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Surface treatments and roughness properties of Ti-based biomaterials

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Nowadays, the use of implanted devices is a well-acknowledged practice in the field of orthopaedic and dental surgery. Scientific research and clinical experience suggest that the successful exploitation of these devices mainly depends on osseointegration, considered as both anatomical congruency and load-bearing capacity. Indeed, the osseointegration process is influenced by a wide range of factors: anatomical location, implant size and design, surgical procedure, loading effects, biological fluids, age and sex, and, in particular, surface characteristics. For this reason, several attempts have been aimed at modifying implant surface composition and morphology to optimise implant-to-bone contact and improve integration.

Preliminary interactions between implanted materials and biological environment are deemed to be governed by the surface properties; they control the amount and quality of cell adhesion on the surface and, consequently, cell/tissue growth. Thus, surface properties govern new bone tissue formation and implant osseointegration.

This paper reviews the state of art in the field of physical, chemical and biochemical treatments commonly used on Ti-based biomaterials for the production of biomedical devices. In particular, roughness characteristics due to physical and chemical techniques are investigated; the development of biologically active surfaces by means of biochemical functionalisation is also considered.

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1. Introduction

From a general viewpoint, the performances of implanted materials rely on the nature of the interaction mechanisms between biomaterials and living cells [1]. A detailed, consistent, and coherent picture of processes and mechanisms at the interface between implant surface and bone is still lacking: a preliminary description of these phenomena on molecular/cell scale is reported by Kasemo and Gold [2] and Puleo and Nanci [3]. These authors argued that the specificity of cell–surface interaction ‘‘derives, at least partly, from how the protein layer is composed and organised, which in turn depends on how the surface binds water, ions, and different biomolecules.’’ The protein layer is produced through a sequence of several biochemical steps; then, living cells can adhere, even though a direct cell–surface contact is not needed to induce specific interactions.

It is worthwhile mentioning that the surface may also determine cell behaviour by releasing ions and/or molecules that can penetrate cell membrane or activate membrane-bound receptors. Positive stimuli can be of organic/inorganic nature, while negative stimuli can be produced by corrosion. Moreover, protein–surface and cell–surface interactions can be influenced by micro-topography, roughness, micromechanical and viscoelastic properties. These factors can enforce or reduce the mechanical stress–strain fields at the interface. Basically, protein–surface and cell–surface interactions are affected by surface composition and roughness [4–6].

As to implant surface composition, biocompatibility is the fundamental requirement for a candidate biomaterial; nevertheless, biocompatibilty does not imply the necessary bioactivity for good tissue regeneration around implant surface, which might exhibit not only osteoconductive but also osteoinductive properties [7].

In brief, surface characteristics directly and indirectly influence the way molecules present in the biological world act: this might ultimately control new tissue formation as cell proliferation and differentiation both depend on the quality of their early adhesion [4, 8].

So far, some authors indicate that the design of oral implants is guided by an aggressive marketing approach rather than by fundamental advances in biomechanics or bone biology [9]; other researchers maintain that the implanted devices are simply developed on empirical basis [10–12]. Anyway, when a metallic implant is surgically placed, its surface comes in close apposition to the exposed biological tissue: this results in a sequence of

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different physico-chemical and biochemical interactions, which involve macromolecules from tissue and body fluids, containing other molecules and inorganics [13].

For all these reasons, several treatments have been suggested and applied with the aim of modifying surface characteristics improving osseointegration [14]. Surface treatments are known to induce modifications in both surface chemical composition and topography [15], and most of surface alterations have been produced in order to achieve greater bone-to-implant contact [16, 17].

The present paper reviews treatments and techniques, and methods of application thereof, currently used in treating titanium and titanium alloys surfaces, which are acknowledged as the most widely used biomaterials for applications in the field of orthopaedic and oral implantology. Treatments will be divided into three main categories: physical, chemical-electrochemical and biochemical. They will be separately presented and their effects on surface composition and morphology will be discussed.

2. Physical treatments

A number of different treatments are currently termed as ''physical'' since they are generally suitable for modifying surface characteristics by the application of external actions.

2.1. Mechanical treatments

Among currently available mechanical treatments, the following three categories will be taken into consideration for their practical importance: cutting and turning; smoothing; blasting.

2.1.1. Cutting and turning

These techniques can be coupled on the basis of the characteristic dimensions of the defects impressed on the metallic surface. A typical cutting device is represented by rotating carborundum disk saw: the operator can usually manage cutting pressure and velocity, so that it is possible to control, at least in a restricted range of properties, the characteristics of the surface. Anyway, when homogeneous, even not smoothed, surfaces are required, cutting device has not to be used as the final treatment: jagged and irregular surfaces with a very low finishing degree have to be expected. Furthermore, cutting devices necessarily induce mechanical deformations on materials such as titanium and titanium alloys, so that cutting edges appear deeply altered. This aspect is often not compatible with the required characteristics of an endosseous implants.

Alternatively, it is possible to utilise turning machine, with stainless steel cutting tool. As well as cutting, turning imposes new morphological characteristics over the original surface, but under more reproducible operating conditions. It is worthwhile mentioning that optical microscope analysis can reveal a high anisotropy in the distribution of surface defects even on micrometric scale: in particular, it is always possible to recognise concentric grooves due to the direction of the turning tool.

In the case of metallic materials, turning devices usually produce deformations: crystalline grains disappear, surface properties are changed and, in general, surface hardness increases. Typical average roughness parameter (R_a) values vary from 0.3 up to 0.6 μm.

2.1.2. Smoothing

In order to obtain finer finishing degrees, surfaces can be exposed to smoothing process, by means of grit-papers and/or diamond clothes. They both support abrasive particles, in general corundum (SiC) of different size: the finest particles are used for the highest finishing levels (polishing), the largest ones for simple roughing treatment (grinding). During smoothing process, it is important to avoid scratching effects that could spoils the surface; for this reason, it is convenient to treat the metallic surface by a sequence of particles with decreasing size.

Due to the mechanical properties of titanium and titanium alloys, abrasive particles should be embedded into the metallic matrix: this phenomenon is commonly indicated as abrasive pollution. For example, chemical analysis on smoothed surfaces can reveal not only the presence of Ti, O, Al, V, Fe, C, N, but also Si, Ca, P, etc. These elements might not be compatible with the applications which treated materials are intended for; consequently, undesired elements have to be removed (i.e. solvent cleaning, sonication,...). As mentioned above, abrasive particles are generally made of carburundum or industrial diamond (particle size even lower than 200 μm). Typical roughness values (R_a) of smoothed surfaces vary from 0.1 (mirrored/polished surface) up to 1 μm (rough surface).

As an alternative, Taborelli *et al.* [18] utilised alumina powder available on both grit-papers and diamond-clothes. In this case, titanium samples reveal surface defects (grooves) on micrometric scale (1–2 μm width, 80 nm depth) after smoothing. Roughness parameters estimated in Taborelli *et al.* [18] are: R_t (maximum height of the profile) 81 nm and R_{ms} (integral mean square root of the deviations of surface profile from the average) 6 nm. In another paper [19], the ratio between real surface area and the geometrically projected one for titanium samples after smoothing with alumina powder, resulted 1.081 ± 0.047: this value, quite close to 1, indicates that surface area increases not in such appreciable way and the surface is clearly smooth.

In another work [20], Ti and Ti6Al4V disks (0.8 mm height, 14.75 mm diameter) have been divided into two classes on the basis of different surface treatments: (1) smoothing with 1200 grit-paper (15 μm average particle size); (2) abrasion with liquid suspension of rough mixed oxides (zirconium oxide and alumina). Surfaces can be distinguished due to the physical treatment rather than their chemical composition: smoothed surface defects seem to exhibit a homogeneous distribution; defects on roughened surfaces are appreciable as large (up to 10–20 μm) and deep scratches. As regards surface roughness, R_a parameter has been evaluated (Table I): the effect of abrasion is more evident on Ti than on Ti6Al4V disks, may be due to the lower Ti hardness.

