In X-ray powder diffraction, the sample consists of an infinitely large number of small crystallites, ideally randomly oriented with respect to each other. Because all orientations are simultaneously present, it is only necessary to vary the angle of incidence and the angle of diffraction.

In a powder diffractometer, the X-rays produced by an X-rays tube pass through primary optical components and then go on the sample surface with an angle θ . After that, they are diffracted by the sample phases, so pass secondary optical components and enter the detector. By varying the diffraction angle between the incident and diffracted beam (2θ) (through the movement of tube or sample and detector depending on diffraction geometry) intensities are recorded to create a diffractogram. Bragg-Brentano diffractometer setup is the most commonly used geometry for phase analysis.

As shown in Figure 3.2, a powder diffractogram displays the scattered intensity versus the Bragg angle (2θ). It contains several peaks (reflections), each of which is distinguished by one intensity, profile (FWHM: Full Width Half Maximum) and position in units of 2θ . The peaks and the background are the sources of all information of the XRD technique. Table 3.1 summarized the main information content in a powder diffractogram.

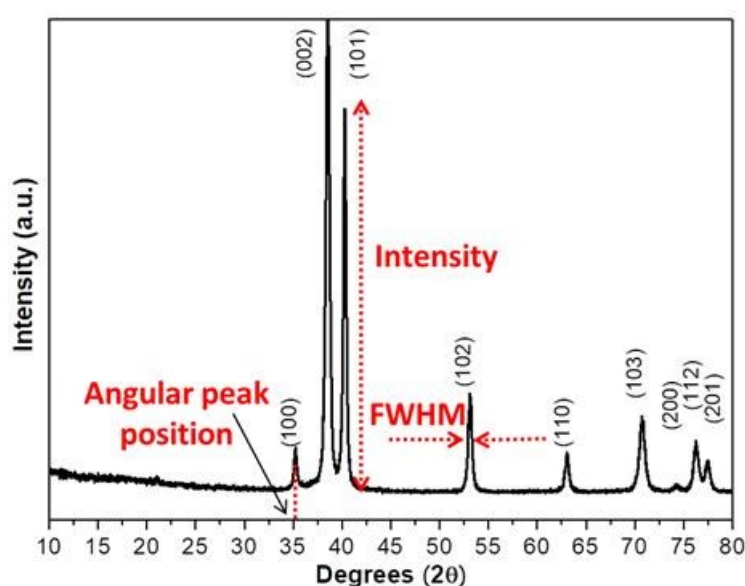


Figure 3.2 XRD spectrum of uncoated Ti machined substrates used in this work.

Reflection	Depends on	Main information
Position	Periodic arrangements of atoms/molecules. λ used	Qualitative phase analysis. Lattice parameters. Residual stresses.
Intensity	Crystal structure. λ used. Sample preparation.	Quantitative phase analysis. Crystal structure. Preferred orientations.
Profile	Lattice distortions.	Microstrain. Crystallite sizes.

Table 3.1 General information content in a powder diffractogram.

The identification of the crystalline phase (when known) can be conducted empirically, by comparing the X-ray diffraction pattern with those of compounds whose structure is known and collected in appropriate diffraction archives. The most common databases are American Society for Testing and Materials (ASTM), International Center for Diffraction Standards (ICDS), and International Centre for Diffraction Data (ICDD). A sample may contain a mixture of phases and so the relative amounts of each phase may be determined. Quantitative phase

analysis is usually carried out using the Rietveld method, which uses a least-squares approach to refine a theoretical profile until it matches the measured profile.

In this work, the crystallographic structures of the prepared samples were determined by means of a Philips X'Pert PW 3710 powder diffractometer, operating in Bragg–Brentano θ – 2θ geometry mode, using Cu K α radiation ($\lambda = 0.154$ nm, 40 kV and 30 mA). Phase identification was performed with the support of the standard 2002 ICDD database files. The relative phase amount and the crystallite sizes were estimated by Rietveld refinement by means of the MAUD (Material Analysis Using Diffraction) software [92] and using COD structures [93].

3.2 Morphological characterization

3.2.1 Scanning electron microscopy

Scanning electron microscopy produces images of a sample by scanning it with a high-energy beam of electrons. Since its development in the 1950s, it has been applied in many areas of study, from medical to physical and material sciences. The essential feature of scanning electron microscope is that it provides enlarged images of the sample surface with high resolution (1–5 nm, depending on the diameter of the electronic beam) and depth of field (i.e. in a wide range of distances around the image focus, the image is still clear), higher than those provided by the optical microscope. Differently from the transmission electron microscopy (where all image information is acquired simultaneously), during a SEM acquisition signals from a single point are collected and the total image is represented by scanning the probe over a sample area. Thus the image is the result of a point by point analysis.

The scanning electron microscope is fundamentally composed of a vacuum chamber and an electron gun that produces a thin beam of high-energy electrons. Schematically it consists of the following essential parts:

- ✓ an electron source (gun), generally a filament of tungsten (W), lanthanum hexaboride (LaB₆) and Field Emission Guns (FEG);
- ✓ an anode towards which the electrons are accelerated, through a potential difference ranging from few keV to 50 keV, with 30 keV as a common value [94];
- ✓ two electromagnetic lenses for focusing electrons;
- ✓ scanning coils to scan the electronic brush on the sample surface;
- ✓ one or more devices to reveal the electrons emitted from the analyzed material.

The most important characteristic of the electron beam is its energy (0–50 KeV) and current (1 pA ÷ 1 μ A). These parameters determine the entity of the damage to the sample (generally due to local thermal damages), the amount of useful signal, and the depth of penetration into the sample. The size of the beam diameter determines the lateral resolution. For example, working with a low current beam improves resolution and decreases damage, but also reduces the detectable useful signal.

To prevent that electrons lose energy and directionality due to collisions with the gas molecules present in the air, the electrons gun, the lenses and the sample are traditionally kept in ultra-high vacuum condition (10^{-6} – 10^{-12} Pa). In recent years, the technology of electronic microscopy instruments has improved considerably, resulting in equipment

capable of making analysis at much higher pressures (until 2000 Pa for ESEM, Environmental Scanning Electron Microscope).

Various SEM methods are differentiated on the basis of what is subsequently detected and imaged. When a specimen is irradiated with a high-energy electron beam, interactions between the incident electrons (primary electrons) and the atoms in the specimen produce various signals (Figure 3.3).

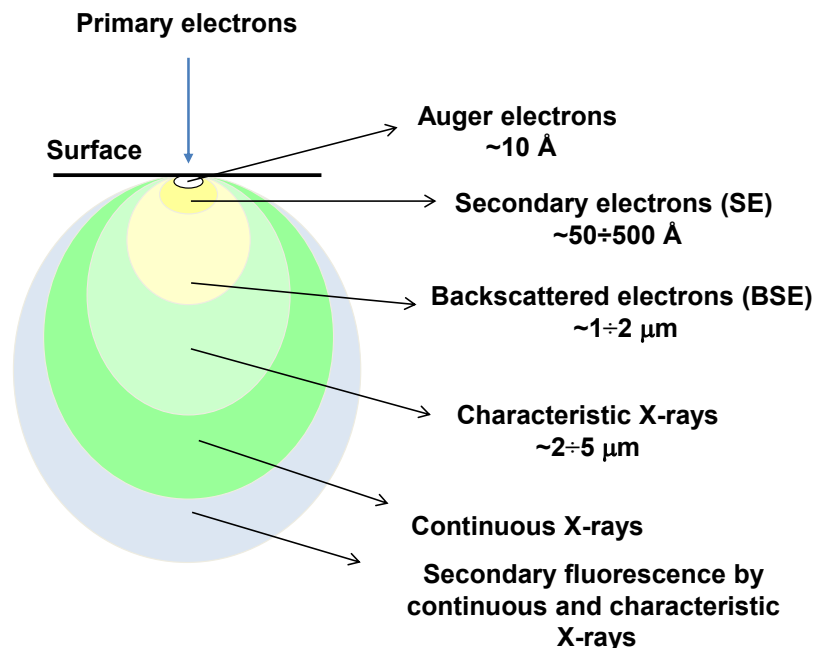


Figure 3.3 Interaction volume of the electron beam with a sample. The depth to which the beam produces a signal depends on the accelerating voltage of the beam. All signals are produced throughout the interaction volume.

The important processes for SEM are:

- Primary electrons lose some of their energy and generate low energy (< 50 eV) secondary electrons (SE) that are used to form the SEM image. Secondary electrons do not provide compositional information and only those produced near the surface can be emitted. The intensity of the SE emitted is related to the superficial morphology of the sample; thus, the number of secondary electrons revealed depends on the material orientation, inclination, and geometry with respect to the incident beam.
- Primary electrons can be backscattered (BSE), which produces images with a contrast that depends on the sample atomic number (Z). The probability of backscattering increases with the atomic number Z of the sample material: larger atoms are indeed stronger scatterers of electrons compared to light atoms, and thus create a higher signal. This dependence helps to distinguish between different phases, providing imaging that conveys information on the sample's composition.
- Primary electrons can also ionize atoms in the sample, which can then relax by electron transitions, which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements present in the top few micrometers of the sample (as described in Section 3.3.2).

While conductive samples are easily studied by SEM, the surface of insulating samples must be made conductor before the analyses, because the primary electron flow could induce the accumulation of charges on the surface material (thus preventing the interaction with other electrons of the beam and/or generating false signals). Isolating samples are therefore coated with few nanometers metal films (such as gold, carbon or aluminum), generally produced by means of vacuum evaporation or sputtering deposition techniques. During these processes, it is important to create thin and uniform coatings, which not mask the details of the underlying surface.

The most peculiar qualities of this technique are:

- ✓ easy sample preparation;
- ✓ simple surface observation;
- ✓ high magnifications (until about $10^5\times$);
- ✓ possibility of choosing the type of signal for image formation;
- ✓ possibility of determining the chemical composition of the explored area.

In this work, two different instruments were used. Specifically, the surface morphology of titania coated surfaces (Ti-TiO_2) was investigated by Sigma Zeiss field emission scanning electron microscope (FE-SEM) with an electron beam acceleration voltage of 5 kV. The surface features of titania/hydroxyapatite coated substrates ($\text{Ti-TiO}_2\text{-HA}$), as well as the bone-like apatite nucleated from simulated body fluids (DPBS), were examined using a FEI Quanta 200 FEG ESEM instrument, equipped with a field emission gun, operating in high vacuum conditions and at an accelerating voltage variable in the range of 20÷25 kV, depending on the observation needs. Before the analyses, the samples were coated with 15 nm of Pt or graphite by Emitech K575X Turbo Sputter Coater.

3.2.2 Profilometry

Stylus profilometer is an instrument that allows the quantitative measurement of the topography of a surface and the thickness of a coating. The principle of mechanical contact profilometer is that a stylus (generally a diamond tip) is vertically moved (Z direction) in contact with the sample and then traversed shifted (X direction) over the sample surface at constant velocity ($1\div 25000\text{ }\mu\text{m/s}$), for specified distance and with definite contact force (from 0.1 to 50 mg). The vertical movements of the tip (attached to a cantilever) as a function of the position are continuously registered, thus obtaining the surface profile: small vertical features (ranging from 10 nanometers to 1 millimeter) can be measured.

Standard profilometer tips generally have a radius of 2 or 10 μm and an angle of 60 or 90 degrees [95]. These geometrical features are of fundamental importance because they determine the smallest pits that the tip can enter and the steepest slopes that may be measured (Figure 3.4): a radius that is too large will result in a loss of information. The stylus profilometer used in this Ph.D. work is equipped with a diamond stylus with 2 μm radius and 90° angle.

To obtain an accurate measurement with this equipment it is necessary to establish a correct baseline. For a two-dimensional scan, two equal points in height define a reference axis, for three-dimensional images the principle is the same and three equal points in the plane should be used.

3-dimensional images (3D maps) are obtained with successive scans in the XY plane: after that one scan is performed, the tip moves back to its starting point, the cantilever is shifted in

the Y direction and another scan is made. The step in the Y direction can be set from 1 to several μm .

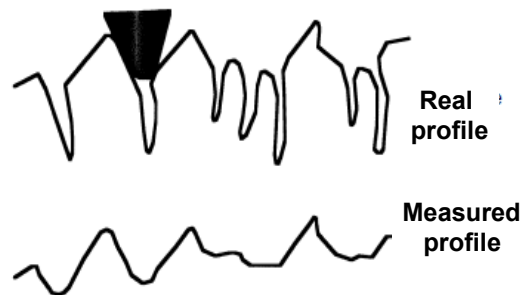


Figure 3.4 Influence of tip radius on the measured profile [95].

Coating thickness can be estimated by evaluating the film-to-substrate gap. The gap can be prepared by masking the surfaces during the deposition or etching it after the coating formation process. Vertical height resolution (Z resolution) depends on the mechanical features of the apparatus and the roughness of the sample; resolution of 1 nm is possible. Soft materials (where the surface may be damaged from the load applied on the tip), substrate roughness and equipment vibration can limit the accuracy of the step measurements.

The evaluation of the surface profile must be carried out considering that surface topography consists of several components: form, waviness, and roughness (Figure 3.5) [95]. Broadly, form describes long-period or non-cyclic deviation; roughness are closely spaced irregularities and waviness are more spaced irregularities. Roughness is what is left when errors of form and waviness are removed [95]. The measuring equipment as well as the cut-off settings, which separate roughness contributions from form and waviness ones, strongly influence the analysis results. Different roughness values can result as a consequence of using different filter sizes; unfortunately, information about which filters have been considered is almost always lacking and wide ranges of roughness are often reported for apparently very similar surfaces [95,96].

To perform correct and comparable roughness assessments, it is advisable to refer to specific regulations (in particular to ISO 3274:1998 and ISO 4288:1998, Table 3.2 and Table 3.3, respectively). These standards define the appropriate analysis parameters, such as the λ_c (roughness/waviness filter cut-off; it removes the longer wavelength components) and the λ_s (micro-roughness cut-off; it removes the shortest wavelength components that are judged not relevant to a roughness measurement) cut-off values, the minimum scan length, the sampling rate, etc. according to the roughness of the examined sample and the size of the instrument tip.

The most commonly used parameter in medical-scientific literature to evaluate the roughness characteristics of a surface is the R_a value. It is the arithmetic average of the absolute values of the profile height deviations from the mean line, recorded within the evaluation length. However, the average roughness can be the same for surfaces with roughness profiles very different because it depends only on the average heights of the profile. Thus, surfaces that have different undulations are not distinguished. For this reason, other parameters have to be used to fully characterize a surface and some examples are described below.

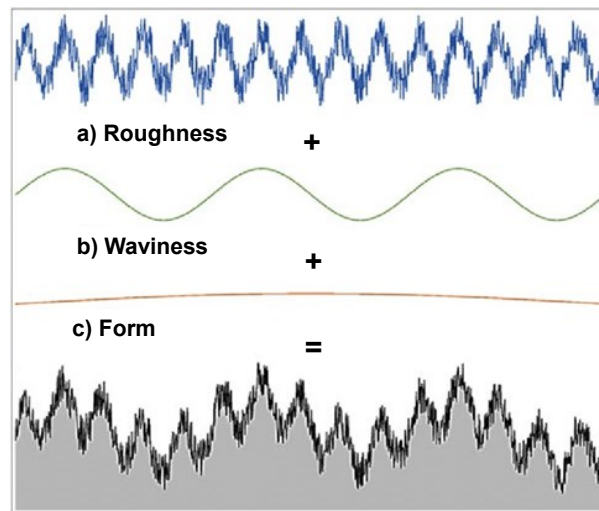


Figure 3.5 Geometric components of a surface profile: (a) roughness, (b) waviness and (c) form [97].

λ_c (mm)	λ_s (μm)	λ_c/λ_s	r tip max (μm)	Maximum sampling spacing (μm)
0.08	2.5	30	2	0.5
0.25	2.5	100	2	0.5
0.8	2.5	300	2	0.5
2.5	8	300	5	1.5
8	25	300	10	5

Table 3.2 From ISO 3274:1998. Relation between λ_c , λ_s , tip radius, and maximum sampling spacing.

Periodic profiles	Non-Periodic profiles		Cut-off	Sampling length/ Evaluation length
Spacing distance RSm (mm)	Rz (μm)	Ra (μm)	λ_c (mm)	λ_c (mm)/L
0.013-0.04	To 0.1	To 0.02	0.08	0.08/0.4
0.04-0.13	0.1-0.5	>0.02-0.1	0.25	0.25/1.25
0.13-0.4	0.5-10	>0.1-2	0.8	0.8/4
0.4-1.3	10-50	>2-10	2.5	2.5/12.5
1.3-4.0	50-200	10-80	8	8/40

Table 3.3 From ISO 4288:1998. λ_c and evaluation length as a function of the sample roughness.

R_q is defined as the root mean square average of the profile heights over the evaluation length. The maximum profile peak height, R_p , is the distance between the highest point of the profile and the mean line within the evaluation length; whereas the maximum profile valley depth, R_v , is the distance between the deepest valley of the profile and the mean line within the evaluation length. R_t (maximum height of the profile) is the vertical distance between the highest and lowest points of the profile within the evaluation length, while R_z

(average maximum height of the profile), is the average of the successive values of R_t , calculated over the evaluation length (generally 5).

In this work, 3D maps and roughness parameters (R_a , R_q , R_p , R_v , R_z , R_t) were calculated using a stylus Profilometer Bruker Dektat XT, according to ISO 3274:1998 and ISO 4288:1998. Considering that R_a of the investigated samples was in the range of $0.1 \div 2 \mu\text{m}$, the analysis parameters were always kept constant and are summarized in Table 3.4. The surface area excess compared to smooth Ti was also calculated (Sdr value, developed interfacial area ratio). It is expressed as the ratio between the actual surface area and the geometrical area.

λ_c / λ_s	800 μm / 2.5 μm	Scan length	4800 μm	Sampling rate	100 Hz
Stylus force	3 mg	Scan time	64 s	Leveling	Polynomial II°

Table 3.4 Analysis parameters used in this work for roughness investigations.

3.3 Chemical characterization

3.3.1 Fourier transform infrared spectroscopy

Although the region of the infrared was discovered more than one century and a half ago by Sir F. W. Hershel, IR spectroscopy is a fairly recent technique for the reason that instrumental difficulties precluded its development and spread until 1940. The infrared (IR) radiation of the spectrum includes radiation with wavenumbers ranging from about 12800 cm^{-1} to 10 cm^{-1} . The majority of analytical applications are, however, mainly restricted to the portion of the spectrum referred to the medium infrared, i.e. between 4000 cm^{-1} and 400 cm^{-1} . Infrared spectroscopy has extensive applications both in qualitative and quantitative analyses. Its most important and characteristic use is that referred to the qualitative identification of compounds: the vibrational transition between two vibrational levels (induced by infrared radiation) is representative of each molecule and provides a unique pattern, which is easily distinguishable from those of all the other compounds.

The term Fourier Transform Infrared Spectroscopy (FT-IR) refers to a development in the manner in which the data is collected as a function of time rather than frequency, and converted (thanks to the Fourier transform) from an interference pattern (time-domain) to a spectrum (frequency-domain). Therefore, instead of viewing each frequency sequentially (as in a traditional dispersive IR spectrometer), all frequencies are examined simultaneously. FT-IR technique has greatly extended the capabilities of infrared spectroscopy and has been applied to many areas that were very difficult or nearly impossible to analyze by the traditionally used dispersive instruments. Employing the FT-IR technique, the signal-to-noise ratio of the spectrum is significantly higher than that of the previous generation infrared spectrometers; furthermore, the accuracy is higher and the error is lower (within the range of $\pm 0.01 \text{ cm}^{-1}$). The scan time of all frequencies is shorter (thus considerably increasing the speed of the analyses) and the resolution is high.

The basic components of a FT-IR system are shown in Figure 3.6. IR radiation from a broadband source is first directed into an interferometer, where it is transmitted to the beam splitter, which splits the incident beam into two beams. One of the split beams is reflected at a 90° angle onto a flat fixed mirror, while the other beam is transmitted to a flat mirror that

moves back and forth at a constant velocity. The two beams are ultimately reflected from their respective mirrors and are recombined as they return at the beam splitter. After passing the sample the measured signal is the interferogram. Performing a mathematical Fourier transform on this signal results in a spectrum identical to that from conventional (dispersive) infrared spectroscopy.

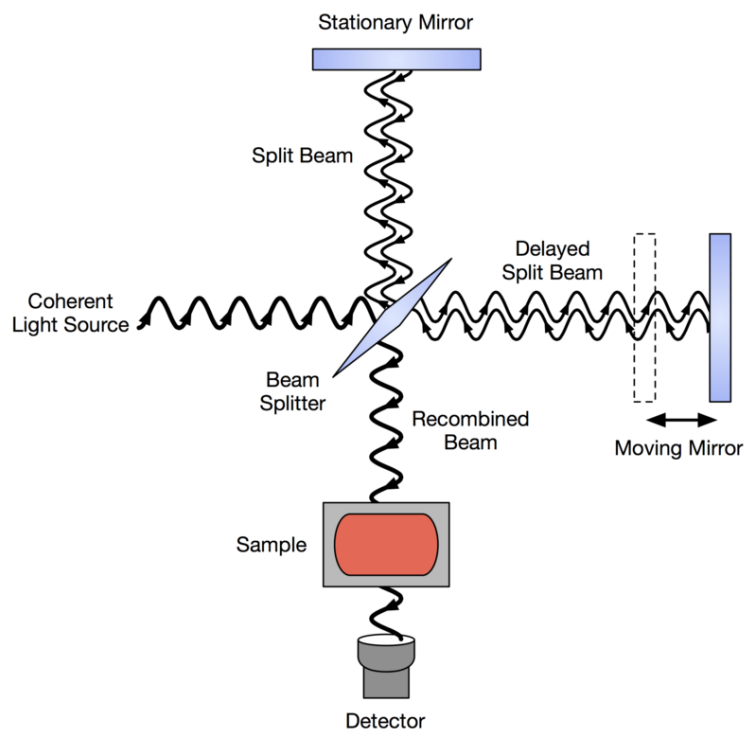


Figure 3.6 Diagram of the FT-IR spectrometer.

Sample preparation is relatively easy. Almost any solid, liquid or gas sample can be analyzed in transmission mode. The sample to be analyzed can be pulverized into the KBr matrix (transparent in the mid-IR region) or dissolved in a suitable solvent (for example CCl_4 and CS_2). Water should be removed from the sample if it is possible. Reflection FT-IR techniques collect the light reflected from an interface (air/sample, solid/sample, liquid/sample), allowing analyses with minimal sample preparation. FT-IR reflection techniques can be Internal Reflection Spectroscopy (attenuated total reflection, ATR), External Reflection Spectroscopy (specular reflection, used with smooth surfaces) and a combination of Internal and External Reflection (Diffuse Reflection, used with rough surfaces).

Molecular vibrations and rotations in the sample are activated by the absorption of infrared light. Different functional groups show vibrations at characteristic energies (different wavelengths) and, consequently, absorb the IR radiation only in a specific range. The infrared light is absorbed when the electric dipole moment in a molecule can be changed and no center of symmetry exists (i.e. only on these conditions the alternating electric field of the incoming radiation can interact with the molecule). These vibrations are called IR active. The electric dipole moment is determined by the magnitude of the charge difference and the distance between the two centers of charge. When molecules absorb IR radiation, transitions occur from a ground state to an excited state. Symmetrical molecules with vibrations symmetrical to the center of symmetry are IR inactive.

The frequency of vibration transition can be described in terms of classical Newtonian mechanics. The theory of vibrations admits that a chemical bond between two atoms can be

compared (as a first approximation) to two-point masses connected by a spring. If the mass is moved to a distance “x” from its equilibrium position, following the application of a force along the binding axis, the returning force (F) is proportional to the shift and it is describable through Hooke's law:

$$F = -kx \quad (3.2)$$

where k is the force constant that depends on the stiffness of the spring (a strong and few flexible spring will have a high value of k, conversely a weak and flexible spring will have a value of small k) and the sign negative indicates that the force is of return (i.e. it acts in the opposite direction to the move).

A molecule composed of N atoms has 3N degrees of freedom, six of which are translations and rotations of the molecule itself. This leaves 3N-6 degrees of vibrational freedom (3N-5 if the molecule is linear). However, the theoretical number of fundamental vibrations is rarely observed, since overtones (multiples of a given frequency) and combination of tones (sum of two vibrations) increase the number of detected bands, while other phenomena as the weak intensity of some bands, their vicinity, spectrometer limitations, and spectroscopic selection rules can reduce them.

The vibrations can be divided into two basic categories: stretching or bending (Figure 3.7). A stretching vibration involves the change of the interatomic distance along the axis between the two atoms and can be symmetrical or asymmetric. Stretching modes are typical of higher energy than bending modes. The bending vibrations are instead characterized by a change in angle between two bonds. There are four types of bend: rocking, scissoring, wagging and twisting.

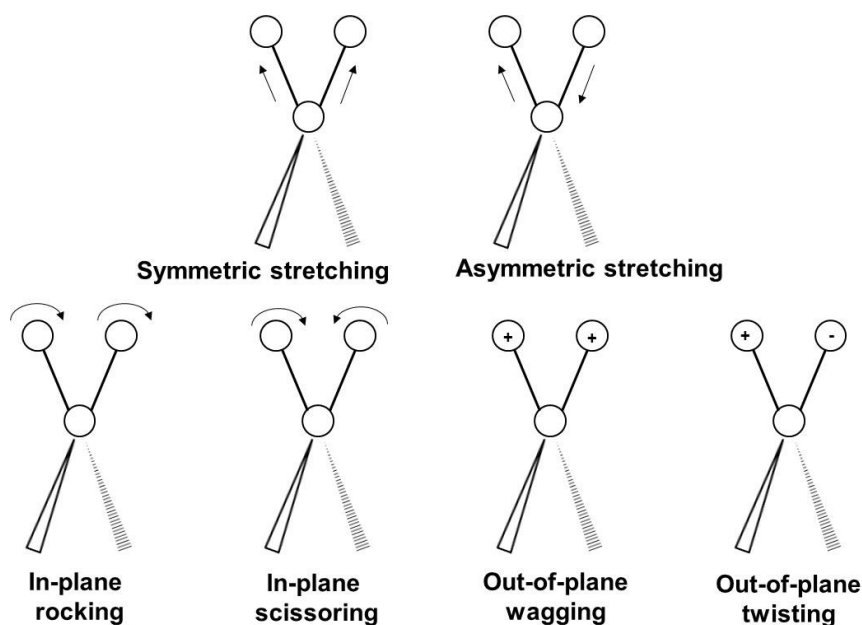


Figure 3.7 Types of molecular vibrations. “+” indicates motion from the page toward the reader, while “-” indicates motion away from the reader.

The frequency absorbed depends on the masses of the atoms in the bond, the geometry of the molecule, the strength of the bond, and several other factors. There are two useful regions in the IR spectrum. The functional group region encompasses 3600 cm⁻¹ to 1200 cm⁻¹ region, while the fingerprint region from 1200 cm⁻¹ to 200 cm⁻¹. The absorptions in the

functional group region provide information about the presence of characteristic functional groups (such as --OH , --NH , C=O , --SH and --C=C-- etc), while the fingerprint region includes molecular vibrations characteristic of the entire molecule or large fragments of the molecule. The absorption pattern in this region is frequently quite complex. However, this region is useful for confirming the identity of a particular molecule by comparing it with the spectral absorptions of the authentic sample.

In this work, the FT-IR technique was employed to identify bone-like apatite nucleation on the surface of Ti disks (uncoated, titania coated and titania/hydroxyapatite) after soaking in Dulbecco's Phosphate Buffered Saline (DPBS) solution. Table 3.5 shows the characteristic value of the wavenumbers related to the absorption peaks of the hydroxyapatite [98].

Band position (cm^{-1}) ($\pm 2 \text{ cm}^{-1}$)	Hydroxyapatite Relative intensity	Attributions
PO_4^{3-} groups (apatitic)		
474 (isolated)	Very weak	$\nu_2 (\text{PO}_4)$: OPO bending
562 and 575 (merged together)	Moderate	$\nu_4 (\text{PO}_4)$: OPO bending
603 (isolated)	Moderate	$\nu_4 (\text{PO}_4)$: OPO bending
962 (isolated)	Weak	$\nu_1 (\text{PO}_4)$: PO stretching
1048, 1090 (both isolated)	Intense	$\nu_3 (\text{PO}_4)$: PO stretching
OH^- groups (apatitic)		
632 (shouldered to $\nu_4 (\text{PO}_4)$)	Moderate/weak	$\nu_L (\text{OH})$: OH libration
3572 (shouldered to water O-H)	Moderate/weak	$\nu_s (\text{OH})$: OH stretching

Table 3.5 FT-IR characteristic bands (PO_4^{3-} and OH^-) for stoichiometric HA [98].

External Reflection Infrared spectra were collected by using a Bruker Alpha spectrometer equipped with an external reflection module. Background spectra were collected on a reference gold mirror, samples spectra (64 scans) were acquired in the $7500\text{--}375 \text{ cm}^{-1}$ range, with a resolution of 4 cm^{-1} . The experiments were performed directly on the sample and the investigated area is $\approx 20 \text{ mm}^2$. All the spectra were smoothed by using a method based on the Savitzky-Golay algorithm.

The used instrument operates in contactless mode, collecting both the specular and the diffuse component. The optical geometry of the instrument tends to maximize the specularly reflected radiation working with an angle of incidence equal to reflected one, $45^\circ/45^\circ$. Anyway, the spectral features can be affected by distortions due to the optical and physical properties of the analyzed materials. The intensity and the type of spectral distortions (i.e. derivative shape, inversion effect, and intensity enhancement) depend on the prevalence of the specular reflection or of the diffuse reflection.

3.3.2 Energy dispersive X-ray analysis

As described in the previous section, the scanning electron microscope is principally designed for producing electron images (strictly related to the electron probe used) of the

sample surface. Energy dispersive X-ray analysis (EDX) is a chemical microanalysis technique, employed in conjunction with scanning electron microscopy. Using the X-ray spectrum emitted by the sample all elements from atomic number 4 (Be) to 92 (U) can be detected in principle.

When the sample is hit by the SEM's electron beam, electrons are ejected from the internal orbitals of the atoms of the sample's surface ($2\div5\text{ }\mu\text{m}$). The resulting electron vacancies are filled by electrons from a higher state, and an X-ray is emitted to balance the energy difference between the two electrons' states. The X-ray energy is characteristic of the element from which it is emitted. The characteristic X-ray lines are named according to the shell in which the initial vacancy occurs and the shell from which an electron drops to fill that vacancy. For instance, if the initial vacancy occurs in the K shell and the vacancy filling electron drops from the adjacent (L) shell, a $K\alpha$ X-ray is emitted. If the electron drops from the M shell (two shells away), the emitted X-ray is a $K\beta$ X-ray.

Qualitative analysis is carried out very quickly by identifying the lines in the X-ray spectrum using tables of energies. Ambiguities are rare and can be generally resolved by taking into account the main element line as well as its additional ones. The minimum detection limits vary from approximately 0.1 at. % to a few atom percent, depending on the element and the sample matrix.

Quantitative analyses are carried out considering that the relative intensity of an X-ray line is approximately proportional to the mass concentration of the element considered. This relationship is due to the fact that the mass of the sample penetrated by the incident electrons is approximately constant regardless of the composition. Nevertheless, to obtain the true concentration, certain corrections are required. For example, the acronym 'ZAF' describes a procedure in which corrections for atomic number effects (Z), absorption (A) and secondary fluorescence (F) are calculated separately, from suitable physical models.

Elemental maps can also be produced by collecting characteristic X-rays from elements in the specimen as the focused electron beam is scanned in a raster across the specimen. These images show elemental distributions on a fine-scale range.

In this work, energy dispersive X-ray qualitative analyses were obtained with an Oxford X-Max system at an accelerating voltage of 15 kV or with an EDAX Genesis energy-dispersive X-ray spectrometer at an accelerating voltage of $20\div25\text{ kV}$.

3.3.3 Inductively coupled plasma atomic emission spectroscopy

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) or optical emission spectrometry (ICP-OES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit characteristic energy at a given wavelength as they return to ground state after excitation by high-temperature argon plasma.

This technique allows the quantitative and qualitative elementary chemical analysis of inorganic or organic materials, both liquid and solid (after their dissolution). Almost all the chemical elements present in the periodic table can be determined, except some light elements and halogens. Detection limits typically range from parts per million (ppm) to parts per billion (ppb), although depending on the type of element and the specific instrument, sometimes less than ppb detection limit can be achieved.

Nowadays, this type of analysis has reached a broad degree of maturity; therefore, thanks to its reliability, multi-element options, and high throughput, it is widely considered as the most

versatile analytical technique in chemistry laboratories, both for routine research and in more specific analysis purposes.

An ICP-AES instrument consists of five basic components: the sample introduction system, the excitation source (plasma), the spectrometer (for wavelength selection), the detector and the readout system (Figure 3.8).

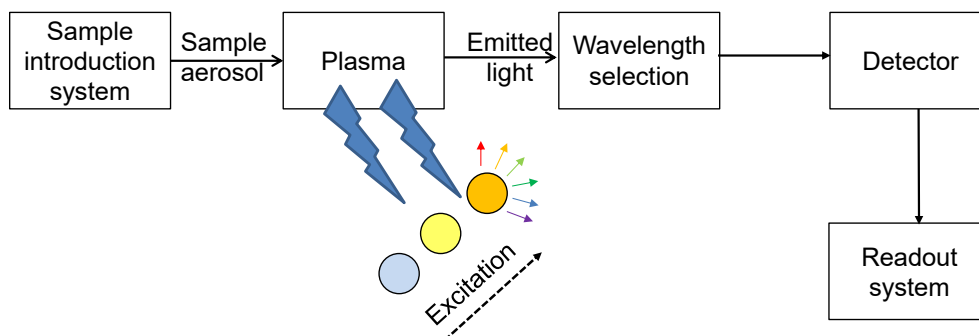


Figure 3.8 ICP-AES emission spectrometer.

The standard sample form for analysis by ICP is the liquid state (not necessarily aqueous). The sample is introduced at a constant rate into the argon plasma as an aerosol; the most common sample atomization systems are pneumatic and ultrasonic ones. In the ICP-AES the plasma is generated at the end of a quartz torch by a water-cooled induction coil through which a high-frequency alternate current flows. Therefore, an alternate magnetic field is made which accelerated electrons into a circular trajectory. The ionization occurs due to collisions between the argon atoms and the electrons, so giving rise to stable and extremely hot ($6000\div 7000$ K) plasma. In the torch desolvation, atomization, excitation, and finally characteristic light emission of the investigated sample take place. The emitted light is then transmitted to the optics, which separate the light according to wavelength through the principle of dispersion and then transmit the light to the detector. The wavelengths used range from the upper part of the ultraviolet (160 nm) to the limit of visible light (800 nm). The detector converts the light energy to electrical current, which is quantified and transformed into concentration units by the readout system. The light intensity can be measured with a photomultiplier tube that works based on the photoelectric effect and can detect specific wavelength(s) at a time, or, in more modern systems, with solid-state charge transfer devices (CTDs). Depending on the structure of the detector and the way in which signals are processed, two types of CTDs are used: charge injection devices (CIDs) and charge-coupled devices (CCDs). CID is a two-dimensional solid-state integrating detector where individual resolution elements (i.e. pixels) are independently and electronically accessible. The here used instrument is composed of a 512 by 512 array of individual detectors on a silicon substrate. Each detector (or pixel) is about $25\text{ }\mu\text{m}$ in size.

Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard. Inductively coupled plasma atomic emission spectroscopy is a "relative" analytical technique by means of it is possible to determine the concentration of the analytes only if they are compared with reference solutions of known concentration. It is assumed that calibrations standards and samples are sufficiently alike to provide unbiased analytical results. If the samples differ from calibration standards, correction procedures are required to improve accuracy. Any characteristic of the sample that causes a variation in the amplitude of the emission signal with respect to the sample

reference generates a disturbance. All potential disturbances that could lead to errors are called "interference". Three main types of interferences are faced during ICP-AES analysis: physical, chemical, and spectral.

Physical interferences are due to differences (such as viscosity, density, or matrix) between the real samples and the calibration standards. These dissimilarities influence sample transport and atomization. The sample dilution can help to overcome physical interferences. Internal standard method (i.e. the addition to all samples and standards of a precisely known amount of some elements) should be employed when the matrix is not entirely known; while the method of standard addition (i.e. the sample is subdivided into three or four parts, one of which is analyzed without modification; the other parts are added with progressively higher amount of the analytes prior the analysis) when the matrix is completely unknown.

Chemical interferences occur when the sample and the calibration standard respond differently to the plasma causing unintended ionization, molecular formation, or plasma loading. Several methods can be employed to lessen these interferences such as the addition of an ionization buffer that mitigate the ionization effects or the optimization of plasma parameters to reduce the plasma loading.

The most common ICP-AES problems are spectral interferences such as stray radiation, overlapping of lines and poor resolution of lines. Stray light is light that does not originate from the analytes and it can be due from reflection inside or outside the spectrometer. There are different ways to deal with spectral interferences such as to use the standard addition method in order to calibrate and analyze the samples or to use the calibration curve method and do corrections for spectral interferences. The correction of the background is typically made by first selecting background points or regions and then applying a correction mode or algorithm. Full frame instruments, in which it is possible to simultaneously analyze the same analyte at different wavelengths, are particularly adapted to check for spectral interference comparing the instrumental response between two or more analytical lines and rejecting that provide abnormal results.

In this work, *in-vitro* titanium, aluminum, and calcium ion release was detected by Thermo Scientific iCAP 6000 DUO, equipped with a CID solid-state detector, which allows the simultaneous reading of wavelengths in the range of 166-847 nm; a DUAL VIEW device (axial view + radial view); an RF generator with software-controlled power-adjustable at 4 values: 750, 950, 1150, 1350 W. Calibration solutions were prepared from Carlo Erba mono-elemental standard solutions (1000 µg/mL). Five solutions with concentration of 0, 1, 5, 10, 15 µg/mL (ppm) for Ca and 0, 1, 3, 6 e 10 µg/mL (ppm) for Ti and Al were used for the calibration curves.

3.4 Electrochemical characterization: potentiodynamic polarization technique

The potentiodynamic polarization technique is a suitable tool for laboratory corrosion testing. Potentiodynamic polarization plot can provide useful information regarding the corrosion and/or oxidation behavior of a material such as its ability to spontaneously form a passive film on its surface in the medium, the potential region over which the specimen remains passive and the corrosion rate in the passive region. This method consists in the creation of the polarization curve, by sweeping (in the cathodic and anodic directions) the potential of the working electrode at a constant rate and monitoring the current density that is produced as a function of the applied potential.

A schematic polarization curve of a passive metal is presented in Figure 3.9 [99]. The typical polarization curve for a passivating material has three distinct regions: active, passive and transpassive. At the beginning of the scan, the applied potential is increased in the noble direction. From the open circuit potential (E_{corr}), the current density increases, indicating increased corrosion activity. However, at and above a potential referred to as the primary passivation potential (E_{pp}) the current density decreases significantly. This current reduction is referred to as the active-passive transition. Above E_{pp} , the current density drops to a very low value (passive current density, J_p). In the passive region, the current density tends to have little dependence on potential. Finally, in certain conditions, current can increase again at a higher potential owing to the breakdown of the passive film. Materials will have good corrosion resistance if they exhibit low current density in the passive state and a wide passive potential region. In contrast, materials are more active when they exhibit a larger current density (are more vulnerable to corrosion attack).

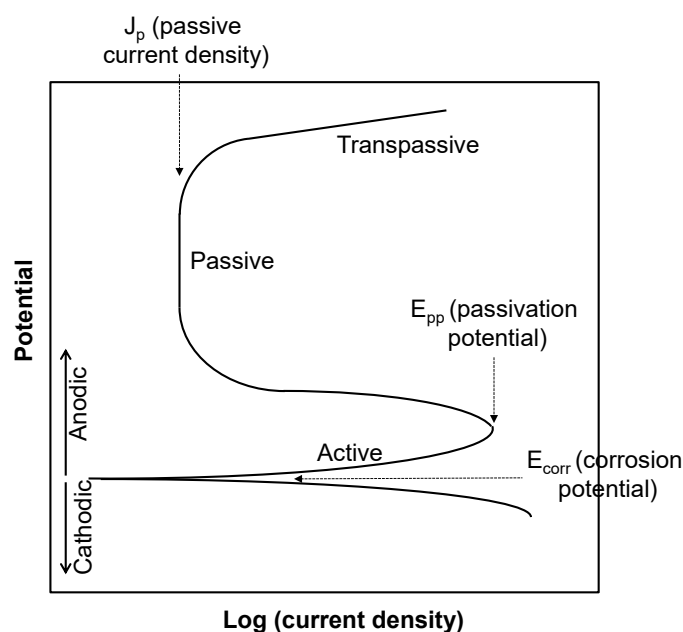


Figure 3.9 Typical polarization curve of a passive metal.

Potentiodynamic polarization curves can be used also to calculate the corrosion current density (J_{corr}) and the free corrosion potential (E_{corr}) of the material in a corrosive environment. Tafel plot technique is the simplest method for this purpose [99].

In this Ph.D. work, EG&G Princeton Applied Research Model 263A potentiostat was used to control a three electrodes cell. The titanium (uncoated and coated) samples were used as the working electrode (WE) and a platinum wire as the counter electrode (CE), the reference electrode (RE) was a standard Ag/AgCl electrode (KCl 1M, -10 mV vs SCE). The working electrode was polarized from -2.5 V to 7.5 V vs SCE at a scanning rate of 2 mV/s, in Artificial Saliva (AS) at 37°C. The AS chemical composition was KCl (0.4 g/L), NaCl (0.4 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.906 g/L), $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.690 g/L), $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (0.005 g/L), and urea (1.0 g/L) [100]. The exposed surface was ϕ 1.4 cm circular zone, for an overall area of 1.54 cm². The current density was calculated considering also the mean roughness of the samples, by exploiting the Sdr (developed interfacial area ratio) parameter obtained from three-dimensional profilometry results. Thus, the actual exposed area was estimated for each surface finishing, taking into account the contribution of surface roughness.

3.5 Tribocorrosion test

Tribocorrosion describes the material degradation resulting from the combined action of the mechanical loading and corrosion attack caused by the environment (chemical and/or electrochemical interaction) [101]. The consequences of coupling mechanical loading and corrosion are complex; mechanical wear might accelerate the corrosion process, or the corrosion attack might accelerate the mechanical wear [102]. Thus, tribocorrosion degradation cannot be considered as the simple addition of wear and corrosion degradation modes, but the effects of the synergies created between them should also be considered.

The total loss due to tribocorrosion effect could be described using the following equation [103]:

$$T = W + C + S = W + C + (WC + CW) \quad (3.3)$$

where T is the total tribocorrosion loss; W is the wear loss in the absence of corrosion; C is the corrosion loss in the absence of wear; and S is the synergic effect between wear and corrosion (i.e. the combination of the effect of corrosion on wear, WC , and wear on corrosion, CW).

When passive materials are subjected to sliding, worn areas are formed where the passive layer is removed (consequently the underlying metal can corrode and its degradation can release metallic ions into the biological media), while the unworn areas remain passive. Generally, the exposed bare metal re-passivates in a short time, recovering its protective effect. Sliding action can also result in the detachment and release of metal particles (mechanical wear). Despite the mechanical nature of this process, corrosion can significantly affect it. For example, passive films may render metals brittle and prone to wear or corrosion products may act as a lubricant.

A schematic illustration of a tribocorrosion set up is shown in Figure 3.10. A potentiostat is assembled to a tribometer, in which a three-electrode electrochemical cell is placed, allowing both tribological and electrochemical parameters to be measured at the same time.

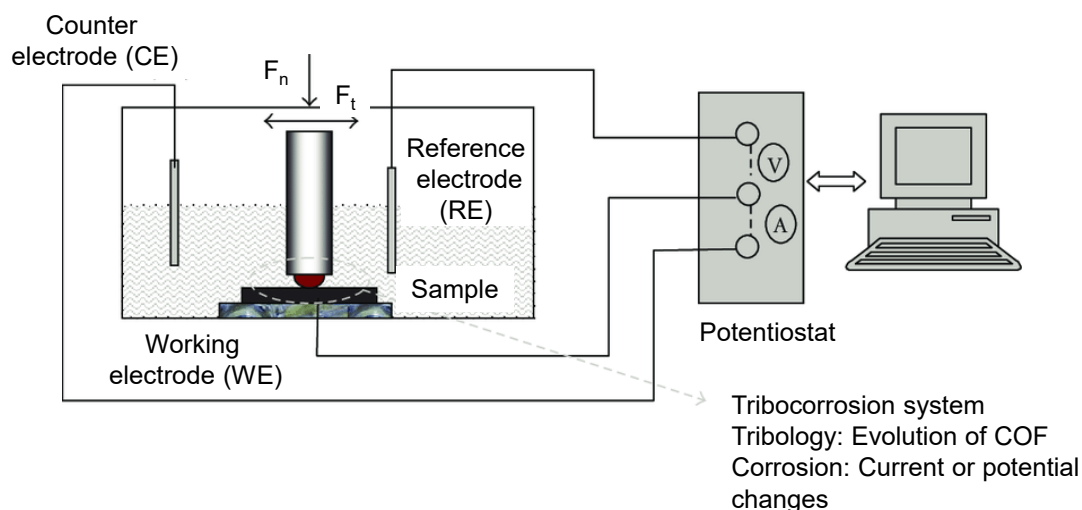


Figure 3.10 Schematic setup for tribocorrosion test [104].

Tribocorrosion behavior is herein investigated by means of open-circuit potential (OCP) measurements and sliding tests with potential recording as a function of the sliding period.

OCP measurements consist of monitoring the potential difference between the sample under investigation (working electrode) and a reference electrode, both immersed in an electrolytic solution (artificial saliva, in our case). OCP is a measure of the tendency to free corrosion of the material under investigation.

