SciMedCentral

JSM Dental Surgery

Review Article

A Biomimetic Surface for Immediate and Early Loading of Dental Implants Surface Characterization and Results from Histological Studies

Albertini M[1]*, Herrero-Climent M[2], Nart J[1], Falcao C[2], and Gil FJ[3]

[1]*Department of Periodontology, International University of Catalunya, Spain*
[2]*Private practice, Porto Dental Institute, Portugal*
[3]*Faculty of Dentistry, International University of Catalunya, Spain*

***Corresponding author**

Albertini M, Department of Periodontology, Universidad Internacional de Catalunya, Campus SantCugat, JosepTrueta, 08195 SantCugat del Vallès, Barcelona, Spain, Tel: 34670485694; Email: malbertini@uic.es

Submitted: 27 October 2016

Accepted: 26 November 2016

Published: 28 November 2016

Keywords

- Dental implants
- Immediate loading
- Early loading; Implant surfaces
- Biomimetic surfaces
- Hydroxyapatite

Abstract

The main purpose of this paper is to describe the characteristics of a biomimetic implant surface obtained by a two-step procedure, which combines grit-blasting with a thermo-chemical treatment. This method produces a crystalline hydroxyapatite with the same mineral content as human bone that is chemically bonded to the titanium surface instead of the amorphous calcium phosphate produced with a plasma-projection treatment. This surface favors an accelerated bone healing and de-novo bone formation at the early phase of healing, making it ideal for immediate or early loading. Chemical treatment, surface characterization, biological mechanism as well as histological results are discussed.

INTRODUCTION

Osseointegration is a well-known biological phenomenon that allows us to restore function and aesthetic in partially or totally edentulous patients through implant-supported prostheses [1,2]. Implant surface characteristics have been demonstrated to be one of the most important factors for early stages of bone healing to reach successful osseointegration [3].

Thanks to advances in technology and bioengineering, development of new implant surfaces has been possible in the last years, leading to a better response of bone cells during healing process, therefore a more predictable osseointegration can be achieved [4].

The development of moderately rough surfaces, through sandblasting and acid-etching procedures, has allowed a faster osseointegration making immediate and early loading more predictable [5].

Implants have recently been provided with bio-active surface treatments by introducing certain molecules which, in contact with blood and bone cells, are able to produce a further enhancement in osseointegration at early phases of bone healing as proved by experimental studies [6-8].

Hydroxyapatite coatings have been used on implant surface due to the increased affinity of the bone to calcium phosphate with good results in the short term [9-11]. However some plasma-sprayed Hydroxyapatite-coated implants have shown a significant failure rate in the long term [12]. This was due to the poor adherence of the apatite layer to the titanium surface, as a result of the high temperatures used in the formation of the amorphous calcium phosphate. This fact led to the bacterial infiltration of the interface and ultimately the progressive bone loss.

Kokubo described an alternative method for a more homogeneous and chemically stable calcium phosphate coating [13,14]. This method allows an in-vitro apatite growth directly bound to the surface, thus achieving a greater adherence and better control in the layer thickness.

Based on Kokubo's studies our research group has recently developed a novel implant surface called Contac-Ti [15, 16], which is based on the combination of a subtraction procedure and an optimized thermo-chemical treatment for moderately rough of titanium surfaces.

The aim of this paper is to describe the biological principals, characteristics and the method used to attain this new implant surface as well as to analyze and discuss the histological evidence of the early phases of bone healing around dental implants provided with this surface.

The evolution of hydroxyapatite coatings

Osseointegration is a time related biological process that allows dental implant to be subjected to functional loading, and implant surface seems to be one of the most relevant factors obtain a predictable bone healing [17].

Recent years have witnessed a progressive development of dental implants and many resources have been invested to improve implant surfaces and improve clinical results when using immediate and early loading procedures.