TABLE I Average roughness values of smoothed and roughed Ti and Ti6Al4V surfaces [20]

Material/surface	R_a (µm)
Ti after smoothing	0.22 ± 0.00
Ti6Al4V after smoothing	0.23 ± 0.00
Ti roughened	4.24 ± 0.13
Ti6Al4V roughened	3.20 ± 0.12

An interesting comparison has been carried out on Ti disks (12 mm diameter) after turning and after smoothing with different grit-papers [21]. Four roughness parameters have been estimated: R_a, R_q (root mean square roughness), R_t and the adimensional S_k (skewness). The data obtained (Table II) confirm the efficacy of smoothing process in removing surface macroscopic defects.

A further comparison has been recently performed between Ti and Ti6Al4V smoothed and roughened surfaces [17], by estimation of a large number of roughness parameters, which can be divided into two groups. Group 1 describes defect dimension: R_a, R_q, R_z (10 points average roughness), S_M (mean spacing); group 2 describes defect frequency (periodicity and organisation): P_c (peak density), LAC (profile autocorrelation function length, adimensional), Order (profile periodicity, adimensional, particularly suitable on micrometric scale), Delta (profile numerical derivability, adimensional). Roughness information has been considered on two different levels: macro- and micro-roughness, above and below 50 µm, respectively, that it has been assumed as typical dimension of human osteoblasts (Table III).

Other materials have been investigated, in particular titanium alloys with Cr, Mo, Nb, Zr, etc., which exhibit improved mechanical properties avoiding the presence (and preventing potential citotoxicity) of Al and V. NiTi binary alloy and TiMoCr ternary alloy have been studied, after different thermal treatment and smoothing conditions [22]. Corresponding roughness parameters are depicted in Table IV: R_a, R_p (maximum profile peak height), R_t and R_{tm} (average value of the maximum heights of profile segments). All the surfaces appear as smoother as finer the grit-paper particle size is; tensional states induced by molding are reduced while process temperature increases.

2.1.3. Blasting

Blasting process requires abrasive particles to be forced against the surface by a dragging fluid; this latter is usually compressed air that, flowing through an ejector, sucks particles up and carries them. Due to the dynamic of the contact between forced particles and surface, blasting treatment can produce higher roughness values and induce mechanical constraints on the metallic surface. The fundamental parameter in blasting is the particle size; some authors stated that particles over 0.1 mm cannot increase roughness values [23], even though for other authors particles up to 900 µm can do [24].

As well as in the case of smoothing, even blasting particles can induce abrasive pollution, which is potentially responsible for modifications in the chemico-physical interaction capacity of blasted surfaces.

TABLE II Roughness parameter values determined on Ti surfaces after turning and smoothing with different grit-papers [21]

Material/surface	R_a (µm)	R_q (µm)	R_t (µm)	S_k (–)
Ti after turning	0.872	1.115	8.090	– 0.462
Ti after turning and smoothing with 320 grit-paper	0.386	0.492	3.953	– 0.145
Ti after turning and smoothing with 4000 grit-paper	0.314	0.392	3.011	0.129

TABLE III Roughness parameter values determined on Ti and Ti6Al4V surfaces after roughing and smoothing [17]

Material/surface	R_a (µm)	R_q (µm)	R_z (µm)	LAC (–)	Order (–)	Density (1/inch)	S_M (µm)	Delta (–)
Ti roughened	0.31 ⟨0.18⟩	3 ⟨2.11⟩	1.99 ⟨1.62⟩	63 ⟨13.5⟩	22 ⟨12.2⟩	451 ⟨726⟩	58.3 ⟨35.1⟩	1.23 ⟨1.28⟩
Ti6Al4V roughened	0.5 ⟨0.17⟩	4.15 ⟨2.15⟩	3.1 ⟨1.53⟩	43.6 ⟨5.02⟩	17.5 ⟨8.15⟩	496 ⟨1356⟩	50.7 ⟨18.62⟩	1.28 ⟨1.39⟩
Ti smoothed	0.07 ⟨0.03⟩	0.73 ⟨0.66⟩	0.45 ⟨0.37⟩	192 ⟨6.61⟩	36 ⟨6.80⟩	1025 ⟨2049⟩	26 ⟨12.1⟩	1.43 ⟨1.49⟩

Values within brackets refer to micro-roughness scale.

TABLE IV Roughness parameter values determined on titanium alloy surfaces rolled at different temperature and smoothed with different grit-papers [22]

Material/surface	Rolling	Grit-paper	R_a (µm)	R_p (µm)	R_t (µm)	R_{tm} (µm)
NiTi	@950 °C	80	0.36 ± 0.21	0.64 ± 0.15	1.43 ± 0.50	0.45 ± 0.05
		600	0.16 ± 0.02	0.63 ± 0.20	0.63 ± 0.44	0.51 ± 0.04
		1200	0.10 ± 0.04	0.16 ± 0.04	0.42 ± 0.08	0.20 ± 0.02
TiMoCr	@850 °C	80	1.48 ± 0.04	3.12 ± 0.58	6.85 ± 0.30	2.57 ± 0.01
		600	1.43 ± 0.17	1.85 ± 1.06	5.82 ± 0.71	1.20 ± 0.14
		1200	0.30 ± 0.03	0.85 ± 0.55	1.35 ± 1.06	0.54 ± 0.16
TiMoCr	@1050 °C	80	0.73 ± 0.05	2.29 ± 0.30	3.74 ± 0.07	0.95 ± 0.05
		600	0.44 ± 0.01	1.10 ± 0.21	2.24 ± 0.32	0.67 ± 0.06
		1200	0.34 ± 0.04	0.58 ± 0.09	1.38 ± 0.01	0.42 ± 0.02

TABLE V Roughness parameter values determined on titanium surfaces turned and blasted with particles of different composition and size [25]

Material/surface	R_a (μm)	R_q(μm)	R_t (μm)	R_{sk} (–)	R_{ku} (–)	Δ_q (°)	λ_q (μm)
Ti turned	0.39 ± 0.16	0.65 ± 0.3	12.33 ± 8.8	– 0.55 ± 1.6	21.94 ± 26.6	25.32 ± 7.9	9.00 ± 1.3
Ti turned + blasted (rutile 25 μm)	0.88 ± 0.2	1.23 ± 0.3	18.47 ± 5.1	0.49 ± 1.0	9.79 ± 6.4	37.08 ± 4.8	11.82 ± 1.1
Ti turned + blasted (alumina 75 μm)	1.27 ± 0.1	1.77 ± 0.2	27.48 ± 7.1	0.05 ± 0.6	9.56 ± 4.3	46.7 ± 2.0	13.66 ± 0.8

TABLE VI Roughness parameter values determined on titanium surfaces turned and blasted with particles of different composition and size [27]

Material/surface	R_a (μm)	R_q(μm)	R_t (μm)	R_{sk} (–)	R_{ku} (–)	Δ_q (°)	λ_q (μm)
Ti turned	0.34 ± 0.1	0.54 ± 0.18	9.00 ± 3.0	0.36 ± 1.7	15.91 ± 6.9	21.40 ± 4.8	8.98 ± 1.6
Ti turned + blasted (rutile 25 μm)	0.96 ± 0.3	1.34 ± 0.53	18.14 ± 6.7	0.44 ± 0.9	8.52 ± 4.3	38.66 ± 8.7	12.17 ± 1.74
Ti turned + blasted (alumina 25 μm)	0.84 ± 0.2	1.23 ± 0.3	19.48 ± 5.4	0.97 ± 1.1	13.43 ± 8.9	37.07 ± 3.6	11.88 ± 2.4
Ti turned + blasted (alumina 75 μm)	1.32 ± 0.3	1.84 ± 0.4	24.53 ± 3.7	0.15 ± 0.66	8.12 ± 1.8	47.50 ± 4.8	13.85 ± 1.27

Particles usually applied in blasting treatments are made of alumina, corundum, rutile, and hydroxyapatite. The following drawbacks are possible:

- hydroxyapatite can enrich metallic surface in Ca and P;
- aluminium oxides residues are possible, but they are easily removed in acidic solutions.