The test sequence was mainly constituted of three stages (Figure 3.11):

- 1) initial stabilization period;
- 2) sliding period;
- 3) final stabilization period.

During the initial stabilization period, OCP values are related to the electrochemical behavior of materials (higher OCP values are associated with nobler behavior of materials) [100,101]. In a sliding dynamic contact, OCP describes the galvanic coupling evolution between the undamaged area (which develops a passive film, in the case of most biomaterials) and the worn region, where the bare metal may be exposed to the solution, due to the failure of the passive film during sliding [101]. For passive metals, the potential values dropped to less noble values, as soon as the mechanical stress starts (onset of sliding), indicating the beginning of wear process with the partial/total destruction of the passive film [8]. An equilibrium is reached depending on both the rate of passive layer removal and the rate of passive layer recovery. Finally, once the stress has been removed, a potential stabilization towards more cathodic values is generally observed [8], due to the restoration of the passive film.

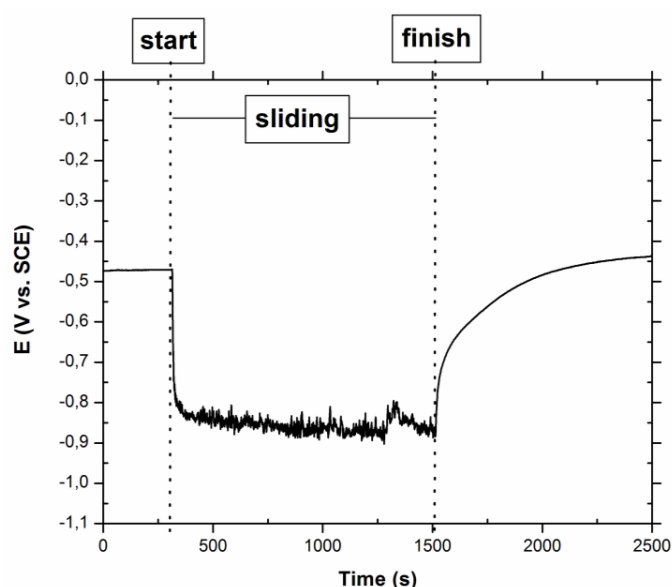


Figure 3.11 OCP monitoring during a reciprocating sliding test [101].

In this work, tribocorrosion characterization was carried out with a Bruker UMT-2 tribotester set for pure sliding and operating in ball-on-flat configuration and linear reciprocating motion mode, equipped with a standard three-electrode electrochemical cell. The titanium samples were used as the working electrode and a platinum wire as the counter electrode, the reference electrode was Ag/AgCl (KCl 1M, -10 mV vs SCE). The reciprocating sliding tests were performed against an alumina ball ($\varnothing$ 5 mm) at an initial Hertzian contact pressure of 400 MPa, sliding speed of 6 mm/s, sliding distance of 3 mm, in order to realize a frequency of 1 Hz. The biological environment was simulated by artificial saliva [100], which was maintained at 37°C during the whole duration of the tests.

During the sliding process, the coefficient of friction (COF) was also simultaneously recorded. COF is influenced from the chemical composition and the topological features of the sliding surfaces, which could changes during the mechanical wear process at the sliding contact [105]. The friction coefficient between any contacting surfaces is defined as [105]:

$$COF = \frac{F_t}{F_n} \quad (3.4)$$

where F_t is the tangential force and F_n is the applied normal load.

In this work, tribocorrosion studies were only carried out on the titania covered Ti surfaces (Ti_TiO_2) and on pristine titanium substrates, since the evaluation of the tribocorrosive performances of the final composite material (made up of a continuous titania interlayer + homogeneous spread and discontinuous HA islands) was not significative due to the discontinuous coverage of the bioactive top coatings on the underlying substrates.

3.6 Mechanical investigation: nanoindentation

The nanoindentation test is a way to estimate the mechanical properties of a material, such as hardness and elastic modulus, at the nano-scale level. Compared with other mechanical characterization methods, nanoindentation requires a quite simple setup and specimen preparation. Also, nanoindentation leaves only small imprints, thus, it can be considered, to a certain degree, as non-destructive [106]. The length scale of the indentation depth is in the nanometer range (in contrast with microns and millimeters in conventional hardness testing): as a result, it can be applied to extract the mechanical properties of small scale specimens, such as thin films.

A force (P) is applied to the surface to be indented by means of a diamond tip with known geometry and the values of the penetration depths (h) are continuously recorded as a function of P . The maximum applied load (P_{max}) and the contact area between the indenter and the sample (A_c) are used to calculate the hardness (H) of the material (equation 3.5), while the elastic properties are calculated from the slope of the unloading curve.

$$H = \frac{P_{max}}{A_c} \quad (3.5)$$

The most common indenter geometry used for nanoindentation experiments is a Berkovich type indenter (triangular based pyramid with a face angle of 65.27°) [107].

Conventional hardness testing uses the residual impression of the indent to determine the contact area. This is not possible with the nanoindentation test because the indents are too small to be measured accurately. As an alternative, the method developed by Oliver and Pharr [108] uses the displacement of the indenter into the material together with the known indenter geometry to calculate the contact area. Figure 3.12a shows a typical load-displacement curve recorded during an indentation experiment, while Figure 3.12b shows the schematic cross-section of an indent during and after the indentation.

The Oliver and Pharr [108] method can be described as follow. The indenter is pushed into the material surface until it reaches a certain maximum load P_{max} and maximum depth h_{max} , causing elastic and plastic deformations under the tip. During the unloading of the indenter, the elastic displacement recovers and the final indentation depth h_f remains. The contact depth (h_c) can be calculated as:

$$h_c = h_{max} - h_s \quad (3.6)$$

where h_{max} represents the maximum displacement and h_s is the displacement of the surface at the perimeter of the contact (Figure 3.12b). h_{max} can be obtained from the load-displacement curve, while h_s depends on the indenter geometry and can be determined using Sneddon's elastic contact theory [109]:

$$h_s = \varepsilon \frac{P_{max}}{S} \quad (3.7)$$

where ε is a geometric constant (for Berkovich indenter $\varepsilon = 0.75$) and S is the contact stiffness. S is obtained from the load-displacement curve: it is the slope of the tangent of the unloading curve at the peak load P_{max} . Usually, S is calculated computer-aided because slight variations of the tangent can lead to significant differences in S values.

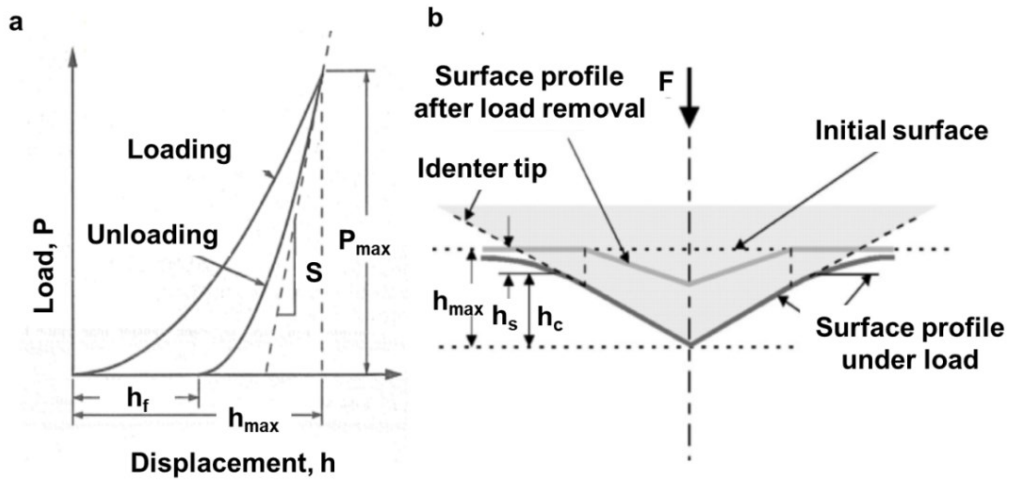


Figure 3.12 Load–displacement curve corresponding to the nanoindentation experiment (a) and schematic cross-section of an indent during and after the indentation (b) [108].

Combining equations 3.6 and 3.7 leads to:

$$h_c = h_{max} - \frac{\varepsilon \cdot P_{max}}{S} \quad (3.8)$$

Thus the contact depth can be determined using only parameters that are obtained from the load-displacement curve and a predefined geometry factor.

Once h_c is calculated, the contact area A_c is determined by the area function:

$$A_c(h_c) = C_0 h_c^2 + C_1 h_c + C_2 h_c^{\frac{1}{2}} + C_3 h_c^{\frac{1}{4}} + C_4 h_c^{\frac{1}{8}} + \dots \quad (3.9)$$

with C_0 describing an ideal indenter geometry ($C_0 = 24.5$ for a Berkovich indenter) and the other constants C_i describing the deviations from an ideal tip. For each tip, these constants must be calculated from time to time by calibrations to take into account the inevitable blunting of the tip after several indents. Finally, the contact area can be used to calculate the hardness using equation 3.5.

The reduced modulus E_r is derived using:

$$E_r = \frac{\sqrt{\pi}}{2\beta} \frac{S}{\sqrt{A_c}} \quad (3.10)$$

where β represents a geometrical constant (for a Berkovich indenter $\beta = 1.034$). The reduced modulus combines the elastic properties of the indenter tip and the tested material through the relation:

$$\frac{1}{E_r} = \frac{1-n^2}{E} + \frac{1-n_i^2}{E_i} \quad (3.11)$$

with E and n , E_i and n_i being Young's modulus and Poisson's ratio of the specimen and of the indenter tip, respectively. For diamond, the most common indenter material, $E_i = 1141$ GPa and $n_i = 0.07$.

As an example, in NanoTest instrumental configuration, the indenter tip is mounted on a movable pendulum that can be controlled by the magnetic field produced by a coil. Depending on the applied voltage, the pendulum applies a certain load on the indenter tip, pushing it into the sample surface. The displacement of the diamond tip is measured by means of a parallel plate capacitor. The indenter could also be equipped with a microscope, allowing precise placement of the indents on the specimen.

In this work, nanomechanical properties were evaluated by NanoTest, Micro Materials Ltd, using a Berkovich pyramid-shaped diamond tip and operating in the depth control mode (30 s loading / 5 s load holding / 30 s unloading, thermal drift measurement 30 s). The maximum depth reached after the loading phase was 30 nm in order to minimize the influence of the underlying substrate. The thermal drift and compliance were automatically corrected by the software. The hardness and the elastic modulus were evaluated using the method of Oliver and Pharr [108]; the results correspond to an average of a minimum of 45 indents.

3.7 Contact angle measurements

The contact angle (CA) is a quantitative measure of the wettability of a solid by a liquid. Wettability studies usually involve the measurement of contact angles, which provide information about the interactions between a solid surface and a liquid (generally water). Calculations based on the measured contact angle values yield the solid surface tension, which quantifies the wettability characteristics of a solid material.

When a liquid drop is put on a flat and horizontal solid surface, the contact angle (Figure 3.13) is defined as the angle formed by the intersection of the liquid-solid interface and the liquid-vapor interface [110].

The interface where solid, liquid, and vapor co-exist is referred to as the "three-phase contact line". Small contact angles are observed when liquid spread on the surfaces, while large contact angles are observed when the liquid bead on the surface. More specifically, a contact angle less than 90° indicates that wetting of the surface is favorable, and the fluid will spread over a large area on the surface; while contact angles greater than 90° generally means that wetting of the surface is unfavorable, so the fluid will minimize its contact with the surface and form a compact liquid droplet. Complete wetting (superhydrophilic condition) occurs when the contact angle is below 5° . Differently, superhydrophobic surfaces have water contact angles greater than 150° , showing almost no contact between the liquid drop and the

surface (lotus effect) [110]. The contact angle is characteristic for a given solid-liquid system in a specific environment [110].

Young's equation describes the geometrical relationship between the contact angle and the interfacial tension:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta \quad (3.12)$$

where γ_{LV} , γ_{SV} , and γ_{SL} represent the liquid-vapor, solid-vapor, and solid-liquid interfacial tensions, respectively, and θ is the contact angle.

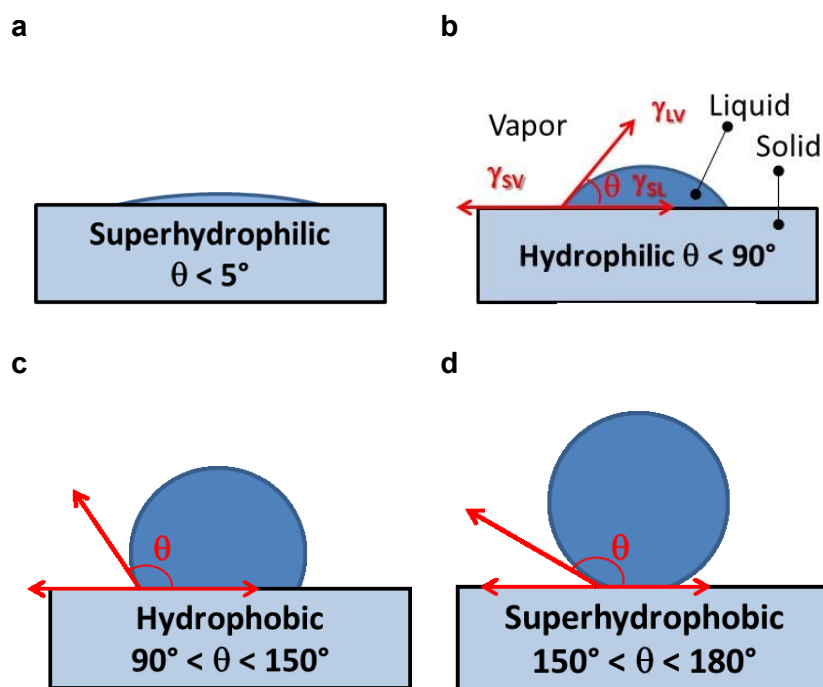


Figure 3.13 Contact angles formed by sessile liquid drops on a smooth homogeneous solid surface.

Sessile drop method is the most widely used technique to evaluate the CA: the contact angle formed by a droplet of liquid placed on a horizontal surface is measured. Sessile CA measurement can be divided into static and dynamic. Static contact angles are measured when the droplet is on the surface and the three-phase boundary is not moving. Static contact angles are utilized in quality control and in research and product development, with an accuracy of approximately $\pm 2^\circ$. When the three-phase boundary is moving, dynamic contact angles can be calculated (advancing and receding contact angles, respectively). Contact angle hysteresis is the difference between the advancing and receding contact angles and arises from the chemical and topographical heterogeneity of the surface, solution impurities absorbing on the surface, rearrangement or alteration of the surface by the solvent.

The sessile drop method equipment consists of a horizontal stage to mount the sample, a motor-driven syringe to distribute the liquid drop, an illumination source, and a camera. A software can also be used to evaluate the contact angles. This method is simple, furthermore small amounts of liquid (a few microliters) and small surface substrates (a few square millimeters) are required for the analyses. Unfortunately, the accuracy and reproducibility of the measures strongly depend on how the operator assigns the tangent line, which can lead

to significant errors and inconsistency between multiple users. Furthermore, CA values below 20° cannot be accurately measured due to the uncertainty of assigning a tangent line when the droplet profile is almost flat [110]. Also, the contact angle estimation could be dependent on the drop size, and surface heterogeneity might cause CA variations.

Ideally, the shape of a liquid droplet is only determined by the surface tension of the liquid. Surface tension tends to minimize the surface area by making the drop spherical. In practice, external forces (usually gravity) deform the droplet; consequently, the contact angle is determined by a combination of surface tension and external forces. Gravity deforms the drop in two ways: by elongating a pendant drop and/or flattening a sessile drop. This balance between surface tension and external forces (such as gravity) is reflected mathematically in the Laplace equation of capillarity, which offers the possibility of determining surface tension by analyzing the drop shape.

ImageJ software (plugin LB-ADSA, Low Boundary Asymmetric Drop Shape Analysis) was used in this work in order to evaluate the contact angle values. The Low Bond Axisymmetric Drop Shape Analysis plugin is an interactive method that uses five variables to manipulate the Young Laplace drop shape (green curve that is superimposed upon the drop image, Figure 3.14). Manipulation of these variables by moving their respective sliders allows a close fit of the drop shape to the drop image. The LB-ADSA plugin should not be used when analyzing drops lacking symmetry. Furthermore, image tilt can introduce significant error, and this plugin will not provide individual values for the left and right contact angles of an asymmetric drop [111].

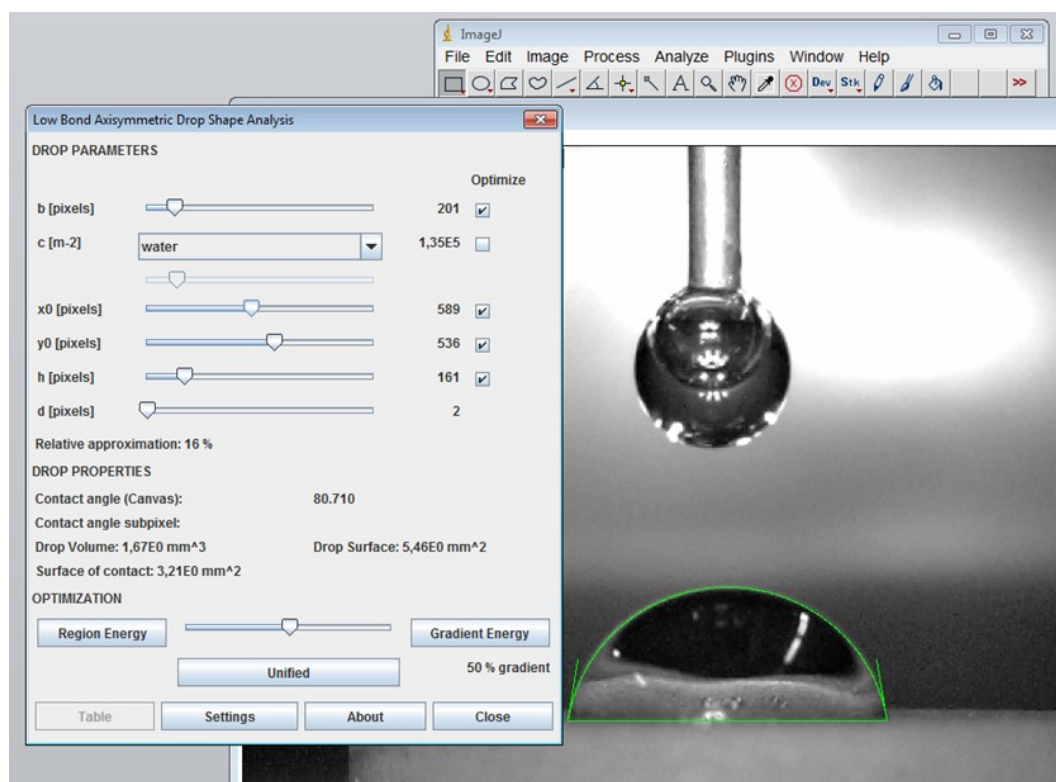


Figure 3.14 Screen-shot of a LB-ADSA contact angle analysis.

In this work, static contact angle characterization (sessile drop method) was used to determine the wettability of uncoated and coated (TiO_2 and TiO_2/HA) titanium substrates, and the aging of wettability with time. Samples were conserved into a vessel at a constant relative humidity ($\sim 43\% \pm 5\%$) and analyzed immediately afterward. Tests were carried out

in ambient laboratory temperature range $20\pm 25^{\circ}\text{C}$. A drop of $10\ \mu\text{L}$ demineralized water was deposited on the surface of the sample and observed by a CCD camera. The equilibrium contact angles were measured after 20 seconds from water drop depositions. The reported values are the average of at least 5 determinations.

Chapter 4

EXPERIMENTAL

4.1 Introduction

The present study deals with the development of a novel two-step deposition route for the osseointegrative functionalization of Ti dental implants, through their coating with suitable ceramic materials. A dense, compact and crystalline titania inter-layer was deposited via Metal-Organic Chemical Vapor Deposition, and a homogeneously spread but discontinuous calcium phosphate ceramic (hydroxyapatite) top-layer was deposited by means of spray pyrolysis. A final thermal treatment at 600°C was found as the best compromise between an adequate crystallinity, bioactivity and composite material features.

Since, besides the surface chemical composition, the morphology and the topography of the Ti surfaces are important factors that influence the rate and quality of the osseointegration process [1,112], three types of Ti substrates were considered. In literature, various surface treatments are proposed to create a certain surface topography and morphology of titanium dental implants [1,2]. In particular, additive procedures (which generally consist in the coating of the pristine surface with suitable particles) or subtractive processes (mostly sandblasting of the surface with alumina, titania or hydroxyapatite particles, chemical etching, or their combination [1,11,12]) are exploited.

In this Ph.D. work, three types of surfaces, with different surficial morphologies and topographies, were used as titanium substrates, all commercial grade IV. Ti surfaces were treated according to the following three different modification procedures, in order to obtain various surface characteristics:

- (a) Machined samples, named Ti_m, were used as-received from Mech & Human Srl (i.e. titanium disks in which the surface appears as at the end of mechanical turning processing);
- (b) Sandblasted samples, named Ti_s, were machined disks, which were sandblasted by Mech & Human Srl with Al₂O₃ particles;
- (c) Sandblasted/acid etched samples, named Ti_{s+a}, were machined disks initially sandblasted (following the same procedure of samples (b)) and then treated with an acid solution according to a procedure, developed at the ICMATE-CNR laboratory, as described below.

4.2 Preparation of the sandblasted and acid etched (“s+a”) titanium surfaces

Preliminary to the pursuing of the main target of the project (i.e. the development of surfaces with enhanced osseointegrative features), the work was initially focused on the developed of high aspect-ratio titanium substrates, with the intent of providing an alternative procedure to that commonly adopted by the industrial partner of the project. The process used by Mech & Human Srl to improve the surface topography of their titanium dental implants involves two successive subtractive treatments to the titanium substrates: the sandblasting with alumina particles and the subsequent acid etching with hydrofluoric acid (HF). This dual step process allows the generation of micro-rough titanium surfaces, with a high specific surface area; nevertheless, this procedure has some limitations such as the use of corrosive and powerful contact poison HF and the potential presence of Al₂O₃ residues.

Figure 4.1 shows scanning electron microscope images, at different magnifications, of a typical dental implant. This titanium implant was subjected to the processes of increasing the surface area by Al₂O₃ sandblasting and hydrofluoric acid etching procedures, sequentially. It can be seen that the implant has a homogeneous morphology in the different parts, with a

micro-rough surface. The compositional analysis of the surface using an EDX microprobe demonstrated the presence, in addition to titanium and oxygen, of traces of aluminum residues (less than 1 wt. %). Alumina residues often remain after the sandblasting procedures. Specifically, aluminum residues in sandblasted surfaces (Ti_s) were detected by means of EDX (about 11 wt. %) and XRD (about 13 wt. %) analyses. Thus, this quantity of residual aluminum was reduced to values below 1 wt. % by the subtractive chemical treatment with hydrofluoric acid.

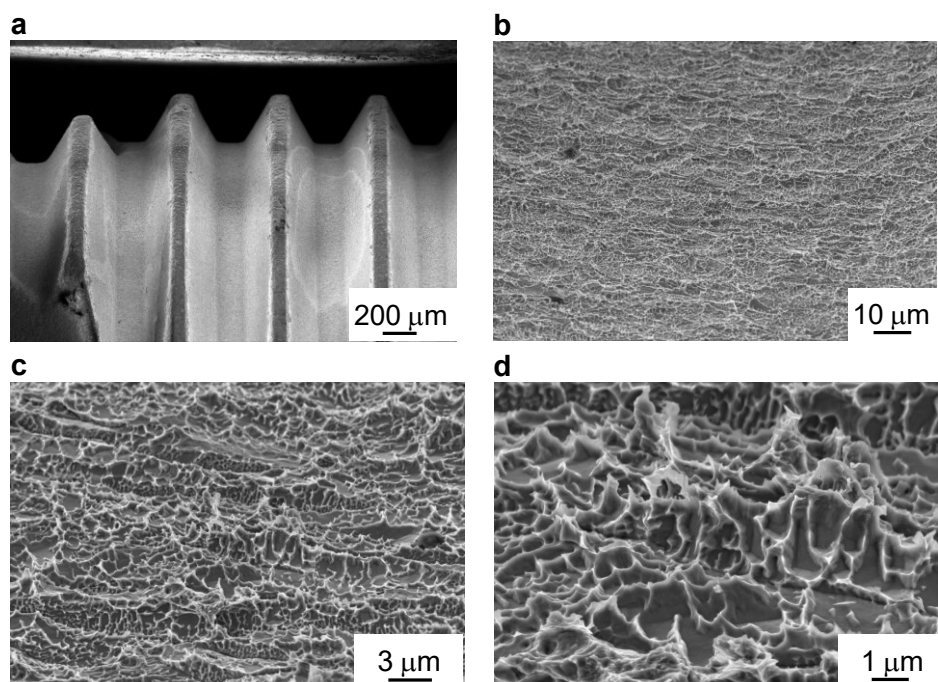


Figure 4.1 Secondary electron SEM images, at different magnifications (a-d), of a titanium implant sandblasted with alumina and chemically treated with HF, supplied by the company Mech & Human Srl.

This research initially aimed to study a method to increase the surface roughness alternative to alumina sandblasting and the subsequent HF etching, in order to avoid the presence of traces of intermediate processing residues on the finished products. Moreover, from an industrial point of view, chemical subtractive processes using hydrofluoric acid are particularly inconvenient due to the considerable cost of the process caused by the high risk associated with the handling of this type of acid.

Taking into account the state of art [1,11,113], it was chosen to treat machined or sandblasted substrates with a subtractive attachment process using a solution of hot strong acids. Specifically, a solution composed of 45 wt. % H_2SO_4 , 15 wt. % HCl , and 40 wt. % H_2O was taken into account (with HCl 37 % from Sigma Aldrich and H_2SO_4 96÷98 % from Carlo Erba). It should be emphasized that this chemical approach using strong acids is undoubtedly safer than similar chemical subtractive methods that use hydrofluoric acid solutions. Following the acid treatment, the samples were always washed with distilled water in ultrasound, in order to favor the elimination of acid residues and possible titanium particles.

It must be taken into account that whereas flat surfaces are easily accessible to laboratory-scale characterization techniques, dental implants have complex shapes (i.e. curvature, threads, steps) making measurements difficult due to inappropriate shape, roughness, too

small or inaccessible areas. For this reason, in this work, most of the studies were carried out on flat substrates (Ti disks, $\phi = 8$ mm) that accurately reproduce the surface features of typical implants.

The determination of the roughness of the substrates following the chemical processes was performed by means of a mechanical stylus profilometer (Bruker Dektat XT) and, in particular, the Ra values were evaluated. The roughness of Ti sandblasted disks treated with HF (Ra: 0.9 ± 0.1 μm) was considered as the value of reference to be reached.

Literature reports that the type of acid, the concentration of the solution, the temperature and the time of the acid attack are influencing factors, which lead to different surface features [65]. Furthermore, the temperature is a relatively dominant factor in deciding the surface texture and roughness compared to time [65].

The effect of the temperature of the acid bath on the final roughness (Ra) of the Ti disks was initially studied. Acid bath temperatures from RT (Room Temperature) to 90°C were taken into account. In the beginning, the acid attack time was kept constant at 30 minutes during the various tests, renewing the acid etching bath at each test.

It should be noted that the aggressive power of the bath depends (in addition to the time spent by the disks in the bath and the bath temperature) also on the ratio between the available titanium surfaces and the volume of the acid solution. Because of this reason, the titanium area to be treated was always kept constant with respect to the acid bath amount.

It was assessed that the agitation of the bath is another parameter of considerable importance. However, due to the small size of the titanium substrates used in this work (disks with diameter 8 mm, 1 mm thick), too vigorous agitations of the bath induced an accumulation of disks (with a not homogeneous disks distribution, frequent disks overlapping and possible disks self-abrasion), with consequent inhomogeneity of chemical etching produced. On the other hand, limited agitation considerably reduced the aggressive power of the acid bath itself because of the establishment of zones where the bath no longer worked, while in other regions the bath had still strong power. In this regard, the scalability of the acid attack process at the industrial level was hypothesized assuming to support the real implants during the acid attack, thus avoiding the problems here encountered with the titanium disks.

Taking into consideration the problems and limitations faced during the acid attack tests, it was nevertheless observed that growing the bath temperature increases the final roughness of the treated surfaces. Furthermore, by operating at room temperature, the acid bath was practically inefficient (the roughness of the treated samples did not vary from that of the untreated ones, within the experimental error). In particular, the thermal effect of the bath became significantly important at temperatures higher than 70°C, and operating at the bath temperature of 90°C the maximum roughness values were obtained. From the practical point of view and considering the feasibility of the industrial transfer of this process, the bath temperature of 90°C was inconvenient because of the bath became more difficult to manage. It was indeed complicated to contain the solution depletion, with the consequent variation of the characteristics of the bath itself over time. In conclusion, acid bath temperatures in the range between 70°C to 80°C seemed to be the most promising.

Following the study of the effect of the bath temperature, the possibility of working at temperatures of 70°C and 80°C and reducing the treatment time was also examined. As it is reported in the work of Lin et al. [65], the etching process is not endless: the surface roughness will finally reach a plateau when micro-pores are uniformly formed. However, the final result strongly depended on the ratio between the available titanium surface and the volume of the acid solution and, eventually, from the agglomeration of the disks. Therefore, it was evaluated that in order to obtain comparable and reproducible roughness results, 30

minutes of acid etching were the best choice. Experiments carried out for lower times produced very different and scattered results probably due to the lack of a suitable bath agitation and disks overlapping (i.e. some samples were strongly etched while other no). Differently, longer etching time allowed the obtainment of more homogeneous samples. Yet, in view of the industrial transfer of the process, a shorter treatment could be reasonably used for time-saving and increasing productivity. It is also worth to point out that, differently from surface roughness, time plays a more dominant role than the temperature in hydrogen absorption. It is known from the literature that the incorporation of hydrogen in Ti occurs during the acid treatment and induces a reduction in the mechanical properties of the material. This effect is much more marked in conditions of long treatment times [65]. That is to say, the longer the time of etching, the more hydrogen diffused into the matrix, especially at high temperatures [65]. In this regard, it is convenient to operate at high temperatures but for reduced times.

Finally, concerning the type of the Ti disk as starting material, sandblasted substrates appeared to be the best substrates for the subsequent acid treatment. It was evaluated by electron microscope analyses that in machined substrates, after the acid treatment, the grain boundaries were particularly evident (Figure 4.2a), index of selective etching. The grain boundaries, typical of polycrystalline materials, are notably particularly sensitive areas, where generally preferential corrosion tends to occur, with localized alteration of the properties of the material. Differently, in SEM images of acid etched sandblasted surfaces, the grain boundaries seemed to be less pronounced (Figure 4.2b).

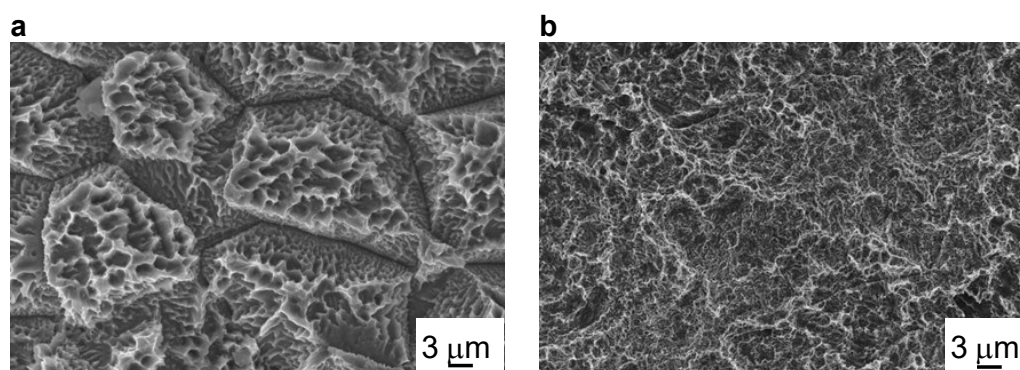


Figure 4.2 Secondary electron SEM images of the machined (a) and alumina sandblasted (b) Ti disks after acid etching at 80°C for 30 minutes.

In conclusion, this research enables the evaluation that there are different possible operating conditions to have roughness comparable to the reference samples (titanium sandblasted with alumina and chemically treated with HF). At this point, the choice of the most convenient procedure must be obtained by making various considerations, such as:

- the service-life of the acid bath;
- the overall process time, linked both to the time that the substrates remain in the acid bath and to the possible need of a previous sandblasting operation;
- the peculiar surface morphology created following the specific acid treatment (defined by time, temperature and starting substrates);
- the possible embrittlement due to the incorporation of hydrogen [65] in titanium.

To summarize, starting from 30 Ti sandblasted disks (diameter: 8 mm) with initial R_a of $0.7 \mu\text{m} \pm 0.1 \mu\text{m}$ and working with 50 g of acid solution (45 wt. % H_2SO_4 , 15 wt. % HCl , and 40 wt. % H_2O with HCl 37 % and H_2SO_4 96-98 %), a bath at 80°C, for 30 minutes and with

moderate bath agitation, it is possible to obtain samples (named Ti_s+a) with a mean final roughness of $1.2 \mu\text{m} \div 0.1 \mu\text{m}$. The surface morphology of these samples is discussed in Chapter 5. Differently from previous investigated sandblasted and HF etched surfaces, EDX analyses on sandblasted and HCl/H₂SO₄ etched samples demonstrated the absence of alumina residues deriving from the mechanical sandblasting process.

4.3 Optimization of the TiO₂ MOCVD process

Low Pressure Metal-Organic Chemical Vapor Deposition (LP-MOCVD) is a widely used technique for depositing crystalline TiO₂ thin films, at relatively low temperature and high growth rates, with moderately low equipment costs and easy scale-up [114]. It is particularly suitable to coat complex-shaped samples, such as dental implants [57,115]. MOCVD technique is successfully applied to titanium dioxide deposition on titanium dental implants, as demonstrated by several biological tests both *in-vitro* and *in-vivo* [25,57,116–119]. Furthermore, by means of *in-vitro* acellular bioactivity experiments, Baryshnikova et al. [23] show that nanostructured TiO₂ grown via MOCVD technique may act as a bioactive material inducing formation on its surface of bone-like apatite. Moreover, besides the intrinsic bioactivity of the crystalline titania thin films, TiO₂ layers on Ti substrates may also be used as a inter-layer for the subsequent deposition of the highly bioactive calcium phosphate ceramics [19,22]. Lastly, crystalline TiO₂ layers may influence on the photoactivity of the Ti implant surface. Indeed, such titania functionalized surfaces would be sterilized simply with the use of UV illumination. In this way, the same coating could perform two tasks: enhance the osseointegration process and contribute to the surface disinfection process [120].

The choice of the best-operating conditions in a MOCVD process is certainly one of the most critical points, as it can significantly influence the quality and the features of the films deposited. Beyond the possible technical limitations, closely related to the specific MOCVD apparatus that is used, the choice of the deposition parameters (such as for example the temperature and pressure of the process, the flows of the reactive and transport gases, the precursor evaporation temperature) principally depends on the nature of the precursor and the substrate, and on the particular type of film that it is intended to deposit. The parameters that should be considered, therefore, are multiple and interdependent, and their correct evaluation and rationalization, in addition to being a function of the individual system under examination, must be done case by case and purely via experiments.

Preliminary MOCVD TiO₂ growth tests were carried out to adjust the process parameters for the production of crystalline, adherent, dense and compact titania films, with optimal conformal coverage of the Ti substrates. In this context, it must be underlined that the knowledge in TiO₂ deposition via MOCVD is well consolidated at ICMATE-CNR and crystalline (anatase and/or rutile phases) titanium dioxide thin films have been extensively studied on different substrates [114,115,117,121]. Therefore, the main CVD parameters such as the precursor nature, its evaporation temperature range, the reactor temperature range, the carrier gas flow, the co-reagents and/or dilution gas flow, and the deposition pressure did not need to be strongly implemented, being part of the institute knowledge [114,115,117,121]. Consequently, just an optimization/verification of the process parameters was carried out.

Working with the proper process conditions it is possible to deposit thin titania crystalline films, simultaneously avoiding the occurrence of the detrimental homogeneous gas-phase

reactions. As it was previously reported, two parallel phenomena could indeed take place during a CVD process:

- a) $(\text{Ti}(\text{OC}_3\text{H}_7)_4)_{\text{gas}} \rightarrow (\text{TiO}_2)_{\text{film}} + (\text{by-products})_{\text{gas}}$
- b) $(\text{Ti}(\text{OC}_3\text{H}_7)_4)_{\text{gas}} \rightarrow (\text{TiO}_2)_{\text{powder}} + (\text{by-products})_{\text{gas}}$

While the heterogeneous reaction (a) represents the process of TiO_2 films and some by-products formation, the parasitic reaction (b) expresses the detrimental reactions for the purposes of the formation of films (but capable of forming powders and other volatile by-products).

In this project, the target thickness of the TiO_2 inter-layer was about 200 nm. The titanium dioxide thickness was chosen, in accordance with literature references [22], in order to obtain the best compromise between the increase of the Ti corrosion resistance (the ability of the titania layer to act as a barrier film against the corrosion is proportional to the deposit thickness) [22] and the optimal coating-to-substrate adhesion features; and at the same time preserving the pristine substrates roughness [118]. In this perspective, taking into account the similar thermal expansion coefficients of Ti and TiO_2 [22], as well as the quite thin titania inter-layer (200 nm), the thermal mismatch can only marginally (or not even) negatively contribute the bonding strength; otherwise an increase in the thickness of TiO_2 layer could result in a decrease in the bonding strength due to the thermal mismatch effect [22]. Therefore, the application of a thinner layer of TiO_2 might be favored.

During the optimization phase, titania depositions were performed on Si (100) substrates. In this regard, preliminary work carried out on silicon substrates helped in determining the TiO_2 thicknesses by profilometer analyses. Indeed, the film thickness evaluation by means of stylus profilometer was very difficult on the effective Ti substrates here used (i.e. Ti_m , Ti_s , and Ti_{s+a}), since their intrinsic roughness prevented the determination of the thickness being of the same order of magnitude of the TiO_2 film deposited (few hundreds of nanometers). Furthermore, because of the ductile nature of the titanium substrate (i.e. not easily sectionable), titania layers deposited on silicon substrates allowed (although with a certain approximation due to the actual diversity of the substrates) the analysis of the coating features along the sections.

As an example, in order to understand how the individual evaluation of each deposition parameter is important during a MOCVD process, the graph in Figure 4.3 shows the thickness of titania films as a function of the substrate position with respect to the reactor inlet and as a function of growth temperature (while the other deposition parameters were kept constant).

By means of this study, it was possible to define the most suitable process temperature to maximize the useful zone of the reactor for the homogeneous and reproducible covering with crystalline TiO_2 of different titanium samples, thus allowing the simultaneous coating of several Ti substrates. In this context, although the temperature inside the reactor is frequently considered constant, it has a Gaussian course. This is mainly due to the inevitable thermal dispersion at the ends of the reactor and to the particular arrangement of the heating resistors. Furthermore, precursor depletion (because of the film growing occurrence) could also happen along the reactor. As can be seen in Figure 4.3, by means of the MOCVD apparatus showed in Figure 2.9 and Figure 2.10, depositing at 390°C it was possible to achieve a fairly homogeneous deposition zone in terms of film thickness in a variable position between 4 and 18 cm from the reactor inlet (in addition, the reaction in homogeneous phase was avoided). Differently, higher deposition temperatures sensibly reduced the useful zone, while lower temperatures resulted in not very homogeneous and largely amorphous coatings.

This temperature, therefore, was subsequently selected to deposit on the planar titanium substrates supplied by the industrial partner.

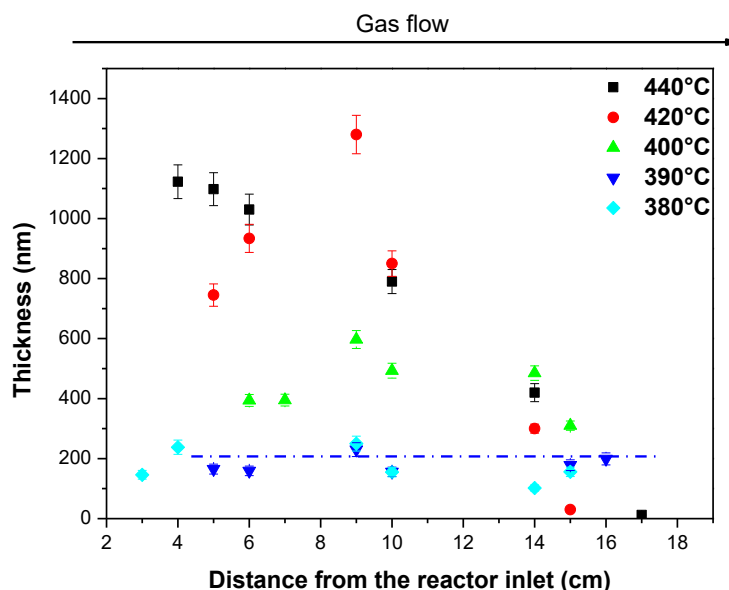


Figure 4.3 Thickness of the titania films on Si (100) substrates as a function of the deposition temperature and the position inside the reactor.

The used process parameters are summarized in Table 4.1. The Ti source was Titanium Tetraisopropoxide (see Chapter 2, section 2.2.5) and no co-reagents were necessary (i.e. the TTIP precursor provides the O and Ti atoms necessary for the TiO_2 growth). The process pressure was 100 Pa; working at higher pressures phase gas reactions became more probable, while lower pressures were unfeasible because of the instrumental features. TTIP evaporation temperature was set at 40°C (working at lower precursor evaporation temperature problems due to precursor depletion along the horizontal furnace were detected, while at higher temperature phase gas reactions were observed). The carrier gas flow was established at 110 sccm, allowing a good compromise between the precursor transport and the working pressure. Adopting these experimental conditions, a titania growth rate of about 25 nm/min was measured. Prior to deposition, all the substrates were subjected to a cleaning process, with soap and ultrasound, and were let dry in air. Generally, the temperature inside the reactor stabilized within about an hour.

Deposition temperature	390°C
Deposition pressure	100 Pa
Ti source	Titanium tetraisopropoxide (TTIP) Aldrich > 97%
Ti precursor temperature	40°C
Carrier flow	N_2 - 110 sccm
Process time	8 minutes

Table 4.1 MOCVD parameters here used to deposit crystalline titania films on titanium substrates.

Figure 4.4 shows uncoated (Ti_m) and 200-nm-titania coated machined titanium substrates (Ti_m_TiO₂), covered according to the experimental conditions reported in Table 4.1. It can be observed that depositing few hundreds of nanometers, the surface acquires characteristic color (Figure 4.4b) due to light interference phenomena taking place at the metal-oxide-air interfaces and that depends on the titania film thickness [122]. Besides, the homogeneous green color further confirms the thickness uniformity.

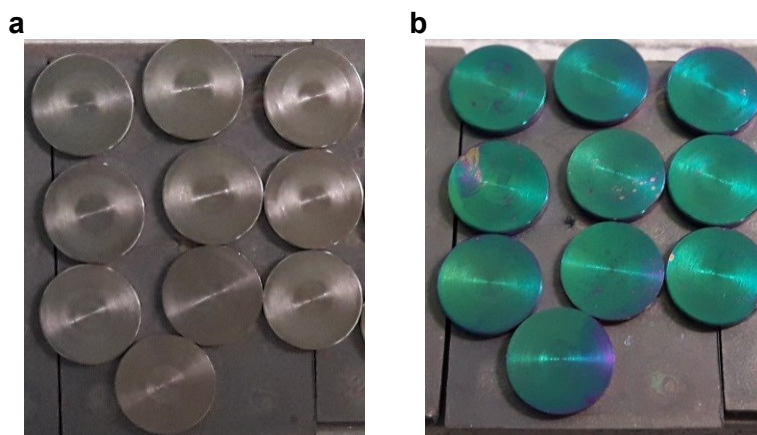


Figure 4.4 Uncoated (a) and 200-nm-titania coated (b) machined titanium substrates.

Simultaneously to the coating features, during this phase of preliminary investigations, particular attention was also paid on the future scale-up of the process, so reproducibility, cost and environmental impact of the method were at the same time evaluated. Concerning these aspects, the chosen relative low growth temperature, as well as the quite simplicity of the MOCVD apparatus (does not require high vacuum conditions, expensive or unavailable reagents, etc.), make this process a reliable procedure for the future composite material scale-up. Furthermore, the precursor decomposition path is quite clean. It is reported that TTIP decomposes at $T \leq 400^{\circ}\text{C}$ through the formation of acetone, isopropanol, and propene as by-products [79]. These by-products can be easily removed from the apparatus (for example by burning them and/or by dissolving them in water and/or by using absorbing carbon cartridge).

4.4 Optimization of the calcium phosphate ceramic deposition process

Spray pyrolysis technique was employed to obtain homogeneously spread and discontinuous calcium phosphate ceramic coatings on titanium substrates with different pristine morphologies. The spray pyrolysis apparatus was specifically projected, assembled and optimized at the ICMATE-CNR laboratory for this purpose.

A variety of methods can be used for the deposition of bioactive calcium phosphate ceramic coatings (mainly composed of hydroxyapatite) [1,6,12,123] on titanium substrates. Most of these procedures are vacuum-based expensive techniques, necessitate of high-cost reagents or targets as starting materials and the films show poor homogeneity and adhesion [123]. Nowadays, the most widespread procedure to coat Ti dental implants with layers of bioactive calcium phosphate ceramics consists in the preparation of hydroxyapatite deposits by plasma spray technique [1,11,124]. Yet, plasma spray calcium phosphate ceramic coatings on metal implants usually suffer from poor adhesion [19]; furthermore difficulties

related to the poor control of the stoichiometry and phase composition of the deposited materials (mainly due to extremely high processing temperatures) are reported [13,125].

