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Possible Role of Microcrystallinity on Surface Properties of Titanium Surfaces for Biomedical Application

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Abstract

Dental implantology has grown tremendously, since the introduction of titanium. To enhance osseointegration, roughening techniques such as grit blasting, chemical etch, electrochemical anodization have been used with good results. An oxide layer mainly composed of TiO_2 covers the surface of dental implants ensuring excellent corrosion resistance and chemical stability. Despite its biological role in achieving bone interlock, surprisingly, little is known about the structure of TiO_2 , which may be either amorphous or crystalline. Furthermore, at least two crystalline polymorph phases can be found at the bone-implant interface: anatase (tetragonal) and rutile (tetragonal). Therefore, besides the recognized importance of surface topography, energy, and charge, a more refined knowledge of surface chemistry is advisable when studying the bone-implant interface. Recently, sophisticated analysis techniques have been applied to dental implants such as Raman spectroscopy and X-ray diffraction to obtain structural-crystallographic characterization.

This book chapter reviews the scientific literature with the scope of assessing what is known about the surface micro-/nanotopography and the crystallographic microstructure of titanium dental implants. Also, the correlation between these surface features and the biological outcomes in vitro and in vivo is a primary object of the manuscript. An electronic search was made in the databases of MEDLINE (through MeSH) and SCOPUS, extended to September 30th 2015, with no linguistic restrictions.

Based on the results of the most recent studies, the surface of titanium dental implants may be constituted of anatase, rutile, and amorphous phases. Anatase seems more present in arc-oxidized implants, alone or with rutile, according to the oxidation conditions (voltage, electrolyte etc.). Rutile and amorphous phases are more frequently found in machined, double-etched, sandblasted, sandblasted acid-etched implants. Particular interest is raised by the possible presence of brookite, which was found on a

commercially available sandblasted acid-etched implant. Taking into consideration the variations in the biological activity of these polymorphs, identification of the TiO_2 phases found in the surface layers of implants should be regarded as fundamental not only by researchers but also by manufacturers.

Keywords: Raman spectroscopy, Dental implants, Nanotopography, Surface micro-crystallography, Surface properties

1. Osseointegration: an overview of clinically used surfaces

Since Swedish orthopedic surgeon and researcher Per-Ingvar Brånemark discovered the particular connection titanium was capable to develop within bone [1], the concept of osseointegration has been developed as a stable and direct interlock between bone and implant [2, 3]. Currently, commercially pure titanium (Grade 4 titanium) and Ti-6Al-4V alloy (Grade 5 titanium) have become the preferred material in implant dentistry, although ceramic materials with the use of zirconium dioxide and innovative metallic alloys are also attracting growing interest in dentistry [4]. Indeed, the number of dental implant brands on the market increased remarkably during the last three decades from 45 systems in 1988 [5], to 225 systems in 2002 [6], reaching an estimate of 1600 systems nowadays.

In such a competitive field, among all the possible approaches experimented in order to improve the properties of titanium implant surfaces, the main route adopted by the research and industry to enhance osseointegration has successfully entailed roughening techniques [7, 8]. Briefly, the different essential types of modification available on the market can be achieved by applying physical or chemical agents on the implant surface, as follows:

- a. blasting (sand, glass or ceramic microspheres accelerated toward the surface);
- b. wet etching (exposition to acid or alkali chemicals);
- c. anodization;
- d. plasma spray;
- e. exposition to laser radiation;
- f. exposition to electron beams.

Other treatments will be briefly outlined including exposition to cold plasmas and inorganic coatings.