Due to its chemical similarity with Ti surface, rutile is often preferred. R_a values impressed by blasting treatments are in a wide range of values: from 0.5–1.5 up to 2–6 μm, depending on the particles size. As to sand-blasting conditions, many contributions are available in literature: Wennerberg *et al.* [25] investigated the effects obtained on the surfaces of 60 identical implants divided into three classes: (i) turned; (ii) turned and blasted with rutile (25 μm); (iii), turned and blasted with alumina (75 μm). Seven roughness parameters have been estimated (Table V): apart from the already known R_a, R_q, and R_t, skewness (R_{sk}, representing the asymmetry of the profile) and kurtosis (R_{ku}, indicating how much the profile is steep) adimensional parameters have been determined. Moreover, Δ_q (square root of the integral mean of square profile slopes) and λ_q (square root of the integral mean of square defect spacing) have been taken into consideration [26]. Turned surfaces which, however, reveal typical defects due to the turning tool scratches, are smoother in comparison with the sand-blasted ones; for these latter, roughness parameter values depend on the particle size.

In another paper, Wennerberg *et al.* [27] evaluated the same roughness parameters for investigating the characteristics of titanium surfaces as obtained by turning and blasting, even with finer alumina particles (Table VI). The results confirm what was previously demonstrated, that is, surface roughness depends on the particle size but does not depend on the nature of the particles: finest particle induces finest roughness and *vice versa*.

Sometimes, blasting treatments utilise a mixture of particles of different dimension (Tables VII and VIII) [28, 29].

An extremely detailed study has been carried out on titanium surfaces after blasting with alumina (25 and 75 μm), with the aim of comparing the roughness parameters in Table IX [30]. Overall results confirm that the larger the particles, the rougher the blasted surface (Table X). These results have been confirmed in a further paper [31] where larger alumina particles have been used: following the quantitative roughness description proposed by Stout *et al.* [32], three parameters have been identified: two (S_a, S_{cx}) describe roughness height and width, and the third parameter (S_{dr}) is hybrid (Table XI).

The effects of rutile particles have been investigated in detail in two papers published by Mustafa *et al.* [23, 33]: titanium surfaces have been sand-blasted with several particles of increasing size, and the surface topography has been described by three roughness parameters (S_a, S_{cx}, S_{dr}). A correlation between roughness amplitude and particles size is still detectable, but it is not dramatically appreciable (Tables XII and XIII). Actually, it seems that the intermediate particle size can only induce a homogeneous distribution of surface defects, whereas largest particles produce little, but frequent, flattened areas on the treated surface: further increase of the particle size does not result in a significant increase of defect dimensions.

TABLE VII Average roughness values of turned and rutile blasted Ti surfaces [28]

Material/surface	R_a (μm)
Ti turned	0.31 ± 0.12

TABLE VIII Average values of defect depth on turned and blasted Ti surface [29]

Material/surface	Values range of defect depth (μm)
Ti blasted (corundum 0.25–0.5 μm)	22–28

TABLE IX Roughness parameters as examined in [30]

Parameter	Description	Unit
S_a	Average roughness	μm
S_q	Root mean square roughness	μm
S_t	Maximum depth of profile defects	μm
S_z	Ten points average roughness	μm
S_{sk}	Profile asymmetry	–
S_{ku}	Profile kurtosis	–
S_{ti}	Isotropy index (from 0 to 1)	–
S_{dr}	Developed surface versus projected one ratio	–
S_{cx}	Integral mean of defect spacing along x axis	μm
S_{cy}	Integral mean of defect spacing along y axis	μm

TABLE X Roughness parameter values determined on titanium surfaces blasted with alumina particles of different size [30]

Material/surface	S_a (μm)	S_q (μm)	S_t (μm)	S_z (μm)	S_{sk} (–)	S_{ku} (–)	S_{ti} (–)	S_{dr} (–)	S_{cx} (μm)	S_{cy} (μm)
Ti blasted with alumina (25 μm)	1.11 ± 0.04	1.61 ± 0.07	20.37 ± 3.5	15.20 ± 1.3	− 0.61 ± 0.4	14.76 ± 21.4	0.51 ± 0.02	1.37 ± 0.02	9.91 ± 0.2	15.72 ± 1.3
Ti blasted with alumina (75 μm)	1.45 ± 0.2	1.97 ± 0.1	25.98 ± 5.47	18.27 ± 2.2	− 0.60 ± 0.3	13.34 ± 18.4	0.53 ± 0.03	1.50 ± 0.04	11.06 ± 0.3	16.77 ± 0.3

TABLE XI Roughness parameter values determined on titanium surfaces blasted with alumina particles of different size [31]

Material/surface	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.96 ± 0.4	8.48 ± 1.2	1.34 ± 0.1
Ti blasted with alumina (25 μm)	1.20 ± 0.4	9.80 ± 0.6	1.44 ± 0.1
Ti blasted with alumina (75 μm)	1.43 ± 0.3	11.63 ± 0.6	1.49 ± 0.1
Ti blasted with alumina (250 μm)	2.20 ± 0.3	13.59 ± 1.1	1.81 ± 0.1

TABLE XII Roughness parameter values determined on titanium surfaces blasted with rutile particles of different size [33]

Material/surface	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.34 ± 0.02	8.17 ± 0.30	1.17 ± 0.02
Ti blasted with rutile (8–88 μm)	0.60 ± 0.03	10.62 ± 0.36	1.33 ± 0.03
Ti blasted with rutile (45–63 μm)	0.78 ± 0.07	11.55 ± 0.11	1.42 ± 0.05
Ti blasted with rutile (63–90 μm)	0.80 ± 0.07	12.04 ± 0.39	1.41 ± 0.04

Other materials have been studied after blasting with glass beads and corundum [34]. Surface topography properties has been expressed in terms of average roughness (R_a) and average value of defect spacing along measured profile (S). As expected, differences are mainly due to particle size rather than to surface composition (Table XIV). Indeed, it is worthwhile pinpointing that only in the case of a titanium surface the parameter S does depend on the abrasive particles; in all the other cases, S can be deemed constant.

A recent contribution by Aparicio *et al.* [24] investigated the effects produced on titanium surfaces by blasting with different particles of different size: large (200, 600, 900 μm) corundum and alumina particles have been utilised. Before blasting, all the surfaces have been partially passivated with 0.1 M HCl, and after blasting they have been analysed by profilometry and BS-SEM microscopy. Apart from the usual average roughness parameter (R_a), other properties have been measured: the amount of abrasive particles embedded into the metallic matrix (CS, %); the real surface area (RSA, mm^2) and the ratio between RSA and the geometrically projected surface area (RSA/A); the real surface area corrected by the presence of abrasive particles (CRSA, mm^2). These parameters may be suitable for foreseeing the extension of the contact between the surface of an implanted device and the surrounding biological tissue. Turning process induces fine roughness, which results in a reduced contact area; on the other hand, surfaces treated with larger particles exhibit a more extended contact area (Table XV). There is an important difference between corundum and alumina particles: the latter are able to produce higher surface roughness. This may be due to a different particle size distribution, as usual for commercially available products. The investigation was extended to the behaviour of the treated surfaces with respect to corrosion processes, mediated by the presence of titanium oxide. By means of electrochemical measurements on the surface electrical potential and polarisation, authors stated that the contribution of oxide layer thickness to electrical resistance is as lower as the abrasive particles are larger. Moreover, abrasive particles size effects also the intensity of electrical current through titanium samples: this is in agreement with the experimental evidence that higher contact surface is produced by larger particles.

3. Chemical treatments

Chemical treatments are suitable for producing modifications in the chemical composition of native materials, with specific regards to the surface layer. Among all available methods, this paper considers the most widely used ones, which will be presented on the basis of the nature of the effects produced on the material surface.