Hydroxyapatite deposition via spray pyrolysis (and its modifications, ultrasonic spray pyrolysis, and electrostatic spray deposition, see Chapter 2) [13,85,123,125–127] can be considered as a simple deposition method, with low setting-up costs and capable of processing at atmospheric pressure, in air and at ambient conditions with good productivity, thus suitable for large scale industrial applications [128]. Furthermore, the homogenous chemical composition of the products and their high-purity (that depends only on the purity of the precursor used) satisfy the major requirements related to the development of hydroxyapatite coatings for dental implant applications such as the preparation of stoichiometric material with exact chemical and phase composition [123,126–128]. Calcium phosphate coatings with thickness varying between 5 μm and 70 μm have been prepared using the spray pyrolysis technique [126].

The objective of this study was firstly to investigate the feasibility of the spray pyrolysis technique to deposit calcium phosphate ceramic (i.e. hydroxyapatite) on titania coated titanium substrates with a precisely targeted arrangement, i.e. homogeneously spread but discontinuous islands (see Preview, Aim of the thesis, Figure 1Ia) that induce both the biomaterials (i.e. hydroxyapatite and titania) to simultaneously contribute to the osseointegrative process.

Different experimental conditions (such as the substrate temperature, the carrier gas pressure, the nozzle-to-substrate distance, the substrate rotation frequency, the number of spray cycles, the continuous or intermittent spray mode) were investigated for this purpose.

During the spray pyrolysis process, the coatings were prepared with an intermittent spray (2 s of spray and stop of 60 s between two cycles of spray) in order to give adequate time for the solvent to evaporate from the precursor solution. In this way, the coatings have much less and little cracks than those prepared with a continuous spray [126] because each layer is completely dried and decomposed during the period of “rest” without the spray, allowing relatively small shrinkage during the successive heating.

Throughout spray deposition, the temperature of the Ti substrate was relatively low (300°C), thus the pyrolysis was partially separated from the process of densification and consolidation of the material. On the other hand, less solvent in the droplets evaporated during the flight and the droplets arrived at the substrate surface with the capability to spread [126], so improving the bonding efficiency of the coatings [126]. After deposition, the films were maintained at 300°C for 3 minutes and then quickly cooled down to room temperature.

In order to delineate the most suitable spray pyrolysis parameters, preliminary tests were conducted on bare soda-lime (SL) glasses and on soda-lime glasses previously coated with crystalline titania (via MOCVD).

During the preliminary experiments, several parameters were considered, such as:

- The nozzle-to-substrate distance (tested distances: 11.0 cm, 19.5 cm, and 25.0 cm).
- The carrier gas (N_2) pressure (tested pressures: 0.7 bar, 1.0 bar, and 2.0 bar).
- The type of substrates: soda-lime or titania coated soda-lime glass substrates.

Differently, other process parameters were maintained constant, such as:

- The precursor solution. It was a calcium phosphate aqueous solution (acidified with HNO_3) with target stoichiometry ($\text{Ca/P} = 1.67$), corresponding to the composition of hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, and in concentration of 0.1 M. These conditions were chosen and adapted from literature studies [123,126]. In detail, 2.36 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and 0.792 g of $(\text{NH}_4)_2\text{HPO}_4$ were separately dissolved in 10 mL of distillate water. Successively, the ammonium hydrogen phosphate solution was

poured into the calcium nitrate tetrahydrate solution while vigorous stirring. Finally, concentrated nitric acid (Fluka, 65%, 0.69 mL) was added to the solution to dissolve the precipitate hydroxyapatite powder.

- Substrate temperature: 300°C.
- Spray time: 2 seconds.
- Delay-time or rest time (in case of multiple sprays): stop of 60 s between two cycles of spray.
- Substrate rotation frequency: 0.5 Hz.
- The roller in front of the trigger of the airbrush Paasche VL was regulated at full scale (in this way the airbrush works as a single action system where it is only possible to regulate precursor solution flow).
- The trigger button during the spray phase was always pushed down at full scale (in order to spray constant amounts of solution in the time unit).

Figure 4.5 shows a photo of the samples obtained on soda-lime glasses and on TiO₂ coated soda-lime glasses, changing the carrier flow pressures (0.7 bar and 1.0 bar) and the nozzle-to-substrate distances (11.0 cm, 19.5 cm, and 25.0 cm), while 1 spray cycle of 2 seconds was always carried out. In order to obtain homogeneous deposits over the entire substrate surface, it was necessary to operate with high nozzle-to-substrate distances (Sample 6 and Sample 7). At reduced distances (less than 20 cm) the sprayed surface was not uniform and a central circular spot with a higher precursor amount was observed (see Sample 1, Sample 2, Sample 3, Sample 4 and Sample 5). Furthermore, at a pressure of 1.0 bar, the amount of the sprayed deposit appeared to be higher than that sprayed at a pressure of 0.7 bar (the coatings in Sample 2 and Sample 3 looked slightly thicker than those in Sample 4 and Sample 5).

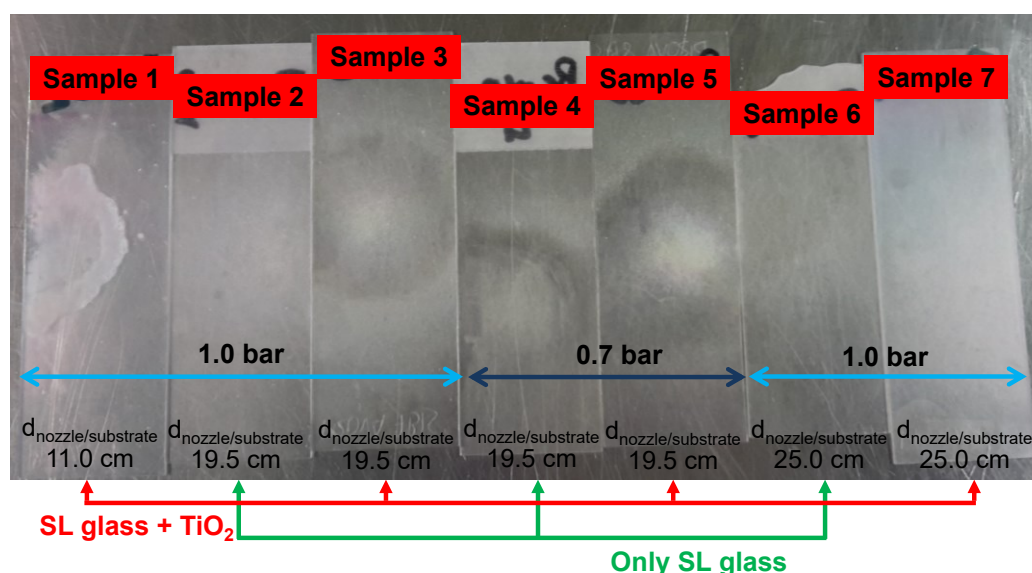


Figure 4.5 Photo of the HA coatings on soda-lime glasses and on titania coated soda-lime glasses, at different carrier flow pressures and nozzle-to-substrate distances (with 1 spray cycle of 2 seconds).

By performing multiple deposition processes (i.e. 1, 2 or 3 spray cycles with spray time: 2 s; delay-time: 60 s), thicker and less porous deposits were obtained, as shown in Figure 4.6. Experiments were also conducted at a carrier gas pressure of 2.0 bar. Under these conditions, however, the airbrush often malfunctioned due to the dripping of the precursor solution from the nozzle. Thus, to avoid this problem, it was later chosen to work at a

pressure of 1.0 bar. Finally, from these preliminary studies, nothing can be predicted about the influence of the titania inter-layer on the glass substrate surfaces.

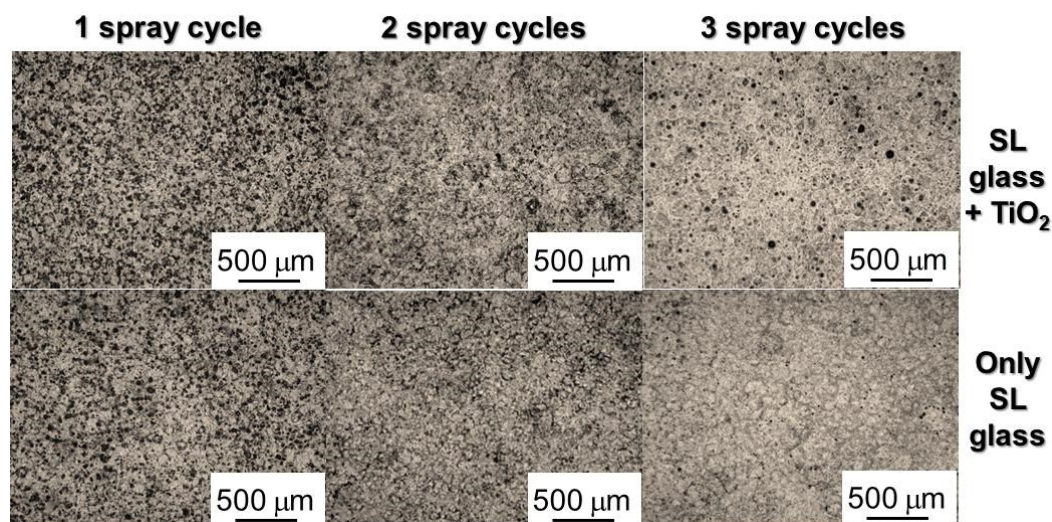


Figure 4.6 Optical microscope images of the HA coated samples obtained on soda-lime glasses and on titania coated soda-lime glasses, as a function of the number of spray cycles (with nozzle-to-substrate distance: 25.0 cm; carrier gas pressure: 1.0 bar).

Once the most suitable spraying parameters were defined, deposits were made on the Ti substrates (Ti_m, Ti_s, and Ti_{s+a}) beforehand coated with 200 nm of TiO₂ via MOCVD. Initially, to obtain homogenous and discontinuous HA coatings (i.e. HA islands), one and two spray cycles were taken into account.

As an example, Figure 4.7 shows the secondary electron SEM images of sandblasted titania coated Ti substrates after 1 spray cycle (a) and 2 spray cycles (b). The white circles highlight some of the HA coated areas.

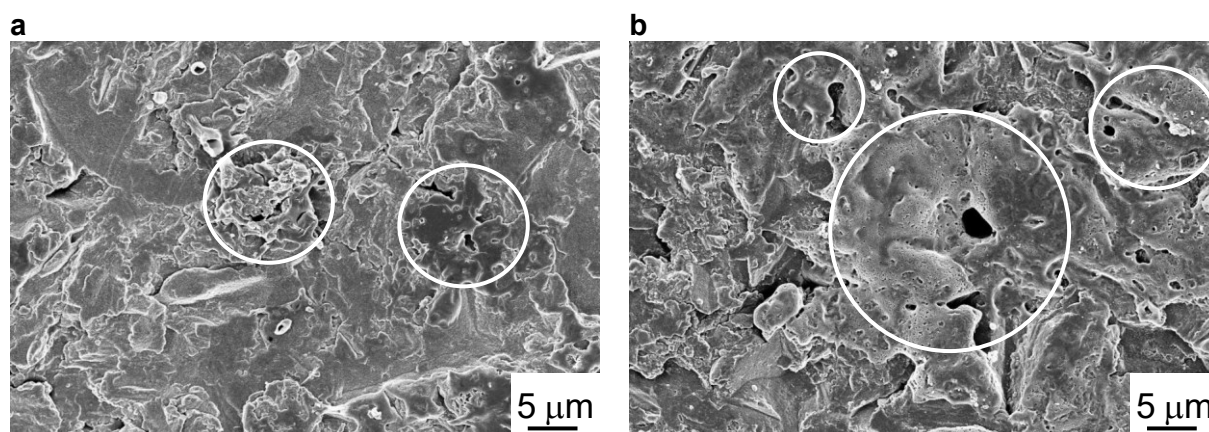


Figure 4.7 Secondary electron SEM images of sandblasted titania coated Ti substrates after 1 spray cycle (a) and 2 spray cycles (b). The white circles highlight some of the HA coated areas.

From optical microscope (Figure 4.6) and SEM images (Figure 4.7), it appeared that just one spray cycle of 2 seconds was not sufficient in order to deposit a sufficient amount of the bioactive component on the titania surfaces (i.e. the HA islands were very dispersed and isolated). The evaluation of the deposited HA amount with 1 or 2 spraying cycles by means of EDX analyses was very arduous due to the low quantity of material deposited. Only the

analyses carried out at high magnification allowed the detection of calcium e phosphorous EDX signals, but, in these circumstances, the results were strongly influenced by the specific area analyzed (being for definition HA coatings discontinuous). Therefore, two consecutive spray cycles of 2 seconds were always carried out.

Table 4.2 summarizes the parameters for the spray pyrolysis deposition process.

Substrate temperature	300°C	Sprayed area	16 cm ²	Rest time	60 s
Nozzle-to-substrate distance	25 cm	Precursor flow rate	0.3 mL/s	Spray cycles	2
Carrier (N₂) gas pressure	1 atm	Spray time	2 s	Substrate rotation frequency	0.5 Hz

Table 4.2 Spray pyrolysis parameters here used to deposit hydroxyapatite.

4.5 Definition of the post-synthesis thermal treatment temperature

Before the spray pyrolysis process, the Ti substrates were pre-coated with a titania inter-layer by means of MOCVD operating at 390°C (first step of the functionalization). The features of the TiO₂ inter-layer (i.e. morphology and topography, thickness, integrity, crystallinity, hardness, etc.) are particularly important in determining the success of the two-step functionalized composite material because the failure of the bi-layered coating could also be the consequence of the degradation of the titania inter-layer. For this reason, the properties of pure titania MOCVD coated titanium materials are the focus of a manuscript [27] and they will be also discussed in Chapter 5.

During the second step of the titanium functionalization (i.e. spray pyrolysis process) the substrate temperature was kept relatively low (300°C). Yet, due to the low deposition temperature, a subsequent heat treatment is necessary to allow the consolidation of the coating, with an increase of integrity and bonding strength. Furthermore, taking into account that the TiO₂ inter-layer is able to improve the hydroxyapatite to titanium adhesion strength only when the hydroxyapatite layer is well crystallized [22], and that, in general, the crystallinity of the HA coatings for biomedical applications is about 65÷70% [29], a post-deposition thermal treatment in order to crystallize (or partially crystallize) the sprayed HA phase was considered. For this reason, some *ad-hoc* samples with higher amounts of the HA solution were prepared, spraying 10 times for 2 s, with 60 s interval between each other (the other deposition parameters are reported in Table 4.2). In this way, it was possible to strengthen the HA XRD signals. After the deposition process, the samples were left as-deposited or annealed at different temperatures, from 400°C to 700°C, in air, for 6 hours, with a heating rate of 10°C/min. After the spray pyrolysis process, the samples were named as Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, and Ti_s+a_TiO₂_HA.

Figure 4.8, Figure 4.9 and Figure 4.10 show the XRD patterns of TiO₂/HA coated Ti, as-grown or annealed at different temperatures, from 400°C to 700°C, in air, for 6 hours, for machined substrates, sandblasted, and sandblasted/acid etched Ti substrates, respectively. The as-grown HA coatings, grown on all the types of substrates (i.e. “m”, “s”, and “s+a”), are composed of poorly crystallized hydroxyapatite (ICDD: 01-084-1998) and monetite (CaHPO₄; ICDD: 00-009-0080). This last phase disappeared after thermal treatment, while only hydroxyapatite, TiO₂, and titanium phases were detected, also suggesting the absence of any chemical reaction among the different components.

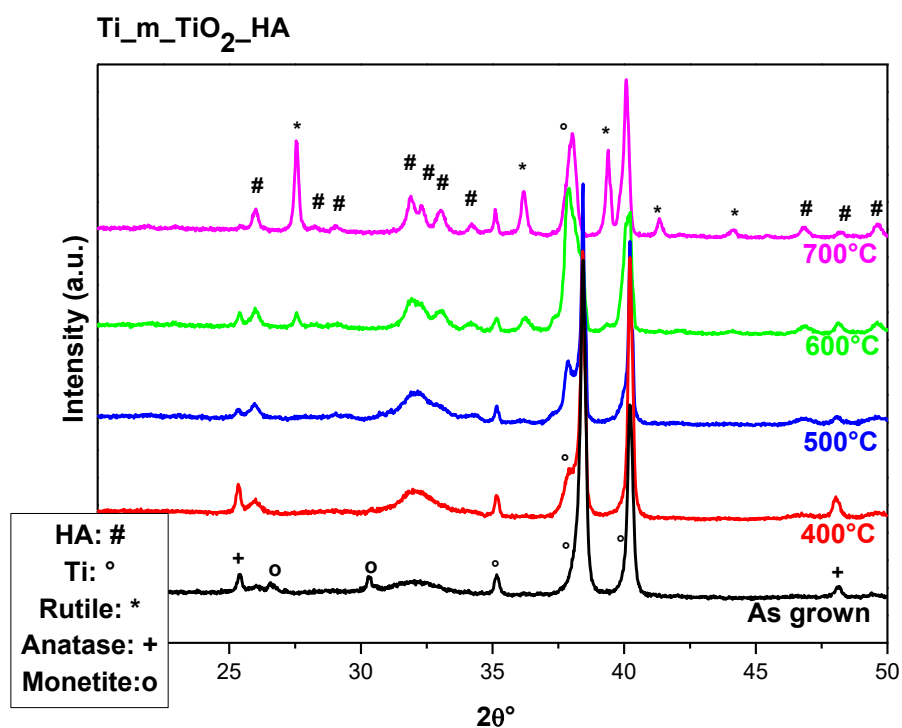


Figure 4.8 XRD patterns of machined TiO₂/HA coated Ti as-grown and annealed at different temperatures, from 400°C to 700°C, in air, for 6 hours.

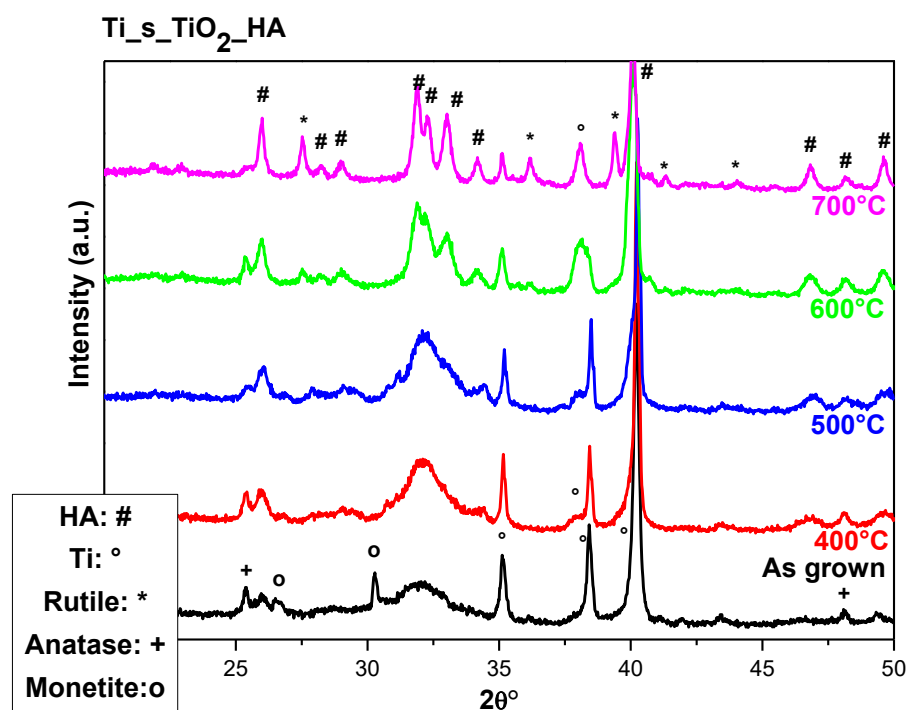


Figure 4.9 XRD patterns of sandblasted TiO₂/HA coated Ti as-grown and annealed at different temperatures, from 400°C to 700°C, in air, for 6 hours.

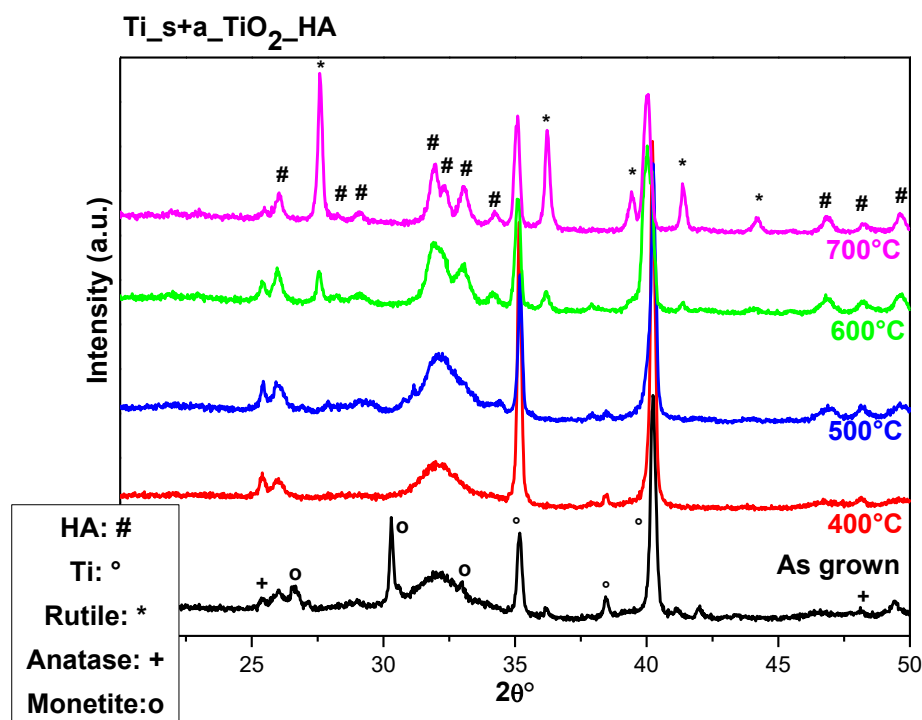


Figure 4.10 XRD patterns of sandblasted/acid etched TiO₂/HA coated Ti as-grown and annealed at different temperatures, from 400°C to 700°C, in air, for 6 hours.

Moreover, increasing the thermal treatment temperature, the HA structure evolved from a low crystallized state to a highly crystallized one. According to other findings [129], well-crystallized hydroxyapatite phase started to form at about 600°C. However, at that temperature, the MOCVD deposited titania in the anatase phase (ICDD: 01-084-1286) partially underwent a phase transition to the rutile phase (ICDD: 01-088-1172). Nevertheless, at high temperatures (typically $\geq 600^\circ\text{C}$, but depending on the specific conditions), it cannot be excluded the formation of rutile deriving from the pristine titanium oxidation [130–132] (as it will further prove in the next chapter by weighing experiments).

Figure 4.11 summarizes the evolution of the hydroxyapatite crystallite size, calculated using the Rietveld method, as a function of the thermal annealing temperature and substrate type. It can be seen that the crystallite size remains almost constant until 500°C, while for higher temperatures the crystallite size clearly increases, confirming that the hydroxyapatite crystallization process strongly improved.

The Ti_{s+a}TiO₂HA sample at 700°C presents a lower value probably due to a finer dispersion of the HA islands during the spray pyrolysis process (see Chapter 5).

Literature *in-vitro* and *in-vivo* studies show that HA coatings with high dissolution rates (i.e. low crystallinity HA) improve bone bonding in the earliest stages of healing [29]. On the other hand, highly crystalline and stoichiometric HA coatings show slow dissolution rates and are more stable *in-vivo* than amorphous ones, but display limited bioactivity [29]. Therefore, in order to maximize the crystallinity of HA coating, meanwhile maintaining adequate bioactivity [29], implant durability and composite material performances, the best compromise of thermal annealing of 600°C, in air, for 6 h was considered from here on.

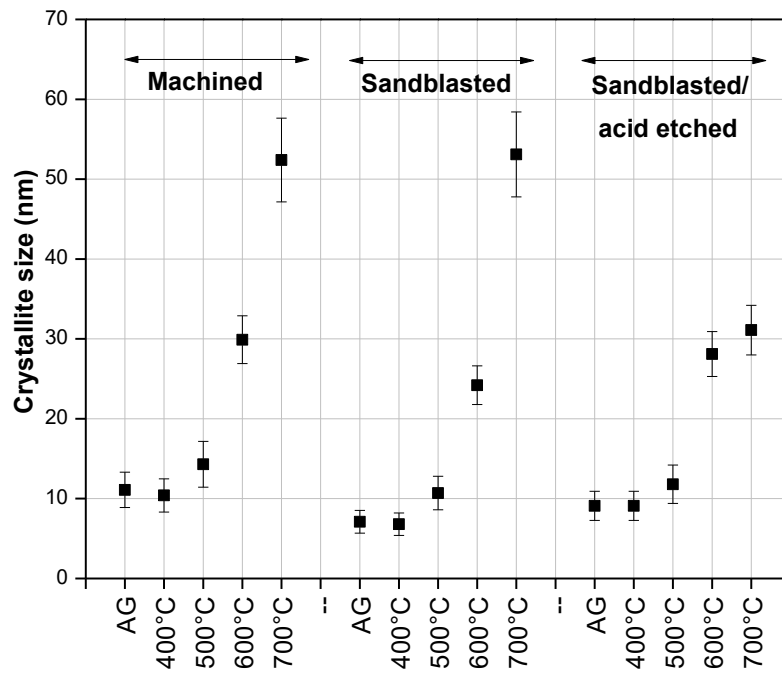


Figure 4.11 Evolution of the hydroxyapatite crystallite size, calculated using the Rietveld method, as a function of the thermal annealing temperature; AG: As-Grown.

**Chapter 5 TiO₂/HA
FUNCTIONALIZED
Ti DENTAL
IMPLANTS**

5.1 Introduction

To increase Ti bioactivity and ensure the short- and long-term success of the implant, titania (TiO_2) - hydroxyapatite (HA) bi-layered coatings, with targeted surface design, were obtained by means of a two-step process on the surface of diverse Ti substrates. Specifically, Metal-Organic Chemical Vapor Deposition was used to deposit adherent, dense, compact and crystalline titania inter-layer, while spray pyrolysis technique was employed to obtain homogeneously spread but discontinuous hydroxyapatite top-layer.

The influence of each treatment step and each substrate typology on the morphology, crystalline structure, and spreading of the coatings, and on the functional performances of the coated materials was investigated. Surface wettability and hydrophilicity preserving, ion release, potentiodynamic and tribocorrosive behavior, nanohardness and qualitative adhesion of coatings were evaluated. Finally, acellular *in-vitro* bioactivity and *in-vitro* cytotoxicity were assessed.

All quantitative data were reported as the mean value $\pm$ standard deviation. For the assessment of the differences among groups, two-sample t-test or One-Way ANOVA were used (Minitab software). Statistical differences between the samples were determined by Tukey's multiple comparison, while the Dunnett method was used to compare each mean with a control group. A p-value < 0.05 was taken to indicate statistical significance.

5.2 Morphological and topographical characterization

The relevance of changes in implant geometry, surface morphology and/or chemistry within the osseointegration process is extensively reported [1,11]. Concerning implant surface morphology, it is described that it has a noticeable effect on cell adhesion, extension, and sequence [133]. In the past, the most common machining technique for oral implants was turning. Nowadays, the market is dominated by implants with surface roughness higher than the turned component ones. Rougher surfaces are generally claimed to promote improved healing, thanks to their better mechanical anchorage to the bone. Many *in-vivo* and *in-vitro* studies show a better osseointegrative response of rougher surfaces when compared to smoother ones [133], and the significance of nanostructured surfaces is documented [133]. In the work of Kubo et al. [134], it is shown that TiO_2 nanonodules onto micro-pitted acid-etched Ti surfaces enhance the attachment, spread, proliferation, and differentiation of osteoblasts. Moreover, Cochran et al. [135] compare micro/nano-porous implants (large grit sandblasted and acid-etched, SLA) with micro-porous implants (i.e. titanium plasma spray, TPS) and find higher bone-to-implant contact for the rougher surfaces (SLA implants). However, rougher surfaces may have clinical drawbacks, such as marginal bone resorption and/or increased ion release [63].

In this work, three types of surfaces with different surficial morphology and topography were used as titanium substrates. Ti discs ($\phi = 8$ mm) or implants (cylindrical implants with truncated cone end, external hexagon geometry, height: 10 mm; $\phi = 4$ mm) were treated according to three different surface modification procedures, to obtain various surface characteristics, as described in Chapter 4. For all the Ti substrates, before the coating processes, a cleaning process, with soap and ultrasound, was applied.

Scanning Electron Microscopy (SEM) images (at different magnifications) of machined, sandblasted and sandblasted/acid etched titanium discs are shown in Figure 5.1, Figure 5.2, Figure 5.3 and Figure 5.5.

Machined substrates (Figure 5.1 and Figure 5.2) have the surface morphology of as-received machined implants, without any treatment, except for the washing procedure. The turning process generated tool marks and the surface is clearly anisotropic with directional irregularities.

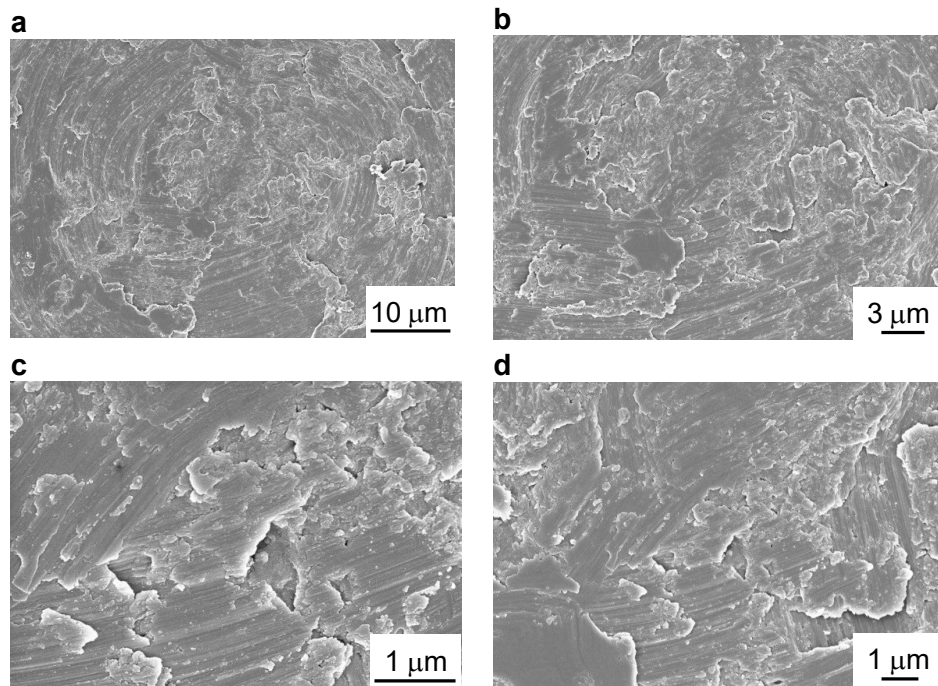


Figure 5.1 Secondary electron SEM images of machined Ti substrates (in the middle of the Ti disk), at growing magnification (a-d).

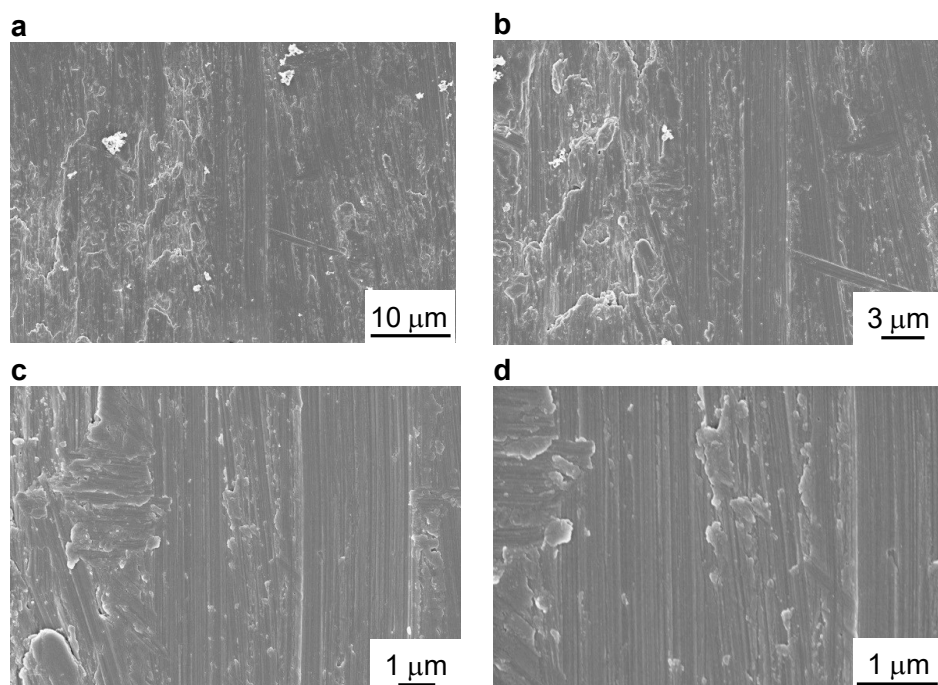


Figure 5.2 Secondary electron SEM images of machined Ti substrates (in the edge of the Ti disk), at growing magnification (a-d).

Different surface directionality can be indeed observed in the middle (Figure 5.1) and in the edge of the machined titanium disks (Figure 5.2). This strong anisotropy must be taken into account because, as it will show later, it markedly influences the spray pyrolysis process.

In Figure 5.3, it can be observed that the Al_2O_3 sandblasting process clearly modified the pristine machined surface morphology and a homogeneous and isotropic surface structure is evident.

Alumina is a commonly used blasting material, yielding a surface roughness that depends on the size of the blasting media [1]. Unfortunately, alumina residues often remain after the cleaning procedures (alumina is insoluble in acid, so it is particularly hard to remove), thus blasting material is frequently embedded into the implant surface. These residues must be particularly taken into account, because they could be released into the implant surrounding tissues (therefore negatively interfering with the osseointegration process) or create chemical heterogeneity that decreases the titanium corrosion resistance [1].

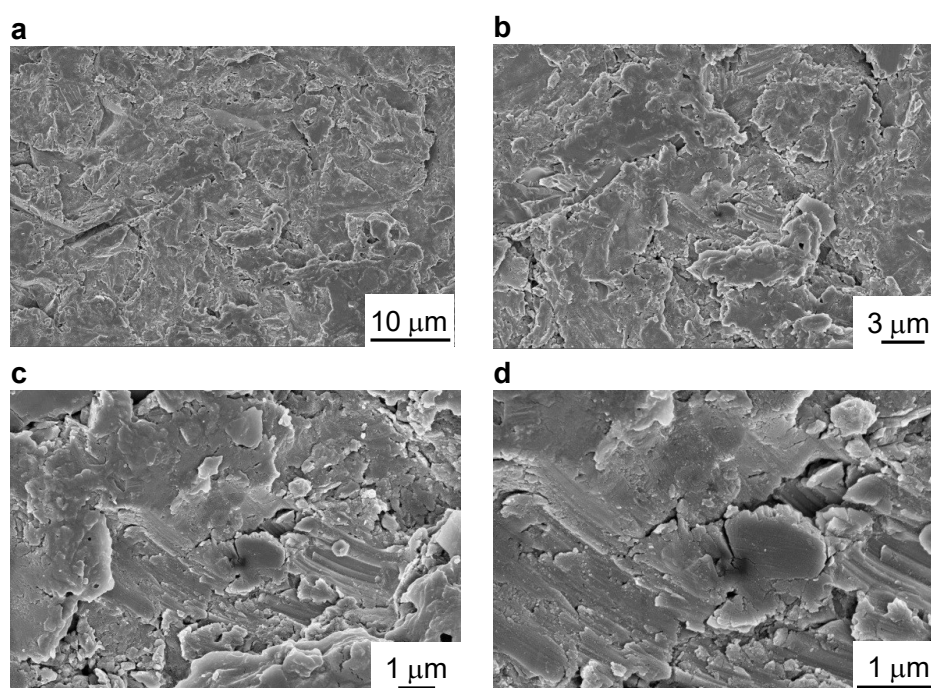


Figure 5.3 Secondary electron SEM images of Al_2O_3 sandblasted Ti substrates, at growing magnification (a-d).

EDX analyses, in sandblasted samples, confirmed the presence of alumina residues (generally about 11 wt. %). The EDX mapping of Ti, Al and O elements (Figure 5.4) revealed that these elements were not homogeneously distributed, as also confirmed by the dependence of the Al/Ti ratio, which varied according to the area analyzed.

The surface morphology of sandblasted and acid etched samples (Figure 5.5) appears more isotropic than that of the turned and sandblasted surface ones. The sandblasting process and the following acid treatment changed the implant surface morphology: multi-level pores (induced from alumina sandblasting and acid etched processes) can be detected. The high magnification images (Figure 5.5c,d) show nanometric grooves resulting from etching procedure. EDX analyses revealed that the acid etching process allowed the whole removal of sandblasting particles, as confirmed also by XRD characterization, discussed later.

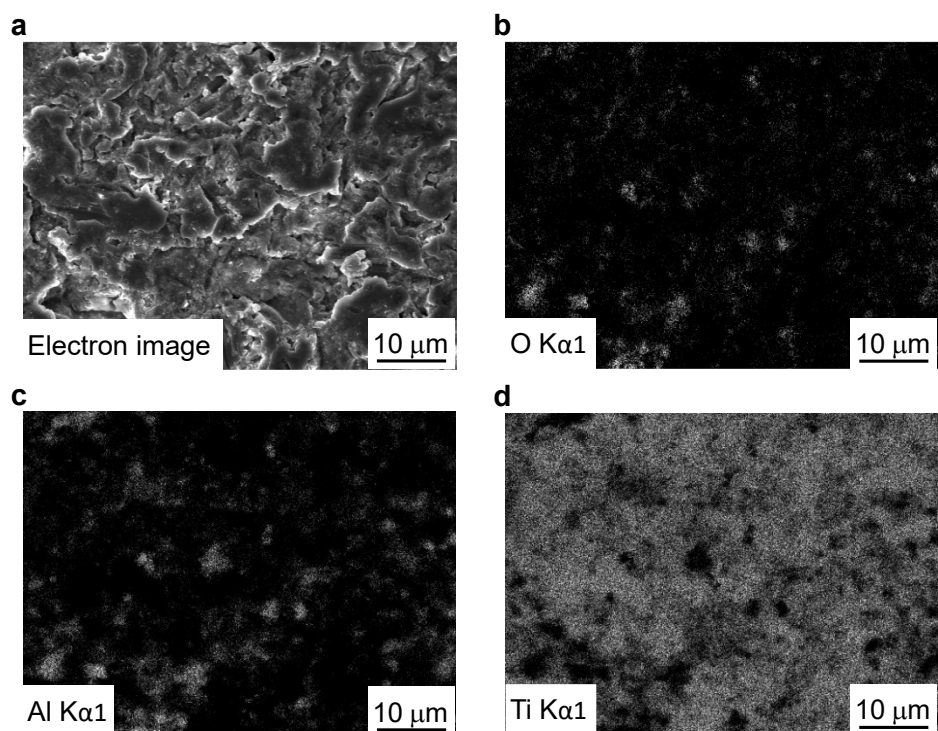


Figure 5.4 EDX surface mapping performed on Al₂O₃ sandblasted titanium disk. Electron image (a), O Kα1 (b), Al Kα1 (c), Ti Kα1 (d).

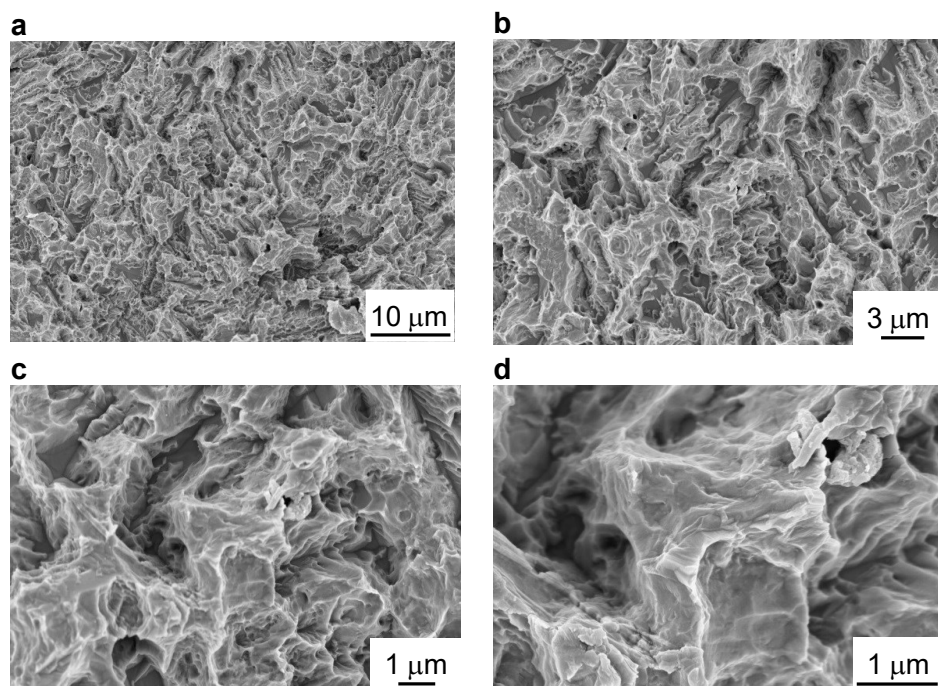


Figure 5.5 Secondary electron SEM images of sandblasted and acid etched Ti substrates, at growing magnification (a-d).

Figure 5.6, Figure 5.7 and Figure 5.8 show SEM images, at different magnifications, of the Ti substrates (machined, sandblasted and sandblasted/acid etched, respectively) after the LP-MOCVD deposition process (with the experimental condition reported in Chapter 4, Table 4.1). MOCVD TiO₂ films were conformal, even though the pristine substrate surface was rough, and the TiO₂ film matched the profile of the substrate below [118]. The typical titania

morphology, with an angular platelet-like structure, was detectable at high magnification. Precisely, in Ti sandblasted coated samples (Ti_s_TiO₂) and in Ti sandblasted/acid etched coated samples (Ti_s+a_TiO₂) the grains appeared slightly less sharp and smaller than that of Ti machined coated ones (Ti_m_TiO₂).

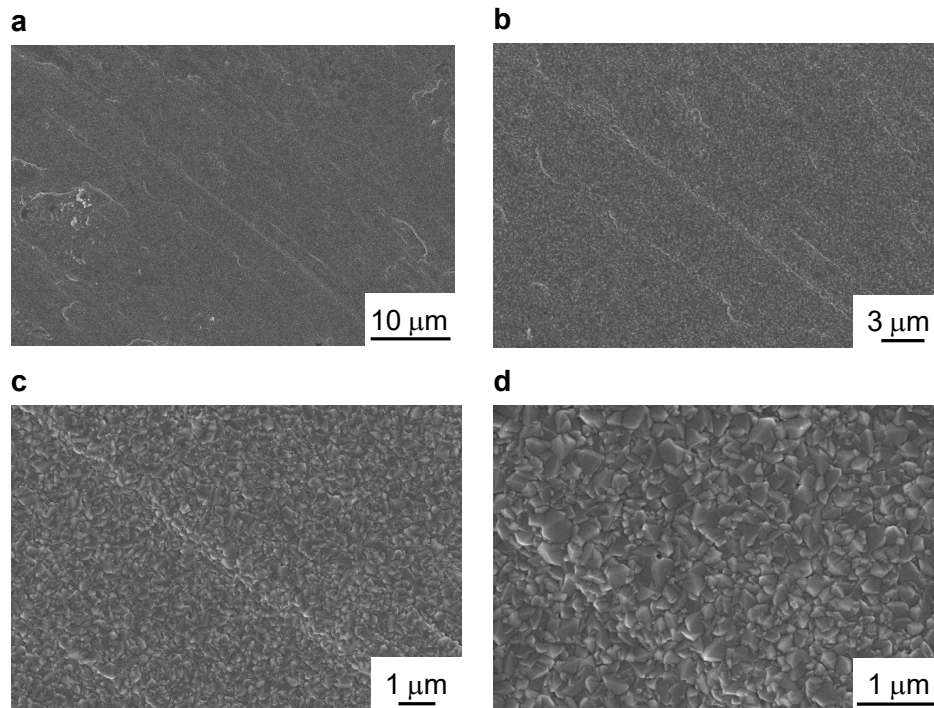


Figure 5.6 Secondary electron SEM images of pristine machined Ti substrates, at growing magnification (a-d), after the MOCVD TiO₂ deposition.

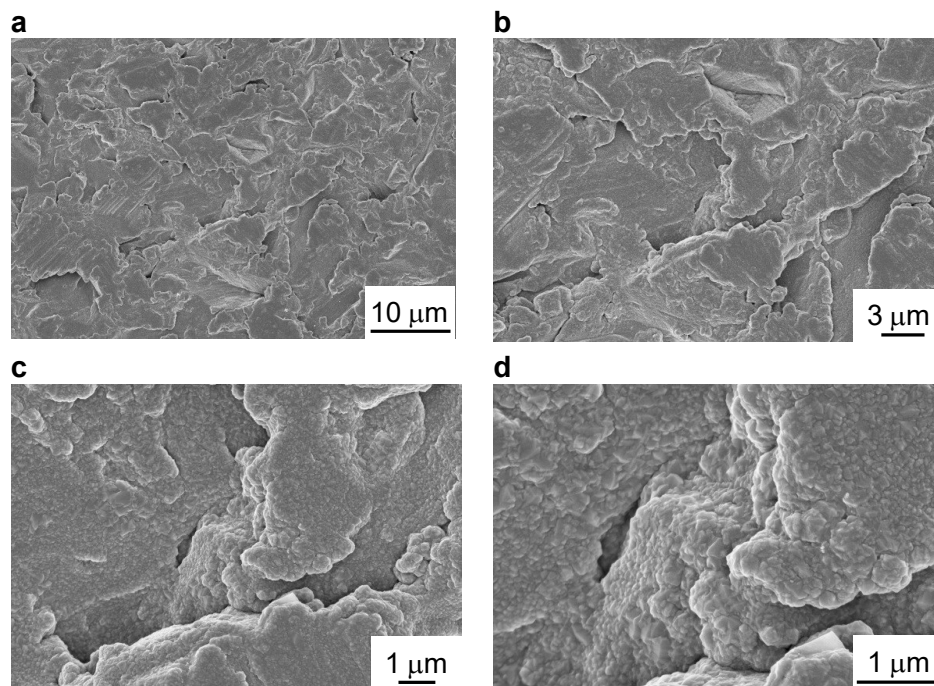


Figure 5.7 Secondary electron SEM images of pristine sandblasted Ti substrates, at growing magnification (a-d), after the MOCVD TiO₂ deposition.