Abrasive blasting (also called sandblasting or grit blasting) is a very common type of surface modification, thanks to the simplicity, low cost, and easy application. Microspheres of diameter in the range 10–540 μm are typically accelerated toward the surface to be treated, using a compressed air or nitrogen blow. Corundum (Al_2O_3) [9, 10], silicon carbide (SiC) [11], titania (TiO_2) [12], hydroxyapatite (HA) [13], zirconia (ZrO_2) [14], silica (SiO_2) [15], and aluminum powders [15] are the most used grit materials. Increasing roughness is the main

effect sandblasting obtains on the morphology of the treated surface. Several parameters contribute to the roughening process, including: the material type, the sphere dimension, the treatment duration, and the energy and angle at the moment of the impact on the surface. The roughness of dental implants normally spans from $R_a = 0.3 \mu\text{m}$ to $R_a = 3 \mu\text{m}$ [15, 16], while polished Ti surfaces assume R_a values lower than $0.1 \mu\text{m}$ [15, 16]. A side effect of the sandblasting process is, however, the contamination of the surface resulting from the material released by the microspheres during their interaction with the surface. Recently, it has been pointed out [15] that the different types of grit materials and the microsphere dimensions can lead to different amounts of surface contamination. In particular, alumina blasting with microspheres of $54 \mu\text{m}$ diameter was found to effectively remove Si contamination from the machined titanium surface, but it was also responsible for the Al contamination as high as ~15%.

Acid treatment is often used to remove contamination and obtain clean and uniform surface finishes. A combination of acids such as HCl, H_2SO_4 , HNO_3 , and HF is frequently used to pretreat titanium. A solution composed of 10–30 vol% of HNO_3 and 1–3 vol% of HF in distilled water has been recommended to be a standard solution for acid pretreatment. To reduce the possible incorporation of hydrogen in titanium and thus the embrittlement of the surface layer, a ratio of nitric acid to hydrofluoric acid of 10–1 is suggested [17]. Acid etching generally leads to a thin surface oxide layer (<10 nm). Although the oxide is predominantly TiO_2 , residues from the etching solution are frequently observed, especially chemicals containing fluorine.

Of great interest is the dual thermo-etching process first proposed by Beatty that has become the paradigm for the dual acid-etched surfaces [18]. Titanium surface is immersed in 15% HF solution and then etched in a mixture of $\text{H}_2\text{SO}_4/\text{HCl}$ (6:1) and heated at 60–80°C for 3–10 min. The main effect of the acid-etching processes is to modify the implant morphology by producing micropits of a few microns diameter on titanium surfaces [16, 19] (**Figure 1A**).

Acid etching is also commonly applied after sandblasting. The complete process, usually referred to as sandblasting and large grit acid etching (SLA) [20], is often considered the reference surface treatment in dental implantology [12, 16, 1920]. This process and its derivatives involve the use of alumina microspheres of 200–540 μm diameter, followed by the etching with a mixture of HCl and H_2SO_4 [16] (**Figure 1B**). The SLA surface treatment combines the

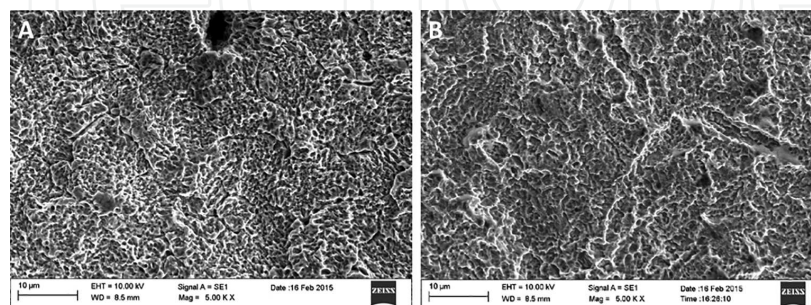


Figure 1. SEM images of a dual acid-etched surface treatment commercially known as DM (A) and a sandblasted acid-etched surface treatment commercially known as SL (B). The samples were provided courtesy of Titanmed s.r.l. (Milan, Italy).

macroroughness generated by the sandblasting process with the microroughness achieved through the acid-etching process [21].