From a general viewpoint, chemical treatments imply the immersion of metallic samples into polar solution (organic or aqueous) of different chemical compounds: inorganic acids produce deeper modifications than

TABLE XIII Roughness parameter values determined on titanium surfaces blasted with rutile particles of different size [23]

Material/surface	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.20 ± 0.02	9.81 ± 0.61	1.06 ± 0.02
Ti blasted with rutile (63–90 μm)	0.72 ± 0.04	14.20 ± 0.65	1.32 ± 0.02
Ti blasted with rutile (106–180 μm)	1.30 ± 0.11	15.48 ± 0.71	1.69 ± 0.07
Ti blasted with rutile (180–300 μm)	1.38 ± 0.14	15.73 ± 0.78	1.76 ± 0.07

TABLE XIV Roughness parameter values determined on titanium and titanium alloys surfaces blasted with glass beads and corundum particles of different size [34]

Material/surface	R_a (μm)	S (μm)
Ti blasted with glass beads (150–250 μm)	1.30 ± 0.20	8.25 ± 0.60
Ti blasted with corundum (300–400 μm)	2.60 ± 0.04	13.00 ± 0.05
Ti6Al4V blasted with glass beads (150–250 μm)	1.20 ± 0.03	10.90 ± 0.04
Ti6Al4V blasted with corundum (300–400 μm)	2.50 ± 0.03	11.80 ± 3.00
Ti6Al7Nb blasted with glass beads (150–250 μm)	1.40 ± 0.30	11.80 ± 2.05
Ti6Al7Nb blasted with corundum (300–400 μm)	2.70 ± 0.04	11.80 ± 0.90

TABLE XVI Average defect depth as determined on titanium surfaces blasted with corundum particles of different size and attacked with two different acidic mixture [29]

Material/treatment	Defect depth (μm)
Ti sand blasted (corundum 0.12–0.25 μm) + HF/HNO_3 attack	6
Ti sand blasted (corundum 0.25–0.5 μm)	22–28
Ti sand blasted (corundum 0.25–0.5 μm) + HCl/H_2SO_4 attack	18–23

solvents such as ethanol or acetone. It is worthwhile mentioning that titanium and titanium alloys exhibit different reactivity which, on turn, depends on the nature of the acidic mixture: thus, it is possible to induce the formation of a protecting layer (passivation), the dissolution of ceramic compounds, the flattening of surface defects, and the formation of micropores surrounded by a gel. Anyway, chemical methods effects are restricted to surface modifications and rarely change the shape of the samples.

3.1. Acid attacks

Under the generically called ''acid attacks'', a variety of treatments are included: they are usually performed by immersion into aqueous, non-oxidant solutions (with the exception of HF/HNO_3 mixture), at room or higher temperature. Chemical effects are due to redox reactions, which are responsible for metal dissolution (as soluble salt) and development of gaseous hydrogen (which adhere to metallic surface, at least partly).

It is important to note that materials such as titanium and titanium alloys normally react with oxygen, which forms an oxide layer responsible for the very low chemical reactivity of the surface. Only few acids are able to react with the oxide: HCl, H_2SO_4 and HF; the latter represents the best solvent; it is extremely reactive and for this reason its use is avoidable.

In literature, several contributions are dedicated to acid attacks on titanium-based materials after mechanical treatments (e.g. turning, smoothing, blasting, ...) which, in general, mainly affect macroscopic surface characteristics. Only highly intensive acid attacks can further modify surface morphology, while acid treatments typically make surface defects flatten. As usual, the operator set temperature, acidic mixture composition (acid concentration) and treatment duration; then acid attack produces little defects: new roughness configuration is created over the original one [35].

For example, acid attack (HCl/H_2SO_4 or HF/HNO_3 mixtures) after blasting with 0.12–0.25 and 0.25–0.5 μm corundum has been performed on titanium implants [29]. More aggressive mixture produces finer surface defect dimensions, while less aggressive acidic solution induces finer roughness distribution (Table XVI).

Another paper considers the same abrasive particles for blasting, followed by acid attack with HCl/H_2SO_4 mixture [36]. A comparison among defect dimensions confirmed the rule of particle sizes in affecting surface morphology; roughness estimation has been performed by evaluating three parameters (Table XVII): Z_m, concerning the average value of defect depth; $R_{\max}$, representing the maximum defect height; V_u, known as ''void volume'' due to surface defects. Actually, no difference is appreciable among differently treated materials: acid attack decreases pollutant presence (i.e. Na, P, Ca, ...) and increases O titer (higher than stoichiometric).

As expected, mechanical treatments can modify surface reactivity to acid attack: it depends on how much original surface area has been increased and on how large is the real surface exposed to acidic mixture. Li *et al.* [35] compared titanium surfaces acid-attacked immediately after turning and after turning and blasting (Table XVIII). Remarkable differences in roughness parameter values are due to different characteristics in original surfaces.

The so-called SLA surfaces (sand-blasted large grit and acid-etched) are characterised by different topography and different chemical composition. In the paper by Wong *et al.* [34], three different materials have been investigated: titanium, Ti6Al4V and Ti6Al7Nb alloys. All the materials have been sand-blasted with corundum particles (300–400 μm) and acid-attacked (HCl/H_2SO_4

TABLE XV Surface properties determined on titanium surfaces blasted with alumina and carborundum particles of different size [24]

Material/treatment	R_a (μm)	CS (%)	RSA/A (–)	RSA (mm^2)	CRSA (mm^2)
Ti turned	1.25 ± 0.20	0.00	1.83 ± 0.30	51.80 ± 8.00	51.80 ± 8.00
Ti blasted with alumina (200 μm)	4.12 ± 0.10	16.10 ± 2.00	3.36 ± 0.30	95.00 ± 9.00	79.70 ± 9.00
Ti blasted with carborundum (200 μm)	3.67 ± 0.10	8.20 ± 1.00	3.56 ± 0.30	100.60 ± 9.00	92.50 ± 9.00
Ti blasted with alumina (600 μm)	5.98 ± 0.80	15.90 ± 4.00	4.28 ± 0.40	121.00 ± 10.00	101.80 ± 11.00
Ti blasted with carborundum (600 μm)	5.62 ± 1.80	10.80 ± 2.00	4.28 ± 0.40	121.00 ± 11.00	107.90 ± 11.00
Ti blasted with alumina (900 μm)	8.28 ± 2.00	14.40 ± 4.00	4.47 ± 0.50	126.40 ± 13.00	108.20 ± 14.00
Ti blasted with carborundum (900 μm)	7.45 ± 0.70	8.30 ± 2.00	4.51 ± 0.60	127.60 ± 17.00	117.00 ± 17.00

TABLE XVII Roughness properties determined on titanium surfaces preliminarily attacked with HF, then blasted with corundum particles, and finally attacked with HCl/H_2SO_4 [36]

Material/treatment	Z_m (μm)	R_{max} (μm)	V_u(μm^3)
Ti attacked with HF	10.31 ± 0.42	19.90	(66.64 ± 2.70) × 1E-4
Ti attacked with HF, blasted (corundum 0.12–0.25 μm) and attacked with HCl/H_2SO_4	9.55 ± 0.17	19.68	(61.09 ± 1.19) × 1E-4
Ti attacked with HF, blasted (corundum 0.25–0.5 μm) and attacked with HCl/H_2SO_4	11.52 ± 0.26	21.90	(74.64 ± 2.02) × 1E-4

TABLE XVIII Roughness properties determined on titanium surfaces acid-attacked after turning and after turning and blasting [35]

Material/treatment	R_a (μm)	R_{zDIN} (μm)	K (–)
Ti blasted (corundum 250–500 μm), acid-attacked	2.18	7.30	11.23
Ti turned and acid attacked	1.57	3.95	1.37

mixture, 5 min at 125–130 °C). Roughness dimensions decrease after acid attack for titanium but not for the alloys (see parameter S in Table XIV and XIX). Titanium surface exhibits higher defect density: little defects due to acid attack are homogeneously distributed on the macro-roughness imposed by blasting. As regards the general properties of SLA materials, the study by Taborelli *et al.* [18] concerns the physico-chemical characteristics of these surfaces; a further contribution comes from Perrin *et al.* [37] who investigated the mechanical performances of this kind of materials. Combination of mechanical and chemical treatments seems to determine anomalous deformability of implant threads. A comparison among implants obtained after blasting and acid attack (hot temperature), implants further exposed at 200 °C (till hydride disappears due to thermal decomposition), and implants intentionally damaged on the thread heads, has been carried out. Even if a detailed explanation of the surface behaviour is still lacking, Perrin *et al.* [37] stated that material deformability can be due to its surface topography, which in turn affects surface hydrophilic properties and, consequently, the capacity of the surface to interact with biological tissues.