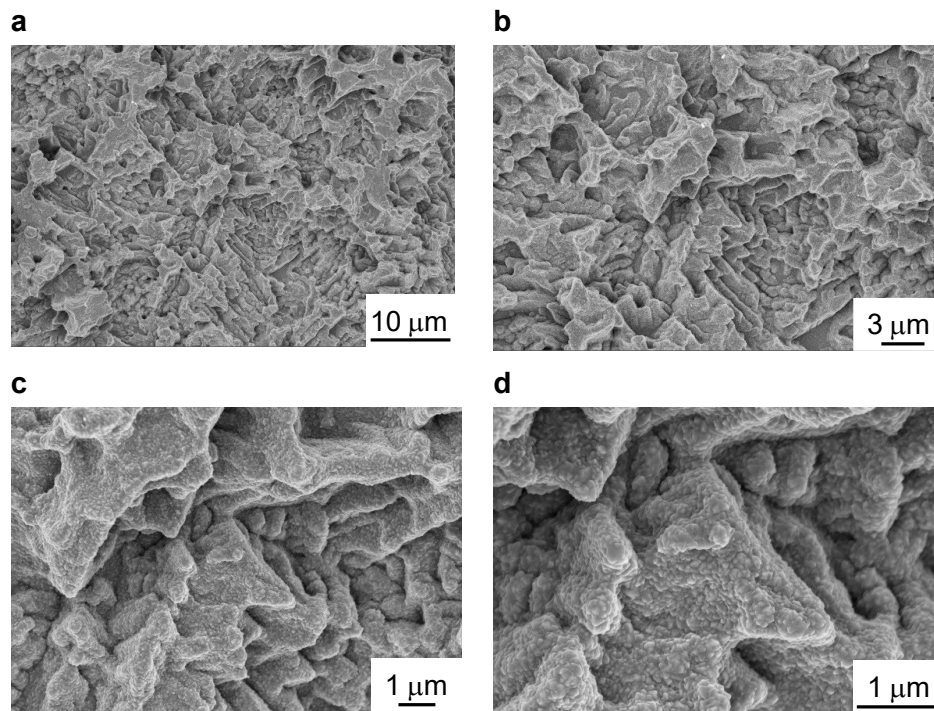


Figure 5.8 Secondary electron SEM images of pristine sandblasted and acid etched Ti substrates, at growing magnification (a-d), after the MOCVD TiO₂ deposition.

Because of the ductile nature of the titanium substrate (i.e. not easily sectionable), in order to analyze the coatings along the sections, titania layers were deposited on Si (100) substrates. From cross-section SEM image (Figure 5.9) of a titania layer deposited on Si substrate, under the experimental conditions of titanium substrates (Table 4.1), a 200 nm titania layer with a compact structure was obtained, with a growth rate of 25 nm/min. This is consistent with previous studies where it is shown that under similar growth conditions, the deposited titania assumed a compact structure as long as the thickness is less than 400 nm, and a columnar structure formed upon increasing the thickness [57,136].

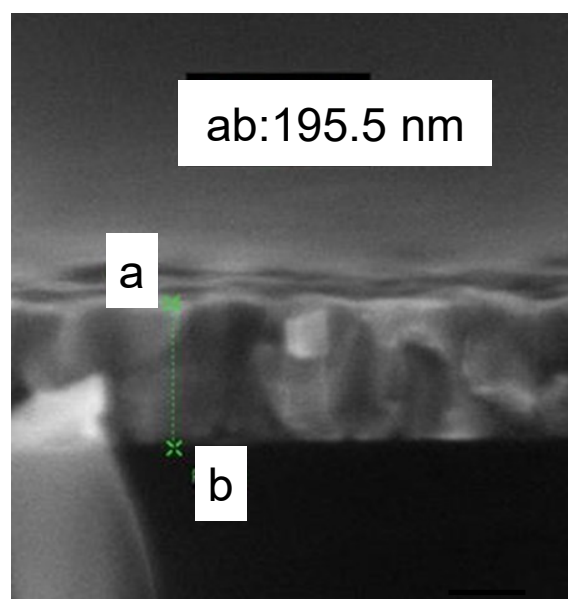


Figure 5.9 Cross-section SEM image of a titania layer deposited on Si (100) substrate, under the same experimental conditions of titanium substrates.

After the spray pyrolysis process (with the experimental condition reported in Chapter 4, Table 4.2) and the thermal treatment at 600°C, for 6 h, in air, the surface changed and a new porous and discontinuous HA top-layer over the titanium dioxide inter-layer was detectable (Figure 5.10, Figure 5.11, Figure 5.12 for Ti_m, Ti_s and Ti_s+a substrates, respectively).

EDX analysis (Figure 5.13), on the Ti_s-TiO₂-HA sample, shows the presence of Ca, P, O and Ti peaks, according to their elemental composition in the coatings, proposing the presence of Ti, TiO₂ and calcium phosphates phases on the substrate, as expected. Furthermore, also aluminum residues from sandblasting process were detected in sandblasted coated surfaces.

Similar results (except for Al identification) were observed for the coatings on machined and sandblasted/acid etched surfaces, not shown for the sake of brevity.

Finally, to investigate the effect of the thermal annealing procedure on the features of the coatings, Figure 5.14 shows titania and HA (2 spray cycles of 2 s, rest period of 60 s) coated Ti sandblasted substrates before (a) and after (b-d) the thermal annealing process, at 600°C, in air, for 6 h. In order to help the reader in the identification of the TiO₂ and HA surfaces, examples of the TiO₂ coated areas are labeled with red ovals, while the HA coated zones with blue ones. It can be observed that there is a strong change in the HA coating morphology due to the thermal treatment; the smooth as-grown HA layer, indeed, re-organizes into a new porous and nanostructured crystalline layer (with grains of few tens of nanometers). Differently, titania morphology seems to be unchanged due to the thermal treatment. Finally, no significant cracking occurred in the coatings after the heat treatment.

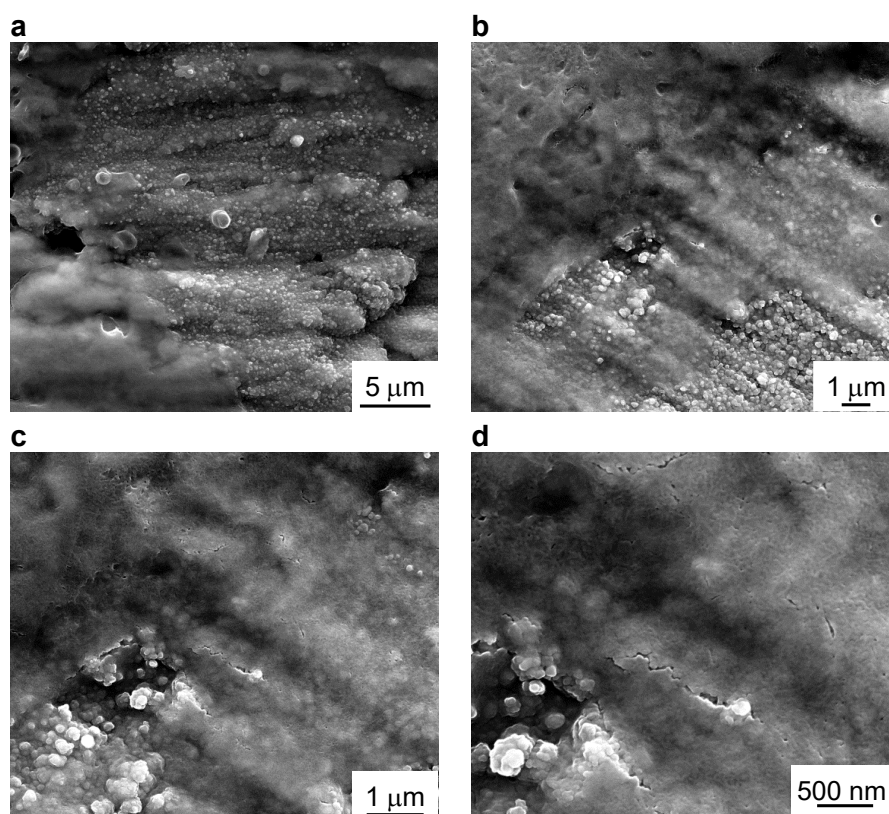


Figure 5.10 Secondary electron SEM images of TiO₂/HA coated machined Ti substrates, at growing magnification (a-d), after thermal treatment at 600°C, for 6 h, in air.

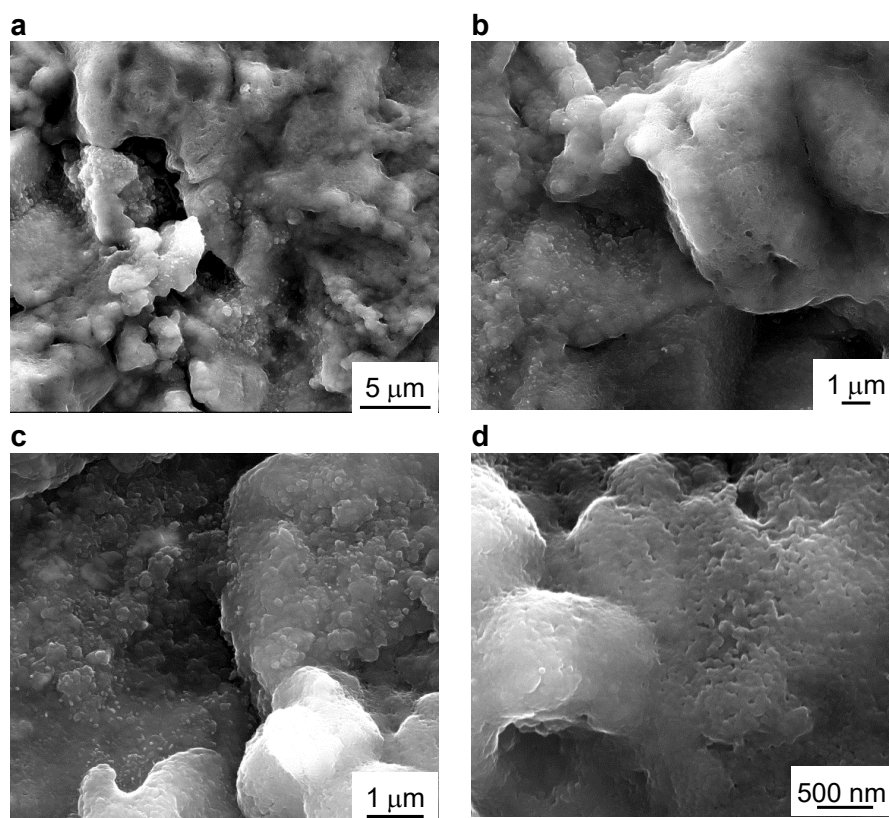


Figure 5.11 Secondary electron SEM images of TiO₂/HA coated sandblasted Ti substrates, at growing magnification (a-d), after thermal treatment at 600°C, for 6 h, in air.

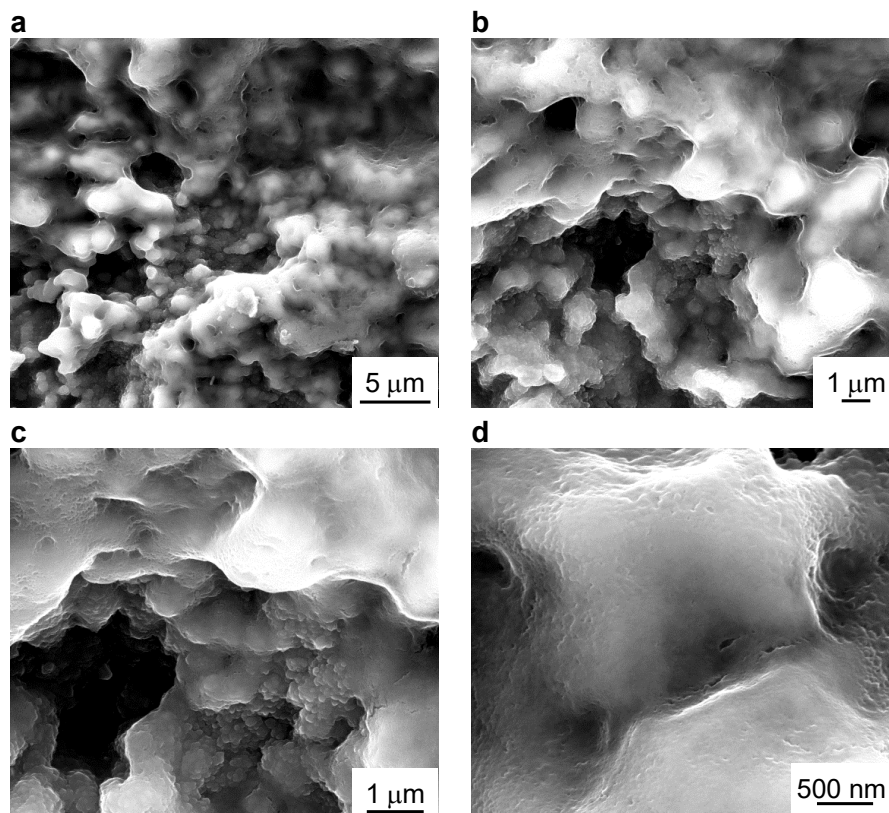


Figure 5.12 Secondary electron SEM images of TiO₂/HA coated sandblasted and acid etched Ti substrates, at growing magnification (a-d), after thermal treatment at 600°C, for 6 h, in air.

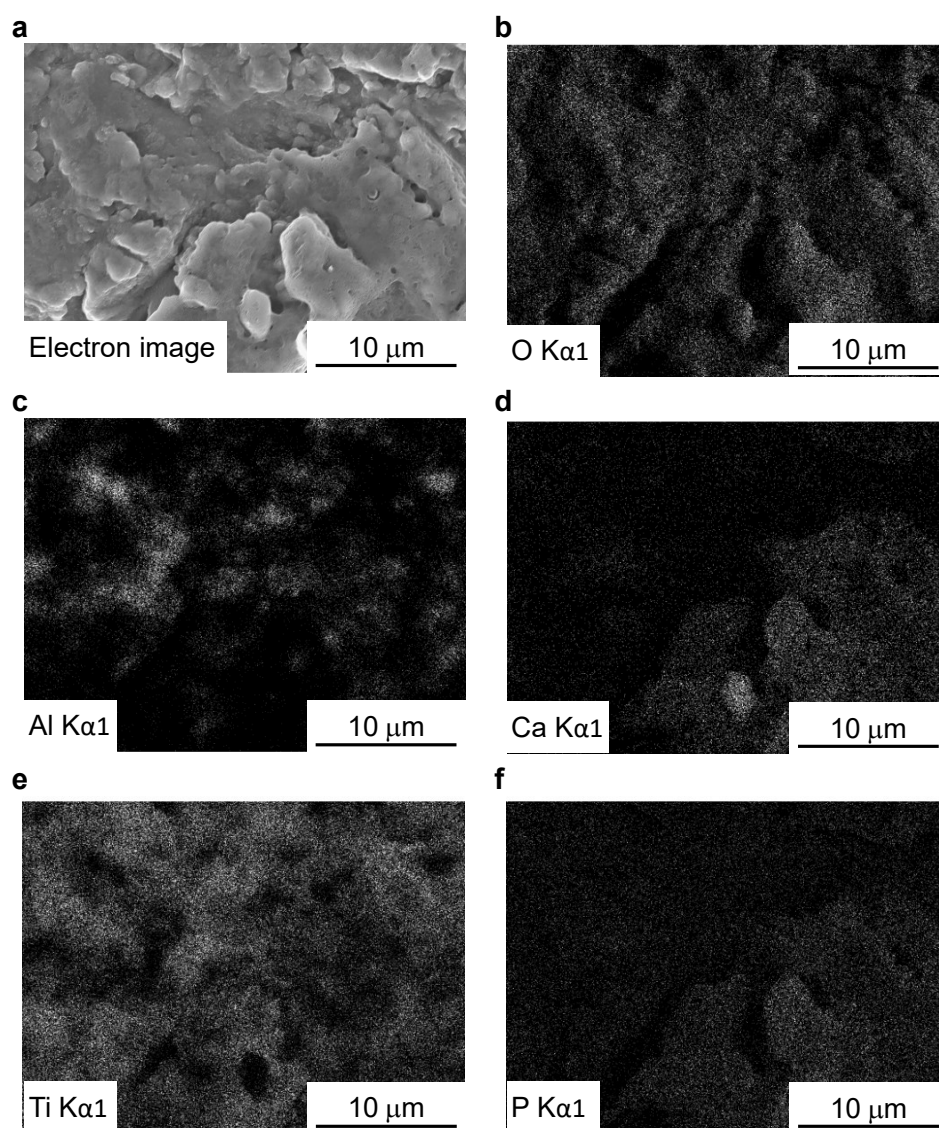


Figure 5.13 EDX surface mapping performed on Al_2O_3 sandblasted titanium disk covered with TiO_2 and HA, and thermally treated at 600°C for 6 h in air. Electron image (a), O K α 1 (b), Al K α 1 (c), Ca K α 1 (d), Ti K α 1 (e), P K α 1 (f).

3D maps and roughness parameters (Figure 5.15 and Figure 5.16) were estimated using a stylus profilometer and according to ISO 3274:1998 and ISO 4288:1998. Apparently, machined and sandblasted uncoated surfaces had similar roughness parameters, with a better homogeneity for the sandblasted ones, while “s+a” samples had the biggest roughness parameters. Specifically, Ti_s uncoated surfaces showed the lowest Ra (arithmetic average roughness) value, while Ti_s+a the highest (at a 0.05 level of significance). Moreover, “s” surfaces had valleys that were more pronounced than the peaks ($R_v > R_p$), conversely for “m” ones. “s+a” uncoated surfaces showed almost the same distribution of valleys and peaks. Rt values of uncoated surfaces were rather equal to Rz ones, indicating that the different samples investigated in the scan length were quite homogeneous (i.e. while Rt describes the maximum height of the profile within the evaluation length, Rz is the average of the five successive values of R_{t_i} calculated over the sampling length). However, machined surfaces presented a bigger shift between these values, confirming their anisotropy (already formerly observed by electronic microscopy images).

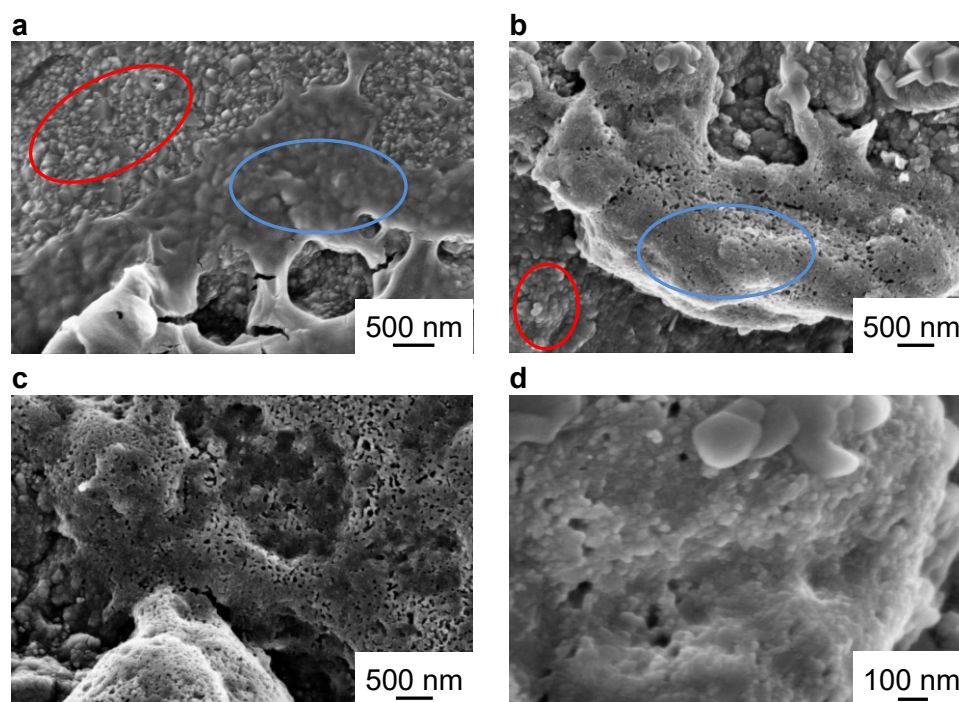


Figure 5.14 Secondary electron SEM images of the TiO₂/HA coated Ti sandblasted substrates before (a) and after (b-d) the thermal annealing process. The TiO₂ coated areas are labelled with red ovals, while the HA coated zones with blue ones.

For dental implant applications, it is important to calculate the effective surface area because this parameter is expected to influence the total amount of surface absorbed proteins [2]. Thus, surface area excess (Sdr) compared to smooth Ti surface was also calculated (Ti_m: 0.01, Ti_s: 0.33, Ti_{s+a}: 0.45, Figure 5.15). As expected, the highest surface area values of uncoated Ti substrates were found in the “s+a” samples, while the lowest ones in the “m” samples. From the comparison of the roughness parameters with the Sdr values, it appears that in machined samples there were grooves, which were responsible for the roughness parameters increasing but did not influence significantly the effective surface area.

After the first step of functionalization (i.e. the MOCVD process), the roughness parameters were preserved, confirming the optimal MOCVD conformal coverage previously detected from SEM analyses (the roughness parameters, for each typology of surface, before and after the MOCVD process were almost the same, at a 0.05 level of significance). Indeed, even if from Figure 5.16a a slight increase of the Ra values of the “m” and “s” coated samples could be observed (2% for Ti_m-TiO₂, 3% for Ti_s-TiO₂), it was restrained and the data analysis did not show any difference between the mean values, at a 0.05 significance level. In accordance with Popescu et al. [118] thin titania coatings (thinner than 1 micron) do not strongly influence the intrinsic substrates roughness and only long deposition times (i.e. thicker film) favor the formation of big crystal clusters that led to increase the roughness.

Also in the second step of the functionalization (HA deposition via spray pyrolysis and thermal treatment, in air, at 600°C) the roughness parameters were quite preserved (at a 0.05 level of significance), except for the case of the machined coated surfaces. It could be supposed that in the machined surfaces, the HA islands were less dispersed and consequently more concentrated in wide agglomerates, so strongly influencing the roughness values. This idea can be also confirmed by observing the corresponding 3D map (Figure 5.15c) where big HA agglomerates above the titania inter-layer are detectable, and

observing the different values between Rz and Rt and their great standard deviations (Figure 5.16b).

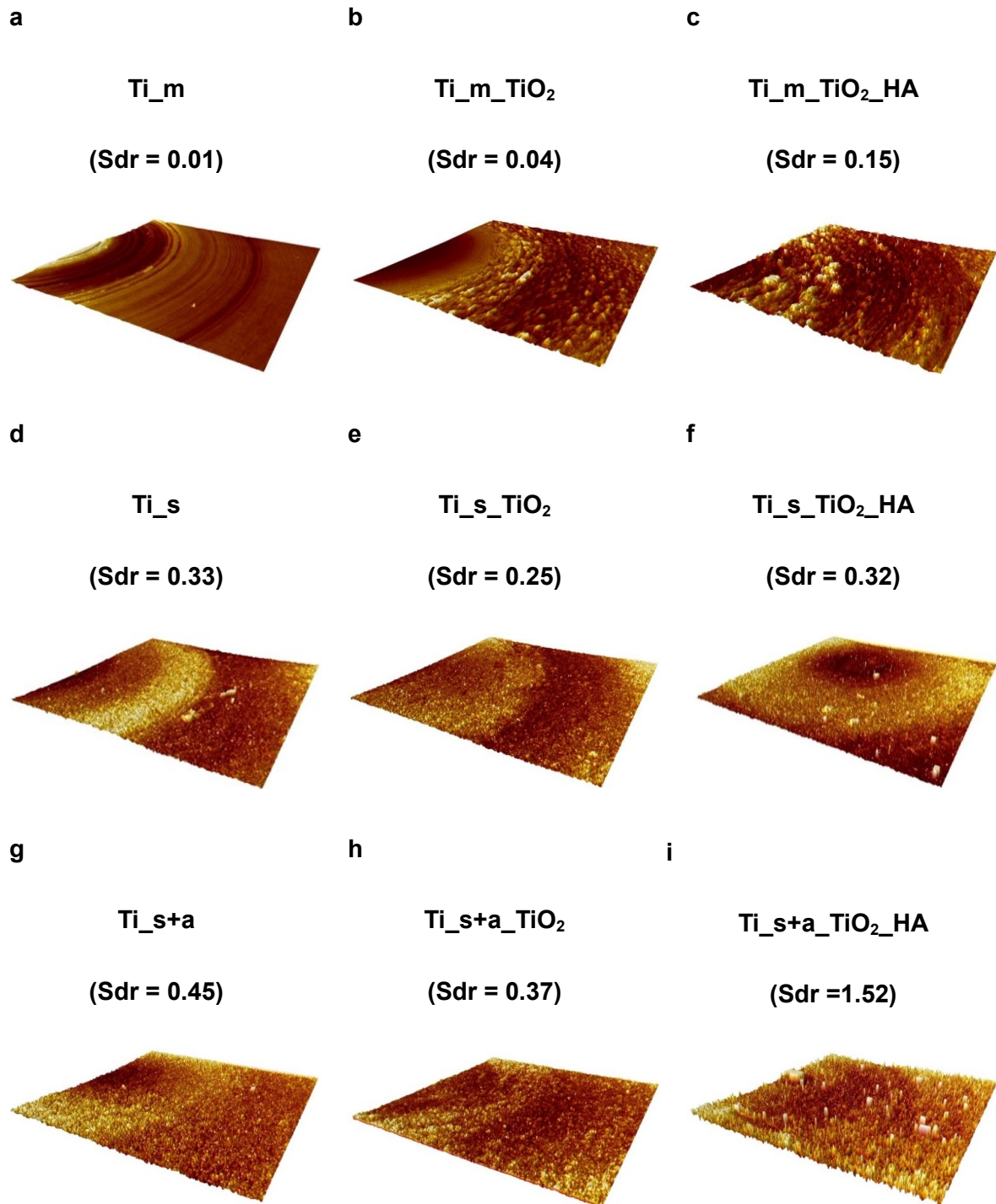


Figure 5.15 2mm × 2mm 3D maps for Ti substrates (Ti_m, Ti_s, Ti_s+a), for the corresponding titania coated Ti substrates (Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂) and for the analogous titania/HA coated Ti substrates (Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, Ti_s+a_TiO₂_HA). Sdr values are also reported (standard deviation ± 0.01).

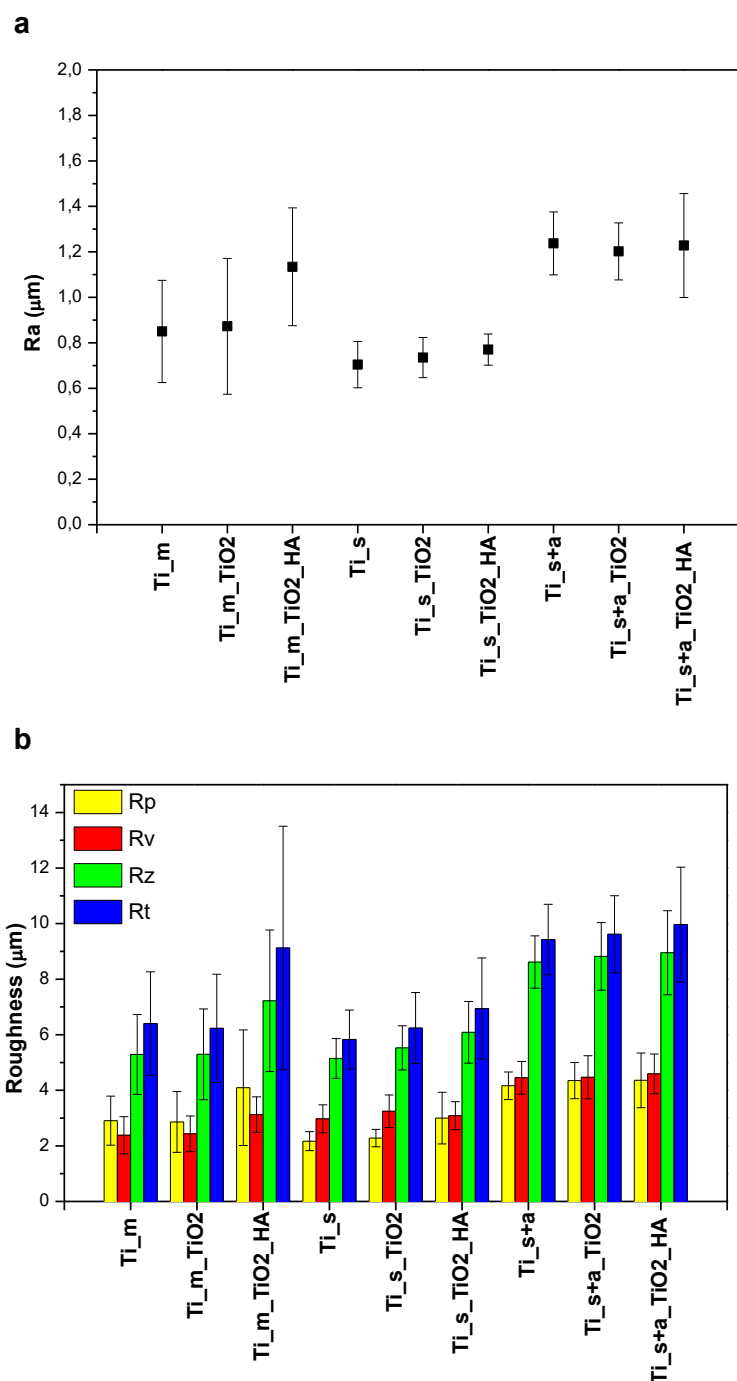


Figure 5.16 Roughness parameters for Ti substrates (Ti_m, Ti_s, Ti_s+a), for the corresponding titania coated Ti substrates (Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂) and for the analogous titania/HA coated Ti substrates (Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, Ti_s+a_TiO₂_HA).

5.3 Structural characterization

X-ray diffraction analyses were carried out on the three different Ti substrates (Figure 5.17) and after the coating deposition processes (Figure 5.18 and Figure 5.19, for titania and titania/hydroxyapatite coated surfaces, respectively).

All the Ti substrates were composed of hexagonal Ti (ICDD: 01-089-5009). Ti_m and Ti_s+a substrates showed also preferential orientation mainly with respect to (002) and (100) planes, correspondingly, as testified by the intensity of the related peaks. Specifically, the

machined substrate was (002) oriented: this feature could be attributed to superficial mechanical stress induced by the turning process. Indeed, no preferential orientation was detected in the Ti_s sample, likely suggesting that the preferential orientation was just located only on the Ti_m surface. Differently, the Ti_s+a substrate could be (100) preferential orientated because this face orientation was chemically more stable and consequently less etched during the chemical subtractive procedure.

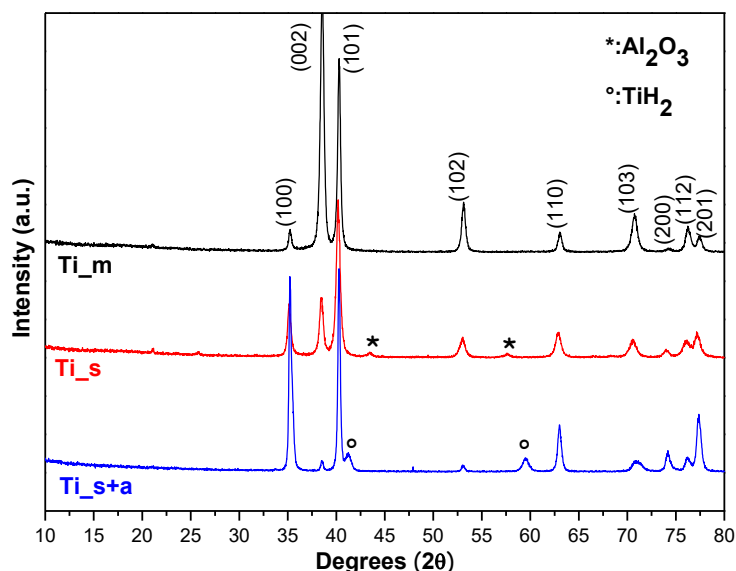


Figure 5.17 XRD patterns of uncoated Ti substrates.

Rietveld refinement of XRD patterns confirms previous EDX observations: Al_2O_3 residues from the sandblasting process (about 13 wt. %) were detected on “s” substrates, while the acid etching process allowed the whole removal of sandblasting particles. In the Ti_s+a substrate titanium hydride (TiH_2 , ICDD: 00-009-0371) was also found after the etching, as reported in the literature [65].

Three different crystalline phases of TiO_2 exist naturally at atmospheric pressure, namely rutile, anatase, and brookite. In all these three forms, titanium (Ti^{4+}) is coordinated to six oxide (O^{2-}) anions, forming octahedric- TiO_6 . Brookite has a rhombohedral structure, while anatase and rutile have a tetragonal one [137]. Rutile is the thermodynamically stable crystalline form of TiO_2 , and it is the most industrially used form. It can be easily formed on titanium surface by heat treatment at high temperature (typically $\geq 600^\circ\text{C}$, but depending on the specific conditions) [131,132]. Differently, brookite and anatase are metastable forms; however, the transition from the anatase to the rutile structure is kinetically disadvantaged so that also anatase crystals are present in nature.

TiO_2 in anatase phase is reported in some works to better assist the bone adhesion due to the similarities of its crystal structure to hydroxyapatite (i.e. the crystal structure of apatite is found to better align with anatase than with rutile phase) [138,139] and the larger number of hydroxyl groups on the anatase surface [139]. Despite the question of whether the anatase phase is the most bioactive in the SBF environment remains essentially open [140,141], anatase crystalline structure of titanium dioxide is reported to be more suitable for the osteo cells nucleation and growth [142].

As can be seen in Figure 5.18, after the MOCVD process, crystalline TiO_2 in the anatase phase (ICDD: 01-084-1286) was detected in all the substrates, without preferred orientation.

The anatase average crystallite size (calculated from Rietveld refinement) was dependent on the substrate typology: about 100 ± 10 nm for Ti_m_TiO₂, while Ti_s_TiO₂ and Ti_s+a_TiO₂ showed similar values: about 50 ± 5 nm. Ti_s_TiO₂ sample also showed TiO₂ in the rutile phase (ICDD: 01-088-1172), 25 wt. % calculated from Rietveld refinement. In this field, it could be supposed that alumina residues catalyze the rutile formation. Furthermore, XRD analysis shows that the MOCVD temperature (390°C) induced the whole decomposition of TiH₂, in accordance with the literature [143].

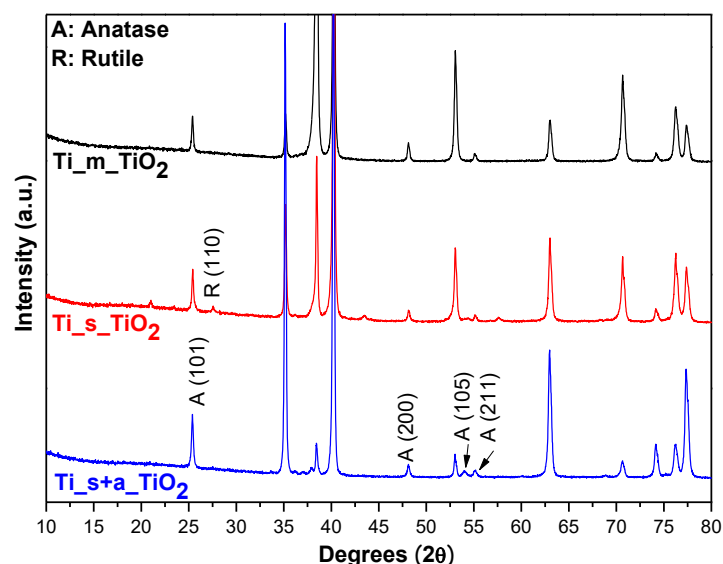


Figure 5.18 XRD patterns of MOCVD TiO₂ coated Ti substrates.

Figure 5.19 shows the XRD patterns of titanium disks covered with TiO₂ via MOCVD, hydroxyapatite via spray pyrolysis (2 spray cycles of 2 s) and thermally annealed at 600°C, for 6 h, in air.

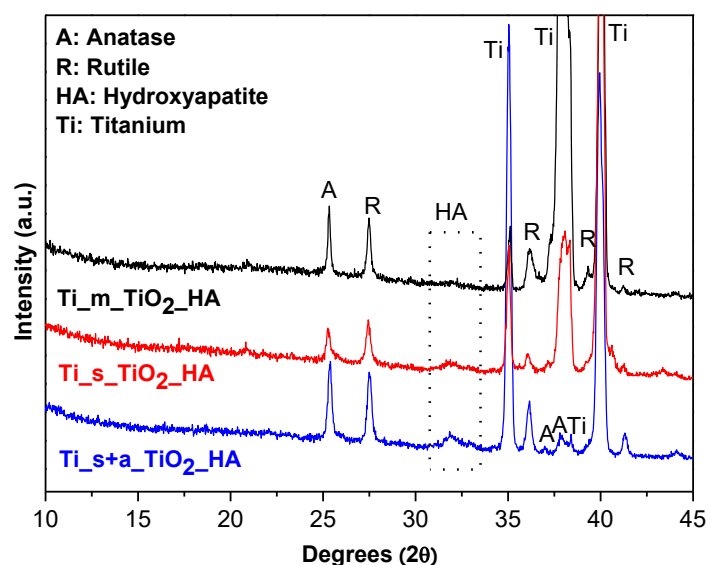


Figure 5.19 XRD patterns of titanium disks covered with TiO₂ via MOCVD, HA via spray pyrolysis, and thermally annealed at 600°C, for 6 h, in air.

A detailed discussion about the XRD analyses of the HA coated samples as a function of the thermal annealing temperature was carried out in Chapter 4 (section 4.5 Definition of the post-synthesis thermal treatment temperature) on samples with higher amounts of the HA solution (10 sprays of 2 s). In comparison with the spectra discussed in Chapter 4, a net reduction on the strength of the HA signals is detectable (as it was expectable taking into account the lower quantity of HA sprayed solution). Indeed, only a large band centered around $2\theta \sim 32^\circ$, and resulting from the overlapping of (121), (112), (300) and (202) reflections, can be identified. Furthermore, it seems that the amount of HA phase sprayed on the machined coated samples was almost nil, thus suggesting that on this surface there was a lesser amount of HA and/or, if present, it was less crystallized. Finally, as it was previously reported, at 600°C the MOCVD deposited titania in the anatase phase underwent phase transition to rutile. However, at this temperature ($T \geq 600^\circ\text{C}$), it cannot be excluded the formation of rutile deriving from the pristine titanium oxidation [130,131] (as it will be later observed from weighing experiments).

5.4 Evaluation of the CPC deposited amount (EDX and weighing)

As described in Chapter 2, the impact and spread of a drop against a solid surface can be conditioned by the properties of the fluid precursor (e.g. viscosity and surface tension), the kinetic process parameters (e.g. drop velocity and diameter), and the solid surface characteristics (e.g. surface energy, morphology and temperature) [83]. Among these conditions, the three typologies of the investigated substrates differ only in surface morphology (being, after the MOCVD deposition process, all TiO_2 coated surfaces). Therefore, if concrete differences in the amount of the deposited bioactive material exist, they could be reasonably due to the surface morphology contribution.

To estimate the amount of hydroxyapatite sprayed on the surface of the titanium substrates with three different superficial topographies and morphologies, two different approaches were taken into account.

The first methodology consisted of evaluating the amount of Ca and P present on the surface of Ti disks by means of energy dispersive X-ray analysis. For this purpose, 5 Ti disks ($\phi = 8$ mm) of each surface (i.e. Ti_m_ TiO_2 _HA, Ti_s_ TiO_2 _HA, Ti_s+a_ TiO_2 _HA) were investigated in three different areas. As previously explained, EDX analyses were very arduous in our samples because of the low quantity of deposited CPC material and its non-homogeneity (being for definition the here discussed HA coatings discontinuous, of course in a small investigated area). Therefore, in order to try to be less influenced by the specific area/point analyzed, EDX analyses were carried out at the relatively low magnification of 500x (for further reduction of the magnification it became often impossible to record the signals of Ca and P). Additionally, it was tried to choose the analyzed areas as random as possible, with the aim of giving a medium value of the CPC amount on the Ti surfaces.

Table 5.1 shows the mean values of the ratio $\text{Ca}/(\text{Ca}+\text{Ti})$ wt. % obtained (5 samples, 3 random areas of each sample, magnification 500x) for Ti_m_ TiO_2 _HA, Ti_s_ TiO_2 _HA and Ti_s+a_ TiO_2 _HA disks. Figure 5.20 displays the corresponding data box plots.

As can be observed from Table 5.1, as well as from the data box plots of Figure 5.20, the results obtained for the ratio $\text{Ca}/(\text{Ca}+\text{Ti})$ wt. % were highly dispersed (as, on the other hand, it was expected taking into account the nature of the deposits). By performing a statistical analysis on the data (One-Way Anova, 0.05 level of significance), no differences among the mean values of the three different surfaces were detected.

These experiments also allowed the estimation of the Ca/P at. in 1.6 ± 0.3 , a value very close to that of 1.67 for stoichiometric hydroxyapatite. Furthermore, no differences (0.05 level of significance) in Ca/P at. ratios were detected as a function of the morphology of the covered substrates.

As a conclusion, EDX studies seem to suggest that there is no dependence of the deposited hydroxyapatite amount from the pristine substrate type.

Ti_m_TiO ₂ _HA	Ti_s_TiO ₂ _HA	Ti_s+a_TiO ₂ _HA
1.9 ± 1.8	2.9 ± 1.4	2.1 ± 1.1

Table 5.1 Mean value of the ratio Ca/(Ca+Ti) wt. % for Ti_m_TiO₂_HA, Ti_s_TiO₂_HA and Ti_s+a_TiO₂_HA disks.

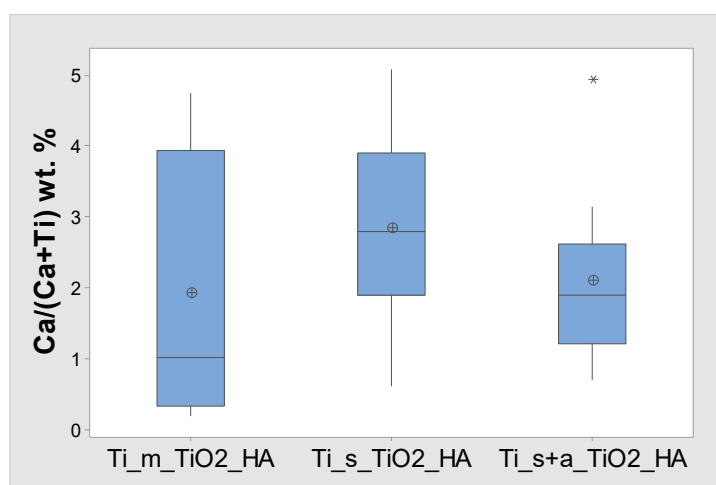


Figure 5.20 Box plot of the ratio Ca/(Ca+Ti) wt. % for Ti_m_TiO₂_HA, Ti_s_TiO₂_HA and Ti_s+a_TiO₂_HA disks. The mean value and the interquartile range box are shown. For the Ti_s+a_TiO₂_HA sample, an outlier is shown by a star symbol.

The amount of HA sprayed on the surface of the titanium substrates with three different superficial features was also evaluated through weighing experiments. A high precision electronic microbalance (Sartorius M2P, standard deviation ≤ 0.001 mg for sample weight < 500 mg) was used for this purpose. During these experiments, since the weight variations (before and after the HA coating process) were very limited, it was necessary to produce and analyze a large number of samples. Indeed, only increasing the number of probed samples, it was possible to perform a statistical analysis of the results. In order to evaluate the quantity of the hydroxyapatite deposited via spray pyrolysis on titanium disks (average weight < 200 mg) previously coated with 200 nm of TiO₂ via MOCVD, 45 titania coated titanium disks (for each type of surface, i.e. “m”, “s”, and “s+a”) were weighed before and after the spray pyrolysis (2 s of spray and stop of 60 s between two cycles of spray) and the thermal annealing (air, 600°C, 6 h) processes. Furthermore, 15 only titania coated Ti disks (for each type of surface) were weighed before and after the thermal annealing process, at 600°C, in air, for 6 hours. Indeed, as it was previously reported, at high temperatures (typically $T \geq 600^\circ\text{C}$, but depending on the specific conditions), it cannot be excluded the formation of titanium dioxide deriving from the pristine titanium oxidation [130,131] (which, as a consequence, would increase the weight of the sample).

Table 5.2 shows the mean values of the weight differences, before and after the thermal annealing process, obtained for not HA coated (“only TT”) and for HA coated (“HA + TT”) Ti_m_TiO₂, Ti_s_TiO₂, and Ti_s+a_TiO₂ disks, respectively. Figure 5.21, 5.22 and 5.23 display the respective data box plots.