Employed together, alkali and heat treatment [22] enable the formation of a biologically active bone-like apatite layer on the surface of titanium [23]. Due to the strong tendency of titanium to oxidize, the heat treatment is performed at a pressure of 10^{-4} – 10^{-5} Torr. Crystalline sodium titanate (when using NaOH as a base) as well as rutile and anatase precipitates after thermal treatment. The whole process generates a surface capable of promoting the HA precipitation in simulated body fluid following Kokubo's test [ISO 23317:2014(E)].

A native oxide layer grows slowly and spontaneously on titanium kept in air, with an estimated rate of 3–6 nm during a 400-day period [24]. To substitute this thin layer with a thick porous layer of titanium oxide, anodization is widely used. This process consists in either a potentiostatic or a galvanostatic electrochemical oxidation, usually carried out in strong acids, such as HNO_3 , H_2SO_4 , H_3PO_4 , and HF [19, 25]. To some extent, it is also possible to choose the phase of the titanium oxide layer among its amorphous, brookite, rutile, and anatase forms [25].

Titanium plasma spraying (TPS) consists in projecting titanium powders onto the implant surface by means of plasma torch at high temperature. Thus, the titanium particles condense and fuse together, forming a film about 30–50 μm thick [4]. The resulting coating has an average roughness of Sa 4 μm [26]. This three-dimensional topography was reported to increase the tensile strength at the bone–implant interface in vivo [20].

In an endless endeavor to improve the properties of Ti surfaces [27–30], laser treatments have also been proposed. As a result of the heating generated by the absorption of the high-density radiation, the main effect of laser radiation on metals, such as Ti, is to produce a localized melting of the material. The melting process involves only a very thin metal layer under the surface, which is quickly recrystallized after the radiation beam is moved to another portion of the surface, while a titanium oxide layer is formed because of the interaction between solidifying metal and air [27]. Although several types of lasers suit for the modification of metals and oxides, including ruby, like Nd–YAG, argon ion, CO_2 and excimer lasers [29], Nd–YAG appears to be the most diffused one for titanium and its alloys in dentistry [27–30]. Laser-treated Ti is usually rougher than machined Ti surfaces, with typical Ra values ranging from 0.5 to 2 μm [29, 30].

Electron beams have been introduced [31, 32] and used mainly as a pretreatment for the deposition of CaP coatings on titanium [31]. The process was found to reduce the roughness while improving the nanohardness of the material [32] and permitting the deposition of smoother CaP layers [31].

As plasma treatments could prove advantageous compared to wet techniques, such as acid etching, owing to the absence of chemical residuals on the surface, the avoidance of chemical waste, and the reduced safety concerns during manufacturing [33], their application has greatly increased recently.

Depending on the pressure conditions at which they are carried out, plasma treatments can be subdivided into vacuum plasma treatments (reduced pressure plasma treatments) and

atmospheric pressure plasma (APP) treatments. APP treatments are simple and user friendly, however, when dealing with industrial application, reduced pressure plasma displays some advantages. At low pressure, a lower power is required to activate a plasma discharge and, even more importantly, the process performed in vacuum ensures a controlled environment less prone to external contaminations. Although plasma processes have mostly been applied for cleaning and sterilizing dental implants, owing to their capacity to strongly affect the surface energy, they have also been tested for the acceleration of osseointegration [33–36] and the application of antibacterial features to implants [33, 34, 37]. To this aim, argon and oxygen were preferably selected [33–38]. Speaking of plasma treatments, plasma immersion ion implantation (PIII) techniques are also noteworthy as a promising future research avenue in intrabony biomaterials. Here, plasma is used as a source of ions, which are accelerated toward the treated surface and there implanted [38]. Very recently, the incorporation of specific chemical elements such as fluorine (F) [39], calcium (Ca) [40], and zinc [41] was described to confer suitable biological properties.