In the paper by Taborelli *et al.* [18] the effects of different mechanical treatments and acid attack on commercially pure Ti have been also evaluated. In particular, Ti surfaces have been smoothed with alumina powder, others have been sand-blasted with particles of 250–500 μm. The defects obtained on the surfaces, before and after acid attack with HCl/H_2SO_4, have been measured with SFM; two roughness parameters have been reported (Table XX). It is worthwhile noting that the original surface after treatment with alumina powder is smooth and the acid attack produces large defects; indeed, larger defects are obtained on sand-blasted surfaces after acid attack: irregular and rough surface properties clearly appear so that a precise scansion of the surface profile is hindered. This fact makes the estimated roughness parameters poorly affordable with specific regard to the micro-roughness properties. In another paper, the enlargement in the surface area of the previously treated samples has been measured (Table XXI) as an estimation of the capacity of each treatment to improve the contact area between biomaterial and biological environment [19].

An interesting investigation has been carried out by Klokkevold *et al.* [38] on titanium surfaces turned and acid-attacked; after SEM analysis at different magnifications, these authors reported roughness values as measured by means of optical profilometry (Table XXII). Two parameters, that is, SA (which describes the surface area) and SAI (which corresponds to the ratio between the real surface area and its geometrical projection), have been measured along the height of cylindrical turned elements, at the top and bottom of the threads. It is interesting to note that acid treatments results in a deep modification of the surface topography, as confirmed by SEM analysis. Roughness parameters, anyhow, are not suitable to appreciate what SEM pictures show: there is a difference in the amount of the attack among surface areas at the top and bottom of the threads. Actually, in this case the profilometry data are not sufficient to describe the real distribution of surface defects on the acid-attacked surfaces.

A similar comparison between turned and acid-attacked surfaces has been illustrated by Abrahamsson *et al.* [39] on titanium-made abutments (8.5 mm length, 3.5 mm diameter). By laser profilometry analysis, 245 μm^2 areas on the samples have been scanned; surface roughness has been described by the parameters (Table XXIII) discussed in Wenneberg *et al.* [30].

3.2. Alkaline attacks

Chemical treatments do not include mineral acids only, but they can be performed with NaOH or other alkaline concentrated aqueous solutions. In this case, the high pH values improve the formation of –OH group on the TiO_2 protected surface; titanate salts form and dissolve so that the surface is progressively covered by a hydrogel that can locally precipitate. The underlying metal topography is then modified and its original roughness properties change. When operating at a higher than room temperature, the chemical reaction kinetic is accelerated and sub-micrometric defects are normally produced: for

TABLE XIX Roughness parameters determined on titanium surfaces after blasting and acid-attack [34]

Material/treatment	R_a (μm)	S (μm)
Ti blasted (corundum 300–400 μm) and acid-attacked	1.80 ± 0.20	4.90 ± 0.50
Ti6Al4V blasted (corundum 300–400 μm) and acid-attacked	2.30 ± 0.20	13.00 ± 3.00
Ti6Al7Nb blasted (corundum 300–400 μm) and acid-attacked	2.10 ± 0.05	11.80 ± 2.00

TABLE XX Roughness parameters determined on titanium surfaces after blasting, and before and after acid-attack [18]

Material/treatment	R_t (nm)	R_{ms} (nm)
Ti smoothed with alumina powder	81	6
Ti smoothed with alumina powder and acid-attacked	2100	190
Ti sand blasted (0.25–0.50 μm) and acid-attacked	3600	660

TABLE XXI Sample areas improvement on Ti samples due to different treatments [19]

Material/treatment	(–)
Ti smoothed with alumina powder	1.081 ± 0.047
Ti smoothed with alumina powder and acid-attacked	1.440 ± 0.247
Ti sand blasted (0.25–0.50 μm) and acid-attacked	2.455 ± 0.146

TABLE XXII Roughness parameters measured at the top and bottom of the threads of Ti cylindrical elements turned and turned and acid-attacked [38]

Material/treatment	R_a (μm)		R_q (μm)		SA (nm^2)		SAI (–)	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Ti turned	0.185 ± 0.069	0.200 ± 0.075	0.241 ± 0.094	0.246 ± 0.085	575.8 ± 41.0	528.5 ± 13.9	1.05 ± 0.03	1.01 ± 0.02
Ti turned and acid-attacked	0.494 ± 0.145	0.380 ± 0.075	0.629 ± 0.242	0.485 ± 0.092	1074.6 ± 192.9	965.5 ± 77.7	2.13 ± 0.38	1.91 ± 0.15

TABLE XXIII Roughness parameters determined on titanium turned abutments before and after hot acid-attack [39]

Material/treatment	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.22	12.3	1.03
Ti turned and acid-attacked	0.45	11.9	1.09

TABLE XXIV Roughness parameters determined on turned titanium implants before and after alkaline attack, and after rabbit's plasma deposition [42]

Material/treatment	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.79	9.35	1.26
Ti turned and covered with rabbit's plasma	0.56	8.59	1.16
Ti turned and NaOH-attacked	0.68	8.74	1.19
Ti turned and NaOH-attacked, and covered with rabbit's plasma	0.55	8.72	1.15

TABLE XXV Average roughness measured on Ti6Al4V implants after smoothing, after passivation and after hot water treatment [50]

Material/treatment	R_a (nm)
Ti6Al4V smoothed	0.99
Ti6Al4V smoothed and passivated	1.29
Ti6Al4V smoothed and exposed to hot water	0.56

TABLE XXVI Roughness parameters measured on Ti implants after mechanical and electrochemical treatments [15]

Material/treatment	R_{rms} (nm)	A_{diff} (%)
Ti turned	30.3 ± 19.8	10.8 ± 7.6
Ti turned and anodised	40.8 ± 14.7	18.0 ± 8.2
Ti turned and electro-polished	2.9 ± 2.9	0.5 ± 0.4
Ti turned, anodised and electro-polished	32.5	23.3
Ti turned, anodised and electro-polished (smooth areas)	2.7 ± 0.2	0.6 ± 0.1
Ti turned, anodised and electro-polished (rough areas)	116.7 ± 40.2	88.0 ± 35.0

this reason, the attack is usually carried out at more than 100 °C; after drying, the surface is no more hydrophobic because of the –OH groups that can be also used to covalently bind other molecules.

The alkaline attack has been performed onto the surface of titanium-made endosseous dental implants (7 mm length, 3.75 mm diameter) as described in Albrektsson *et al.* [40] and Adell *et al.* [41]. Another study has been presented in a recent paper [42] comparing turned and alkaline-attacked implants; moreover, turned and attacked surfaces have been separately covered with rabbit's plasma (100 μm average thick-

TABLE XXVII Roughness parameters measured on Ti and Ti6Al4V surface after electro-erosion at different conditions [17]

Material/treatment	R_a (μm)	R_q (μm)	R_z (μm)	LAC (–)	Order (–)	Densità (1/in)	S_M (μm)	Delta (–)
Ti electro-eroded (3 A)	3.5 ⟨1.42⟩	24.6 ⟨11.87⟩	20.16 ⟨9.96⟩	20.4 ⟨6.19⟩	11.3 ⟨10.29⟩	457 ⟨949⟩	54.8 ⟨26.5⟩	1.19 ⟨1.31⟩
Ti6Al4V electro-eroded (3 A)	3.29 ⟨1.35⟩	23.35 ⟨11.51⟩	19.1 ⟨9.61⟩	20.1 ⟨6.05⟩	10.9 ⟨9.52⟩	462 ⟨955⟩	54.0 ⟨26.4⟩	1.15 ⟨1.23⟩
Ti electro-eroded (0.25 A)	3.0 ⟨1.25⟩	0.73 ⟨11.33⟩	16.7 ⟨8.97⟩	17.4 ⟨6.26⟩	10.0 ⟨10.15⟩	539 ⟨981⟩	50.8 ⟨25.9⟩	1.20 ⟨1.33⟩
Ti6Al4V electro-eroded (0.25 A)	2.22 ⟨1.23⟩	15.8 ⟨10.17⟩	13.3 ⟨8.60⟩	12.6 ⟨5.95⟩	8.7 ⟨9.78⟩	598 ⟨966⟩	41.9 ⟨26.1⟩	1.16 ⟨1.25⟩

Micro-roughness values are given within brackets.