	Only TT	HA + TT
Ti_m_TiO₂	(63 ± 8) µg	(64 ± 13) µg
Ti_s_TiO₂	(65 ± 10) µg	(93 ± 17) µg
Ti_s+a_TiO₂	(102 ± 24) µg	(126 ± 29) µg

Table 5.2 Mean values of the weight differences before and after the thermal annealing process (only TT), and before and after the spray pyrolysis and the thermal annealing processes (HA + TT), for machined, sandblasted and sandblasted/acid etched titania coated titanium disks, respectively.

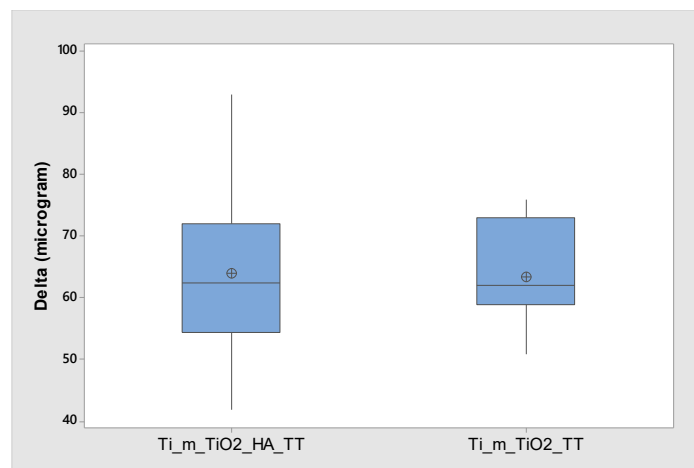


Figure 5.21 Box plot of the weight differences detected for titania coated machined Ti disks before and after the spray pyrolysis and the thermal annealing processes (Ti_m_TiO₂_HA_TT), and before and after the thermal annealing process (Ti_m_TiO₂_TT). The mean value and the interquartile range box are shown.

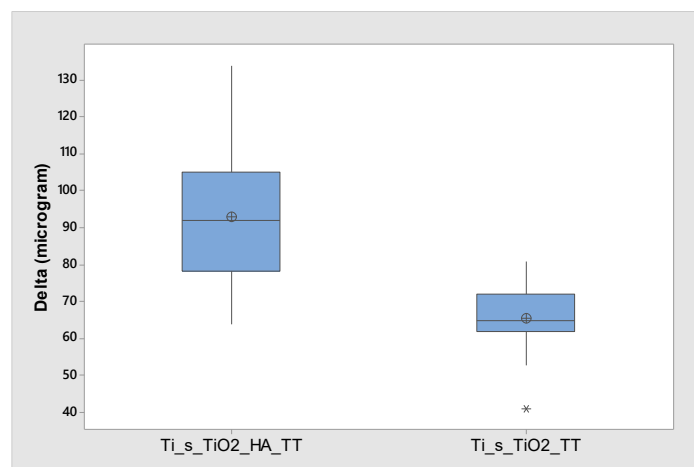


Figure 5.22 Box plot of the weight differences detected for titania coated sandblasted Ti disks before and after the spray pyrolysis and the thermal annealing processes (Ti_s_TiO₂_HA_TT), and before and after the thermal annealing process (Ti_s_TiO₂_TT). The mean value and the interquartile range box are shown. An outlier is revealed by a star symbol.

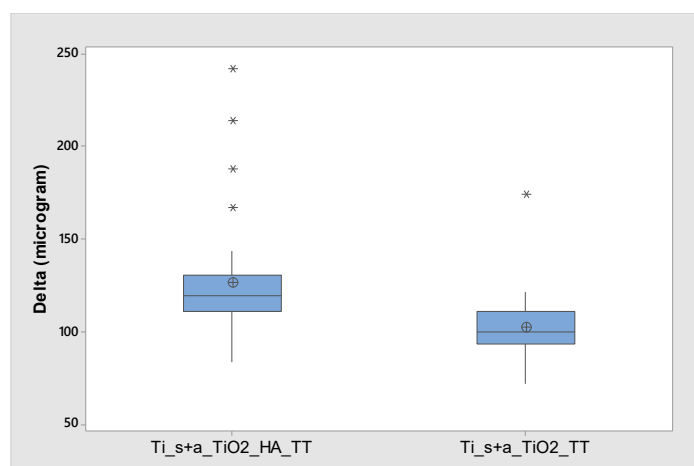


Figure 5.23 Box plot of the weight differences detected for titania coated sandblasted/acid etched Ti disks before and after the spray pyrolysis and the thermal annealing processes (Ti_s+a_TiO₂_HA_TT), and before and after the thermal annealing process (Ti_s+a_TiO₂_TT). The mean value and the interquartile range box are shown. Some outliers are revealed by star symbols.

By performing statistical analysis of the data (2-Sample t-test, 0.05 level of significance), no differences among the weight variation (before and after the thermal annealing process) of uncoated (Ti_m_TiO₂) and HA coated machined substrates (Ti_m_TiO₂_HA) were detected. Differently, for “s” and “s+a” surfaces, statistical difference (2-Sample t-test, 0.05 level of significance) in the weight mean variation values between uncoated and HA coated surfaces were calculated (i.e. “only TT” vs. “HA + TT”), thus suggesting that the amount of HA blocked during the spray pyrolysis process on “s” and “s+a” surfaces was statistically higher (0.05 level of significance) compared to that retained by the smoother (machined) ones, like proposing that sprayed droplets were easily rebounded from smooth surfaces.

Besides, these experiments revealed also a disk weight increase due to the thermal annealing process (i.e. only titania coated surfaces after the thermal annealing process showed a weight increase). As it was previously reported, this could be reasonably due to the titanium thermal oxidation at high temperature ($T \geq 600^{\circ}\text{C}$) [130,131,138]. Furthermore, from the statistical analyses of the data (One-Way Anova, 0.05 level of significance) when no HA was sprayed (i.e. only titania coated surfaces before and after the thermal annealing process), it was observed that the roughest surfaces (i.e. “s+a”) presented, after the thermal treatment, the highest weight increase; thus suggesting a superior degree of oxidation of the titanium surface, most likely due to the greater surface area that allows superior availability of Ti to be oxidized.

As a conclusion, while EDX investigation declared that there were no significant differences among the amount of hydroxyapatite deposited on the different typologies of surfaces (most likely due to the high dispersion of the EDX values), weighing experiments suggested a higher HA blocking capability for the textured ones (i.e. “s” and “s+a”).

5.5 Wettability

Surface topography and morphology, as well as surface chemical composition and energy, are reported to influence the bone formation process [96]. However, differently from surface morphology and roughness, literature lacked of systematic studies concerning the surface energy or wetting properties of the dental implants until the beginning of the 21st century [96].

It is reported that surface wettability influences the early stage of the osseointegration process [65,144], guiding the degree of contact with the physiologic environment. Therefore, increased wettability should enhance the interaction between the implant surface and the biologic environment [113]. In literature, it is described that hydrophilicity presents major benefits during the initial stages of wound healing and during the subsequent osseointegrative events, facilitating the bone integration process [113]. Zhao et al. [145] show that osteoblasts cultivated in hydrophilic and chemically pure surfaces have higher levels of differentiation markers than those cultured in hydrophobic surfaces. Furthermore, *in-vivo* [113] and clinical studies [146] show that hydrophilic surfaces improve the bone-to-implant contact (BIC) and the bone anchorage during the early stages of bone healing. Surface wettability is affected by numerous factors; among them, it is worth to mention roughness, morphology, and chemical composition. Many investigations analyze the influence of surface roughness on wettability, and the conclusion is that roughness increases the water contact angle if its value on the un-roughened surface of the same material is higher than 90° , and decreases it if the contact angle is lower than 90° (i.e. roughness makes hydrophilic surfaces more hydrophilic, hydrophobic ones more hydrophobic) [147]. Acid etched surfaces are generally described to be hydrophobic [65]. Another factor strongly affecting the surface wettability is the contamination of the surface. Generally, wettability is governed by the number of surface hydroxyl -OH groups [148]: the more -OH groups, the higher wettability, protein adsorption and cell adhesion [149]. Conversely, a decrease in wettability is observed due to the increasing contamination by atmospheric hydrocarbons (functional -OH groups may be inhibited by hydrocarbon covering) [65]. Additionally, cleaning processes and autoclave sterilization could further increase hydrophobicity [145].

Starting from these considerations, in this work, it appeared to be of high relevance the investigation of the wettability features of the pristine titanium substrates and of the corresponding functionalized samples. Static water Contact Angles (CA) measurements were carried in ambient laboratory temperature range $20\pm 25^\circ\text{C}$; during all the experiments the samples were conserved into a vessel at a constant relative humidity ($\sim 43\% \pm 5\%$) and analyzed immediately afterward. The equilibrium contact angles were measured after 20 seconds from water drop deposition.

Figure 5.24 shows the wettability evolution, as a function of the time ageing (until about 14 months from sample preparation), after the first step of the syneric approach (i.e. titania MOCVD deposition), while Figure 5.25 shows the wettability evolution with time (until about 14 months) after the complete two-step functionalization process (i.e. titania MOCVD deposition + HA spray pyrolysis deposition and thermal treatment in air, at 600°C , for 6 h).

From Figure 5.24 it can be seen that uncoated Ti substrates had characteristic wettability: $83^\circ \pm 5^\circ$ for Ti_m, $79^\circ \pm 5^\circ$ for Ti_s and $133^\circ \pm 4^\circ$ for Ti_s+a. A possible explanation for the higher hydrophobicity of Ti_s+a samples is in the topography generated by the acid attack [65]. After TiO_2 deposition, the contact angle of the water droplets plummeted to zero, suggesting a superhydrophilic behavior for all the samples. However, TiO_2 -coated samples stored in air were easily contaminated due to organic pollutants [65] and the wettability slowly decreased (in laboratory air environment, recontamination of high-energy surfaces is unavoidable). As organic molecules were progressively absorbed, the initially hydrophilic surfaces gradually turned into hydrophobic or less hydrophilic ones. This process modifies the surface composition and surface reactivity, affecting firstly the protein absorption, subsequently the cell attachment, proliferation, differentiation and finally the osseointegration process [113,150]. Furthermore, it can be observed that the initial (first hours) loss of superhydrophilic features was faster for the “m” and “s” titania coated samples in comparison

with the “s+a” coated ones. This is likely due to the higher effective surface area of the “s+a” titania coated samples (higher Sdr): greater surface area means a larger number of attached -OH groups and, consequently, more hydrophobic molecules are needed to cut down the hydrophilic behavior. However, after about 20 days, the surface wettability of the MOCVD coated samples becomes comparable with that of the corresponding uncoated ones.

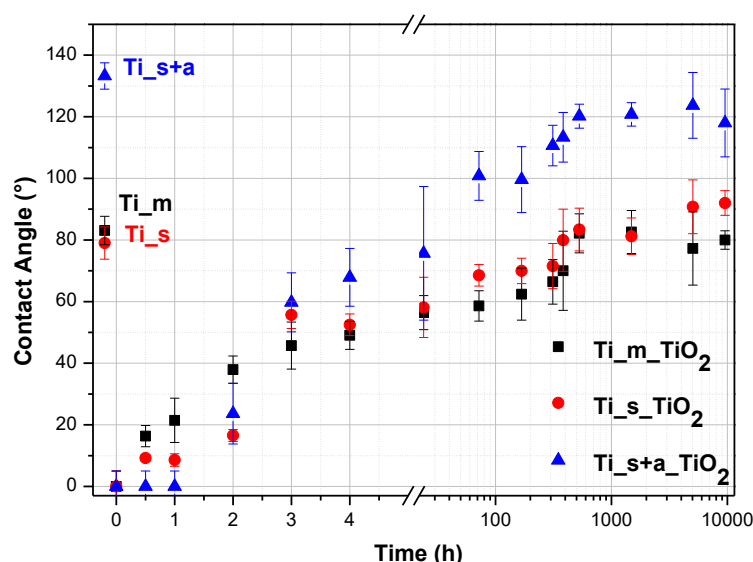


Figure 5.24 Contact angle before (pristine substrates Ti_m, Ti_s and Ti_s+a), just after the TiO₂ MOCVD process (time = 0) and as function of time ageing for machined (Ti_m-TiO₂), sandblasted (Ti_s-TiO₂), and sandblasted/acid etched (Ti_s+a-TiO₂) Ti substrates.

In Figure 5.25 it can be observed that just after the HA deposition and the thermal treatment, the titanium two-step functionalized surfaces were not completely superhydrophilic, however, the contact angle values were close to zero (with average values near to 10°). For the TiO₂/HA functionalized surfaces, it could be thought that the HA islands slightly reduce the titania superhydrophilic behavior. Like for titania coated titanium surfaces, the wettability was lost as a function of time aging due to hydrocarbon contamination, and the initially hydrophilic surfaces gradually turned into hydrophobic or less hydrophilic ones. These processes change the surface composition and surface energy and, therefore, must be seriously taken into account.

Comparing Figure 5.24 and Figure 5.25, the loss of wettability appears to be slower in the two-step functionalized samples (i.e. Ti-TiO₂-HA) when compared to the pure titania coated ones (i.e. Ti-TiO₂). Furthermore, it must be underlined that, differently from titania MOCVD coated samples of Figure 5.24, after few months of air storing, the wettability of the titania/hydroxyapatite coated samples with different morphologies appears quite similar, without substantial difference regarding the pristine substrate morphology (i.e. among “m”, “s” and “s+a”). Besides, lower final average CA values (over the investigated period) were detected for the two-step functionalized surfaces with respect to the titania coated or uncoated titanium surface (especially for the “s+a” surfaces). This aspect could be explained by supposing that the hydroxyapatite islands slightly shield the titania surface from hydrocarbon contamination (therefore resulting in less hydrophobic final materials). Finally, differently from the TiO₂ coated samples, no relevant variations in the loss of wettability rates among the different two-step functionalized surfaces were observed.

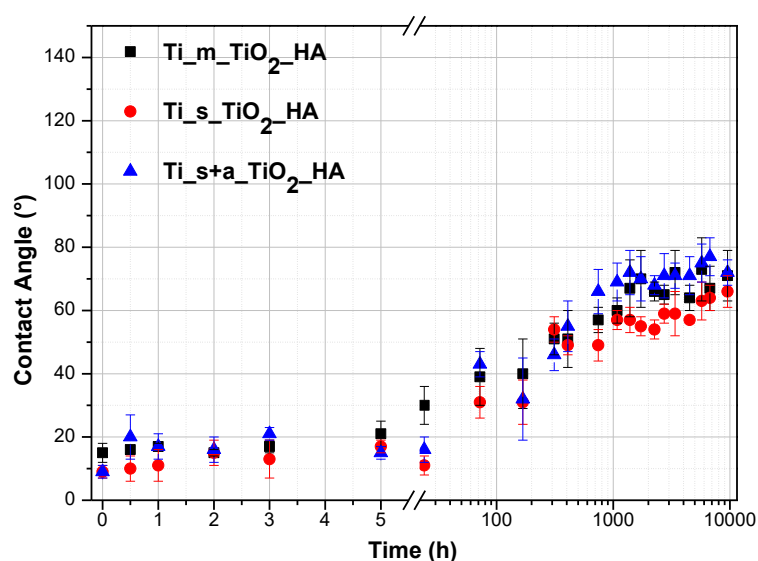


Figure 5.25 Contact angle just after the thermal annealing process (time = 0) and as a function of time aging for TiO₂/HA coated machined (Ti_m_TiO₂-HA), sandblasted (Ti_s_TiO₂-HA), and sandblasted/acid etched (Ti_s+a_TiO₂-HA) Ti substrates.

Nowadays, implant shelf life is principally related to the maintenance of their sterility. Conversely, other components (i.e. hydrocarbons or C- and O- species from air pollution) could progressively absorb on the titanium surface. Theoretically, an implant surface is perfectly clean only if it uniquely consists of Ti and O with chemical bonds between them, but other elements were often identified in all the commercial implants. As a consequence of organic surface contamination, implant failure could also be due to an inappropriate implant surface composition [150]. It follows that it is crucial to control the steps between implant surface functionalization and surgical application. Storage, being the last step before application, directly influences the *in-vivo* performance of implants. Taking into account these considerations, different approaches could be followed to have durable clean hydrophilic surfaces while, in the meantime, maintaining the topography, the morphology, the chemical composition and the crystalline structure of the pristine substrates. In this Ph.D. work, to maintain high energy surfaces, two industrial-scalable strategies were positively set-up. These are i) the storing of the products underwater, or ii) the restoration of the hydrophilicity by UV treatment. The following sections 5.5.1 and 5.5.2 describe these diverse approaches and their applicability to the here functionalized samples.

5.5.1 Storing in water

Concerning the titania MOCVD coated Ti surfaces (i.e. Ti-TiO₂) in order to have durable superhydrophilic surfaces two industrial-scalable strategies can be taken into account. The first approach is the isolation of the freshly coated (clean) titania superhydrophilic surface by preservation under liquid media [133,150–153]. Indeed, in theory, it could be possible to avoid atmospheric poisoning by storing the sample in deionized water, to protect the surface from hydrocarbon contamination [133,151], thus maintaining a hydroxylated superficial state and, therefore, superhydrophilicity. In contrast to other cleaning strategies, this approach avoids implant contaminations ab-initio.

For this purpose, the just MOCVD coated samples (i.e. freshly prepared titania coated Ti surfaces) were stored in plastic bowls filled with Milli-Q water. Milli-Q water was used as the simplest aqueous medium and in order to avoid the influence of other inorganic ions [152,153]. The experiments (Figure 5.26), carried out for about 15 months, showed that this approach permits high hydrophilicity and the slight wettability worsening in Ti_m_TiO₂ and Ti_s_TiO₂ surfaces could be due to the hydrocarbon contamination of the storage solution with time, although the release of organic compounds from the plastic vessel (where the samples were stored) cannot be completely excluded. Moreover, “s+a” surfaces presented longer superhydrophilic behavior. As it was previously observed in Figure 5.24 regarding the loss of the superhydrophilic behavior of Ti_{s+a}_TiO₂ surfaces, again “s+a” titania coated samples stored in water have reduced loss of superhydrophilic behavior when compared with smoother surfaces (i.e. maintain high wettability also after 15 months of storing). Once again, this is likely due to the higher effective surface area of the “s+a” coated samples (i.e. higher amount of attached -OH groups and, consequently, more hydrophobic molecules are needed to cut down the hydrophilic behavior). Finally, it is worth to underline that the slight oscillations of the low contact angle values (CA < 15°) are ascribable to the intrinsic instrumental uncertainty.

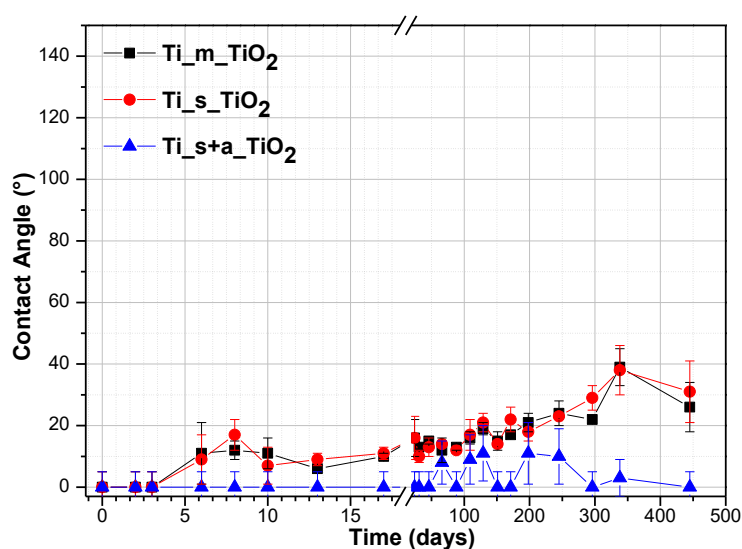


Figure 5.26 Evolution of the contact angle with aging time for the titania coated Ti substrates stored in Milli-Q water.

It must be pointed out that similar previous studies [133,150–153], performed in water or in saline solutions, on titanium substrates or on titania functionalized Ti surfaces, are always carried out for shorter aging times, not comparable with the implant shelf life. The purpose of these experiments was instead to study the wettability behavior on intervals comparable with the implant storage life.

Unfortunately, this approach presents an important limitation concerning its feasibility on the two-step functionalized surfaces (i.e. titania and hydroxyapatite coated substrates). For Ti₂TiO₂_HA samples, indeed, the storage in solution to avoid hydrocarbon contamination cannot be applied because of the HA dissolution in aqueous media [14] (as it will be shown below by means of Ca release test). For this reason, in this work, the storing of the two-step composite materials in water was not taken into account.

As a conclusion, the sample storing in water appears simple, cost-effective and allows the preservation of the products with high hydrophilic behavior for several months, although it does not completely guarantee the superhydrophilic characteristic, especially over long periods of conservation, comparable with sterility shelf life (about 5 years). Furthermore, this approach cannot be applied to the here developed two-step functionalized bioactive surfaces.

5.5.2 UV re-activation

The second approach investigated to have high energy surfaces takes advantage of two different processes that happen on the crystalline titania surface when it is exposed to a suitable UV irradiation ($\lambda < \text{ca. } 400 \text{ nm}$, $E_g > 3.2 \text{ eV}$): the photocatalytic oxidation process and the photoinduced surface reorganization process (surface wettability conversion) [154,155]. In other words, an UV treatment could re-establish the titania (super)hydrophilic behavior and, in the meantime, clean the surface thanks to the photocatalytic removal of organic substances [154].

These phenomena originate when a semiconductor is irradiated by photons with adequate energy, which cause that electrons (e^-) are promoted to the conduction band, leaving holes (h^+) in the valence band. In the photocatalytic oxidation process, e^- and h^+ can react with electron acceptors (O_2 as an example) and donors (H_2O as an example) molecules [156,157], therefore starting the oxidation process and causing the mineralization of the organic and/or inorganic pollutants and obtaining carbon dioxide, water and inorganic inert salts as final products [156]. In the photoinduced surface reorganization process, electrons and holes are still produced, but they react differently: electrons reduce the Ti(IV) cations to the Ti(III) state, and the holes oxidize the O^{2-} anions. In this process, oxygen molecules are ejected, creating oxygen vacancies. Therefore, water molecules can occupy these oxygen vacancies, thus producing adsorbed $-\text{OH}$ groups on the titania surface and, as a consequence, making it hydrophilic [157].

Starting from the titania MOCVD coated samples stored in air for at least 20 days (and consequently with stable CA values, see Figure 5.24), it was found (Figure 5.27) that two hours of UV treatment (irradiance: 58 W/m^2 at the level of the samples, UVC light, λ : 254 nm) were sufficient in order to completely restore the surface superhydrophilicity, with contact angle values close to zero for all the typologies of substrates. However, in the dark and in air, titania films returned to a more hydrophobic state and it was due both to the organic re-contamination of the surfaces and to the reversible change in titania surface structure under UV illumination (indeed the photo-generated $-\text{OH}$ groups absorbed on the titania surface after UV irradiation are thermodynamically metastable [155]). Finally, a slightly reduced loss of wettability appeared in UV activated samples of Figure 5.27 when compared to the freshly deposited MOCVD TiO_2 coated surfaces (Figure 5.24), and it was likely due to the contribution of the photoinduced surface reorganization to the global wettability. Again, like for the aging in air and the sample storing in water (Figure 5.24 and Figure 5.26, respectively), $\text{Ti}_s+\text{a-TiO}_2$ surfaces showed the best wettability behavior: longer time was necessary in order to lose the superhydrophilic behavior after UV re-activation.

Differently from the sample storing in water, this approach can also be applied on the two-step functionalized surfaces (i.e. titania/hydroxyapatite coated substrates). Once again (as for the titania coated surfaces), starting from the titania and hydroxyapatite coated samples stored in air for at least 40 days (and consequently with stable CA values, see Figure 5.25), it

was found (Figure 5.28) that two hours of UV treatment (irradiance: 58 W/m² at the level of the samples, UVC light, λ : 254 nm) were sufficient in order to completely restore the surface hydrophilicity, with average CA values close to 10° (as for the correspondent freshly prepared samples).

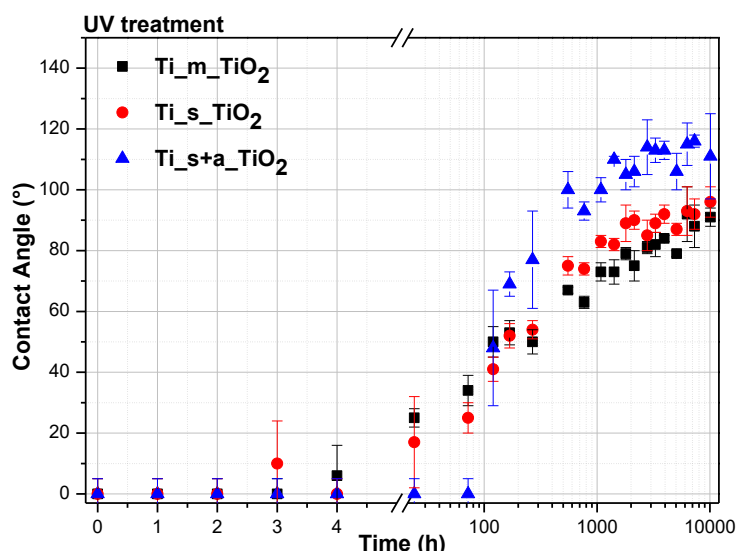


Figure 5.27 Re-establishment of the superhydrophilicity by UV treatment and evolution of the contact angle with aging time for the titania coated Ti substrates stored in air.

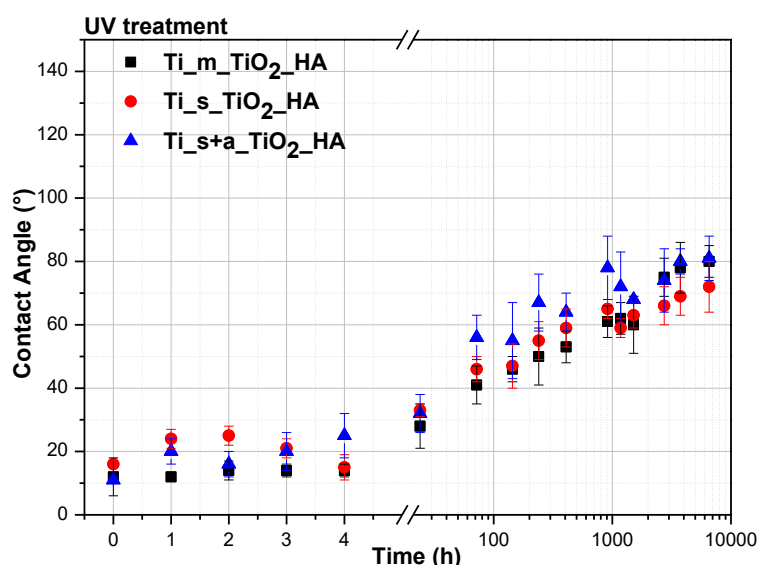


Figure 5.28 Re-establishment of the hydrophilicity by UV treatment and evolution of the contact angle with aging time for the titania/hydroxyapatite coated Ti substrates stored in air.

For these samples, even further prolonging the UV lighting time or increasing the UV source irradiance, did not produce lower CA values (as it was imaginable considering the wettability values of the freshly titania/hydroxyapatite composite samples). In the dark and in air, TiO₂/HA coated Ti substrates returned to a more hydrophobic state (like for the pure titania coated samples) and it was most likely due to the organic re-contamination of the surfaces as well as to the reversible change in titania surface structure under UV illumination. However, differently from the titania coated surfaces (i.e. freshly prepared titania coated

surfaces compared to titania coated surfaces after UV re-activation), the loss of wettability appeared comparable with that of the freshly prepared two-step functionalized coated surfaces (Figure 5.25), thus suggesting that the contribution of the photoinduced surface reorganization to the global wettability is limited because of the presence of HA that partially covers the TiO₂ exposed surface.

As a conclusion, UV reactivation treatment, although it requires an UV light source, appears to be of high relevance because, in addition of being applicable also on the two-step TiO₂/HA coated surfaces, it can be seen as an “on-demand” restoration technique, i.e. to be used just before the use of the product (i.e. before the implantation).

5.6 Ion release

Even if titanium is vastly used as a biomaterial thanks to its high corrosion resistance, theoretically, no material is 100% inert. An essential surface feature of Ti is its capability to form a thin (5÷10 nm thick) [158], stable, and amorphous TiO₂ layer under exposure to the atmosphere and/or physiological fluids. Although this passive layer is widely described as a protective layer, it is too thin and its chemical barrier action is not longtime effective under the action of body fluids. This makes the bulk material to undergo a slow but lasting metal ion release in the neighboring tissues [60], especially at low pH and in presence of fluoride (toothpaste for instance) [61]. It is reported that Ti release affects the cellular differentiation processes, negatively influencing the whole process of healing and osseointegration [61]. Wachi et al. [159] propose that Ti ions may induce inflammatory reaction and bone resorption processes around the implant. Moreover, Barao et al. [160] reveal that pathogenic bacteria are more easily accumulated on corroded surfaces and, therefore, inflammatory reactions around the implant could take place.

Additionally, given the long dental implant life, its failure could also be caused by adverse body reactions to titanium (such as Ti allergy). Even if some clinical studies on this topic have been carried out [61], nowadays there is not enough data on the hypersensitivity to titanium and, consequently, more studies are necessary. In this context, there may be a risk of greater ion release for surface-increased implants than conventionally machined ones.

Starting from these considerations, this investigation aimed to evaluate the beneficial performance of the coating processes and whether there is a relationship between ion release (i.e. Ti and Al) and surface roughness. Moreover, the amount of Ca released from the two-step functionalized surfaces was also investigated.

In this work, *in-vitro* titanium, calcium and aluminum release was evaluated by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). For these experiments cylindrical implants with truncated cone end, with ϕ : 4 mm, length: 10 mm; weight: 372 ± 10 mg were used. Three of each type of specimen (Ti_m, Ti_s, Ti_s+a; Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂, Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, Ti_s+a_TiO₂_HA) were separately immersed in 10 mL of 1% lactic acid aqueous (LA) solution (pH = 2.4) or in 10 mL of 0.1 mol/L phosphate buffered saline (PBS) solution (pH = 7.4), in order to analyze implant corrosion at different pH levels. In particular, the former was used as a more aggressive medium, while the second to simulate biological environment pH [63]. Furthermore, blank tests without samples, treated in the same manner and always in triplicates, were carried out. Each specimen was placed in its individual plastic bottle with a cap. The entire specimen was completely submerged in the release test solution, kept at 37°C and shaken in a reciprocating shaker for 28 days at 60 cycles/min. After this time, the ion concentration (Ti and Al) released from the specimens into

each solution was analyzed. Calcium release was calculated only from the two-step functionalized samples. The amounts of released ions were calculated as micrograms per implant. The mean quantity and standard deviation were calculated for the three flasks.

According to Wennerberg et al. [63], it was found that lactic acid (LA) solution is a more severe environment for testing the corrosion resistance of all specimens used (uncoated and coated Ti substrates): aluminum and titanium release was only detected from lactic acid solutions and no detectable titanium or aluminum release was measured after soaking in phosphate buffered saline solution (for 28 days of incubation at 37°C).

Figure 5.29 shows the amount of Ti or Al released (μg released per implant) in lactic acid aqueous solution for machined, sandblasted and sandblasted/acid etched uncoated, TiO₂ and TiO₂/HA coated dental implants.

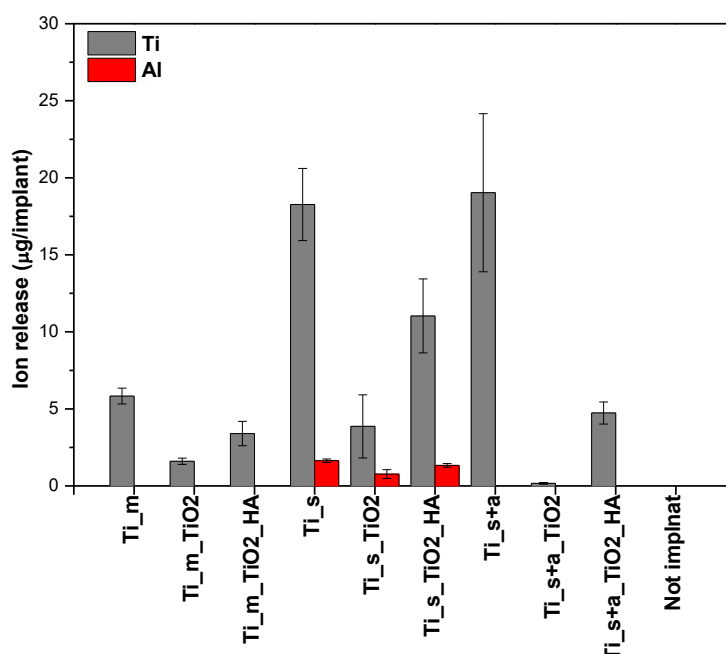


Figure 5.29 Amount of Ti or Al released (μg per implant) in lactic acid aqueous solution (pH=2.4) for uncoated, TiO₂ coated and TiO₂/HA coated implants. The implants had exactly the same geometrical size and weight.

The here used implants (uncoated and coated) had exactly the same geometrical size and weight. Nevertheless, it must be stressed that considering the different superficial finishing (diverse Sdr values) of machined, sandblasted and sandblasted/acid etched surfaces, the implants present diverse effective surface area exposed to the corrosive media.

Before the MOCVD processes, differing from previous work of Wennerberg et al. [63], a significant difference (at 0.05 confidence level) in ion release in LA among the three different surfaces (“m”, “s”, and “s+a”) was detected. On bare substrates, Ti release in lactic acid solution followed almost the increasing of surface area: rougher surfaces seem to be associated with higher ion release (Ti_s and Ti_s+a surfaces have higher Sdr values than that of machined ones). Furthermore, Al release was detected only in the sandblasted implants, confirming only in this sample the presence of Al₂O₃ residues from the sandblasting process.

Considering the effect of the titania coatings, it can be observed that thin TiO₂ films reduce, for all the sample typologies, ion release with respect to the corresponding uncoated

samples (at 0.05 confidence level), with a non-linear trend for the different types of surfaces. Ti dissolution rose from machined to sandblasted surfaces and plummeted on the roughest surfaces ("s+a"). This could be explained considering synergistic effects. Firstly, the increasing of ion release due to the enhancement of the surface area, secondly the effectiveness of the titania film as a protective layer. This second aspect showed its best effect in the "s+a" coated sample where the multi-level superficial structure, joint with a perfectly cleaned surface due to the acid etching, increased the titania coating adhesion features with a probable better mechanical (film-to-substrate interlocking) and chemical synergic contribution [161,162], while in the sandblasted sample the presence of alumina residues could negatively influence the first titania nucleation steps. Finally, as for bare Ti substrates, Al release was found only in the sandblasted implants and TiO₂ coating did not totally prevent the Al release in LA. To sum up, the functionalization of the titanium surface with the titania layer can be a good strategy to reduce the ion release and consequently achieve a potential better osseointegrative capability.

After the two-step functionalization process (Ti_TiO₂_HA samples), it can be seen that the two-step procedure here developed permitted the reduction of the ion implant release for each surface with respect to the corresponding uncoated Ti implants (at the 0.05 level of significance). Nevertheless, ion release, after the second step of functionalization, increased (at the 0.05 level of significance) with respect to the analogous pure titania coated sample. According to previous findings concerning the TiO₂/HA systems after thermal treatment in air at 600°C, i.e. increasing of the TiO₂ rutile phase amount and weight raising only due to the thermal treatment procedure (that is also in absence of the spray pyrolysis phase), ion release testifies a partial degradation/modification of the titania inter-layers induced by the annealing procedure. However, it is worth to point out that the performances of the TiO₂/HA materials were, in any case, improved with respect to those of bare titanium substrates. This aspect will be further detected and discussed in section 5.7.2 concerning the potentiodynamic polarization analyses. Finally, comparing the two-step functionalized surfaces, Ti_s_TiO₂_HA substrates showed higher Ti release, further suggesting that the presence of alumina residues could negatively influence the first titania nucleation steps.

To recapitulate, the functionalization of titanium matrix can be a good strategy to reduce Ti and Al release (with respect to bare titanium substrates) and consequently achieve better biocompatibility.

Figure 5.30 shows the Ca ions released from the two-step functionalized surfaces. Differently from Ti and Al release, Ca release was observed from both corrosive media, but again LA is a more severe environment. The large amount of calcium released in lactic acid aqueous solution is due to the higher hydroxyapatite dissolution rate in acid conditions [14,16].

Finally, a higher Ca amount (at 0.05 level of significance) was released in LA from "s+a" surfaces. This result can be due to two different reasons or to their combination. The first explanation could be the higher amount of the HA solution deposited on these surfaces during the spray pyrolysis process. Indeed, rougher surfaces can maintain (i.e. block) a higher quantity of the HA solution during the spray process and, on the contrary, sprayed drops can bounce easily off smooth surfaces. This hypothesis was verified by means of the previously reported weighing tests. A second hypothesis could be that the HA islands in the "s+a" surfaces are finer dispersed (as it was seen in the 3D maps). Consequently, they have larger surface area exposed (i.e. in contact) to the corrosive media, so higher Ca dissolution is produced.

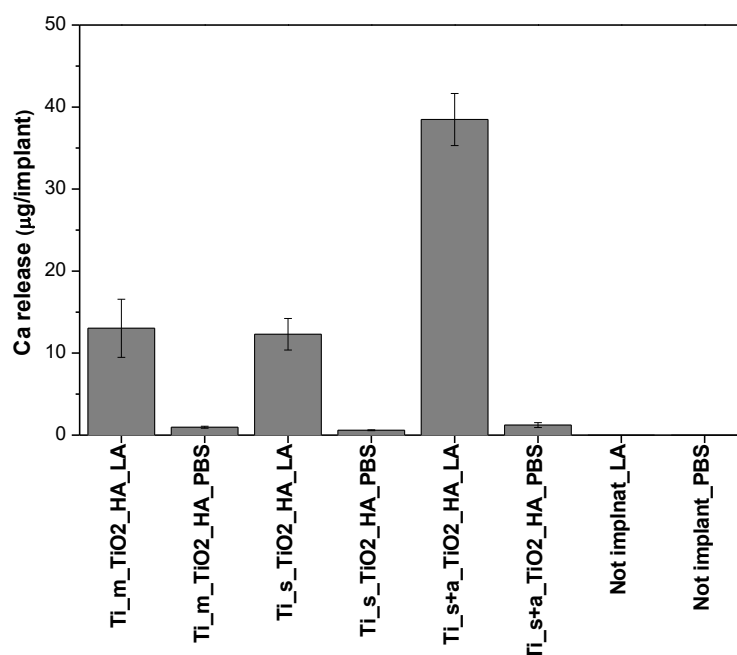


Figure 5.30 Amount of Ca released (μg per implant) in lactic acid (LA) aqueous solution and in phosphate-buffered saline (PBS) solution from TiO₂/HA coated implants. The implants had exactly the same geometrical size and weight.

5.7 Tribocorrosion and potentiodynamic polarization analyses

5.7.1 Tribocorrosion experiments

In the oral cavity, dental implants are exposed to a complex degradation process that involves interrelated mechanical, electrochemical, and microbiological actions (Figure 5.31). Particularly, during mastication, dental implants are subjected to different forces, which may create micro-movements at several interfaces (such as implant/crown, implant/abutment, abutment/crown, and implant/bone) [163]. In these situations, wear and corrosion of the system can occur simultaneously. Tribocorrosion describes the material degradation resulting from the combined action of the mechanical loading and corrosion attack caused by the environment. For passive metals, when two surfaces rub against each other, their protective passive film could be removed, leading to surface corrosion and release of corrosion products into the system [163]. The composition and characteristics of the oxide film as well as the chemical composition of the surrounding media have a strong effect on the wear and corrosion behavior of the materials [164].

In this work, tribocorrosion studies were only carried out on the titania covered Ti surfaces and on pristine titanium substrates, since the evaluation of the tribocorrosive performances of the final composite material (made up of a continuous titania inter-layer + homogeneous spread and discontinuous HA islands) was not significative due to the discontinuous coverage of the underlying surfaces by the bioactive top coatings (i.e. HA islands).

Tribocorrosion behavior was investigated by means of Open Circuit Potential (OCP) measurements and sliding tests with potential recording as a function of the sliding period. OCP measurements consist of monitoring the potential difference between the sample under investigation (working electrode) and a reference electrode, both immersed in artificial saliva

as an electrolytic solution. The electrochemical potential is a measure of the tendency to corrosion of the material under investigation: higher OCP values are related to nobler behavior of materials [100].

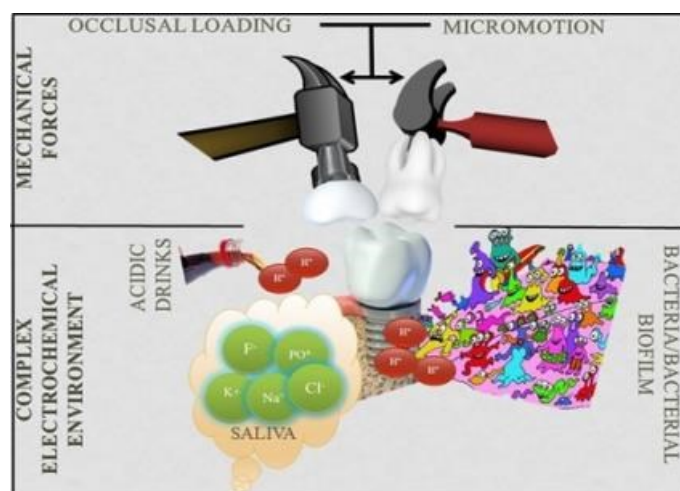


Figure 5.31 Drawing showing the various factors acting on a dental implant in the oral environment [165].

Figure 5.32 shows the OCP evolution for uncoated and MOCVD titania coated machined, sandblasted and sandblasted/acid etched samples, immersed in AS at 37°C, before, during and after the sliding phase.

Different OCP values were observed before the sliding phase between uncoated and TiO_2 coated samples, which demonstrated the beneficial increase of electrochemical nobility for coated surfaces in comparison to native passive films (i.e. thin and compact titania layers act as a more stable protective layer than the native passivation film). The uncoated samples showed low OCP values (i.e. -0.16 V, -0.10 V, and -0.03 V for Ti_m , Ti_s and Ti_{s+a} , respectively), thus revealing a higher tendency to corrosion in AS, while the corresponding coated sample exhibited nobler features (+0.04 V for $\text{Ti}_m\text{-TiO}_2$, +0.08 V for $\text{Ti}_s\text{-TiO}_2$, and +0.05 V for $\text{Ti}_{s+a}\text{-TiO}_2$). Hence, it is evident that MOCVD treatment could enhance the corrosion resistance of Ti surfaces in AS.

In a sliding dynamic contact, OCP describes the galvanic coupling evolution between the undamaged area (which develops a passive film, in the case of most biomaterials) and the corroded region, where the bare metal of substrate is exposed to the solution, due to the failure of the passive (or deposited) film during sliding [101]. For all samples (uncoated and coated), the potential values dropped to less noble values, as soon as the mechanical stress starts (onset of sliding), indicating the beginning of wear process with the partial/total destruction of the passive/deposited film [8]. Among the uncoated substrates, Ti_s demonstrated to be the noblest one, probably because of the presence of Al_2O_3 residuals from the sandblasting process. Ti_{s+a} uncoated sample exhibited an irregular trend with two different phases, that could be ascribed to the initial presence and subsequent removal of the superficial acid etched layer. Finally, the Ti_m sample was the less noble surface. During sliding, OCP values of titania coated samples were always higher than those of corresponding uncoated ones; therefore TiO_2 films demonstrated to effectively protect the underlying Ti material from the direct contact with AS as long as they were in contact (it seems that for titania coated samples, the substrate surfaces were not exposed to the environment, or at least not in significant extension, and just a partial deterioration of the

films occurred). In particular, titania coatings on Ti_m substrates (Ti_m-TiO₂ sample) appeared to possess better coating behavior. Indeed, during the rubbing, OCP decreased only a few tenths of millivolts (from 0.04 V to -0.01 V), which could be associated to the wear of the topmost film layers.

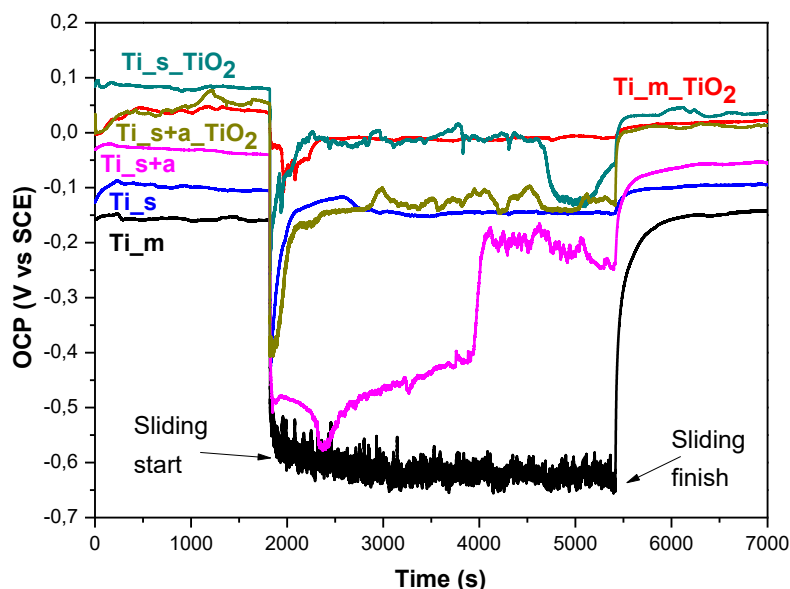


Figure 5.32 Open circuit potential (OCP) evolution for uncoated and MOCVD titania coated samples, immersed in AS at 37°C, before, during and after the sliding phase.