For the sake of completeness, it is convenient to briefly outline some additive surface modifications, in spite of their limited human use. Calcium phosphate (CaP)-based alloys [42, 43] including HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] [42] and calcium phosphate cements (CPC) [43] result among the most studied coating materials for the enhancement of osseointegration. Several methods have been tested for the deposition of CaP coating on Ti implants, including plasma spray, sputtering, sol–gel deposition, and electrophoretic deposition processes, but plasma spray is considered the most successful so far [33]. Plasma-sprayed coatings can be deposited with a thickness ranging from a few micrometers to a few millimeters, which are characterized by their own roughness and show low density and high porosity [44]. Within the body fluids, these materials lead to the formation of HA nanocrystals. Calcium plays a relevant role in binding biologically active proteins as in its ionized form it adsorbs to the TiO_2 surface and further to macromolecules with high affinity for Ca^{2+} [45, 46].

Plasma-sprayed HA coatings are usually composed of large crystalline HA particles embedded into a highly soluble amorphous calcium phosphate phase. Numerous clinical studies were reported for HA-coated implants [47–49]. Unfortunately, plasma-sprayed HA-coated dental implants have been associated with clinical problems [50–54], due to the possible delamination of the coating from the bulk underneath. This break at the implant-coating interface obviously implies the implant failure despite the fact that the coating is well attached to the bone tissue [50–53]. Coating delamination has been described when the efficacy of plasma spraying was not optimal owing to the size of the dental implants [50]. Loosening of the coating has been reported, especially when the implants have been inserted into dense bone. Inflammatory reaction caused most of long-term failures. Tsui et al. [55, 56] associated the presence of metastable and amorphous phases in the HA coating during the plasma-spraying process to the low crystallinity of HA coating and to the deriving poor mechanical strength [57]. Despite their negative reputation in dental practice, a meta-analytic review could not detect significantly inferior long-term survival rates of plasma-sprayed HA-coated dental implants compared to other dental implants [51].

2. Key surface features

Accurate surface characterization is a fundamental topic in material science. Several relevant surface parameters can be characterized easily using standard analytical methods, such as contact or optical profilometry, electron microscopy and contact angle determination, independently of the production process. This permits to classify the surface of a given implant based on two key characteristics:

- i. topography at the microscale (roughness) and nanoscale;
- ii. wettability

2.1. Topography

At the microscale, the topography of an implant surface is supposed to increase the contact surface and thus the biomechanical interlocking between bone and implant [58]. However, as bone is continuously remodeled [59], the functional osseointegrated area is lower than the theoretical surface developed area [60]. The effects of the various microtopography patterns on bone apposition are still unclear and require more investigations. The quantitative description of surface topography is usually based on roughness, which can be determined either as a profile (2d) or evaluating the whole area (3d). In the former case R_a , R_z , and R_{ms} are the key parameters, while in latter case, it occurs to mention S_a , S_{ds} , and $S_{dr}\%$. Height deviation amplitude (S_a) is conveniently used for classifying osseointegrated implants into four categories: smooth 0–0.4 μm , minimal 0.5–1 μm , moderate 1–2 μm , and maximal $>2 \mu\text{m}$ [58, 61]. As for spatial density, surfaces are either rugged ($S_{dr}\% > 100\%$) or flattened out ($S_{dr}\% < 100\%$), while the morphology of the microstructures may be described as rough, patterned, or particled, with respect to the number of dimensions. Specifically, following Dohan Ehrenfest et al., “microrough surfaces have one micrometric dimension (the peak heights). Micropatterns have two micrometric dimensions (dimensions of the repetitive pattern), such as the micropores created by anodization (...). Microparticles have three micrometric dimensions.”