TABLE XXVIII Roughness parameters measured on Ti surfaces after anodisation and coating with rabbit's plasma [42]

Material/treatment	S_a (μm)	S_{cx} (μm)	S_{dr} (–)
Ti turned	0.79 ± 0.2	9.35 ± 1.7	1.26 ± 0.17
Ti turned and coated with rabbit's plasma	0.56 ± 0.1	8.59 ± 0.8	1.16 ± 0.45
Ti anodised	1.08 ± 0.2	10.98 ± 0.8	1.37 ± 0.09
Ti anodised and coated with rabbit's plasma	1.20 ± 0.2	10.88 ± 0.7	1.42 ± 0.12

TABLE XXIX Roughness parameters measured on Ti surfaces after pickling and electro-polishing [36]

Material/treatment	Z_m (μm)	$R_{\max}$ (μm)	V_u (μm^3)
Ti decapped	10.31 ± 0.42	19.90	(66.64 ± 2.70) X 1E-4
Ti decapped and electro-polished	5.02 ± 0.16	9.56	(32.02 ± 1.15) X 1E-4

TABLE XXX Roughness parameters measured on Ti surfaces after blasting with rutile and after HA deposition [62]

Material/treatment	R_a (μm)	S_m (μm)
Ti blasted with rutile	1.3	21.2
Ti blasted with rutile and HA-coated (0.1 μm)	1.3	19.0
Ti blasted with rutile and HA-coated (1 μm)	1.4	21.3
Ti blasted with rutile and HA-coated (4 μm)	2.1	21.5

TABLE XXXI Average roughness parameter values measured on Ti surfaces after blasting with rutile and after HA deposition [28]

Material/treatment	R_a (μm)
Ti turned	0.31 ± 0.12
Ti blasted with rutile (10–53 μm)	0.61 ± 0.03
Ti blasted with rutile (10–53 μm) and HA-coated	1.89 ± 0.15

TABLE XXXII Roughness parameters measured on Ti6Al4V surfaces after blasting with glass beads and corundum and after HA deposition [34]

Material/treatment	R_a (μm)	S (μm)
Ti6Al7Nb blasted with glass beads (150–250 μm)	1.40 ± 0.30	11.80 ± 2.05
Ti6Al7Nb blasted with corundum (300–400 μm)	2.70 ± 0.04	11.80 ± 0.90
Ti6Al7Nb blasted with corundum (300–400 μm) and HA-coated	6.48 ± 0.29	20.83 ± 0.60

TABLE XXXIII Roughness parameters measured on Ti surfaces after pickling, blasting and TPS coating [36]

Material/treatment	Z_m (μm)	$R_{\max}$ (μm)	V_u (μm^3)
Ti decapped	10.31 ± 0.42	19.90	(66.64 ± 2.70) × 1E-4
Ti decapped, blasted (0.25–0.5 μm) and TPS-coated	18.28 ± 1.01	39.80	(117.92 ± 7.02) × 1E-4

ness), which required a preliminary treatment with rabbit's fibrinogen [43, 44]. Different sample areas $(245 \times 245\ \mu m^2)$ taken at different positions on the implant surface, have been scanned by laser profilometry obtaining the roughness parameters depicted in Table XIV. Artifacts due to implant geometry have been corrected by a Gaussian filter [45]. The results obtained suggest that there is no significant influence of the biological layer covering the metallic surface, even turned or attacked; anyway, the biological layer reduces surface roughness dimensions. Original surfaces are different: the turned one is rougher than the attacked one.

3.3. Other chemical methods

It is worthy to mention that titanium is not always the material of choice in biomedical application; sometimes, titanium alloys are preferred due to their improved mechanical performances. In these cases, it is necessary to evaluate the possibility of ion release from the surface: many contributions are available, investigating the rule and the amount of ion release when the alloy is in deep contact with the biological environment. For a short discussion on this topic see Callen *et al.* [46], Amstutz *et al.* [47], Jacobs *et al.* [48] amd Liao *et al.* [49]. It is a general rule to apply opportune treatment on the material surface to reduce and/or avoid ion release with potential toxic effect able to evoke adverse responses in the patient's body. For example, it is possible to induce a chemical passivation using HNO_3, to heat the surface by exposure to hot air or hot water. These treatments, which usually require a prolonged application, can introduce –OH groups on the surface, so modifying its chemical properties. On the other hand, neither hot treatments nor chemical passivation can deeply modify surface topography. In a paper by Ku *et al.* [50] the average roughness of different Ti6Al4V based substrates is examined (Table XXV); this parameter cannot reveal great differences on the differently treated surfaces, while AFM analysis can.

Further studies [51, 52] investigated the differential effects of hot water and HNO_3 on Al^{+++} release: hot water does reduce (0.84 μM) the amount of ion release after simple passivation (5.55 μM). Furthermore, the presence of vanadium ions in the biological environment is appreciable.

3.4. Electrochemical methods

From a general point of view, the electrochemical processes are performed connecting the metallic device to the positive pole of an electrical circuit. The device is immersed in an electrolytic solution (not always aqueous) containing ionic substances or oxidants. In this situation, the surface of the device is the anodic electrode and the potential applied from external supply governs the course of the process.

Many methods were originally set for the electrochemical production of aluminium and they were successively extended to the treatments of titanium-based materials. Three different methods can be identified: electro-erosion, electro-polishing, and anodisation. The first method produces a localised melting of the metallic material, the second one provides for a fine dissolution of surface defects, with a smoothing effect on micrometric scale. The third method (anodic oxidation) allows the oxide layer to grow from the usual 5–10 nm of atmospheric oxidation up to 40 μm. In the specific case of titanium substrates, it is likely to appreciate that electrochemical methods can be applied to obtain a wide range of surface properties with a variety of finishing degrees due to the opportunity of a fine tuning of the process conditions. Larsson *et al.* [15] illustrated the different surface modifications obtained on the same kind of titanium implant: 96 dental implants (4 mm length and 3.75 mm diameter) were mechanically turned and threaded, and thoroughly washed and sonicated in different solvents. Afterwards, implants have been divided into four groups and differently processed (24 samples have been used as control). The electro-polishing treatment has been performed into a cryogenic mixture (MeOH : n-BuOH : HCl at 240 K), applying 22 V at the anodic substrate. Surface defects dissolution concerns a 100 μm depth layer, thus involving the metallic material stressed by plastic modification due to previous mechanical processes. Chemical residues (i.e. alcohol, acids, and dissolution products) are removed by methanol washing.

On the other hand, anodic oxidation is carried out in 1 M acetic acid solution, at 20–25 °C, applying 80 V as anodic potential, avoiding electrical discharge phenomena; oxide layer grows linearly with a constant α of 2–3 nm/V. After anodisation, samples have to be washed to remove electrolyte residues from the surface. Surface characteristics are then determined by AFM measurement of two roughness parameters: R_{rms} (integral root mean square of the deviations of surface profile from the average line) and A_{diff} (representing how greater than unity is the ratio between the real surface area and its geometrical projection). The first parameter directly describes surface roughness while the second represents how much the surface is extended by the presence of defects whose depth values vary from few nanometers (instrumental resolution) up to 1 μm (1 μm^2 is the area of the scanned surface samples). Surface chemical composition has also been determined by Auger spectroscopy but it does not reveal great differences among the differently treated materials. Ti, O and C are the major components: in particular, the presence of C is mainly due to pollutants of organic origin. The O : Ti ratio is over the stoichiometric value, and this is probably because of the presence of –OH and =C=O groups. Other chemical elements found are: Si, S, P, Ca. It is worthwhile noting that the thickness of the surface layer is minimum for the electro-polished surface (< 5 nm), and maximum for the anodised one (180–200 nm). Furthermore, SEM analysis shows that there is a pattern analogy between turned and anodised surfaces: these latter maintain the typical profiles of the turning machine tool and can only superimpose a finer roughness (not over 1 μm scale). These patterns can be removed by electro-polishing; in particular, on the anodised and electro-polished samples it is possible to distinguish between smoother and rougher areas. Anyway, only electro-polished surfaces are clearly different from the others as reported in Table XXVI.