Once the stress has been removed, a potential recovery towards more cathodic values was observed for all samples (uncoated and titania coated) [100]. It is necessary to specify that the collected OCP values after sliding test represent a sum of different contributions given by both the wear track, where a damaged and/or missing protective film could be ascribed, and the surrounding unperturbed area, where the deposited and/or native film remained untouched (that was the larger area), which supplied the highest contribution.

In Figure 5.33, the evolution of the coefficient of friction (COF) with the sliding time for uncoated and titania coated Ti samples, is shown. Generally, COF values for coated Ti substrates were lower and their fluctuations were smoother than that of un-treated Ti samples, with a maximum gap of about 0.25 for the “m” and “s+a” substrates. These differences in the COF values could be partially associated with the peculiar surface features of uncoated and coated samples [8], in particular, the chemical nature and mechanical properties of the deposited coating, and also indicate that MOCVD coatings improved the anti-friction characteristics of the final material. The relation between the surface and the counter body material was tribologically favorable in the case of MOCVD titania coated sample with respect to the native oxide that spontaneously forms on untreated Ti surface. Therefore, TiO₂ functionalized samples exhibited lower COF reasonably ascribable to the chemical modification of surfaces, whereas physical properties were the same for each type of sample surface (uncoated and coated).

For both “s” and “s+a” uncoated types, the COF exhibited an initial increase due to the enlargement of the contact surface as a result of wear effect on roughness ridges. The rise of surface available to couple with the counterpart generally increases the adhesive forces responsible for wear phenomena, thus enhancing COF values. The same was not observed in the corresponding coated samples, indicating that the presence of a low-friction coupling

was able to avoid, or at least slowdown, the process of wear on roughness ridges. Moreover, in the case of the “s+a” finishing ($\text{Ti}_{\text{s+a}}\text{TiO}_2$), the coating operated an effective and positive action during the overall tribological test, while s-type sample ($\text{Ti}_{\text{s}}\text{TiO}_2$), although titania coated, reached a failure point with a sudden increase of COF after 2500 s. More in detail, for the $\text{Ti}_{\text{s}}\text{TiO}_2$ sample, the COF value had a sudden increase exactly when the OCP curve (Figure 5.32) showed a rapid reduction of the OCP value. For this sample a partial coating breakage was possible: after this event the COF value for the $\text{Ti}_{\text{s}}\text{TiO}_2$ sample became very similar to that of Ti_{s} one. Also, m-type finishing in the coated sample ($\text{Ti}_{\text{m}}\text{TiO}_2$) exhibited good behavior and showed only a small instability of COF.

To conclude, these experiments demonstrate that TiO_2 coating represents a promising surface modification towards improved tribological properties of the underlying titanium material.

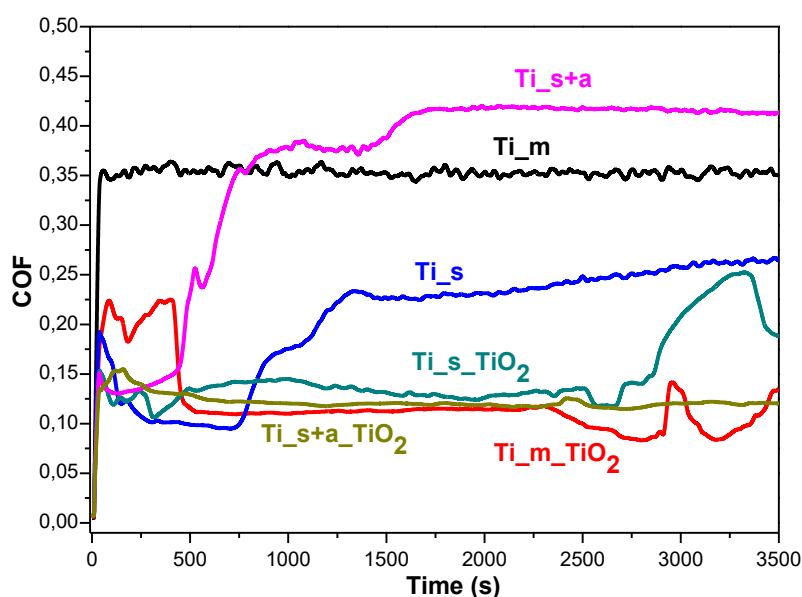


Figure 5.33 Coefficient of friction (COF) during the sliding phase for uncoated and MOCVD titania coated samples, immersed in AS at 37°C.

5.7.2 Potentiodynamic polarization analyses

Potentiodynamic experiments were carried out to observe the effect of the functionalization on the corrosion resistance of the Ti substrates.

Potentiodynamic polarization curves of the pristine Ti substrates, TiO_2 coated Ti substrates and TiO_2/HA coated Ti substrates are plotted in Figure 5.34, Figure 5.35 and Figure 5.36 for “m”, “s” and “s+a” substrates, respectively. Plots are obtained by scanning the applied voltage from -2.5 to 7.5 V vs SCE (scanning rate: 2 mV/s), in artificial saliva (pH ~ 6). The electrolytic cell was thermostated at 37°C. J_0 was calculated considering the Sdr values obtained from profilometer results. Thus, the actual exposed area was evaluated for each sample, taking into account the contribution of surface texture.

All the curves show the typical shape of an active-passive behavior: all substrates demonstrate a passive region that extends from OCP potential value to several volts, depending on the kind of sample.

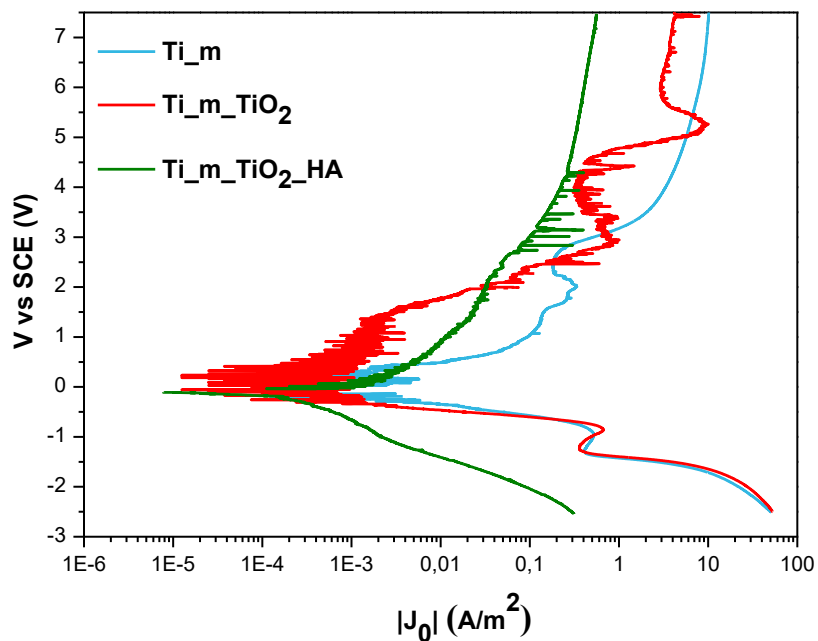


Figure 5.34 Potentiodynamic polarization curves in AS at 37°C for Ti_m, Ti_m_TiO₂, and Ti_m_TiO₂-HA substrates.

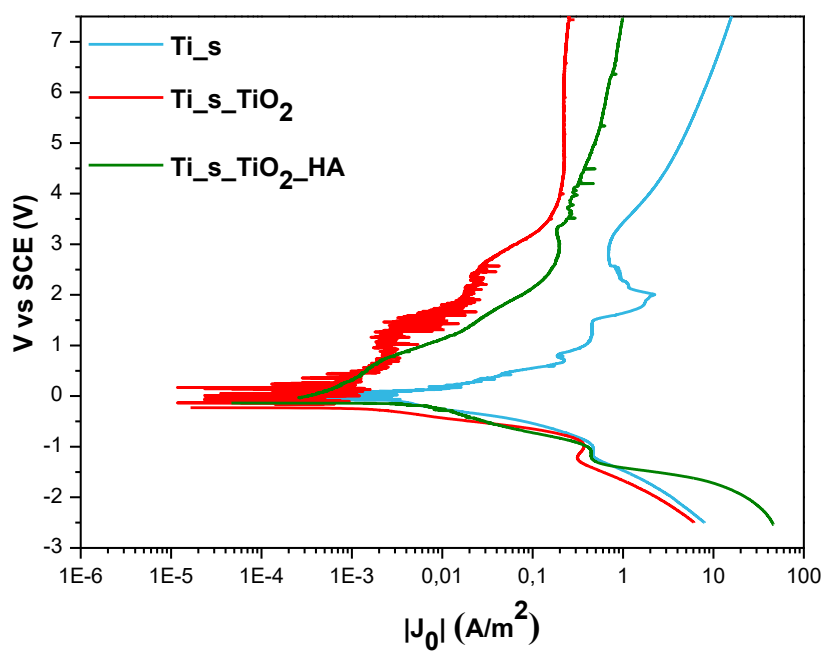


Figure 5.35 Potentiodynamic polarization curves in AS at 37°C for Ti_s, Ti_s_TiO₂, and Ti_s_TiO₂-HA substrates.

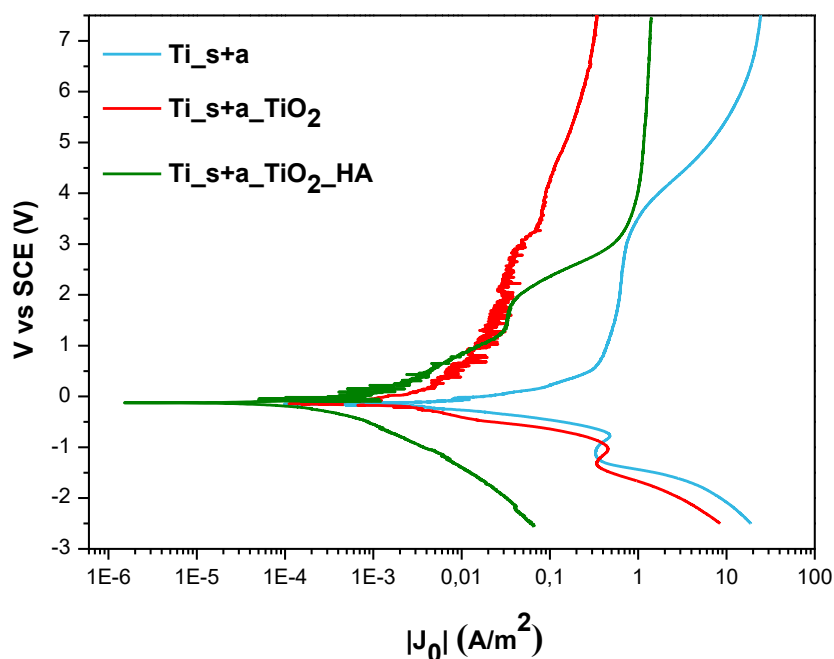


Figure 5.36 Potentiodynamic polarization curves in AS at 37°C for Ti_s+a, Ti_s+a_TiO₂, and Ti_s+a_TiO₂_HA substrates.

When compared to bare Ti, coated samples exhibit a slightly nobler corrosion potential (E_{corr}). Because of the difficulty of revealing clear linear regions due to the high curves noise, the Tafel extrapolation method is hardly applicable to interpret the electrochemical response of the samples here investigated [166]. Therefore, in such circumstances, the corrosion behavior can be assessed by the passive current density (i.e. the current which passes through the passivating film) that becomes a measure of the performance of the protective film [166]. It can be seen that coated samples exhibit lower j_{pass} (passive current density) and a wider passive region, thus confirming the improved corrosion resistance of the film/substrate systems with respect to the substrates alone.

Finally, it can be observed that the potentiodynamic curves, after the second step of functionalization (spray pyrolysis process and thermal annealing, in air, at 600°C, for 6 h), seem to some degree to get worse with respect to those of the only titania coated surfaces. Considering that the improvement of the electrochemical behavior of the coated systems was due to the contribution of the titania inter-layer (i.e. HA islands did not improve the corrosion resistance because of their discontinuous nature), it is reasonable to suppose that the thermal annealing process slightly degraded the anticorrosive performance of titania inter-layer. Previous findings regarding the titania/hydroxyapatite coated systems (after thermal treatment at 600°C) with respect to only titania coated surfaces revealed that i) there was an increase of the TiO₂ rutile phase amount after thermal annealing; ii) there was a weight increase after the thermal treatment procedure and in absence of spray pyrolysis process; iii) there was an increase in metal ion release after the two-step functionalization processes and with respect to the only titania coated surfaces. Potentiodynamic experiments gave again evidence of partial degradation of the titania inter-layer induced by the annealing procedure. Nevertheless, even if the thermal treatment implicated the observed problems, it must be underlined that it was unavoidable to crystallize and, in the meantime, consolidate the HA sprayed phase. Nevertheless, it is important to stress that the performances of the two-step functionalized materials were always enhanced with respect to those of bare titanium

substrates and the partial properties worsening did not reduce the *in-vitro* bioactivity of the final two-step functionalized composite materials (see section 5.9).

5.8 Mechanical investigations

5.8.1 Nanoindentation

Since implants (and therefore the corresponding coatings) are subjected to stress in the oral cavity (during the surgery procedure and the loading phase), it is significant to know their mechanical behavior and, in the case of coated materials, it is also fundamental to know the coating mechanical features [167]. The indentation test is a way to estimate the mechanical properties of a material at the nano-scale level. Nanoindentation studies were very arduous on the investigated samples since several factors (i.e. indentation size effect, surface roughness, nanostructured surface, corrective factors, etc.) must be taken into consideration in order to avoid errors and obtain reproducible and reliable data [60]. For these reasons and principally taking into account that in the developed samples the surface roughness is of the same order of magnitude of the thickness of the titania MOCVD films, nanoindentation tests cannot be directly performed on the “m”, “s” and “s+a” surfaces. Therefore, smooth (uncoated and coated) Ti samples were arranged specifically for this characterization. Thus, *ad-hoc* Ti substrates were prepared by smoothing, via lapping, the Ti machined substrates to a $60 \div 80$ nm of residual Ra, and subsequently coating them with the above-described deposition routes (obtaining the sample named 200 nm TiO₂ coated Ti and 200 nm TiO₂ + HA coated Ti, respectively). Under this condition, nanoindentation results should not be influenced by the surface roughness of the pristine Ti substrates, but only by the coatings contribution. On the other hand, it must be mentioned that Bückle’s rule predicts no influence on the mechanical properties of the film due to the substrate if the indentation depth is lower than 1/10 of the overall film thickness [107]. Considering that the used instrumental apparatus does not allow for the accurate control of the penetration depths lower than 30 nm, the mechanical properties detected were influenced, to a certain extent, by the contribution from the underlying metallic substrate. Because of this reason, a specific sample was prepared by smoothing the Ti substrate as previously described and coating it by MOCVD with a thicker TiO₂ layer (i.e. 340 nm).

With reference to the 200 nm TiO₂ + HA coated Ti sample, this surface was obtained via the traditional spray pyrolysis procedure. However, as it was previously observed, sprayed drops can bounce easily off from smooth surfaces. Therefore, being this surface also smoother than the machined ones, the amount of HA deposited on this substrate is realistically supposed to be very low. Therefore, for this sample, the detected nano-mechanical properties will be the result of different effects deriving from the titania inter-layer (stronger contribute) and the HA islands (supposed only marginally influence the final mechanical properties being the HA islands very dispersed and isolated). However, the investigation of this type of sample was in any case very challenging, because it allowed to evaluate if the previously detected partial worsening of the two-step functionalized surface features with respect to those of the only titania coated one (induced by the annealing procedure) also affects the mechanical properties of the composite material at nanoscale level.

Figure 5.37 shows the mean values of the hardness (H) and elastic modulus (E) detected for all the surfaces here investigated. The average values of H and E for the uncoated titanium

were higher than those reported in the literature for bulk Ti [142,167]. Clearly, the pristine Ti had lower hardness, while TiO₂ films (both 200 nm and 340 nm thick) increased the Ti mechanical properties. In literature, the hardness of titania thin films varies from 2 to 13 GPa [168] as a function of the thin film deposition method and/or the crystalline phase [169]. Comparing the H values of bare substrates with the MOCVD treated ones, higher H values (at 0.05 level of significance) can be observed for the coated samples (both 200 nm and 340 nm). This is reasonable because, generally, oxide materials are harder than metallic ones, due to their covalent chemical nature. Finally, lower mean H value (at 0.05 level of significance) was detected in 200 nm TiO₂ coated samples in comparison with 335 nm TiO₂ coated ones. As it was mentioned before, in the case of thin films and for penetration depth higher than 10% of film thickness, the mechanical response is a combination of the contribution from the coating and the substrate. The mean penetration depth for the 200 nm TiO₂ coated samples (31.8 ± 0.8 nm) was higher than a tenth of the film thickness (~ 32 nm > ~ 20 nm), consequently, the detected values were (at least partially) influenced by the underlying Ti substrate.

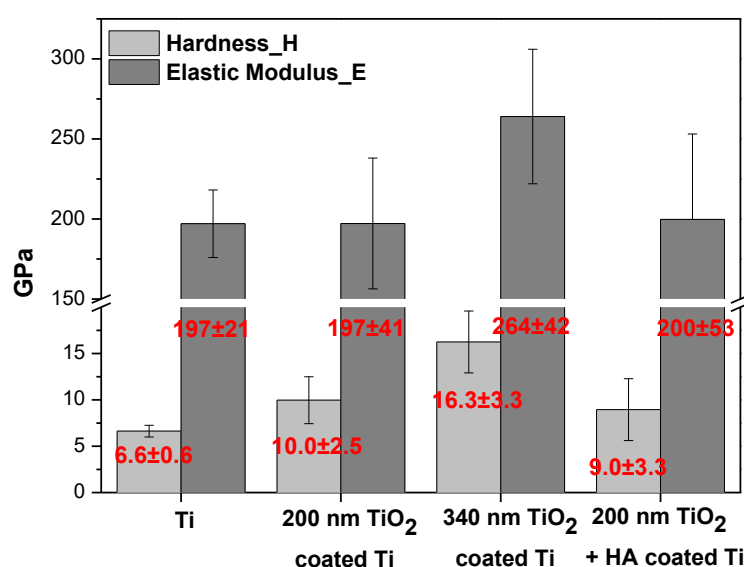


Figure 5.37 Mean value of hardness (H) and Elastic Modulus (E) for uncoated Ti, 200 nm TiO₂ coated Ti, 340 nm TiO₂ coated Ti and 200 nm TiO₂ + HA coated Ti.

Furthermore, as it was previously declared, from the comparison of “200 nm TiO₂ coated Ti” and “200 nm TiO₂ + HA coated Ti”, it appears that the nanohardness improvement happened after the first step of the functionalization (i.e. it was due to the presence of the titania inter-layer), while none additional difference (at 0.05 level of significance) in hardness could be detected after the whole two-step modification process. This result is not surprising because the HA layer is discontinuous and, as previously reported, HA islands (especially on a smooth surface) are only slightly spread on the titania surface, so they cannot or can only marginally contribute to the modification of the mechanical properties of the final bi-layered material. Furthermore, hydroxyapatite films are known to have lower nanohardness than titania ones [170]. Finally, differently from previous findings concerning the partial degradation of the titania inter-layer induced by the annealing procedure, nanoindentation experiments did not evidence worsening in the mechanical properties of the two-step functionalized surfaces after the thermal annealing process.

Concerning the elastic modulus, there were no statistical differences (at 0.05 level of significance) in the E values of 200 nm TiO₂ coated Ti, 200 nm TiO₂ + HA coated Ti and uncoated Ti substrate, providing good conditions for the composite material mechanical deformation. While the Elastic modulus (E) of the titania coatings on the Ti substrates appeared very similar to the values reported in the literature for anatase TiO₂ [60,167,171], the reported elastic modulus values range from 3 to 180 GPa for hydroxyapatite. Difference in density, pore distribution, crystallinity, grain size, and purity are the main factors suggested contributing to the spreading of the reported elastic modulus values [172].

To sum up, the surface mechanical properties of the bulk titanium were also improved with the deposition of TiO₂ and TiO₂/HA films and the observed outcomes can suggest interesting positive effects on the actual investigated samples (Ti_m, Ti_s, Ti_{s+a}).

5.8.2 Adhesion tests

As described in Chapter 1, several techniques are used to create bioactive HA coating on metallic implants [1,2,12,67]. Common limitations in HA coatings are the high film porosity, unsatisfactory thickness uniformity, not controllable crystallinity and phase impurity, and poor adhesion [67]. Among these, the low coating-to-substrate adhesion is the major critical factor, limiting the extensive use of HA coatings for implants at a commercial scale [67]. Consequently, the improvement of adhesion strength between the metallic substrate and ceramic coating is a general requirement, regardless of the techniques used. In general, the bonding strength between hydroxyapatite and metal substrate depends on various factors such as surface chemical and physical interactions, film thickness, coating and substrate microstructures, substrate surface roughness, and deposition technique [21,173].

The adhesion strength of hydroxyapatite coating is widely studied by using the scratch test [67]. It consists of introducing stresses deforming the surface by means of indentation of a moving diamond tip. The applied load is increased, either stepwise or continuously, until the film detachment. Acoustic, friction and tip position data are collected by appropriate sensors attached to the indenter holder. The failure of the coating reflects in the data recorded by the sensors. Several attempts were done in this Ph.D. work to characterize the adhesion of the HA coatings by quantitative scratch tests. However, since the distribution of the HA phase onto the Ti substrates is not homogeneous, the scratch test could not provide reliable and reproducible results, not even increasing the statistics and the number of tests. Other conventional adhesion tests often require defined sample characteristics (i.e. minimum coating thickness, porosity, suitable sample size, potential surface machining to level the coating thickness, etc.), which cannot be present in the studied samples (due to the targeted discontinuous nature of the HA islands) without strongly modify the morphology/architecture of the here planed bi-layered coatings, thus obtaining, in case, not reliable and significant results.

Therefore, with the aim to estimate if the overall systems are adequately joined, compare the behavior of the different functionalized surfaces among themselves, and obtain a qualitative indication of the best treatment among those realized in this work, simple, quick and low-cost tape tests were carried out. The tests were performed according to ASTM D3359-09 “*Standard test methods for measuring adhesion by Tape Test*”. It describes the procedure for assessing the adhesion of coating films (thickness lower than 125 μm) to a metallic substrate by applying and removing pressure-sensitive tapes over cuts that are made in the film. It should be said that this test is also used in literature to assess the adhesion of calcium

phosphate bioactive coatings on titanium substrates [173–176], and it is often employed in industrial practice.

Each test was executed as follows. Firstly, a lattice pattern, with three cuts both in horizontal and vertical direction, was made in the film to the substrate (ASTM D3359-09: test method B). Then a pressure-sensitive tape (transparent 3M Brand Scotch) was applied over the lattice and the tape was smoothed into place by using a pencil eraser over the area of the incisions and let for 90 ± 30 s. After that, the tape was removed by pulling it off rapidly back over itself as close to the angle of 180° . Finally, damage of the coating was evaluated visually by optical microscopy images and the adhesion was classified into six grades, as defined in Table 5.3.

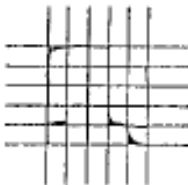
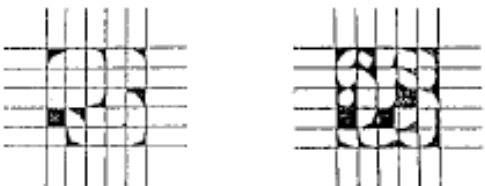
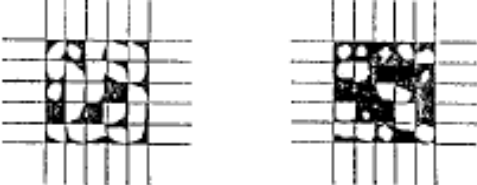
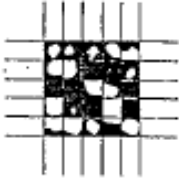
Classification	Area removed %	Surface of cross-cut area
5B	0 % - None	
4B	Less than 5 %	
3B	5 – 15 %	
2B	15 – 35 %	
1B	35 – 65 %	
0B	Greater than 65 %	

Table 5.3 Classification of adhesion test results (ASTM D3359-09).

It must be underlined that this method presents important limitations. As it is reported in the commentary section of ASTM D3359-09, this test does not define the absolute adhesive strength but it gives an indication of relative adhesion performance. Also, it has low sensitivity and it is applicable only to coatings of relatively low bonding strengths (indeed it does not distinguish between higher levels of adhesion). Furthermore, in multi-coated systems adhesion failure may occur also between the coatings, so that the adhesion of the coating system to the substrate is not determined. Also, the accuracy and the precision of the obtained results depend largely upon the skill of the operator (i.e. operator ability influences the angle and the rate of tape removal, and the visual assessment of the tested samples). Besides, the type of tape, the temperature, the humidity, the flatness of the tested surface, etc., may also influence the obtained results.

In this Ph.D. work, to carry out the tape tests, *ad-hoc* samples were prepared. The here investigated conventional (2 spray cycles) HA coated samples could not be evaluated by means of tape test because the low amount of the sprayed HA made it impossible to observe the coating modification after the tape test. Therefore, customized samples with higher HA amounts were prepared. In detail, samples were obtained spraying 5 times for 2 seconds, with 60 s interval between each other. 5 spray cycles were chosen as the best compromise between the necessity to strengthen the HA optical signal (i.e. visible white spots) and, at the same time, not to produce too thick HA coatings. In this context, it should be underlined that the adhesion strength of these coatings is surely worse of that of the traditional 2 spray cycles samples because the adhesion strength of a coating gets worse when the coating thickness increases (coating cracking and delamination decrease with the decreasing of thickness) [21,173,176,177]. Furthermore, to evaluate if the insertion of dense and compact titania inter-layers improved the adhesion strength between the Ti substrate and the HA layer, adhesion tests were performed (in addition to the traditional titania/HA coated surfaces) also on HA (5 spray cycles) coated titanium substrates without titania inter-layer.

Before to analyze the adhesive behavior of the HA coated samples, the pure titania coated surfaces were tested by means of tape test (i.e. Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂). These experiments qualitatively confirmed the good adhesion features of the MOCVD titania coatings on titanium substrates. In this regard, different theoretical/literature considerations support a good adhesion between the titania MOCVD film and the Ti substrate. It is well-known that the MOCVD process produces uniform films, with good reproducibility and adhesion, at reasonably high deposition rates [73]. Furthermore, the high chemical affinity of TiO₂ to Ti improves the coating/substrate adhesion strength [19]. Moreover, considering the low thickness of the here deposited MOCVD TiO₂ films (about 200 nm) and the similar thermal expansion coefficients of Ti and TiO₂ [22], the thermal mismatch can only marginally (or not even) negatively contribute to the bonding strength.

Figure 5.38 shows Ti_m_TiO₂, Ti_s_TiO₂, and Ti_s+a_TiO₂ surfaces before (a, c, e) and after (b, d, f) the tape tests. It can be observed that all the samples showed a high level of adhesion, with no surface changes after the qualitative mechanical test. All the surfaces investigated were, therefore, classified into category 5B (see Table 5.3).

Concerning the adhesion tests on the hydroxyapatite coated surfaces, the adhesion of the HA coatings was evaluated both in the presence and in absence of the titania inter-layer. The tests were performed on six separate samples. Different results were obtained as a function of the typology of the pristine titanium substrates (i.e. machined, sandblasted and sandblasted/acid etched).

Concerning the HA coated machined surfaces, Figure 5.39 and 5.40 show the optical microscopy images (at different magnifications) of HA coated machined substrates (5 spray

cycles) before (a, b) and after (c, d) the tape test, without and with the titania inter-layer (Figure 5.39 and 5.40, respectively).

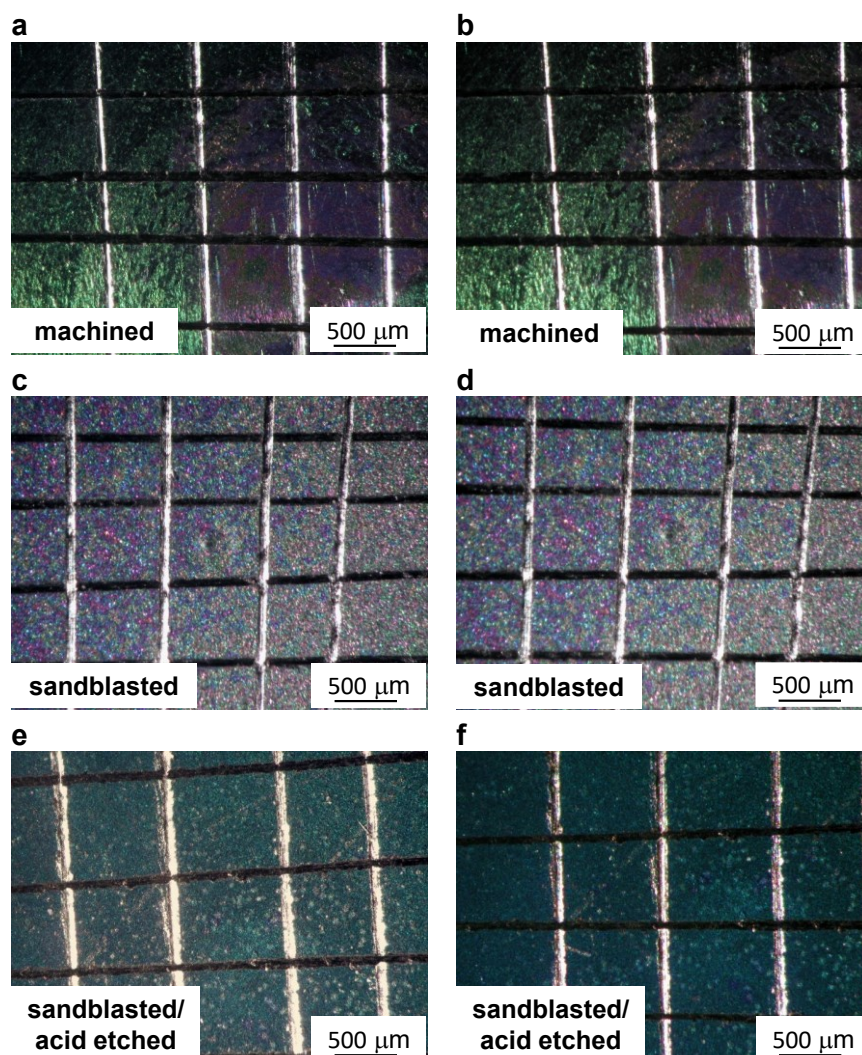


Figure 5.38 Optical microscopy images of $\text{Ti}_m\text{-TiO}_2$, $\text{Ti}_s\text{-TiO}_2$, $\text{Ti}_s+\text{a-TiO}_2$ substrate before (a, c, e) and after (b, d, f) the tape test (according to ASTM D3359-09).

First of all, it can be noted that, as previously observed from profilometer and SEM studies, as a consequence of the anisotropy and directional irregularities of machined substrates, HA islands were not homogeneously spread/dispersed on these surfaces (both in the absence and in presence of the titania inter-layer). The substrate anisotropy markedly influenced the spray pyrolysis process and, therefore, the HA islands were concentrated in wide agglomerates and preferentially located on the rougher zones (rougher surfaces can maintain/block higher amounts of the HA solution during the spray process). Finally, it can be observed that the presence of the titania inter-layer seems not influence or not strongly affect the amount of the deposited HA.

From the comparison of the photos of Figure 5.39a and b to c and d (before and after the tape test), and of those of Figure 5.40a and b to c and d, it can be noted that HA coated surfaces were seriously modified after the tape tests, and the big agglomerates seemed to be the most damaged. Indeed, the upper part of the large HA agglomerates remained on the tape, while the underlying layer remained on the substrate. It appears that the adhesion of the basic layer is higher than the strength of the big agglomerates. Anyway, the HA adhesion

on the substrates does not seem to be the main reason for the coating failure, and the primary cause is related to the non-self-consistency of too large HA islands.

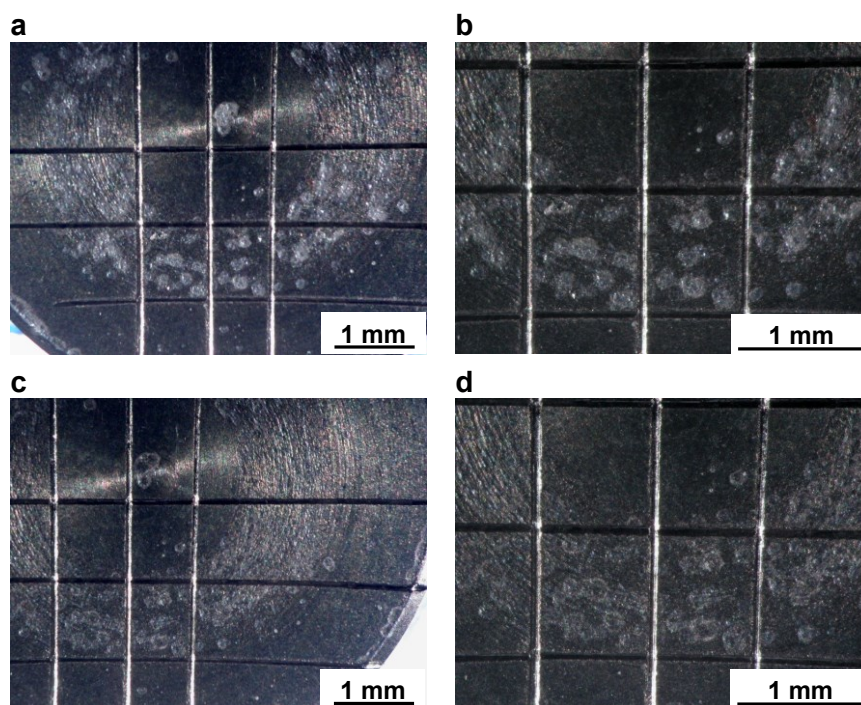


Figure 5.39 Optical microscopy images of HA (5 spray cycles) coated and thermal annealed machined Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

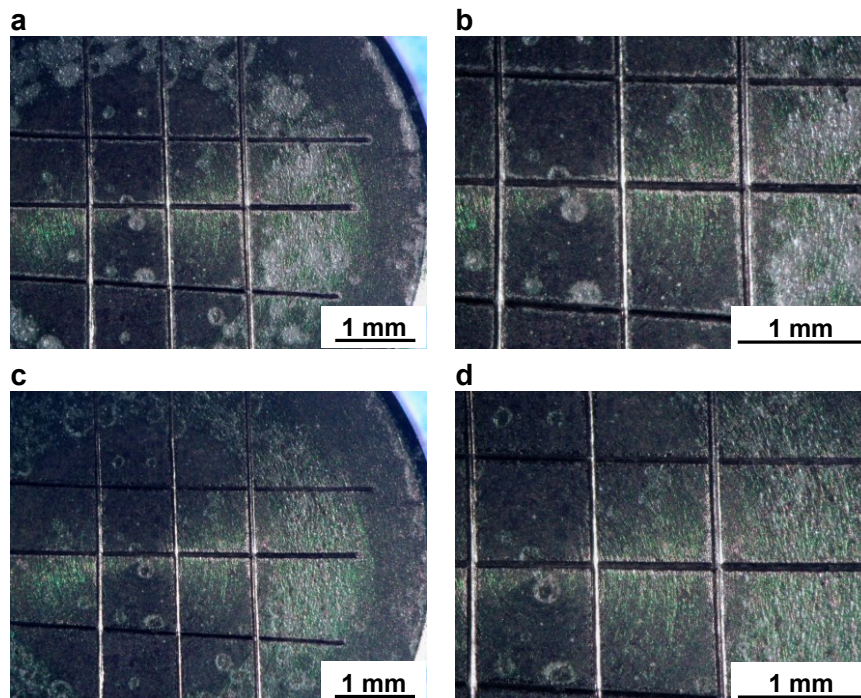


Figure 5.40 Optical microscopy images of titania/HA (5 spray cycles) coated and thermal annealed machined Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

Similar results were obtained testing the other five samples investigated (not shown for sake of brevity). Following the indications of Table 5.3, machined substrates without titania inter-layer were classified as 1B and/or 2B (result deriving from the 6 surfaces investigated), while

machined substrates with titania inter-layer were classified as 0B and/or 1B. Therefore, as a conclusion, machined surfaces showed insufficient mechanical properties with very low HA retention, independently from the presence or the absence of the titania inter-layer.

Concerning sandblasted substrates, Figure 5.41 and 5.42 show the optical microscopy images of HA coated sandblasted substrates (5 spray cycles) before (a, b) and after (c, d) the tape tests, without and with the titania inter-layer (Figure 5.41 and 5.42, respectively).

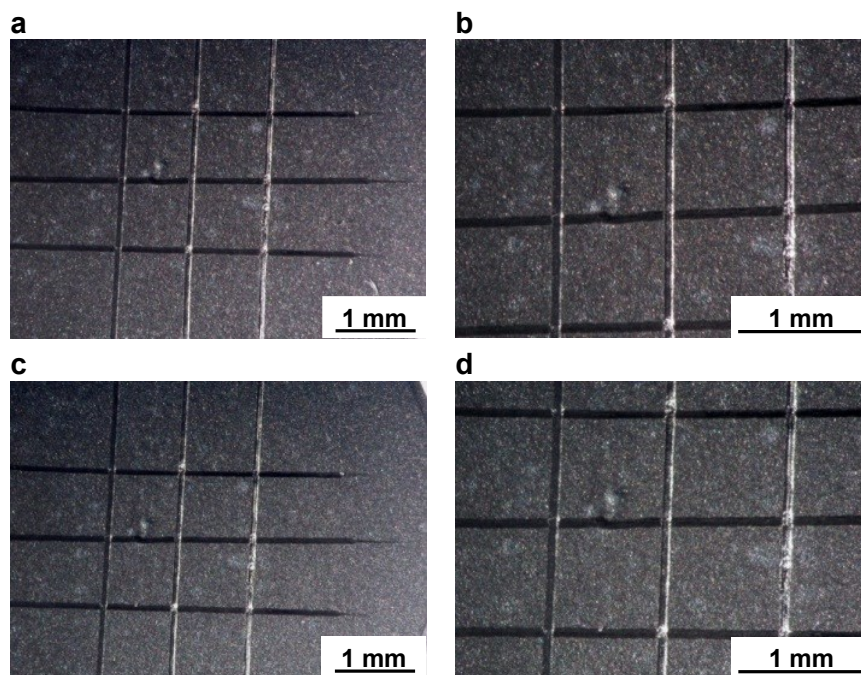


Figure 5.41 Optical microscopy images of HA (5 spray cycles) coated and thermal annealed sandblasted Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

Firstly, differently from “m” substrates, it appears that the HA islands were more homogeneously spread on sandblasted surfaces (both in the absence and in presence of the titania inter-layer). In this case, the surface isotropy allowed a better and regular distribution of the HA islands. Furthermore, it can be observed that the presence of the titania inter-layer strongly influenced the amount of the deposited HA islands (compare Figure 5.41a,b with Figure 5.42a,b): TiO_2 coated sandblasted surfaces showed higher amounts of sprayed HA (i.e. the surface appeared clearly whiter). When sprayed drops impact against a solid surface they can spread in different ways and deposition, rebound, and drop fragmentation may occur [83]. As described in Chapter 2, three conditions mainly affect the drop impact characteristics: the fluid properties, the kinematic parameters, and the substrate surface characteristics [83]. Considering that the fluid properties, the kinematic parameters, surface morphology, and temperature are the same for uncoated and titania coated Ti_s substrates, the higher amount of the deposited HA on titania functionalized surfaces could be explained supposing a more favorable chemical affinity of HA with respect to crystalline TiO_2 than Ti. However, this chemical affinity contribution was not exhibited by machined surfaces, most likely because of the prevalent strong influence of the substrate anisotropy.

From the comparison of the photos of Figure 5.41a and b to c and d (before and after the tape test), and of those of Figure 5.42a and b to c and d, it can be noted that sandblasted HA coated surfaces appeared poorly modified (high HA retention) after the tape tests (both with or without the titania inter-layer), suggesting good adhesion. Following Table 5.3,

sandblasted substrates, with and without titania inter-layer, were classified as 4B. The better HA adhesion on sandblasted surfaces with respect to machined ones could be explained considering different contributions: firstly the finer dispersion of HA islands (avoiding big agglomerates that would result in less adhesion). Secondly, the bonding strength of hydroxyapatite coating is improved by substrate with high surface roughness, thanks to a better film-to-substrate mechanical interlocking [161,162].

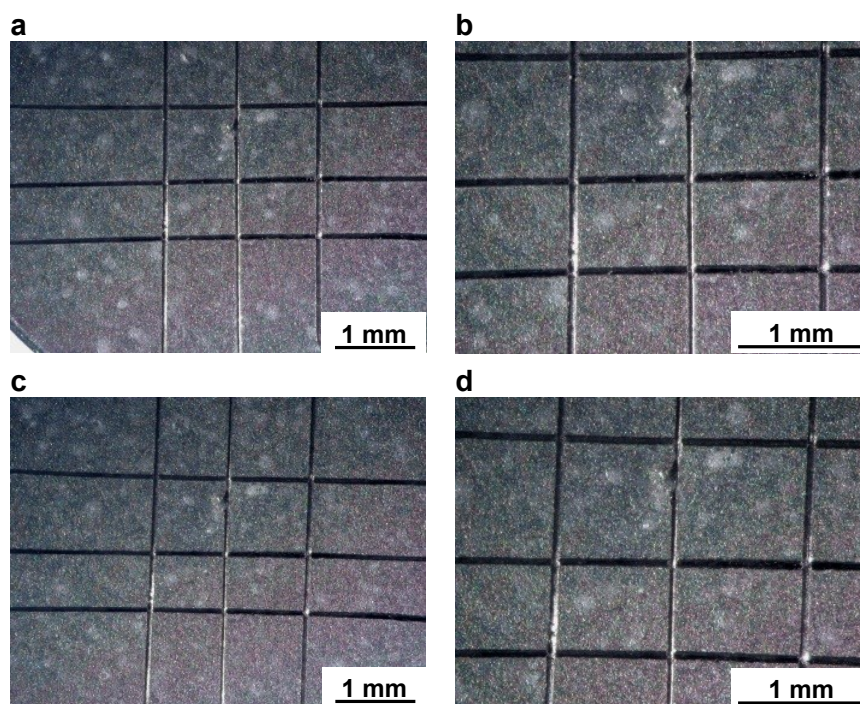


Figure 5.42 Optical microscopy images of titania/HA (5 spray cycles) coated and thermal annealed sandblasted Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

Concerning Ti_{s+a} substrates, Figure 5.43 and 5.44 show the optical microscopy images of HA coated sandblasted/ acid etched substrates (5 spray cycles) before (a, b) and after (c, d) the tape tests, without and with the titania inter-layer (Figure 5.43 and 5.44, respectively). First of all, differently from machined substrates and equally to sandblasted surfaces, HA islands were homogenously spread (both in the absence and in presence of the titania inter-layer). As for sandblasted surfaces, it appears that the presence of the titania inter-layer strongly influenced the amount of the deposited HA, that is titania coated sandblasted/acid etched surfaces showed the higher quantity of sprayed HA. This behavior can be argued considering the more favorable chemical affinity of HA with respect to crystalline TiO₂ than Ti. Moreover, comparing qualitatively sandblasted and sandblasted/acid etched surfaces, it seems that “s+a” surfaces permitted to block higher HA amounts (both in the presence and in absence of the titania inter-layer). As it was detected from the morphological and the topographical analysis, “s+a” surfaces present the highest roughness parameters, therefore they could block the highest amount of HA solution during the spray pyrolysis process. Differently, previously results regarding weighing experiments did not report significant differences in the blocking capability of the rougher surfaces (“s” and “s+a”).

From the comparison of the photos of Figure 5.43a and b to c and d (before and after the tape test), and of those of Figure 5.44a and b to c and d, it can be noted that HA coated surfaces were not seriously modified after the tape tests, thus the adhesion was very good. Following Table 5.3, sandblasted/acid etched HA coated substrates (with and without titania

inter-layer) were classified as 4B, independently from the presence or the absence of the titania inter-layer. Like for “s” surfaces, the finer dispersion of HA islands and the better film-to-substrate mechanical interlocking [161,162] could describe the improved adhesion of the HA coatings to “s+a” surfaces with respect to “m” ones.