At the nanoscale, a more textured surface topography is known to increase the surface energy. The higher the surface energy the greater becomes the wettability. To an increased, wettability is due to the improved adsorption of fibrin and matrix proteins on the surface, which, in turn, favors cell attachment, tissue healing, and eventually the osseointegration process. Nanotopography might also directly influence cell behavior through the influence nanopatterning has on the cytoskeleton [62–66]. Even though all surfaces have their own nanotopography, by definition, not all of them possess significant nanostructures. A nanostructure is conventionally defined as an object of size comprised between 1 and 100 nm. Dealing with nanostructures, it may be helpful to specify the number of nanoscale dimensions. One dimension at the nanoscale implies the concept of nanoroughness [67], while nanopatterns are endowed with two nanoscale dimensions, like the nanotubes produced by anodization [68, 69], or the chemically produced nanopatterned surfaces [64, 70] (**Figure 2**).

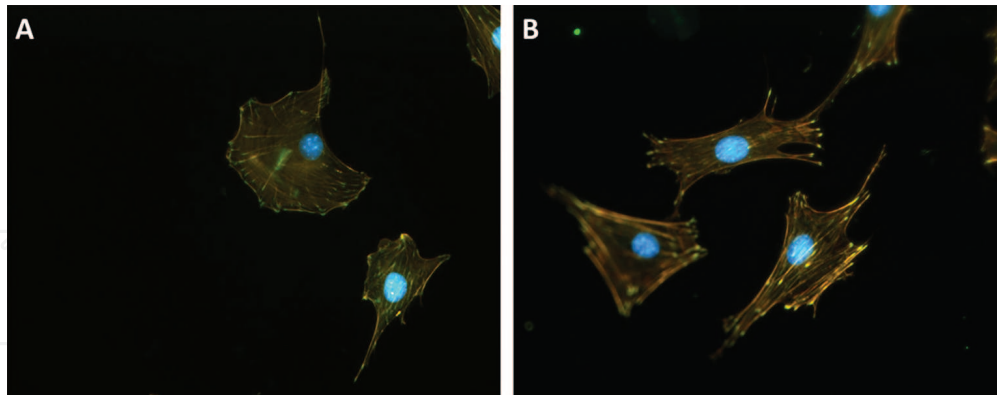


Figure 2. The picture depicts morphologically the cytoskeleton arrangement of murine osteoblasts (MC3T3-E1) grown, respectively, on smooth (A) and nanostructured titania surfaces (B). Cells were stained with Rodamine-phalloidin (red), anti-paxillin antibody (green), and DAPI (blue). The effect of the surface pattern on the cells is clearly appreciable from the number of focal adhesions as visualized by marking paxillin in green.

The presence of three nanoscale dimensions is typical of the nanoparticles. If nanostructures are not clearly detectable (no patterns, no particles, insignificant texture) or not homogeneous and repetitive, the surface should be considered as nanosmooth. Repetitiveness and homogeneity are indeed important yet difficult to define—morphological parameters that may be deemed qualitative.

2.2. Wettability

The wetting features of a solid material are usually determined through the sessile drop technique. Briefly, a drop of a given liquid is placed on the surface sample and the angle between the tangent of this drop at the three-phase boundary and the solid surface is measured. Thus, the contact angle CA expressing the surface wetting is quantified according to the liquid employed. For instance, if water is used, the CA will characterize the hydrophilicity of the surface. In principle, the CA can assume values from 0° to 180° , in case of complete spreading or beading of the drop, respectively. Water CAs lower than 90° ascribe surfaces a hydrophilic feature, while water CAs above 90° designate surfaces as hydrophobic. As the “the drop rests on an ideal homogeneous and flat surface in thermodynamic equilibrium, the drop shape with the characteristic ideal CA θ is formed as a result of the liquid/vapor γ_{lv} , solid/liquid γ_{sl} , and solid/vapor γ_{sv} interfacial tensions, according to Young’s equation”. (...) Surface tension is caused by the asymmetry of the cohesive forces of molecules at a surface compared to molecules in the bulk where each molecule has surrounding partners resulting in a net force of zero. Correspondingly, the surface energy is minimized in the bulk, whereas at the surface, the energy is increased due to the missing surrounding molecules. Therefore, to reduce surface energy, the surface area has to be minimized, thus resulting in phenomena like spherical water drops or the spreading of aqueous liquids on higher energetic surfaces.” [71]

High energetic solid surfaces enhance wetting, which has been associated with improved implant success [72] (**Figure 3**).