A different technique (i.e. electro-erosion) is examined in Bigerelle *et al.* [17]. In this paper, cylindrical metallic samples are treated in deionised water and the Cu–Zn wire (0.25 mm diameter) is moved closer to the substrate. Due to the electric power applied to the wire, a local melting of the substrate is produced so that it is cut. Generally, this process is performed twice: a 3 A current is used for cutting, a lower current (0.25–1 A) for refining the material. Disk samples are divided into four groups: two treatments and two materials (Ti and Ti6Al4V), examined under contact profilometry. A set of eight parameters have then been measured on macro- and micro-scale (Table XXVII): the first three parameters concern defect depth, the other five amplitude, periodicity and organisation of surface defects. In order to reduce the artifacts originated by sample shape, a Gaussian filter was imposed at 50 μm (considered the average size of human osteoblast cell). Surface chemical composition and phase distribution were evaluated by means of X-ray diffractometer. As confirmed also by SEM analysis, there are no significant differences among different surfaces on macro–metric scale. Electro-eroded surfaces appear to be highly homogeneous.

At present, endosseous Ti-made implants are commercially available (7 mm length, 3.75 mm diameter) whose threaded surfaces have been anodised: their morphology is characterised by 1–2 μm pore distribution and 2–7 μm thickness of the surface layer [53].

An interesting comparison on differently treated Ti samples (turned, anodised and coated with rabbit's plasma) has been discussed in Göransson *et al.* [42]. Surface topography has been described by three roughness parameters: anodised samples are clearly different from the others, but the plasma layer does not influence the original surface topography (Table XXVIII).

In a further paper [54] the effects of different anodising operative conditions were investigated in terms of TiO_2 layer growth on Ti substrates. The following variables have been taken into consideration: temperature (14–42 °C), electrolytes (H_2SO_{4aq}, H_3PO_{4aq}, CH_3COOH_{aq}, $NaOH_{aq}$ and $Ca(OH)_{2aq}$) and their concentrations (0.1–1 M), stirring velocity (250–800 rpm), anodic potential (20–130 V), current density on anodic surface (5–40 mA/cm^2), and the anodic/cathodic surfaces ratio (1–9%). On the other hand, four variables describe the behaviour of the oxide layer: growth constant α (nm/V), the growth rate u (nm/s), oxide layer thickness d (nm), and the anodic efficiency $\varepsilon(\mathrm{nm} * \mathrm{cm}^2/\mathrm{C})$. The substrates used for the experimental trials are represented by plane ($30 \times 10 \times 1\,\mathrm{mm}^3$) or cylindrical (18 mm length, 3.75 mm diameter) elements. Opportune teflon-made supports allow to hold the samples (titanium samples and platinum counter-electrodes) avoiding electrical dispersion which could change current density on the electrodes. During the anodising treatment, electrical potential is continuously monitored; afterwards, a complete set of physico-chemical characteristics is taken into consideration: luminosity, hue and saturation of the surface by spectrophotometric means; surface chemical composition by Auger spectroscopy equipped with ion etching probe. The oxide layer thickness is determined as the depth which corresponds to the chemical transition from TiO_2 to TiO (spectroscopic O signal is halved).

Electrochemical methods allow already HF-treated surfaces to be altered. In Martin *et al.* [36] commercially pure titanium discs (1 mm thickness) have been thoroughly washed after chemical attack and then sterilised. Afterwards, disc surfaces have been electropolished and their characteristics (chemical composition, morphology and topography) have been examined by means of SEM and optical microscopy analysis, LSM profilometry and Auger spectroscopy. While optical microscopy images demonstrate a smoothed surface, SEM investigation (1000 ×) reveal surface defects as typical of the acid attack. Profilometry data include average and maximum values of defect depth (Z_m and $R_{\max}$, respectively) and volume included by the defects themselves (V_u); these parameters are taken as means of five measurements on different sample areas ($256 \times 256\,\mu\mathrm{m}^2$). In Table XXIX profilometry parameter values are summarised, which indicate that electropolishing can dramatically reduce defect dimensions. As regards chemical composition, Ti, O and C are the predominant elements: Ti and O belong to the oxide formed on the surface, even though they are present in the stoichiometric ratio only at 100 Å under the external layer; C is due to environmental pollutants.

3.5. Deposition methods

Other methods are usually performed to change surface properties of several materials used for biomedical application: these processes are based on the deposition of foreign chemical substances on the surface under treatment. Metallic compounds (e.g. Ti), oxides and ceramics are often applied by being volatilised due to the exposure to an energy supplying source, and then transported onto the surface. Considering the composition of the mineral part of the bone tissue, since the 1970s endosseous implants have been coated with an hydroxyapatite (HA) layer suitable for improving implant integration with the surrounding biological environment. The integration process is promoted by surface roughness due to the newly deposited external layer that even represents a Ca source; furthermore, HA is converted into the more stable fluorapatite.

As mentioned above, volatilisation of coating compounds requires high energetic sources which can be obtained by means of combustion (C_2H_2/O_2 mixture), electro-voltaic arc and plasma spraying. Many papers suggest that the improvement in the surface roughness due to chemical deposition treatments, allows a parallel improvement in terms of implant biomechanical performances [25, 27, 29, 30, 55–57]. More recently, efforts in the direction of applying ''bioactive'' substances on the surface of endosseous implants have been proposed. As already acknowledged in the scientific literature, bioactive compounds are those able to activate specific interaction mechanisms between implant and biological tissue, thus stimulating new bone tissue growth around implanted devices. In this sense, HA, which is deemed to be one of the most effective bioactive materials, is usually applied by means of magnetron sputtering techniques [58–61]. In the work of Vercaigne *et al.* [62] rutile-blasted Ti discs are used as substrates for HA deposition under different experimental conditions, achieving HA surface layer of 0.1, 1 and 4 μm depth. After HA deposition, performed under low pressure (0.5 Pa) and with a rate of 100–150 nm/min, discs are sonicated and sterilised; disc surfaces are then examined with a laser profilometer and SEM, and HA coatings are analysed with X-ray difractometer. From profilometry data (R_a and S_m) in Table XXX, it is possible to appreciate that only the thickest coating can appreciably improve surface roughness. X-ray analysis reveals that HA structure is amorphous-crystalline with the presence of tetra-calcium phosphate.

So far, roughness is acknowledged as a crucial factor in promoting implant integration with biological tissue [56] and to stimulate the healing process [55, 63]; an interesting study has been published comparing two techniques used for improving surface roughness properties: that is, blasting and ceramics coating [28]. The first one operates by subtracting surface materials, the second one by adding foreign compounds. Ti implants (10 mm length, 3.5 mm diameter) have been turned, then blasted with rutile of mixed particle sizes, finally coated with HA by ionised gas gun [64]. Plasma deposition allows obtaining an HA average thickness value of 55 ± 15 µm; roughness characteristics have been determined by laser profilometry (Table XXXI). SEM images reveal that both blasted and HA coated surface appear rough, but defect dimensions are largely different.

For a more detailed comparison of surface characteristics, other authors proposed to evaluate not only the intrinsic roughness values, but also the spacing between surface defects: this latter can been assumed as the volume available on the material surface to allow bone cells attaching and adhering. Wong and coworkers [34] compared few surface treatments applied on Ti6Al7Nb cylinders (10 mm length, 3.55 mm diameter) (Table XXXII). SEM images show differences on surface topology: glass bead-blasted surfaces appear irregular, while corundum-blasted ones are more homogeneous. HA-coated samples are characterised by the typical aspect of the ceramic material. As to roughness properties, it is worthwhile pinpointing that all the surfaces under investigation are rather rough, but the HA-coated ones exhibit the highest values in terms of average roughness and spacing. With specific regard to the latter parameter, it is to note that blasted surfaces have overlapping values.

In 1991 Buser *et al.* [29] compared surface characteristics in terms of defect spacing between HA-coated and TPS-treated cylindrical Ti samples (10 mm length, 3.55 mm diameter): both the surfaces have defect dimensions from 30 up to 50 µm, but HA-coating largely modifies surface composition.