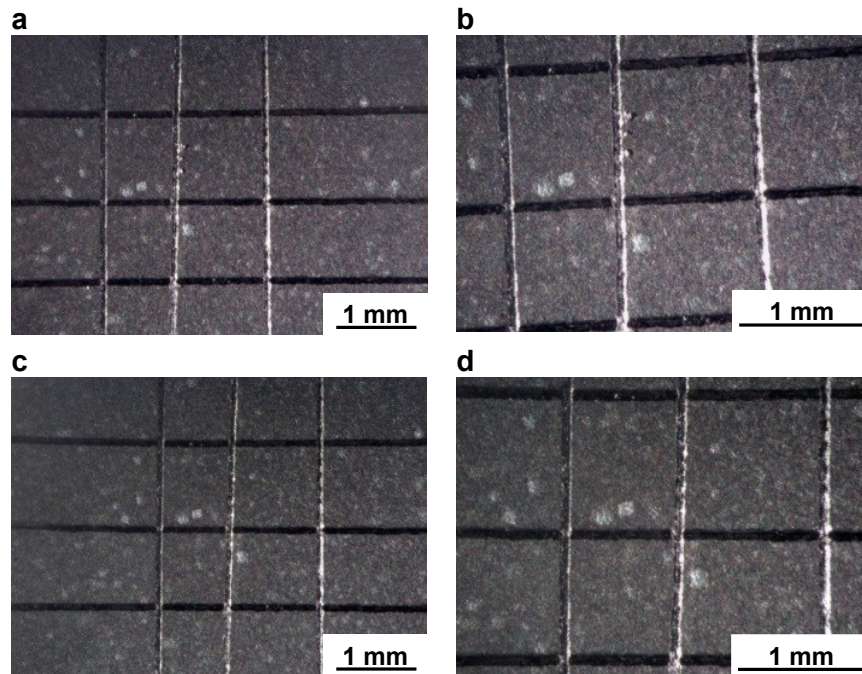


Figure 5.43 Optical microscopy images of HA (5 spray cycles) coated and thermal annealed sandblasted/acid etched Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

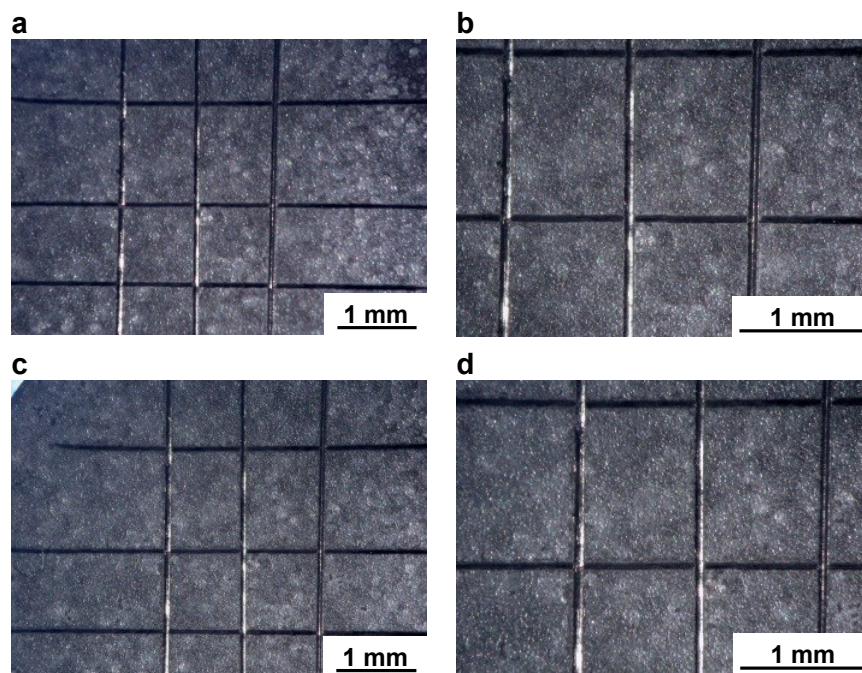


Figure 5.44 Optical microscopy images of titania/HA (5 spray cycles) coated and thermal annealed sandblasted/acid etched Ti substrates before (a, b) and after (c, d) the tape test (according to ASTM D3359-09).

To summarize, according to the ASTM D3359, tape test experiments indicated an adequate adhesion of the HA coatings to the underlying substrate for sandblasted and sandblasted/acid etched surfaces. Differently, machined substrates demonstrated unsatisfactory results probably due to the presence of big HA agglomerates and/or a less coating-to-substrate mechanical interlocking contribution. Also, the adhesion of the HA coatings was evaluated both in the presence and in absence of the titania inter-layer, but no differences in the HA adhesion were observed (probably due to the low sensitivity of the qualitative method).

5.9 Acellular *in-vitro* bioactivity

Bioactive materials (e.g. bioglass, calcium phosphate, glass-ceramic) are currently used in clinical applications thanks to their ability to form (after implantation) an apatite layer on their surfaces, which is thought to be the key issue to bond to the living bone [178]. This superficial apatite layer has similar composition, structure, and morphology to the bone mineral; thus bone-producing cells (i.e. osteoblasts) may well preferentially proliferate and differentiate on this apatite surface layer. By the osteoblasts activity, collagen and biological apatite will also be produced, so allowing new bone generation in contact with the originally formed apatite layer. Definitely, a chemical bond between the bone apatite and surface apatite layer will be established [178].

Based on the process just described, the ability of a material to form an apatite layer on its surface is proven also in simulated body fluid (SBF), i.e. inorganic medium (without organic substances and cells), but with ion concentrations almost equal to those of human blood plasma. Therefore, a material able to form an apatite layer on its surface in SBF may, ideally, bond to the living bone through the apatite layer that forms on its surface (obviously if it does not release any component that induces toxic or immune responses in the surrounding tissue). As a result, *in-vivo* bone-bonding ability of a material can be estimated via *in-vitro* tests, which are based on the degree of hydroxyapatite nucleation on the surface of the material when it is soaked in acellular body-like fluid with pH value, temperature, and ion concentrations similar to the inorganic fraction of human blood plasma, e.g. Hank's balanced salt solution (HBSS), Kokubo's Simulated Body Fluid (SBF), and Dulbecco's Phosphate Buffered Saline (DPBS) solution [179,180]. The inorganic composition of blood plasma and different simulated body fluids are reported in Table 5.4 [139].

Ion	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HPO ₄ ²⁻ / H ₂ PO ₄ ⁻	SO ₄ ²⁻	HCO ₃ ⁻
Blood plasma	142.0	5.0	1.5	2.5	103.0	1.0	0.5	27
SBF	142.0	5.0	1.5	2.5	148.5	1.0	0.5	4.2
PBS	145.0	4.2	0.49	0.91	143	9.6	-	-
5 x SBF	710	25	7.5	12.5	741	5	2.5	21
10 x SBF	1000	5	5	25	1065	10	-	-
HBSS	141.7	5.7	0.8	1.7	145.6	0.7	0.8	4.2
DPBS	145	4.2	0.50	0.5	143	9.6	-	-

Table 5.4 Inorganic composition (mM) of blood plasma and different simulated body fluid [132,139].

However, particular attention must be taken into account before deducing conclusive declarations on the “bioactivity” of the material only based on such laboratory tests (made out of the body, without cells and proteins). Such experiments can (and must) only be useful for a preliminary “classification” of bioactivity performance of the materials [98] and with the aim to, initially, reduce the number of animals as well as the duration and the cost of experiments. However, additional data are needed (i.e. cell behavior assessments and then *in-vivo* confirmations) to draw a more accurate picture of the real bioactivity and biological behavior of the material [98].

Concerning the chemical surface composition of the here investigated samples, i.e. made of titania (both native or MOCVD deposited) and titania/hydroxyapatite, the following mechanisms have been reported in the literature for the apatite growth in simulated body fluids [132,178].

In relation to TiO_2 surfaces, literature reports slightly different points of zero charge values for TiO_2 : from 5.1 to 6.8 [156]. Regardless, at SBF pH of 7.4, deprotonation of the titanium oxide surface happened and negative $\text{Ti}-\text{O}^-$ groups are formed on the surface (see Figure 5.45) [178]. These negative sites attract Ca^{2+} ions from the body fluid that bond to the surface. A layer of amorphous calcium titanate is hence formed and, therefore, the surface becomes slightly positively charged as the layer grows due to the Ca^{2+} ions. This surface will then attract negatively charged phosphate ions, which bond to the surface, and a metastable phase of calcium phosphate is formed. This layer is then eventually crystallized into bone-like apatite because it is thermodynamically more favorable for the amorphous calcium phosphate to adopt a crystalline structure in a wet environment [132].

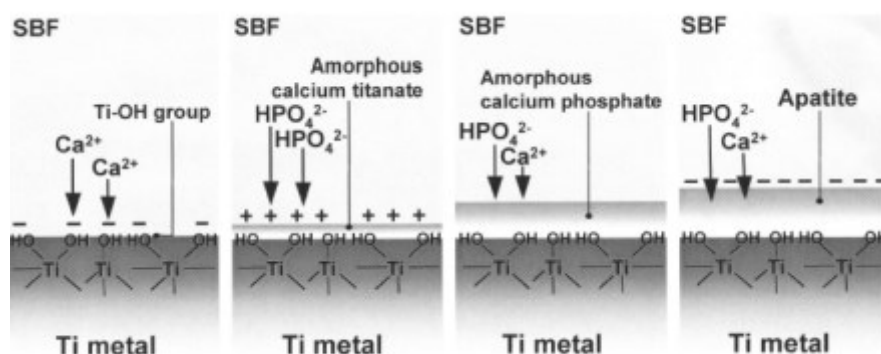


Figure 5.45 Mechanism of bone-like apatite formation TiO_2 surface in simulated body fluid [178].

For synthetic hydroxyapatite, the mechanism is almost similar, except for the initial activating surface. In SBF, hydroxyapatite is initially negatively charged (isoelectric point from 5 to 7, i.e. lower than the pH of SBF) [181]. Thus, the negatively charged HA (deriving from exposed hydroxyl and phosphate units in the HA crystal structure) combines with the positively charged Ca^{2+} ions present in the surrounding fluid. Therefore, Ca-rich amorphous calcium phosphate is formed on the surface of synthetic hydroxyapatite. As previously described for the titania coated surface, the Ca^{2+} ions progressively accumulate and the hydroxyapatite becomes positively charged on its surface. Then, it reacts with the negatively charged phosphate ions. As a result, Ca-poor amorphous calcium phosphate stratum is formed which is eventually transformed into the more stable nanosized crystalline bone-like apatite [178].

As it is reported in [98,139,178,180], several types of acellular solutions with a pH and ion concentrations similar to those of human blood plasma are proposed and used for these

bioactivity tests. In this Ph.D. work, even if the normed BS ISO 23317:2014 procedure uses the SBF solution, the Dulbecco's Phosphate Buffered Saline solution was employed as inorganic simulated body fluid medium. Firstly, because it is commercially available: stable DPBS tables can be found in the market. Thus, for example, for the specific case of Gibco-BRL Life Technologies DPBS, the acellular solution can be easily prepared by simply mixing the powder DPBS with water at room temperature (15°C to 30°C) and finally adding 0.1 g calcium chloride/L. Differently, preparing the SBF solution in the laboratory, experimental mistakes (principally due to the weighing of the hygroscopic starting salts necessary for preparing the supersaturated solution) may lead to undesired immersion conditions and then to nucleated phases other than apatite [98]. Furthermore, SBF solution requires the incorporation of pH-buffering agents like tris hydroxymethyl aminomethane [182], that it is found effective to allow stable pH during the experiments, but this implies the addition to the medium of a "foreign" organic compound, which may not always appear appropriate [98]. On the other hand, pH variations must be carefully taken into account. Indeed, if pH has uncontrolled variations other phases (instead of apatite) may stably form. For example, in acid pH conditions, brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), monetite (CaHPO_4) or octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) are more stable than hydroxyapatite [98].

In this work, *in-vitro* bioactivity was evaluated according to the amount of hydroxyapatite growth ability on the surface of sample disks after soaking in DPBS solution. Dulbecco's Phosphate Buffered Saline (calcium/magnesium-free) and reagent grade CaCl_2 were purchased from Gibco-BRL Life Technologies. DPBS and reagent grade CaCl_2 (100 mg/L) were dissolved in Milli-Q water to prepare the solution. Each sample (disk, $\phi = 8$ mm) was immersed into 5.0 mL of the DPBS solution and kept at 37°C for different times (30 minutes, 1, 15 and 24 h). After the soaking procedure, each disk was removed from the DPBS solution, rinsed thoroughly with Milli-Q water and left to dry in air. The morphology, the crystalline structure and the chemical composition of the nucleated deposits were investigated by means of SEM-EDX, XRD and FT-IR analyses.

5.9.1 Acellular *in-vitro* bioactivity experiments

Scanning electron microscopy observations were initially carried out for assessing the presence of hydroxyapatite deposits on the surface of the samples after immersion in DPBS solution and to determine their morphological features. Figure 5.46, Figure 5.47, and Figure 5.48 show the scanning electron micrographs of uncoated Ti, TiO₂ coated Ti and TiO₂/HA coated Ti surfaces, incubated in DPBS solution at 37°C for determined intervals (30 min, 1 h, 15 h, and 24 h), for machined, sandblasted and sandblasted/acid etched samples, respectively. To compare the hydroxyapatite nucleation process on the different surfaces, all the images are shown at the same magnification (40000x). It can be seen that, following incubation in DPBS solution, the two-step functionalized surface (i.e. Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, and Ti_s+a_TiO₂_HA) exhibited a newly formed homogeneous apatite layer just after incubation for only 30 minutes, while for the same duration, no morphology modifications were detected on uncoated and on TiO₂ MOCVD coated surfaces. Indeed, the surface of Ti_m, Ti_s, Ti_s+a, Ti_m_TiO₂, Ti_s_TiO₂, and Ti_s+a_TiO₂ samples appeared just like those of samples in Figure 5.1, 5.3, 5.4 for uncoated Ti substrates and in Figure 5.6, 5.7, 5.8 for titania coated Ti ones.

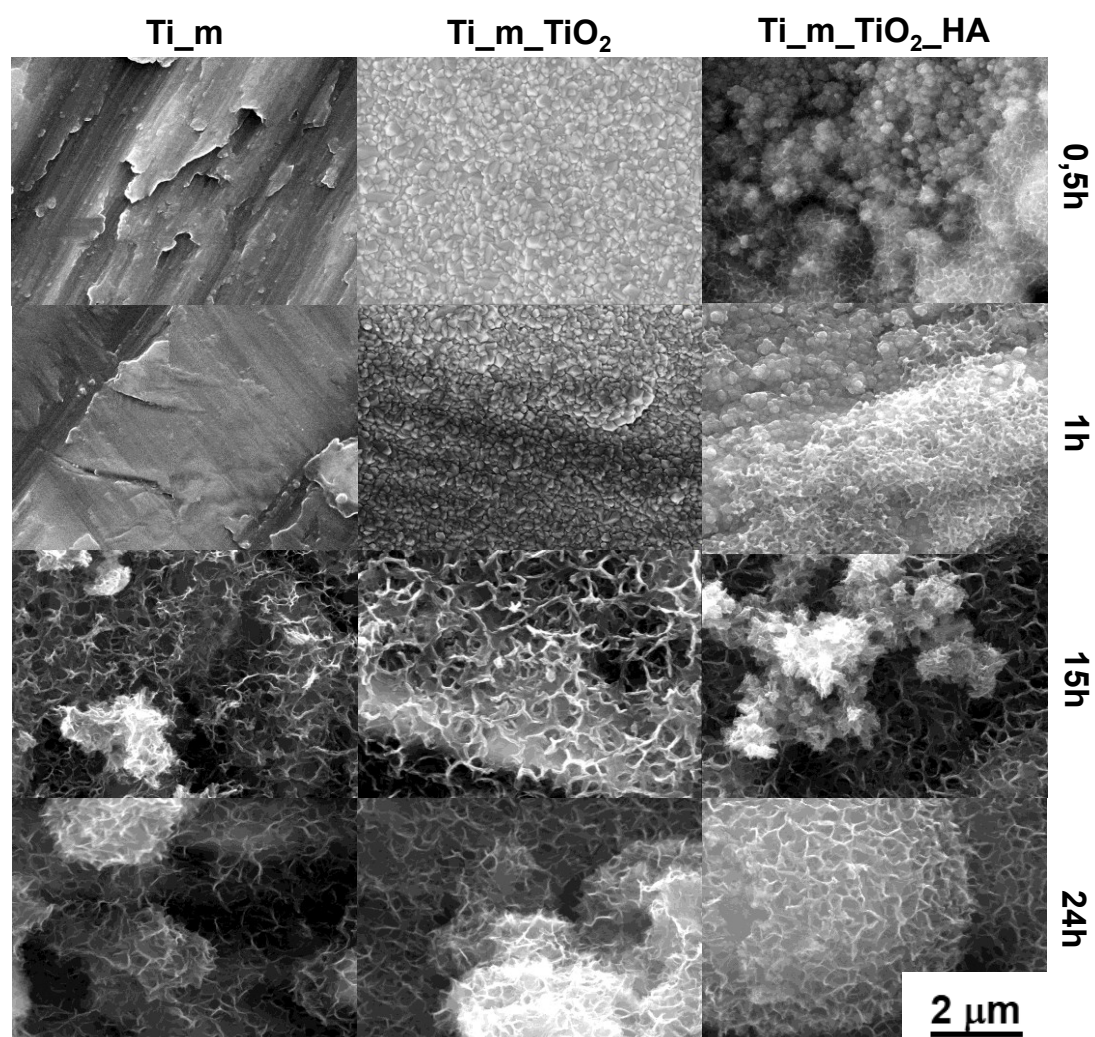


Figure 5.46 SEM surface morphologies (40000x) of the machined Ti substrates (Ti_m), titania coated machined Ti substrates (Ti_m_TiO₂), and titania/HA coated machined Ti substrates (Ti_m_TiO₂_HA), after immersion in DPBS, at 37°C, for 0.5, 1, 15 and 24 h.

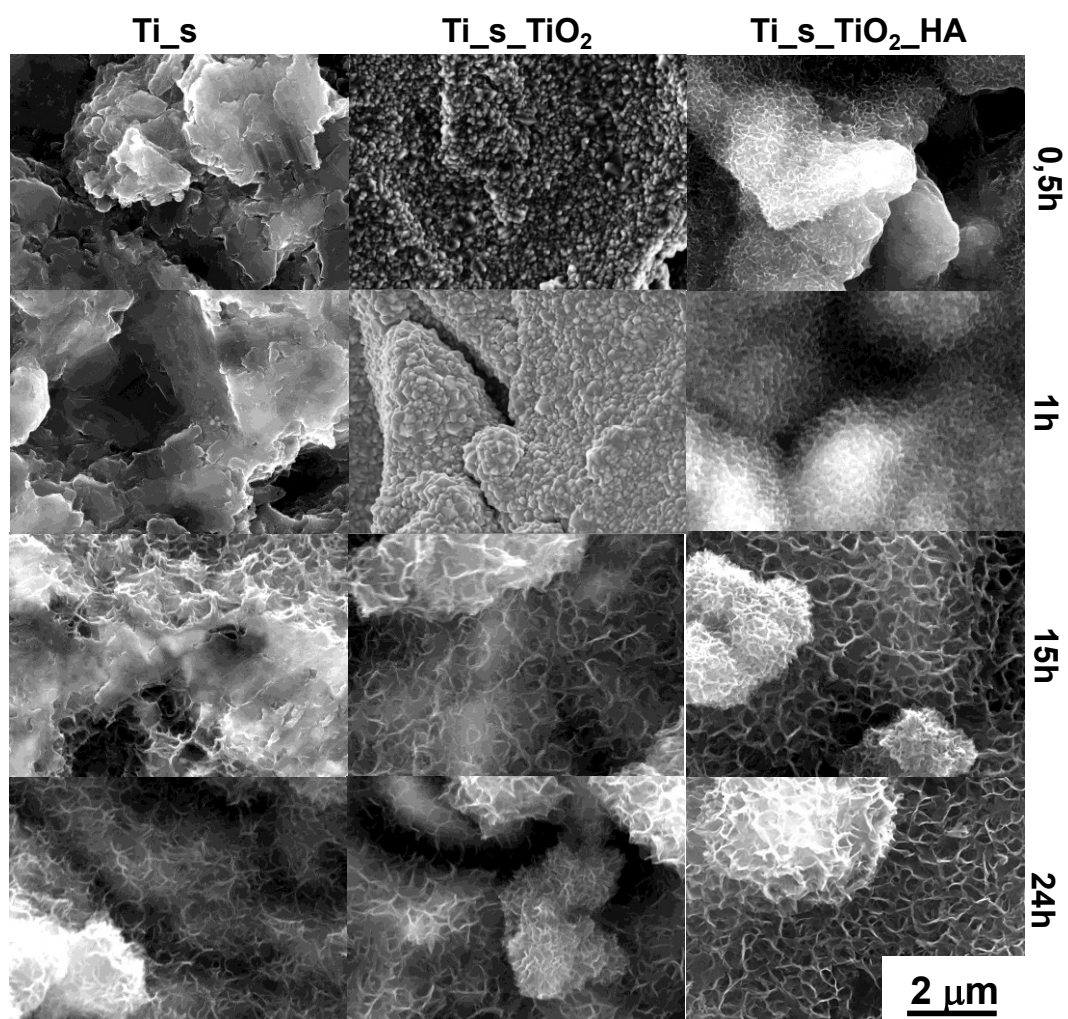


Figure 5.47 SEM surface morphologies (40000x) of the sandblasted Ti substrates (Ti_s), titania coated sandblasted Ti substrates (Ti_s_TiO₂), and titania/HA coated sandblasted Ti substrates (Ti_s_TiO₂_HA), after immersion in DPBS, at 37°C, for 0.5, 1, 15 and 24 h.

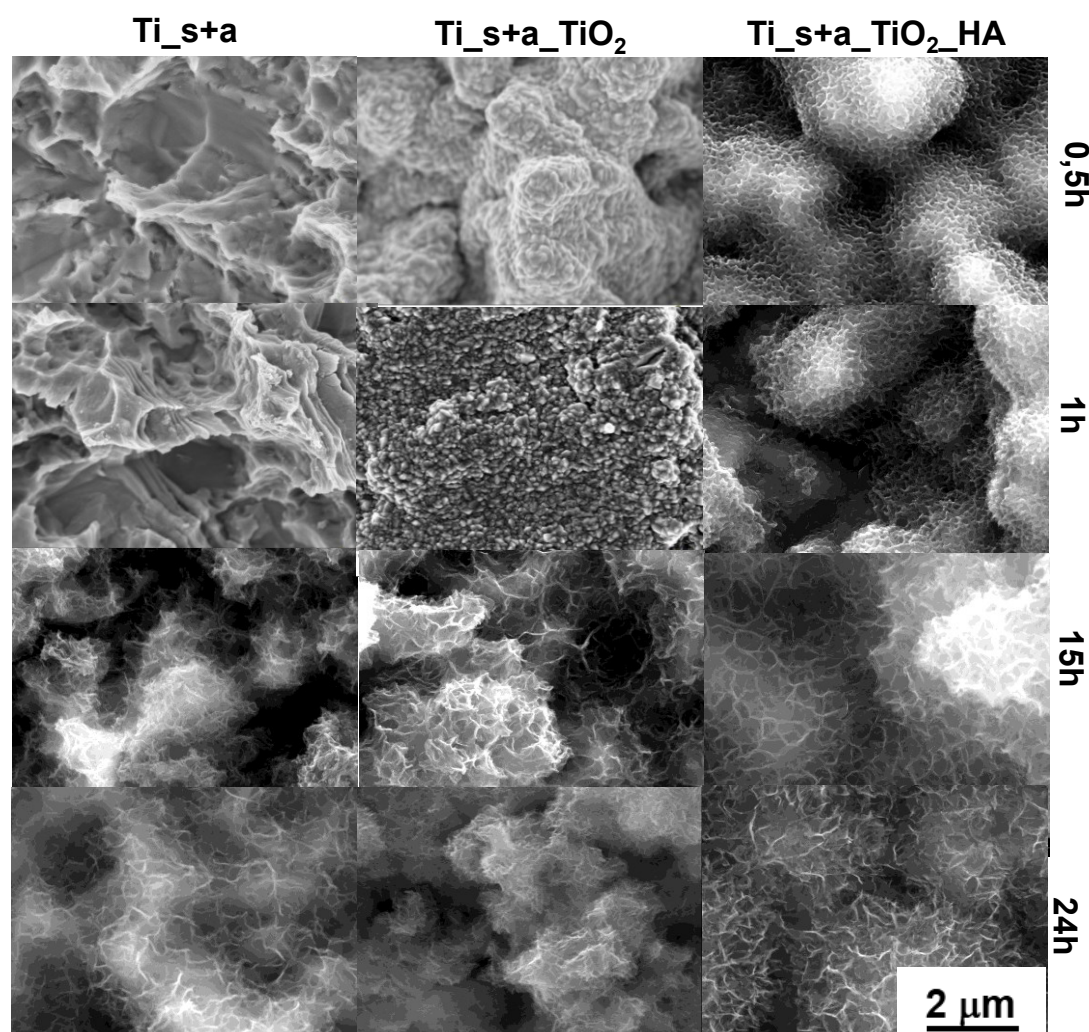


Figure 5.48 SEM surface morphologies (40000x) of the sandblasted/acid etched Ti substrates (Ti_s+a), titania coated sandblasted/acid etched Ti substrates (Ti_s+a_TiO₂), and titania/HA coated sandblasted/acid etched Ti substrates (Ti_s+a_TiO₂_HA), after immersion in DPBS, at 37°C, for 0.5, 1, 15 and 24 h.

Figure 5.49 shows the SEM images, at 10000x, of the different surfaces (uncoated, titania coated, and titania/hydroxyapatite coated; “m”, “s”, and “s+a”, respectively) after incubation in DPBS for 15 hours. Bigger flake-like HA crystals (which are typical for HA formed from DPBS [180]) appeared on the two-step functionalized surfaces, so confirming the great improvement of the bioactivity performances.

As it can be seen from Figure 5.46, 5.47, 5.48, bone-like apatite was deposited on the uncoated and titania coated surfaces (“m”, “s”, and “s+a”, respectively) after 15 hours of incubation. Furthermore, higher hydroxyapatite nucleation rates were detected in the TiO₂ coated samples, confirming the improvement of bioactivity of the titania coated substrates with respect to the uncoated ones. This result can also be observed in Figure 5.50 where the surface morphologies of the pristine sandblasted Ti substrates (Ti_s) and titania coated sandblasted Ti substrates (Ti_s_TiO₂), after immersion in DPBS, at 37°C, for 15 h, are reported. From Figure 5.50, it clearly appears higher hydroxyapatite nucleation on Ti_s_TiO₂ surface with respect to Ti_s one (a vs. b). Specifically, in Figure 5.50c a central area where no HA was nucleated can be seen (i.e. where the typical titanium morphology can be observed), while, at the same magnification, the Ti_s_TiO₂ sample appears homogeneously

HA covered (Figure 5.50d). These results, obtained for sandblasted surfaces (uncoated and coated), were also observed for the machined or sandblasted/acid etched surfaces (not shown for sake of brevity).

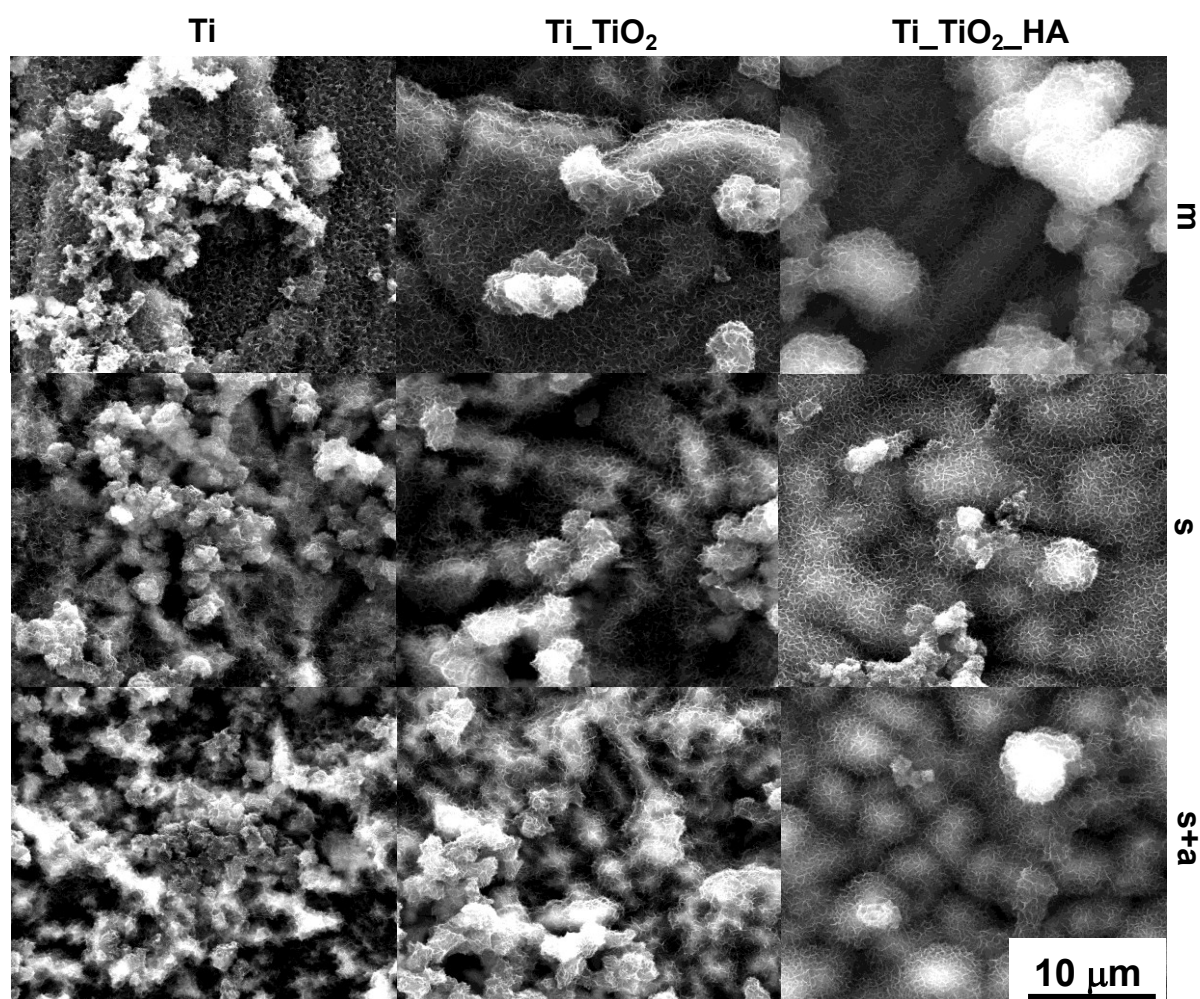


Figure 5.49 SEM surface morphologies (10000x) of the pristine titanium substrates, titania coated Ti substrates, and titania/HA coated Ti substrates (“m”, “s”, and “s+a”, respectively), after immersion in DPBS, at 37°C, for 15 h.

Ca/(Ca+Ti) weight % relationship was obtained from EDX analyses mapping different areas (magnification 500x, at least 3), over the surface of the samples, after immersion in DPBS at 37°C for 0.5, 1, 15 and 24 h (Figure 5.51). These data further confirmed the better bioactivity of the two-step coated samples. Concerning machined surfaces, the statistical analysis (after 15 and 24 h of incubation) did not reveal differences among the samples (i.e. Ti_m, Ti-TiO₂, and Ti_m-TiO₂-HA). This result can be explained considering the higher Ca/(Ca+Ti) wt. % standard deviation on Ti_m-TiO₂-HA surfaces. As previously observed, in these surfaces the HA islands were not homogeneously dispersed, but concentrated in large agglomerates. Consequently, the bone-like precipitation rate was strongly influenced by the point/area of the sample (i.e. there were different apatite nucleation rates in the HA sprayed area and in the not HA coated area). Finally, even if it could appear that two-step functionalized sandblasted surfaces had slightly higher bioactivity, the statistical analyses (performed on the samples soaked for 24 h) did not reveal differences among Ti_m-TiO₂-HA, Ti_s-TiO₂-HA, Ti_s+a-TiO₂-HA surfaces (at 0.05 level of significance).

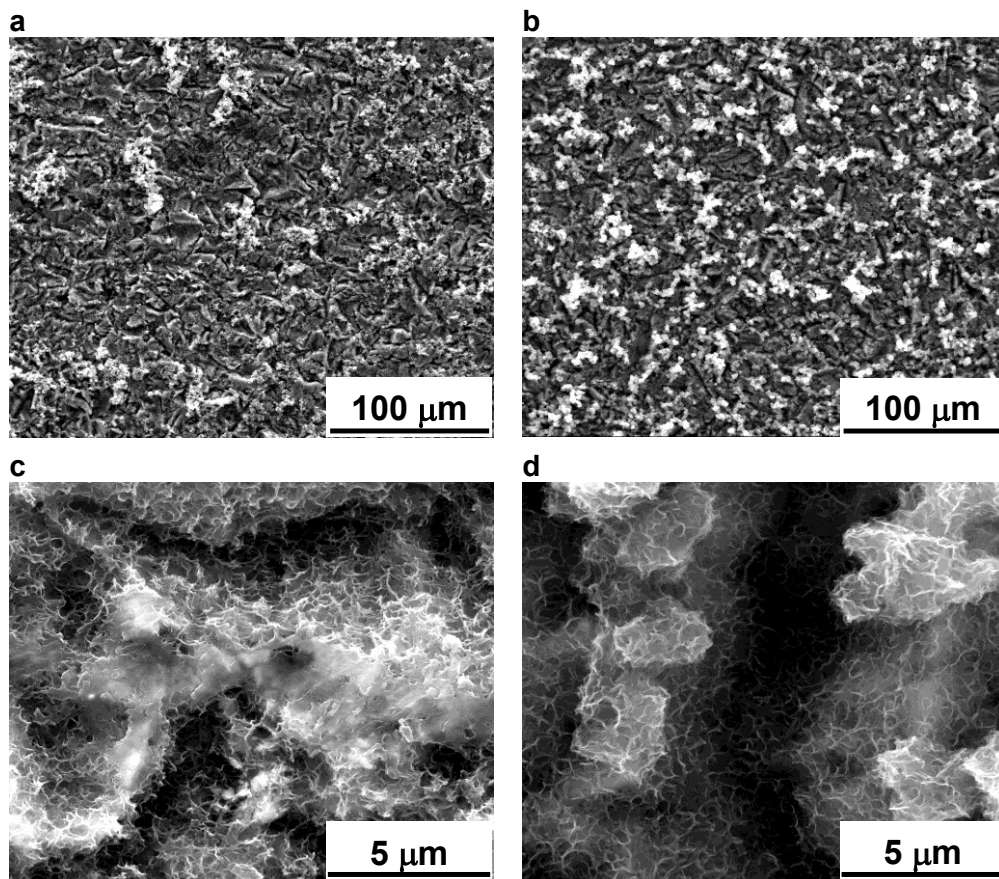


Figure 5.50 SEM surface morphologies of the pristine sandblasted Ti substrates (a, c) and titania coated sandblasted Ti substrates (b, d), after immersion in DPBS, at 37°C, for 15 h.

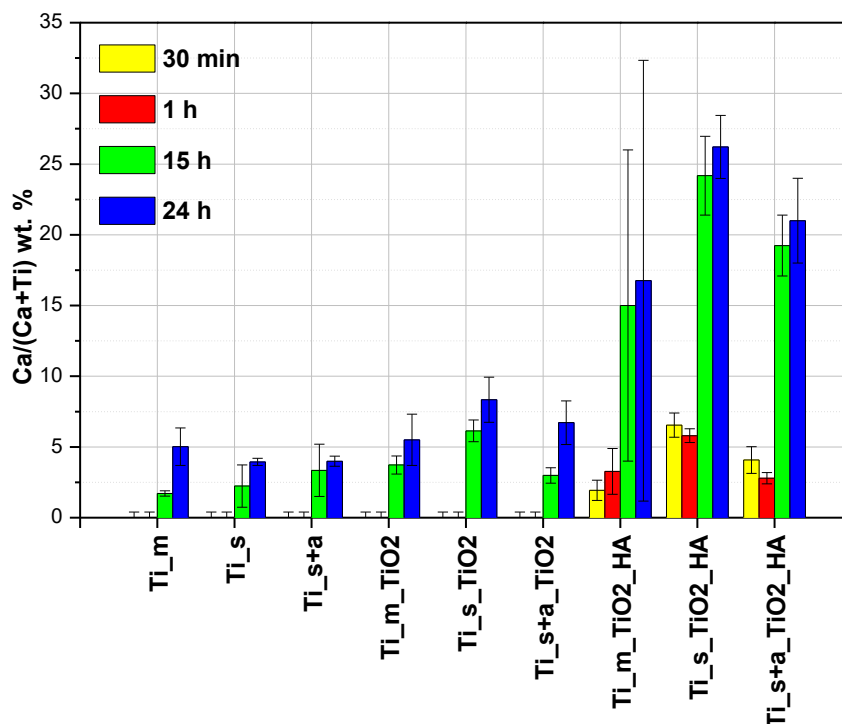


Figure 5.51 Ca/(Ca+Ti) wt. % obtained from EDX analyses, on different areas (magnification 500x), after immersion in DPBS, at 37°C, for 0.5, 1, 15 and 24 h.

Concerning uncoated and titania coated surfaces, on average, higher Ca amount was detected on titania coated samples (Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂) with respect to the uncoated ones (Ti_m, Ti_s, Ti_s+a), additionally confirming the slight bioactivity improvement due to the presence of the titania thin film.

Finally, comparing (two-sample t-test) the Ca/(Ca+Ti) wt. % for TiO₂/HA coated samples stored in DPBS solution for 30 minutes with those obtained in section 5.4 (i.e. Evaluation of the CPC deposited amount), statistical (0.05 level of significance) higher Ca/(Ca+Ti) wt. % values were obtained on the “s” and “s+a” samples incubated in DPBS solution for 30 minutes, so further confirming the bone-like apatite nucleation. Again, because of the high sample inhomogeneity, no statistical difference was evaluated on the machined functionalized surfaces soaked (for 30 minutes) and not soaked in DPBS solution.

The atomic ratio Ca/P was approximately the same for all the samples investigated and varied within the range 1.3-1.5. This value is different from the Ca/P value for stoichiometric HA (1.67). However, bone-like apatite is often largely non-stoichiometric, exhibiting a non-apatitic surface layer, hosting mostly bivalent ions such as HPO₄²⁻ instead of PO₄³⁻ [98]. Therefore, the Ca/P ratio cannot be exploited to unequivocally determine the nature of the calcium phosphate phase (e.g., apatite with a Ca/P ratio as low as 1.30 have been prepared) [98]. According to the studies of Drouet et al. [98], other calcium phosphates phases may also exhibit flake-like morphologies (i.e. monetite, CaHPO₄, brushite, CaHPO₄·2H₂O, and octacalcium phosphate, Ca₈(PO₄)₄(HPO₄)₂·5H₂O) and also present similar elemental composition. Therefore, EDX analysis can be considered as a precious tool regarding compositional aspects (but still not sufficient). It may, for example, rule out the presence of NaCl crystals, while the observation of calcium and phosphorus lines is then in favor of the formation of a calcium phosphate phase. On the other hand, it must be underlined that the observation of distinctive flake-like apatite morphology, as well as the appropriate Ca/P atomic ratio obtained from the EDX analyses, are promising factors for the first characterization statements, but they cannot be exclusively used to confirm the apatitic nature of the precipitated phase, so, necessarily, other additional characterization must be used [98].

XRD is undeniably an important characterization tool for identifying precipitated crystallized phases. In contrast, amorphous compounds (sometimes present beside a crystallized phase) will only lead to large halos, which are unfortunately sometimes ignored [98].

Figure 5.52 shows X-ray diffraction spectra of the sandblasted substrates (uncoated, titania coated and titania/HA coated) after 1 h (a) and 24 h (b) of incubation in DPBS. For the need of synthesis here are only reported the XRD spectra of sandblasted surfaces, but the other typologies of surfaces gave the same results. It can be observed that two-step coated sample stored in DPBS for 1 h (Figure 5.52a, green line) does not show distinct bone-like HA peaks, even if HA agglomerates were clearly detected on the surfaces from SEM images. For this sample, only the weak signal due to synthetic HA is detectable. This phase assignment was done considering the analogy of the spectrum in Figure 5.52a (green line) with that of the pristine material not soaked in DPBS (Figure 5.19), and considering that there is a large microstructural difference between bone-like apatite and synthetic HA. Indeed, synthetic HA is generally isotropic and the observed large band, centered around $2\theta = 32^\circ$, is the result of the overlapping of (121), (112), (300) and (202) reflections. Differently, biological HA has a preferential growth along the (002) direction [16]. These XRD results, after the early stage of incubation (1 hour), are in accordance with earlier findings [183], where the XRD spectra from the early stage of HA growth rarely show distinct HA peaks (either because the coatings are too thin or because the crystallinity increases with aging time)

[183]. However, XRD spectra after 24 h of incubation showed the characteristic peaks of bone-like apatite for all the surfaces and, again, higher HA nucleation rate was detected on the two-step functionalized material.

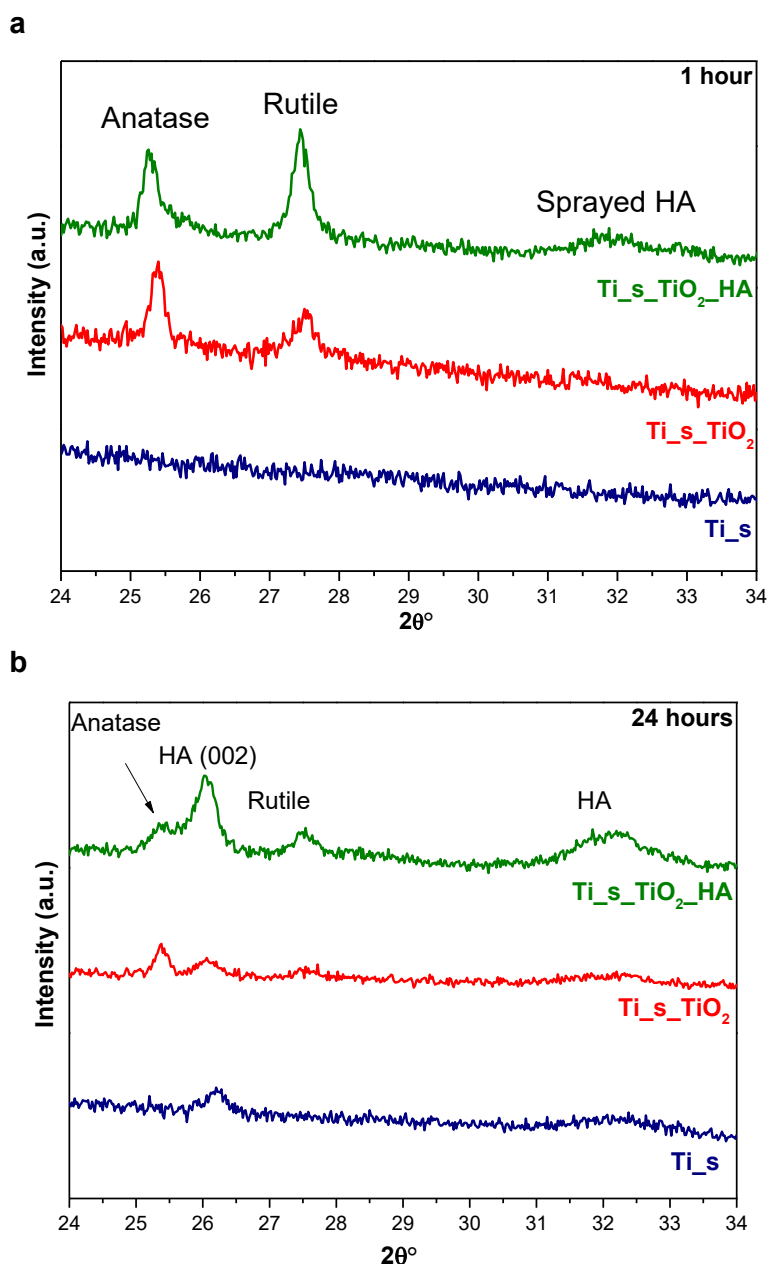


Figure 5.52 XRD analyses of the bone-like apatite layer on the surface of Ti_s , $\text{Ti}_s\text{-TiO}_2$, and $\text{Ti}_s\text{-TiO}_2\text{-HA}$, after immersion in DPBS, for 1 h (a) and 24 h (b).

Vibrational spectroscopy, and in particular FT-IR, represents a suitable tool for bone-apatite identifications in CPC systems [98]. Figure 5.53 shows external reflection FT-IR spectra of the sandblasted substrates (uncoated, titania coated and titania/HA coated) after 0.5 (a), 1 (b), 15 (c) and 24 h (d) of soaking in DPBS. For need of conciseness, here are only reported the FT-IR spectra of sandblasted surfaces, but the other typology of surfaces gave similar results. With the exclusion of the titania/HA coated sample (green lines), the absorptions due to the fundamental modes of the PO_4^{3-} group in the hydroxyapatite structure are detectable only when the DPBS soaking is longer than 1 h. In particular, the strong absorption centered

at $\approx 1032\text{ cm}^{-1}$, exhibiting a shoulder at $\approx 1092\text{ cm}^{-1}$, can be ascribed to the ν_3 modes and the doublet, at ≈ 601 and $\approx 564\text{ cm}^{-1}$, to the ν_4 . The situation is rather different considering the titania/HA coated sample. Indeed, the presence of a pristine HA coating hinders a good estimation of the bone-like HA nucleation when the soaking was lower than 15 h. As reported in Figure 5.53a and 5.53b, the spectra of Ti_s_TiO₂_HA substrate (green lines) are very close to the one collected on the un-soaked substrate (magenta dotted line in Figure 5.53a). The PO₄³⁻ spectral features become more defined in the samples soaked for 15 h and 24 h. The onset of the ν_1 , at $\approx 961\text{ cm}^{-1}$, and a better resolution of the ν_4 doublet are clearly evident in these samples. Literature FT-IR spectra of nanocrystalline (biomimetic) apatite [98] show modified features when compared to that of well-crystallized and stoichiometric HA. These modifications, evident also in the collected FT-IR spectra, mainly involve a band broadening due to the rather low degrees of crystallinity. For instance, these differences are particularly evident in the Ti_s and Ti_s_TiO₂ substrates: the low crystallinity of so obtained HA nanoparticles induces a band broadening, especially in ν_4 , and partial suppression of the ν_3 splitting (Figure 5.53c and 5.53d).