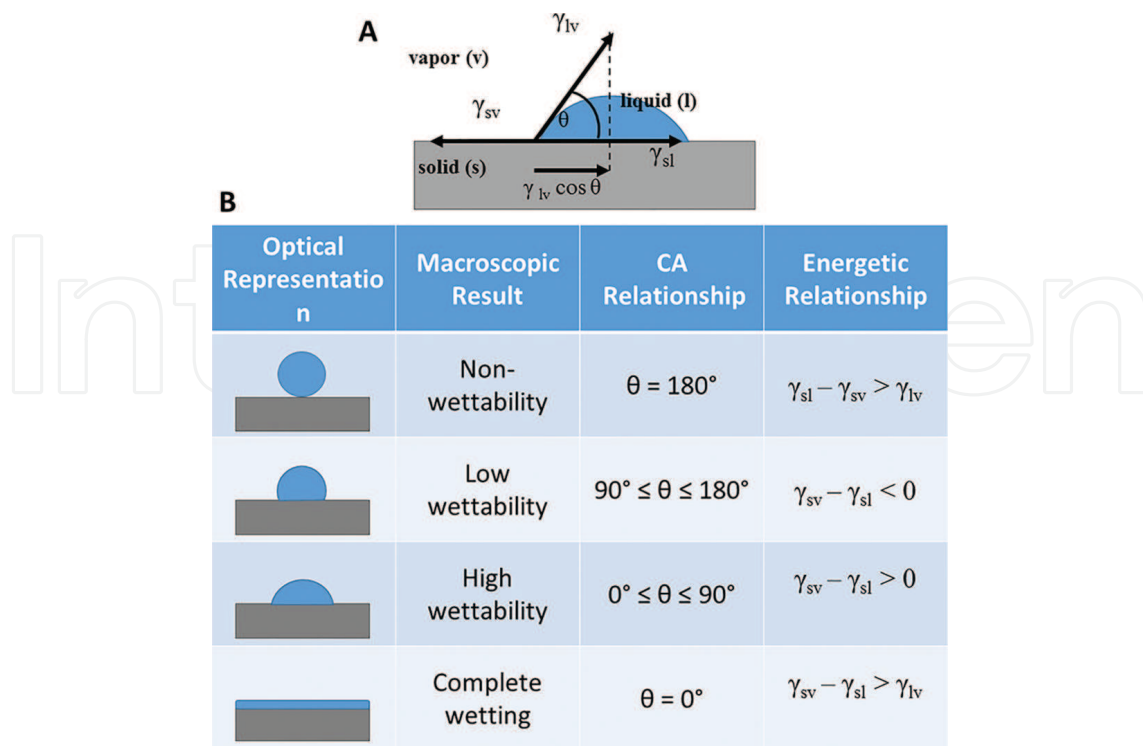


Figure 3. (A) Schematic diagram depicting contact angle CA as measured by sessile drop technique, along with the graphical derivation of Youngs equation. (B) Relations between wetting tension and the wetting of a solid surface. Figure concept has been taken from Rupp et al. [71].

3. The possible role of microcrystallinity state of the titanium surface

The outstanding chemical inertness, repassivation ability, corrosion resistance, and ultimately biocompatibility of titanium result from an oxide layer that is usually only a few nanometers thick. As titanium exists in many different stable oxidation states and oxygen is highly soluble in titanium, titanium oxide is known to have varying stoichiometries. Among the common compounds, there are Ti_3O , Ti_2O , Ti_3O_2 , TiO , Ti_2O_3 , Ti_3O_5 , and TiO_2 [73]; however, the most stable titanium oxide is TiO_2 , also known as titania. TiO_2 is thermodynamically very stable and the Gibbs free energy of formation is highly negative for a variety of oxidation media, such as water or oxygen containing organic molecules.