Further investigations on TPS surfaces have been carried out by Martin *et al.* [36] comparing roughness properties of Ti surface after pickling, and after blasting and TPS coating (by volatilisation of TiH_2 powder). Surface state has been analysed by means of optical and electronic microscopy (SEM) and laser profilometry; Auger spectroscopy allowed determining surface chemical composition. Optical microscopy images reveal that TPS surfaces are highly irregular and rough in comparison with not-treated samples that are smoother. At higher magnification, SEM images demonstrate that the surface is also characterised by several cracks separating globular (10–20 µm) and sheet-shaped defects; other defects are of 0.1 µm. In Table XXXIII average and maximum values of surface defect depth, and the V_u volume are reported, confirming that TPS samples are rougher than the others. As to chemical composition, three are the most abundant elements: Ti, O and C, but also Ca, P, Cl and Na appear as microelements, whose presence is due to chemical treatments and washings.

It cannot be neglected that the surface roughness values are not sufficient to explain the biological responses of endosseous implants [65]: in fact, some authors stated that not surface roughness but microtopography is the critical parameter in stimulating interaction mechanisms which osseo-integration is based on (Klokkevold *et al.* [38]). SEM images and profilometry data have been taken on endosseous threaded implants (4 mm length, 3.25 mm diameter) turned and then TPS-coated. Table XXXIV illustrates profilometry parameter values considering defect height (R_a and R_q) and the developed surface due to roughness improvement (SA and SAI): measurements have been taken both at the top and bottom of implant threads. SEM images reveal that turned surfaces are largely less rough than TPS treated ones, which are covered by globular defects separating by cracks and carvings. Differences between the two kinds of surfaces are appreciable also in terms of defect organisation, which characterises surface topography: TPS-coated surfaces exhibit a more complex surface structure.

4. Biochemical treatments

To complete this review the so-called biochemical treatments must be mentioned, which have been recently addressed by a large number of interesting investigations, both *in vitro* and *in vivo*.

Basically, these treatments are aimed at controlling/guiding the complex sequence of biochemical phenomena that take place at the interface between implanted devices and biological tissues, thus improving osseointegration. Therefore, it is necessary to possess at least a rough description of these phenomena and to identify the molecules involved. Even though a detailed picture of the interaction mechanisms governing osseointegration is not yet available, a preliminary explanation has been provided by Kasemo and Gold [2]. These authors described the cascade of events, on molecular and on cell scale, which follow the surgical insertion of an implant into the bone tissue. The behaviour of the bone cells after adhesion, until new tissue formation, has also been investigated by Ramires *et al.* [1]. It is well acknowledged that the characteristics of the implant surface can determine the amount of cell adhesion, proliferation and differentiation, and finally the quality of the tissue growth [4, 8]. As a consequence, the biomechanical performances of an implanted device largely depend on the properties of its surface, in terms of both chemical composition and roughness [5, 7, 13, 16, 17, 66].

TABLE XXXIV Roughness parameters measured on the top and bottom of the threads of Ti samples after turning and TPS coating [38]

Material/treatment	R_a (µm)		R_q (µm)		SA (nm^2)		SAI (–)	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Ti turned	0.185 ± 0.069	0.200 ± 0.075	0.241 ± 0.094	0.246 ± 0.085	575.8 ± 41.0	528.5 ± 13.9	1.05 ± 0.03	1.01 ± 0.02
Ti turned and TPS coated	7.01 ± 2.09	10.02 ± 2.76	9.46 ± 2.72	13.11 ± 3.39	51326.7 ± 35907.7	82475.4 ± 32592.9	20.70 ± 12.32	30.84 ± 12.19

Many efforts have been recently aimed at enriching an implant surface with specific biomolecules, investigating the physiological process of osseointegration; this procedure allows obtaining bioactive surfaces, able to improve integration. These molecules (i.e. growth factors and adhesive proteins) are normally present on cell membrane and extra-cellular matrix, or both [5, 67]. Among the other proteins, many studies have been focused on the family of transforming growth factors beta (TGF-β) and bone morphogenetic proteins (BMPs) for their potential applications *in vitro* and *in vivo* [68–71]. Indeed, several drawbacks are related with the application of an entire protein mainly due to the low chemical stability and solubility into the biological environment, and the cost; these reasons suggest an alternative approach that is termed ''peptide-mimicry''. It consists in the identification and application of the biologically active sequences belonging to the longer proteins: these sequences, also known as ''bioactive peptides'', are more stable and soluble, and can be reproduced by chemical synthesis at lower costs.

One of the most investigated biologically active peptides is the Arg–Gly–Asp (RGD) amino acidic sequence, known to be the minimal cell-recognisable sequence in many adhesive proteins [72, 73]. Up to now, many other peptides have been studied and utilised for both *in vitro* and *in vivo* experimental trials [73, 74].

Basically, three different methods are available for the biochemical treatment of a metallic surface: (i) simple physico-chemical adsorption of the active molecule on the surface; (ii) covalent binding, direct or through a spacer; (iii) enrichment of a biocompatible and bioresorbable carrier with the active molecule and its application as coating material on implant surface.

4.1. Adsorption

This is the easiest, and cheapest, approach to the biochemical functionalisation of a surface: it is performed by a simple immersion of the metallic sample into a bioactive peptide containing solution. Of course, this method does not allow a controlled deposition of the peptide, thus hindering a precise determination of its surface density, which is of critical importance in controlling the interactions with biological tissues. Furthermore, molecules merely adsorbed onto implant surface can be displaced and eventually diffuse away from the site and, perhaps, may even distribute sistemically [75].

4.2. Covalent attachment

From a general viewpoint, this method is based on the exploitation of the chemical functionalities already present on the material surface, to covalently bind the bioactive peptide, either directly or through a spacer. Usually, –OH groups on the materials surface are utilised; many strategies have been developed to improve the number of the reactive groups [75–78]. A particular approach has been proposed by Ferris *et al.* [79] by covering the metallic surface with gold: cystein-containing peptides exhibit high affinity for the gold atoms and a covalent binding forms by overnight immersion of the metallic sample into a solution of the peptides. This technique rarely controls surface density of the peptides, which can affect biological response to implant insertion [80].

4.3. Peptide inclusion into carrier materials

This technique precisely controls the amount of bioactive peptide introduced into a carrier material, which is used to coat implant surface. Many materials are currently used as carriers/coatings: polylactic and polyglycolic acids [81] and co-polymers thereof [82], hydrogels [70], hyaluronic acid [83], polypyrrole [84]. All of them must be biocompatible, biodegradable and bioresorbable, and must not elicit unfavourable body responses. Carriers can be simply impregnated with the biologically active molecules or these molecules can be covalently bound to the carrier structure.

5. Final remarks

Nowadays the relationship between the surface of an endosseous implant, its reactivity with biological tissue constituents, its long-term integrity and clinical efficacy are not clearly understood. Nevertheless, titanium and titanium alloys are largely used as biomaterials for orthopaedic and dental applications. Many studies have independently focused surface characteristics (i.e. topography and chemistry), identifying the factors which mainly affect implant performances. In particular, it has been already proved that the surface roughness and the chemical composition of the outer layer play a key role in determining tissue response. In fact, these properties directly influence (i.e. promote or hinder) the phenomena occurring at the interface between implanted device and biological environment.

For these reasons, a huge number of scientific contributions, many of which have been cited in the present paper, are centred on how and how much surface properties, not independently but simultaneously, can be modified to promote osseointegration, thus improving implant performances.

It must be appreciated that implantology research has recently moved towards the development of a new generation of biomaterials for surgical applications, able not only to optimise interactions with tissue cells, but also to promote controlled, guided and rapid osseointegration. This is possible by means of biochemical methods of surface modification, which allow the immobilisation of biologically active molecules on biomaterials for the purpose of inducing specific cell and tissue responses.

It is possible to foresee that two principal areas of research will be developed in the near future:

1. Many efforts will be aimed at achieving a deeper understanding of the phenomena involved in the interactions between tissue and materials: a detailed knowledge of the biochemical mechanisms governing body's responses to endosseous implant insertion will surely open new perspectives in the experimental activities.

2. As a consequence, it will be possible to identify new molecules to be used as biochemical signals promoting cell adhesion and growth, thus improving implant integration.

In conclusion, the creation of biomimetic materials able to direct the formation of tissue surrounding implants, represents one of the most promising challenges of research in the field of biomaterials.

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