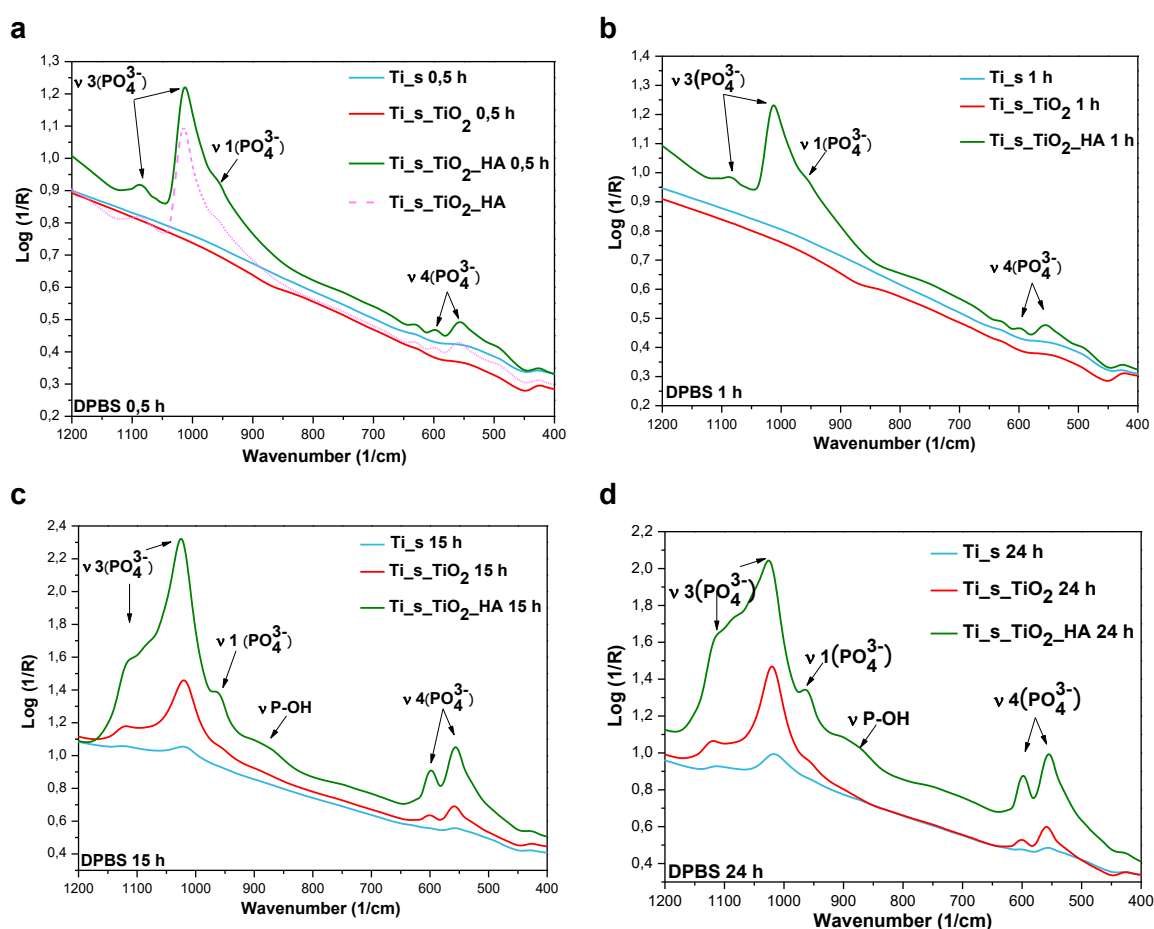


Figure 5.53 External reflection FT-IR spectra of uncoated, titania coated and HA/titania coated sandblasted Ti substrates, after immersion in DPBS, at 37°C, for 0.5 (a), 1 (b), 15 (c) and 24 (d) h.

Furthermore, nanocrystalline apatite is generally non-stoichiometric, with Ca and OH vacancies, and the limited amount of OH⁻ ions in apatitic channels leads to added difficulty in detecting such ions. In the investigated samples, the $\nu(\text{OH})$, at $\approx 3572\text{ cm}^{-1}$ (not shown in the figure), was really weak or not well resolved. Finally, nanocrystalline apatite also exhibits

supplementary bands that are due to the presence of non-apatitic ionic environments within a surface layer on the nanocrystals (for example bands due to non-apatitic/labile HPO_4^{2-} and PO_4^{3-} as well as CO_3^{2-} ions are, in particular, identified) [98]. For instance the weak band at $\approx 875\text{ cm}^{-1}$ (Figure 5.53c and 5.53d) is ascribable to the $\nu(\text{P-OH})$ in the HPO_4 bearing apatite (non-apatitic surface). Finally, the comparison of the intensities of the peaks from FT-IR spectra of different samples clearly showed a higher HA nucleation rate in the two-step coated samples.

The above described XRD and FT-IR results obtained for sandblasted surfaces (uncoated and coated) soaked in DPBS, were also observed for the machined or sandblasted/acid etched surfaces (not shown for sake of brevity).

To conclude, complementary techniques (i.e. XRD and FT-IR) further confirm the precipitation of bone-like apatite on the investigated samples, supporting the morphological and chemical characterization.

5.10 *In-vitro* cytotoxicity

The characterization of biomaterials for medical uses (i.e. medical devices) and intended for contact in the short, medium and long term with the body (i.e. biological fluids, tissues, and organs) cannot be completed without an assessment of their "biocompatibility". There is the need to perform biocompatibility tests that evaluate the potential negative effects of the medical device during the actual use and demonstrate its biocompatibility or not (i.e. they have to determine whether the medical device performs as intended without causing any adverse or harmful effects for patients or users). This assessment should be conducted in order to examine the performance of the biomaterial in conditions similar to those of the biological environment.

ISO 10993-2009 (*Biological evaluation of medical devices*) is the fundamental standard protocol used for medical device biological testing. As described in one of its 20 parts, ISO 10993-1:2009 (*Part 1: Evaluation and testing within a risk management process*), the extent of the test protocols to be followed depends on numerous factors, such as the type of the medical device (i.e. surface device, external communicating device or implant device), the type of patient/medical device contact (for example with skin, circulating blood, tissue/bone, etc.) and the duration of the patient/medical device contact (limited if lower than 24 h, prolonged from 24 h to 30 days, and permanent if longer than 30 days).

The verification of biocompatibility consists in the application of *in-vitro* tests (with cells and tissues), *in-vivo* tests (on animal models), and clinical trials. Before the performance of a biomaterial is tested *in-vivo*, a screening regarding the response of a biological system to the material is commonly conducted *in-vitro*. *In-vitro* tests provide first insights in biological events occurring in the presence of a biomaterial, without the need to take ethical considerations into account that come along with animal experiments. The main advantages of *in-vitro* tests are related to the low costs, the small size of the required equipment and the relative speed of execution, allowing the quickly reproducible evaluation of many materials and devices in a controlled environment.

ISO 10993-5:2009 (*Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity*) describes the test methods to evaluate the *in-vitro* cytotoxicity of medical devices. The term "cytotoxicity" means to cause toxic effects (cell death, alterations in cellular membrane permeability, enzymatic inhibition, etc.) at the cellular level [184], therefore ISO 10993-5:2009 assesses the biological effects of leachables (releasable

substances), which can be drawn out of the devices, on living mammalian cells. Cytotoxicity tests are rapid, standardized, sensitive, relatively inexpensive and useful for screening materials that may be used in medical devices. The ISO 10993-1 standard considers cytotoxicity tests so important that they are prescribed for every type of medical device, along with sensitization and irritation testing. This is a good first step toward ensuring the biocompatibility of a medical device. However, it certainly does not evidence that a material can be considered biocompatible, but it is simply the first step.

According to ISO 10993-5:2009, the test material may be placed directly in contact with the cells, ensuring that it occupies one-tenth of the total surface (direct contact) or extracted in an appropriate solution that is subsequently placed on the cells. "Extract" means a liquid vehicle which, after having a contact for a certain period and at a certain temperature with the medical device, extracts the active molecules. The choice of method varies with the characteristics of the test material, the rationale for doing the test, and the application of the data for evaluating biocompatibility [184].

As said by ISO 10993-5:2009, cytotoxicity effects can be evaluated via qualitative methods (measure microscopically of morphological cell changes such as general morphology, membrane integrity, cell lysis, etc.) and quantitative methods (e.g. Neutral Red Uptake (NRU) cytotoxicity test, MTT cytotoxicity test, XTT cytotoxicity test and colony formation cytotoxicity test).

"Cell lines" are usually used as experimental models in *in-vitro* investigations because, unlike from primary cells (i.e. cells just isolated from the organs), they guarantee greater reproducibility, efficiency, and availability. The human osteoblast-like (bone-forming cells) cell line MG-63 was used in this work as cellular model. This cell line, originally isolated from human osteosarcoma (the most common primary malignant bone tumor), has similar physiology and adhesive properties to human osteoblasts [185], it is well characterized and largely used in *in-vitro* biocompatibility tests [185,186].

In this work, before the *in-vitro* tests with cells, all substrates were sterilized by gamma radiation, according to the procedure that the industrial partner Mech & Human Srl applied to the dental implants.

The evaluation of the cytotoxicity on uncoated, titania and titania/hydroxyapatite coated titanium disks was performed at the University of Ferrara, at the "Dipartimento di Scienze Chimiche e Farmaceutiche", by Prof. Antonella Caputo and Dr. Mariaconcetta Sicurella.

5.10.1 Analysis of cell cytotoxicity using the neutral red uptake (NRU) assay

Neutral red uptake assay provides a quantitative estimation of the number of living cells in the culture. The test is based on the principle of quantifying the live cells through the incorporation of a dye (the neutral red, 3-amino-7-dimethylamino-2-methylphenazine hydrochloride) which colors the lysosomes of living cells, while it is unable to penetrate the dead cells. Subsequently, the cells are fixed and permeabilized with the desorption solution. The amount of dye released is proportional to the number of live cells and can be measured quantifying the optical density (OD) at 540 nm using a spectrophotometer.

Test execution conditions

MG-63 osteoblast-like cells (Sigma Aldrich) were used. MG-63 were cultivated in Dulbecco's Modified Eagle's (EuroClone), complemented with L-glutamine, penicillin, streptomycin and 10% of fetal bovine serum (Sigma Aldrich). All cells were grown in standard cell culture

conditions, at 37°C, in a 5% $\pm$ 1% CO₂ humidified air environment. The cells were cultivated up to 80% of confluence (calculated via microscope as cellular density), then were treated with trypsin and resuspended in the culture medium. Before using, the normal growth and multiplication of the cells were ascertained for two successive changes of culture medium, while the absence of mycoplasma was established using the PCR (polymerase chain reaction) method (Euroclone).

Cytotoxicity tests were carried out by direct contact, i.e. uncoated and coated Ti disks (diameter 5 mm) were placed directly in contact with the cells, ensuring that each disk occupies one-tenth cell layer surface, according to ISO 10993-5:2009. The experiments were performed in triplicate and each test was repeated on three independent days.

For viability tests, cells (7.5×10^4 cells/well) were seeded in 1 mL of culture medium in 24-multiwell plates and incubated overnight (ON) at 37°C and 5% CO₂. The following day, the different Ti disks (i.e. uncoated Ti, TiO₂ coated Ti and TiO₂/HA coated Ti) were overlaid on the cell monolayers and incubated for 24 h $\pm$ 2 h (as indicated in the ISO 10993-5:2009), 72 h $\pm$ 2 h and 5 days $\pm$ 2 h at 37 $\pm$ 1°C and 5 % CO₂. The ideal number of cells to be used per disk was determined in preliminary calibration experiments. All the tests were conducted under sterile conditions and all the operations were carried out under a laminar flow hood equipped with a HEPA filter (WBH 48MP).

Each test always included a negative control (material which does not produce a cytotoxic response, i.e. cells incubated with medium alone without any disk, NC) and positive control (material which provides a reproducible cytotoxic response, i.e. cells grown with dimethyl sulfoxide, at 30 %, capable of inducing cell death, PC). As an example, Figure 5.54 shows the diagram of the two 24-multiwell plates used during an experiment.

	1	2	3	4	5	6
A	NC	NC	NC	PC	PC	PC
B	Ti_m	Ti_m	Ti_m	Ti_s	Ti_s	Ti_s
C	Ti_m_TiO ₂	Ti_m_TiO ₂	Ti_m_TiO ₂	Ti_s_TiO ₂	Ti_s_TiO ₂	Ti_s_TiO ₂
D	Ti_m_TiO ₂ _HA	Ti_m_TiO ₂ _HA	Ti_m_TiO ₂ _HA	Ti_s_TiO ₂ _HA	Ti_s_TiO ₂ _HA	Ti_s_TiO ₂ _HA

	1	2	3	4	5	6
A	NC	NC	NC	PC	PC	PC
B	Ti_s+a	Ti_s+a	Ti_s+a			
C	Ti_s+a_TiO ₂	Ti_s+a_TiO ₂	Ti_s+a_TiO ₂			
D	Ti_s+a_TiO ₂ _HA	Ti_s+a_TiO ₂ _HA	Ti_s+a_TiO ₂ _HA			

Figure 5.54 Diagram of the 24-multiwell plates used during an experiment.

After 1, 3 and 5 days of growth, MG-63 cells appearance and morphology were evaluated by means of optical microscope (Nikon Eclips TS100). The cell monolayers incubated with the substrates were comparable to untreated cells.

The cytotoxic effect (cell viability) was quantitatively evaluated by measuring the neutral red uptake (Sigma Aldrich). Briefly, neutral red dye was dissolved in H₂O at 25 µg/mL and added to the cells for 3 hours at 37°C. After incubation, the cells were washed with PBS and lysed with 1 mL of desorption buffer (49 mL H₂O, 50 mL 95% Ethanol, 1 mL glacial acidic acid,

Sigma Aldrich). 150 µL of each sample was transferred into a 96-well plate and the optical density of the released dye was read at 540 nm (Sunrise-Tecan spectrophotometer).

For each sample the % of cell viability was calculated as (equation 5.1):

$$\% \text{ of cell viability} = \frac{\text{Sample OD}(540 \text{ nm})}{\text{NC OD}(540 \text{ nm})} \times 100 \quad (5.1)$$

where “Sample OD (540 nm)” is the measured optical density at 540 nm of the sample and “NC OD (540 nm)” is the measured optical density at 540 nm of the negative control. Thus, untreated cells were considered as 100% of cell viability. According to ISO 10993-5:2009, the lower the “% of cell viability” value, the higher the cytotoxic potential of the test item. If the viability is reduced to < 70 % of the NC reference, the sample has a cytotoxic potential (in another way, if cell viability is ≥ 70 % of the control group, the material shall be considered non-cytotoxic).

Comparisons were made by one-way ANOVA, the Dunnett method was used to compare each mean with the control group (negative control). Statistical significance was considered at a probability $p < 0.05$.

Figure 5.55, Figure 5.56 and Figure 5.57 show the % cell viability compared with negative controls after 24 h, 3 days and 5 days of incubation, respectively. A positive control is also reported. The red dashed lines show the 70% viability limit.

Starting from Figure 5.55, the two-step functionalized surfaces (Ti_m_Ti_TiO₂_HA, Ti_s_Ti_TiO₂_HA, and Ti_s+a_Ti_TiO₂_HA) showed a marginal increase in cytotoxicity (less cell viability) than the negative control. Indeed these samples showed significant differences from the control group NC (Dunett method, 0.05 level of significance). Differently, the other tested samples revealed cell viability values comparable to those found for negative controls (cells cultured in untreated medium). However, none of the investigated samples were detected to reduce cell viability after 24 h under the 70% NC threshold.

We have tried to evaluate if prolonged incubation time allowed the extraction of the highest level of toxic substances. Therefore, incubation times of 3 days and 5 days were also tested. Considering 3 days of incubation (Figure 5.56), Ti_s, Ti_m_TiO₂, Ti_s_TiO₂, Ti_m_Ti_TiO₂_HA, and Ti_s_Ti_TiO₂_HA surfaces exhibited significant difference than the control group NC (Dunett method, 0.05 level of significance), while the other samples revealed cell viability values comparable to those of the negative controls. As for 24 h of incubation, no one of the investigated samples was detected to reduce cell viability after 3 days under the 70% NC threshold.

Finally, concerning 5 days of incubation (Figure 5.57), Ti_m_TiO₂, Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, and Ti_s+a_Ti_TiO₂_HA displayed significant difference than the control group NC (Dunett method, 0.05 level of significance), while the other samples revealed cell viability values equivalent to those of the negative controls. As for the samples incubated for 24 h and 3 days, none of the investigated samples was detected to reduce cell viability after 5 days under the 70% NC threshold, thus suggesting that none of the samples can be considered cytotoxic (0.05 level of significance).

In conclusion, even if some samples (and especially the hydroxyapatite coated surfaces) exhibited slight worse cell viability with respect to the negative controls, all these dissimilarities are lower or almost equal than the toxicity threshold. Therefore, no toxic effect on the exposed cell population was observed for the titanium, titania and titania/hydroxyapatite coated samples at any time, thus suggesting that none of the here used substrates (Ti_m, Ti_s, and Ti_s+a), as well as none of the functionalized surfaces

(Ti_m_TiO₂, Ti_s_TiO₂, Ti_s+a_TiO₂, Ti_m_TiO₂_HA, Ti_s_TiO₂_HA, and Ti_s+a_TiO₂_HA), are cytotoxic (or, at least, not sufficiently to cause acute toxic effects) in the investigated periods.

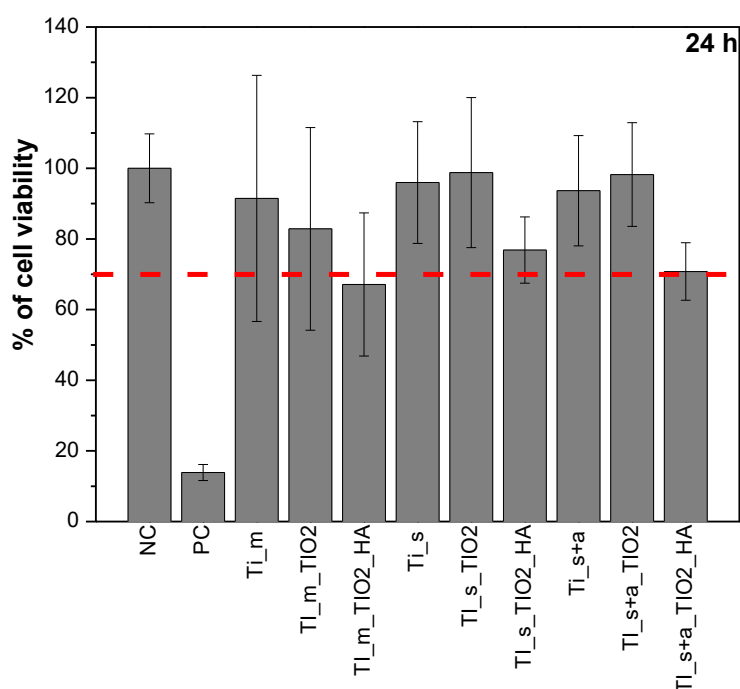


Figure 5.55 Effect of the different substrates on the MG-63 osteoblast-like cell viability, after incubation for 24 h (direct contact), in comparison with negative (NC) and positive controls (PC). Untreated cells were considered as 100% of cell viability. The red dashed line shows the 70% viability limit.

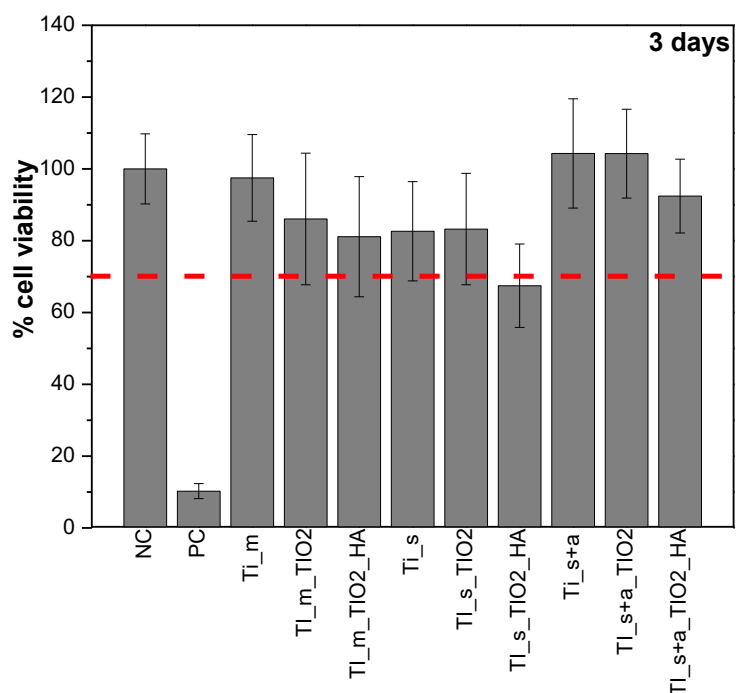


Figure 5.56 Effect of the different substrates on the MG-63 osteoblast-like cell viability, after incubation for 3 days (direct contact), in comparison with negative (NC) and positive controls (PC). Untreated cells were considered as 100% of cell viability. The red dashed line shows the 70% viability limit.

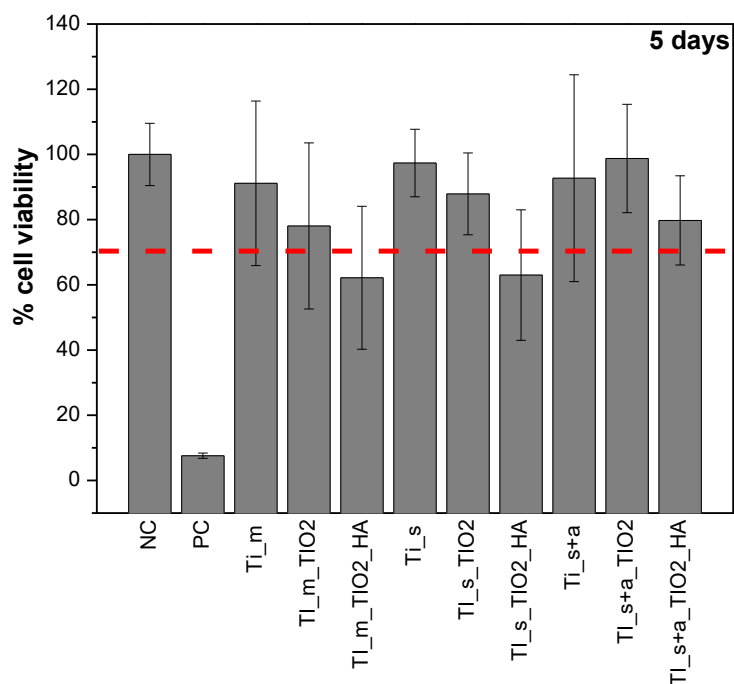


Figure 5.57 Effect of the different substrates on the MG-63 osteoblast-like cell viability, after incubation for 5 days (direct contact), in comparison with negative (NC) and positive controls (PC). Untreated cells were considered as 100% of cell viability. The red dashed line shows the 70% viability limit

Chapter 6

CONCLUSIONS

Teeth are important tools for the processing of food, but they also profoundly influence speech, comfort, facial contour, health, and esthetics. The goal of modern dentistry is to restore edentulous patients to normal functions; dental implants are biocompatible anchors surgically positioned in the jaw bone to support artificial crowns.

The advancement of technologies linked to the improvement of the characteristics of dental implants is a field of primary importance; the engineering of efficient dental implants, with enhanced osseointegrative performances and increased short- and long-term prospects of success, could absolutely lead to an improvement of the quality of life.

Titanium is widely used as starting materials for dental implant applications. However, despite its excellent biocompatibility, titanium has an intrinsic limit due to reduced bioactivity, a property that is fundamental to trigger a specific biological response. Modifying dental implant surface morphology and/or chemistry can be a key issue for improving osseointegration; therefore, the titanium surface is often functionalized.

The goal of this work was the set-up of a new typology of titanium surfaces for enhancing the osseointegration aptitude of Ti dental implants.

Metal-organic chemical vapor deposition technique was employed to deposit a dense, compact, conformal and crystalline TiO_2 inter-layer on Ti substrates, while a discontinuous but homogeneously spread hydroxyapatite top-layer was deposited on the titania inter-layer by means of spray pyrolysis. A final thermal treatment at 600°C was carried out in order to consolidate the composite material and achieve adequate crystallinity and bioactivity. The proposed TiO_2 -HA bi-layered coating should both increase the Ti surface bioactivity and ensure the short- and long-term success of the implant. The further goal is obtained thanks to the presence of a homogeneously spread but discontinuous HA layer that allows to both the ceramic materials to be simultaneously exposed to body fluids and then contribute to the healing process: a bioactive, biocompatible but not resorbable titania inter-layer is available for the long-term integration, whereas a more bioactive but resorbable HA top-layer permits the short-term anchoring.

The influence of each treatment step (i.e. first step: titania deposition; second step: hydroxyapatite deposition and thermal annealing) on the properties and functional performances of the coated materials was investigated. Furthermore, considering that besides the chemical composition, the morphology and the topography of the Ti surfaces are significant parameters that affect the rate and quality of the osseointegration process, three types of Ti substrates with different morphologies and topographies (i.e. Ti machined – Ti_m , sandblasted – Ti_s , and sandblasted/acid etched – Ti_{s+a} , all of commercial-grade IV) were considered.

From SEM and profilometer analyses, it was shown that MOCVD is a reliable technique for coating Ti substrates, with optimal conformal coverage, and the TiO_2 films perfectly matched the profile of the substrate below. After the second step of the functionalization, HA islands were easily spread onto the titania inter-layers, with a spatial arrangement and amount depending on the morphology of the Ti substrates. Specifically, the substrate anisotropy markedly influenced the spray pyrolysis process: in machined surfaces the HA islands were concentrated in wide agglomerates and preferentially located on the rougher zones, while the bioactive component was found to be finer dispersed on the (isotropic) “s” and “s+a” titanium superficies. Furthermore, also the amount of HA solution blocked on the substrate surfaces during the spray pyrolysis process depended on the substrate morphology and topography: rougher surfaces (i.e. “s” and “s+a”) maintained higher amount of the HA solution during the spray process and, on the contrary, sprayed drops bounced easily off from smooth (machined) surfaces.

X-ray diffraction analyses revealed that, after the MOCVD process, the TiO_2 layers grew as anatase, while only in the sandblasted substrates the rutile phase was also observed. After the second step of the functionalization, the as-grown HA coatings were composed of poorly crystallized hydroxyapatite and monetite. This last phase disappeared after thermal treatment in air, while only hydroxyapatite, titanium dioxide, and titanium phases were detected. Furthermore, increasing the thermal treatment temperature (from 400°C to 700°C), the HA structure evolved from a low crystallized state to a highly crystallized one. Well-crystallized hydroxyapatite phase was detected from 600°C .

Wettability studies suggested improved hydrophilic behavior (and even superhydrophilicity in the case of pure titania coatings) for the freshly coated surfaces compared to bare Ti substrates, thus allowing a better degree of contact with the physiological environment. However, coated samples stored in air decreased their wettability in an age-dependent manner due to hydrocarbon contamination. To maintain the high-energy surfaces, two industrial-scalable strategies were positively set-up: i) storing the products underwater or ii) the restoration of the hydrophilicity by UV treatment. The sample storing in water appeared simple, cost-effective and allowed the preservation of the products with high hydrophilic behavior for several months, but it did not completely guarantee the superhydrophilic characteristic, especially over long periods of conservation, comparable with sterility shelf life. Furthermore, this approach could not be applied with the titania/hydroxyapatite coated Ti substrates because of the HA dissolution in aqueous media. Radiating the contaminated samples by means of an UV treatment (2 hours, irradiance: 58 W/m^2 , λ : 254 nm) allowed to completely restore the surface wettability of the freshly prepared samples. Accordingly, UV surface re-activation, although it required an UV light source, appeared to be of high relevance because it can be seen as an “on-demand” restoration technique, i.e. to be used just before the use of the product (before the implantation).

Ion (Ti and Al) release studies in lactic acid (37°C ; for 28 days of immersion) showed that the functionalization of titanium matrix can be a good strategy to reduce ion release and consequently achieve better biocompatibility. Nevertheless, ion release, after the second step of functionalization, increased with respect to the analogous pure titania coated samples, thus testifying a partial modification of the titania inter-layers induced by the annealing procedure.

Tribocorrosion experiments in artificial saliva at 37°C indicated that MOCVD TiO_2 films were able to effectively protect the Ti surfaces from direct contact with artificial saliva; furthermore, better COF values were also detected for the titania coated surfaces in comparison with the uncoated ones.

Potentiodynamic polarization experiments in artificial saliva at 37°C showed that coated materials (both titania and titania/hydroxyapatite) had improved electrochemical behavior when compared to bare titanium. Furthermore, according to ion release experiments, potentiodynamic experiments gave again evidence of a partial modification of the titania inter-layer induced by the annealing procedure. Yet, even if the thermal treatment involved the observed drawbacks, it is unavoidable to crystallize and, in the meantime, consolidate the HA sprayed phase. Nevertheless, it is important to stress that the electrochemical performances of the TiO_2 /HA two-step functionalized materials were always enhanced with respect to those of bare titanium substrates.

Comparing the nanohardness (H) values of the bare Ti with those of the coated ones, higher H values were observed for the coated samples: this means that the titanium superficial mechanical properties were improved with the deposition of TiO_2 and TiO_2 /HA coatings. Concerning the elastic modulus, no differences in the E values of uncoated and coated Ti

substrates were observed, providing good conditions for the mechanical deformation of the bi-layered coated materials.

In agreement with industrial practice, HA-coating adhesion was qualitatively assessed by tape test experiments, according to the ASTM D3359-09. Different results were obtained for the diverse morphologies of substrate surfaces: machined surfaces showed reduced HA retention (probably due to the presence of big HA agglomerates and/or a less coating-to-substrate mechanical interlocking contribution), while HA coatings better adhered on sandblasted and sandblasted/acid etched substrates.

Acellular *in-vitro* bioactivity of all species was tested evaluating the ability of the materials to form a bone-like apatite layer after immersion in Dulbecco's Phosphate Buffer Saline solution (DPBS) at 37°C. These investigations suggested enhanced *in-vitro* bioactivity for the two-step coated surfaces when compared to the uncoated or pure titania coated ones. Furthermore, a higher bone-like apatite nucleation rate was detected in TiO₂ coated samples with respect to bare Ti, then confirming the improvement of bioactivity of the pure titania coated surfaces with regard to the uncoated ones.

According to ISO 10993-5:2009, cytotoxicity effects of uncoated, titania and titania/hydroxyapatite coated Ti surfaces were evaluated quantitatively (Neutral Red Uptake) on human osteoblast-like cell line MG-63, by direct contact. Cell viability, investigated after 24 h, 3 days and 5 days of incubation and compared with negative and positive controls, evidenced no toxic effect on the exposed cell population for the titanium, titania and titania/hydroxyapatite samples (at any time), thus suggesting that none of the used substrates, as well as none of the functionalized surfaces, released relevant cytotoxic compounds in the investigated periods.

The two-step functionalization on the different Ti substrates ("m", "s", "s+a") did not evidence a significant trend in functional properties. However, the overall obtained results showed that Ti_{s+a}_TiO₂ and Ti_{s+a}_TiO₂_HA surfaces had lower Ti release in lactic acid, while Ti_{s+a}_TiO₂ samples had reduced loss of hydrophilicity. Furthermore, adhesion tests revealed that Ti_{s+a}_TiO₂_HA samples exhibited adequate HA adhesion (differently from machined ones). Therefore, "s+a" surfaces should be chosen, also thanks to a better and homogeneous HA distribution, the clean surface (no presence of residues, as for the sandblasted surfaces) and the high surface area able to favor a good mechanical interlocking with bone.

In conclusion, the improvement of the functional properties of the two-step coated materials together with the simplicity, eco-compatibility, and low cost of the processes, make this two-step synergic procedure very promising for dental implant applications.

Chapter 7
FUTURE
PERSPECTIVES

The improvement of the bio-functional and mechanical properties of the traditionally used titanium dental implants is a particularly important goal in the medical field. Therefore, two different surface modification techniques (MOCVD and spray pyrolysis) were tested in this thesis with regard to their applicability to chemically functionalized titanium dental implant surfaces for improving the osseointegrative properties.

Based on the results found in this thesis, there are many possible approaches for future work, delineated below.

a) Implementation of two-step composite materials with antibacterial agents.

One of the main problems arising from dental implants is related to bacterial infections that can occur. Bacterial adhesion on dental implants may cause peri-implant diseases (i.e. mucositis and peri-implantitis) [187], which are triggered by the presence of a biofilm at the implant surface. Mucositis is an inflammation of the soft tissue surrounding dental implants (gingival), it is shown by changes in mucosal color and contour, and bleeding upon gentle probing. This condition is not accompanied by bone loss around the implants, thus peri-implant mucositis can be successfully treated and the process is reversible [187]. However, mucositis is often considered to be an indication of peri-implantitis [188]. Indeed, if left untreated, mucositis progresses vertically, reaching the bone tissue surrounding the dental implant (alveolar bone) and causing bone loss around the osseointegrated implant, with consequent decrease of the implant stability and, eventually, loss of the implant.

Dental implant failures might be divided into early and late types [187]. While early failure is associated with implant failure during the osseointegration process, late failure refers to issues occurring only after implant loading. Peri-implant diseases (due to the presence of bacteria surrounding the implants) are considered as the most common cause for late implant failures [187,189].

For these reasons, there is an increasing need to modify the surface of biomaterials by means of the development of new strategies to prevent the biofilm formation on the implant surface and, consequently, evading infections [187,190]. This can be achieved by preparing dental implants containing antimicrobial materials that exhibit anti-adhesive properties (i.e. inhibit the adhesion of bacteria) and/or antibacterial capacities (i.e. cause cell damage to the adhering bacteria) [190].

Concerning titanium dental implants, although titanium is widely used for dental implant applications due to its excellent combination of strength-to-weight ratio, corrosion resistance, and biocompatibility, it does not exhibit any antiseptic qualities; thus, it is crucial to develop modified titanium surfaces with enhanced antimicrobial capability [190].

There are numerous methods proposed by the literature for the functionalization of surfaces with antibacterial properties, such as the chemical modification of the surface with protein and bacteria-repellent coatings; the coating of the surfaces with quaternary ammonium compounds; the incorporation and release of antibiotics from the surface; the use of UV-activable surfaces and the use of noble metals [189–191].

In this context, the inclusion of a suitable antibacterial agent in the here developed two-step functionalized bi-layered materials could result in a further drastic improvement of the overall performance of the final systems. Concerning the Ti-TiO₂-HA materials and considering the need of an easy scalability of the process, the implementation of the final material with suitable antibacterial agents could ideally be carried out during one of the two deposition steps here proposed (i.e. during the MOCVD route and/or the spray pyrolysis process), therefore avoiding to further increase the number of phases necessary to obtain the final surface.

b) Industrial scale-up of the designed materials and processes.

The industrial scalability of the designed materials and processes is surely another possible theme for future work. This should be carried out in constant collaboration with the industrial partner of the project (Mech & Human Srl), allowing the development of an optimized synergic procedure that takes into account both the requirements of the final composite materials as well as the industrial necessities.

Concerning the developed materials, it must be underlined that before the clinical use of the two-step functionalized dental implants, further *in-vitro* and *in-vivo* tests are necessary. In this work, the *in-vitro* cytotoxicity effect has been investigated using the Neutral Red Uptake assay, but further biological tests are necessary to completely evaluate the material biocompatibility (according to ISO 10993). The complete verification of biocompatibility consists indeed in the application of *in-vitro* tests (with cells and tissues), *in-vivo* tests (on animal models) and, finally, clinical trials.

Concerning the industrial scalability of the two-step process, it necessitates of an industrial system that allows different requirements, such as:

- **Productivity.** As suggested by the industrial partner of the project, an initial productivity range could be about 4000 dental implants per week. These may be managed in one or more production batches. As it has been described in Chapter 4, MOCVD and spray pyrolysis processes are high deposition rate procedures, therefore, also considering the limited size of the dental implant (maximum: 20 mm height, 5 mm diameter), restricted size systems but with high productivity efficiency (number of implant/time) could be reasonably projected.
- **Reproducibility.** The industrial system should guarantee high deposition reproducibility. Furthermore, it should be equipped with control systems able to evaluate eventual problems during the processes of functionalization and testify the conformity of the final products.
- **Automation.** Considering that the final material is built up by two consecutive deposition processes and a thermal annealing procedure, the automation of the whole process should be time and cost-effective. Furthermore, a user-friendly interface, that allows the utilization also for non-technical staff, could be helpful.
- **Abatement system for reaction by-products.** The TTIP precursor decomposition path is quite clean. It has been found that TTIP decomposes at $\sim 400^{\circ}\text{C}$ through the formation of acetone, isopropanol, and propene as by-products [79]. These by-products can be easily removed from the apparatus (for example by simply burning and/or by dissolution in water and/or using absorbing carbon cartridge). Differently, concerning the spray pyrolysis phase, the process effectiveness and efficiency could be strongly improved using additional electrical source governing the droplet's trajectory (i.e. ultrasonic and electric spray deposition) [88]. In this way, electrical force drives the droplets to the substrate allowing better coating adherence and compactness as well as a reduced waste of precursor. Concerning the spray pyrolysis by-products, their removal could be carried out via aerosol and NO_x control.

c) Further improvement of the effectiveness of the materials.

The further improvement of the effectiveness of the composite materials should include more characterization and optimization of the discontinuous and homogeneously spread hydroxyapatite top-layer. In detail, the relationship between hydroxyapatite layer crystallinity and the short- and long-term implant performance. In fact, a suitable crystallinity degree is crucial in terms of HA dissolution rate (useful to induce the faster precipitation of bone-like

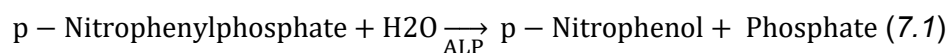
apatite) and implant durability (highly crystallized HA coatings are more stable *in-vivo* than amorphous ones). The right crystallinity degree will both promote faster initial bone fixation and degrade at a controlled rate over the lifetime of the coating. In this work, to optimize the HA dissolution rate, the implant durability and the composite material performances, a thermal annealing of 600°C has been taken into account. However, it has been shown that the thermal annealing in air, at 600°C, for 6 hours involves some drawbacks (increased ion release in lactic acid and reduced corrosion resistance in artificial saliva for the two-step functionalized surfaces with respect to the only titania functionalized ones). Therefore, different post-synthesis thermal treatment temperatures and durations could be considered in order to further investigate the best conditions in relation to the material properties (maximize the crystallinity of HA coating while maintaining adequate bioactivity and corrosion resistance) and the thermal load required for the process.

d) Analysis of cell differentiation via alkaline phosphatase activity.

As described in Chapter 5, MG-63 cells viability on the different substrates has been previously assessed, according to ISO 10993-5:2009. Nevertheless, the only evaluation of cell viability is not sufficient to express a certain indication of the new bone formation capacity of the developed coatings. Implant surface properties, such as morphology, topography, chemistry, superficial energy, etc. can influence cell functionality and activity, at least at an early stage of differentiation [22]. Therefore, further and dedicated analytical methods need to be carried out to evaluate the tendency of the developed functionalization to stimulate the osteointegration of the implant.

Alkaline phosphatase (ALP) is one of the most widely recognized biochemical markers for osteoblast activity, ALP activity is commonly used as an indicator of osteogenesis around the titanium implants [192]. The precise role of ALP in bone mineralization is not completely known yet. Its functions could be related to a calcium-binding action, the generation of free phosphate, or the degradation of mineralization inhibitors [193].

Preliminary studies concerning the ALP activity of MG-63 osteoblast-like cells incubated on the two-step functionalized surfaces here developed have been recently carried out. Specifically, to evaluate ALP activity, MG-63 cells have been seeded onto each sample surface (uncoated - titania coated - titania/hydroxyapatite coated), at the same density. After 24 h, 3 days and 5 days of incubation, ALP activity has been evaluated by colorimetric method. Briefly, the alkaline phosphatase enzymes catalyze the hydrolysis of colorless p-Nitrophenylphosphate (p-NPP) into the chromogenic p-Nitrophenol (in alkaline medium) and inorganic phosphate, as described in equation 7.1. Thus, ALP activity can be measured by the amount of p-Nitrophenol formed at 405 nm using a spectrophotometer.



ALP activity has been normalized to total protein content, determined using a commercial BCA Protein Assay Kit.

Preliminary results have shown that the alkaline phosphatase activity of osteoblasts seeded on uncoated, titania coated and titania/hydroxyapatite coated surfaces (“m”, “s”, and “s+a”, respectively) is (on average) not significantly diverse from that of the negative control NC (i.e. cells incubated with medium alone, without any substrate). This result is basically due to the high error and dispersion associated with the obtained ALP activity values, that make the medium values statistical equal for the different surfaces. However, it should be underlined

that observing the ALP activity tendency, the two-step functionalized surfaces appear to have higher values, especially the rougher ones (Ti_s+a_TiO₂_HA). Further experiments are undoubtedly necessary in order to increase the number of the examined samples, strengthen the statistical results and, therefore, confirm the preliminary outcomes.

List of publications and presentations

Below are listed the publications and the presentations given at conferences since the start of the Ph.D. course, including those deriving from the participation to other research projects.

Publications

A. Galenda, **F. Visentin**, R. Gerbasi, S. Battiston, N. El Habra, “*Effective and low-cost synthesis of Sulphur modified-TiO₂ nanopowder with improved photocatalytic performances in water treatment applications*”, Water Air Soil Pollut. (2017) 228:416.

<https://doi.org/10.1007/s11270-017-3600-5>.

A. Galenda, **F. Visentin**, R. Gerbasi, M. Favaro, A. Bernardi, N. El Habra, “*Evaluation of self-cleaning photocatalytic paints: are they effective under actual indoor lighting systems?*” Applied Catalysis B: Environmental 232 (2018) 194–204.

<https://doi.org/10.1016/j.apcatb.2018.03.052>.

F. Visentin, A. Galenda, M. Fabrizio, S. Battiston, N. Brianese, R. Gerbasi, V. Zin, N. El Habra, “*Assessment of synergistic effects of LP-MOCVD TiO₂ and Ti surface finish for dental implant purposes*”, Appl. Surf. Sci. 490 (2019) 568–579.

<https://doi.org/10.1016/j.apsusc.2019.06.067>.

F. Visentin, N. El Habra, M. Fabrizio, N. Brianese, R. Gerbasi, L. Nodari, V. Zin, A. Galenda, “*TiO₂-HA bi-layer coatings for improving the bioactivity and service-life of Ti dental implants*”, Surf. Coat. Technol. (2019) In Press.

<https://doi.org/10.1016/j.surfcoat.2019.125049>.

Presentations given at conferences

M. Zanatta, N. El Habra, **F. Visentin**, G. Pozza, A. Zambona, N. Elvassore, M. Giomo, G. Brusatin, “*Local measurement of pH gradient on microstructured electrodes using SICM*”, 4th Ertl Symposium on Chemical Processes on Solid Surfaces, Berlin, Germany, 11-13/10/2016.

F. Visentin, M. Fabrizio, S. Battiston, V. Zin, N. El Habra, R. Gerbasi, A. Galenda, “*Morphological, structural and tribocorrosion behaviour of TiO₂ MOCVD coating on Ti substrates with different morphology*”, Joint EuroCVD 21 – Baltic ALD 15 Conference, Linköping, Sweden, 11-14/06/2017.

F. Visentin, N. El Habra, M. Fabrizio, S. Battiston, R. Gerbasi, A. Galenda, “*Bioactivity property of TiO₂ MOCVD coatings on Ti substrates with different pristine morphology*”, 21st International Conference of Solid State Ionics (SSI-21), Padova, Italy, 18-23/06/2017.

N. El Habra, **F. Visentin**, M. Fabrizio, R. Gerbasi, M. Sambì, A. Galenda, “*Exploitation of ALD potentiality for the realization of long-range ordered structures*”, 21st International Conference of Solid State Ionics (SSI-21), Padova, Italy, 18-23/06/2017.

N. El Habra, **F. Visentin**, A. Galenda, R. Gerbasi, “*CVD Techniques for Surface Functionalization: ALD Potentiality for Long-Range Periodic Structures*”, Conferenza di Dipartimento 2017 DSCTM, Alghero, Italy, 19-20/10/2017.

F. Visentin, N. El Habra, R. Gerbasi, M. Fabrizio, A. Galenda, “Osteointegrative Functionalization of Dental Implants: a New Synergic Approach”, Conferenza di Dipartimento 2017 DSCTM, Alghero, Italy, 19-20/10/2017.

F. Visentin, N. El Habra, M. Fabrizio, V. Zin, S. Battiston, R. Gerbasi, A. Galenda, “*Osteointegrative functionalization of titanium substrates for dental implant applications*”, E-MRS Spring Meeting 2018, Strasbourg, France, 18-22/06/2018.

F. Visentin, A. Galenda, M. Fabrizio, R. Gerbasi, N. Brianese, L. Nodari, N. El Habra, “*Synergic deposition route for the functionalisation of biomedical devices*”, AIM 2018, Padova, Italy, 5-7/09/2018.

A. Galenda, **F. Visentin**, R. Gerbasi, M. Favaro, A. Bernardi, N. El Habra, “*Self-cleaning photocatalytic paints: efficiency under indoor lighting systems*”, Conferenza di Dipartimento 2018 DSCTM, Assisi, Italy, 24-26/09/2018.

N. El Habra, R. Gerbasi, **F. Visentin**, A. Galenda, “*Exploitation of Atomic Layer Deposition (ALD) technique for the synthesis of inorganic nanostructured thin films*”, ICMATE-CNR, Conferenza di Istituto 2019, Area della ricerca di Padova, Italy, 21-22/05/2019.

A. Galenda, R. Gerbasi, **F. Visentin**, N. El Habra, “*T.A.R.A.N.T.O. project: supported TiO₂ MOCVD thin films and doped TiO₂ powders for photocatalytic water remediation*”, ICMATE-CNR, Conferenza di Istituto 2019, Area della ricerca di Padova, Italy, 21-22/05/2019.

F. Visentin, A. Galenda, M. Fabrizio, V. Zin, N. Brianese, L. Nodari, R. Gerbasi, N. El Habra, “*New synergic deposition route for the osteointegrative functionalisation of titanium substrates for dental implant applications*”, EuroCVD 22-Baltic ALD 16 | 2019, Luxembourg, 24-28/06/2019.

A. Galenda, **F. Visentin**, M. Fabrizio, V. Zin, N. Brianese, L. Nodari, S. Battiston, R. Gerbasi, N. El Habra, “*Improvement of dental implants properties by surface dual-step functionalisation*”, Conferenza di Dipartimento 2019 DSCTM, Bressanone, Italy, 28-30/10/2019.

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