Although the fundamental biological role of titania in osseointegration has attracted a lot of interest, there is limited knowledge regarding its structure, especially on commercially available products. TiO_2 exists in three crystalline polymorph phases: rutile (tetragonal), anatase (tetragonal), and brookite (orthorhombic), but only rutile and anatase phases are practically important. Brookite is the largest phase, with eight titania groups per crystal unit cell, anatase possesses four groups per unit cell, and finally, rutile has two groups per unit cell. Rutile is the most diffused and stable isoform. In all phases, a six-coordinated Ti participates in unit cells [74]. Titania may be found on implant surfaces in either the amorphous or crystal

phase with heterogeneous results [75], as a consequence of the surface treatment the implants underwent [76, 77]. X-ray diffraction (XRD) is the technique of choice whenever the crystalline structure is to be investigated, for instance, in terms of main crystal orientation, grain size, crystallinity, and strain [78]. X-ray photoelectron spectroscopy (XPS) is instead used to determine the quantitative mean atomic composition of wide and thin surface areas (typically 300 nm in diameter, 5–7 nm depth). When XPS is applied to pure titanium samples exposed to the atmosphere at room temperature after milling, beside the stable titania film, hydroxide, and chemisorbed water bond with Ti cations are detected on the surface. In addition, some organic species like hydrocarbons adsorb and alkoxides or carboxylates of titanium also exist on the outmost surface layer. Currently, microcrystallinity has almost never been assessed in commercially available surfaces [79, 80].

During implant manufacturing, anatase, rutile, or amorphous TiO_2 are produced depending on the conditions. Upon heating, amorphous titania converts to anatase (<400°C) and then to rutile (600–1000°C) [81, 82]. The two crystalline phases, and especially anatase, have been studied as regards photocatalysis and photon–electron transfer [83], hydrophilicity [84], and biological decontamination capacity [85]. Notwithstanding its increased biological activity [79, 86], anatase has been claimed to be more prone to ionic dissolution in than rutile [87]. On the other hand, rutile renders surfaces hydrophobic, whereas anatase improves wetting [85], which may be beneficial for the healing process at early stages.

Recently, these properties have attracted growing interest, as they may provide a synergistic effect to the wide range of the surface treatments used. As mentioned above, the information available on the TiO_2 phases formed on the implant surfaces present in the market is surprisingly limited. The rapid growth of the oxide layer during manufacturing is thought to lead to an amorphous phase on implant surfaces [88]. Despite the well-documented interaction of amorphous TiO_2 layer with bone, HA cannot readily grow on such a surface, in simulated bodily fluid, which may be due to the arrangement of the oxygen portions. In rutile and anatase, however, oxygen groups match better the hydroxyl groups of HA, resulting in deposition of biomimetic apatite, thus possibly facilitating osseointegration [88]. As these phases require additional treatments to be grown from native amorphous TiO_2 , Gaintantzopoulou et al. [89] hypothesized that the various surface treatments performed on titanium implants to enhance osseointegration were aimed at creating anatase and rutile crystalline domains. Briefly, they found that anatase is more pronounced in arc-oxidized implants, alone or with rutile, dependent on the oxidation conditions. Rutile and/or amorphous phases are more common in machined, double-etched, sandblasted, sandblasted acid-etched.

4. Conclusions

Distinct minorities of the implant manufacturers have undertaken basic, animal and human research when designing new or altering the components of existing implant systems. Consequently, many currently commercially available dental implants have insufficient, questionable, or simply totally lacking scientific justification of the product designs and

material compositions. Potential alterations of the implants include surface chemical and biochemical properties, corrosion characteristics and wear debris release, surface energy and wettability as well as topography on micrometer and nanometer scales. Considering the possible role in their biological activity, the identification of the titania phases found in the surface layers of implants should be deemed unavoidable by the manufacturers and the scientific community.

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