The use of coatings with similar composition of the human bone is an attractive strategy in the development of so-called bioactive surfaces, which provide an accelerated osseointegration during the earliest healing stages. Particularly, calcium phosphate apatite has the same chemical composition as the mineral bone phase, so that complete acceptance by the organism and no inflammatory reaction occurs [9]. Many researchers have applied coatings on titanium implants by different techniques such as hydroxyapatite plasma spraying [8]. As demonstrated by clinical studies [11], this treatment produced a quicker osseointegration at early stages after implant placement but an accelerated bone loss due to a bacterial micro-leakage between the hydroxyapatite layer and the titanium has been observed in the long term [12]. Furthermore, additive techniques such as hydroxyapatite plasma spraying do not allow the formation of crystalline apatite like in human bone, but amorphous calcium phosphate due to high elaboration temperatures [18]. The properties of this layer are not considered appropriate for dental implants, since they are extremely soluble and titanium only achieves mechanical retention, not true adhesion.

Bioactivities of titanium: the thermo-chemical method

Bioengineering studies have recently proven that alternatives methods to obtain phosphate calcium coating with higher homogeneity and chemical stability are possible [19]. These methods, propose apatite growth directly bound to the surface as a result of a precipitation reaction in the human body fluid, thus achieving true chemical adhesion and layer-thickness control.

Human body fluid is supersaturated in apatite even under normal conditions and the prerequisite for apatite formation on an artificial material in a living body is the presence of functional groups that could be an effective site for apatite nucleation on its surface [20].

Based on this principle, Kokubo [14], proposed a method to provide implants with a bioactive surface based on a thermo-chemical procedure where titanium, is first chemically treated with alkali solutions and then subjected to heating at high temperatures. The aim of this treatment is to reproduce the in vivo formation of crystalline hydroxyapatite on implant surface therefore accelerating bone healing and osseointegration.

The chemical treatment, as described by the author, consist of soaking the implant in a 5-10M NaOH aqueous solution at 60°C for 24h and then a gentle washing with distilled water. The thermal procedure consists of heating the implants in an electrical furnace to various temperatures below800°C at a rate of 5°C·min-1, kept at the temperature for 1h and allowed to cool to room temperature in the furnace.

Biomimetic formation of hydroxyapatite on the implant surface

Titanium is generally covered with a thin TiO2 passive layer, which provides chemical stability and durability. During the soaking phase of the chemical treatment, the TiO2 layer gets in contact and reacts with the NaOH solution forming a hydrated TiO2 gel, which can be stabilized as an amorphous sodium titanate by a suitable heat treatment.

Sodium titanate layer is expected to form many Ti-OH- groups on its surface in the living body via the ion exchange of its Na+ ions from the surface with H3O- ions in the surrounding body fluid. These Ti-OH- groups make a highly negatively-charged surface that initially combine with positive Ca2+ ions -coming from human plasma- to form amorphous calcium titanate in the surface environment, and later the calcium titanate combines with the negative phosphate ions to form amorphous calcium phosphate, which, at the SBF-pH of 7,4 (simulated body fluid ph -7,4-), eventually transforms into bone-like apatite.

Indeed, nucleation of hydroxyapatite is the consequence of a reaction of precipitation between titanate (which contains Na+ ion) and serum which is normally saturated with Ca2+(calcium) and (PO4)3- (phosphorus) producing calcium phosphate (Ca3 (PO4)2) thus Hydroxyapatite (Figure 1).

Simulated body fluid (SBF) has been used in in-vitro experimental studies to reproduce human plasma and ideal ions concentration has been recently described by Kokubo et al. [20] (Table 1).

Nucleation of hydroxyapatite at implants with thermo-chemical treatment submerged in SBF has been observed by electronic microscope and x-ray diffraction by several authors [13,14,18,22] and our research group has confirmed these results [23,24] (Figure 2-3).

This method can be said to provide a biomimetic surface, since the implant-covering sodium titanate layer can, thanks to Na+ ion bioactivity, and once it gets in contact with biological fluids, form on its own a hydroxyapatite layer without the need of osteoblasts taking part.

Once the hydroxyapatite layer on implant surface has formed, osseointegration process continues with the selective adsorption of fibronectin from human plasma followed by migration, adhesion, proliferation and differentiation of osteoblasts, which starts bone apposition on the surface (Figure 4).

Chemically bonded bone to the implant: a new concept of osseointegration

The classic Osseointegration, as described by Branemark [1] is a clinical concept referred more to the stability of the implant toocclusal forces and in close contact with the bone rather than to a true microscopic surface bond of the bone tissue to the implant surface.

SciMedCentral

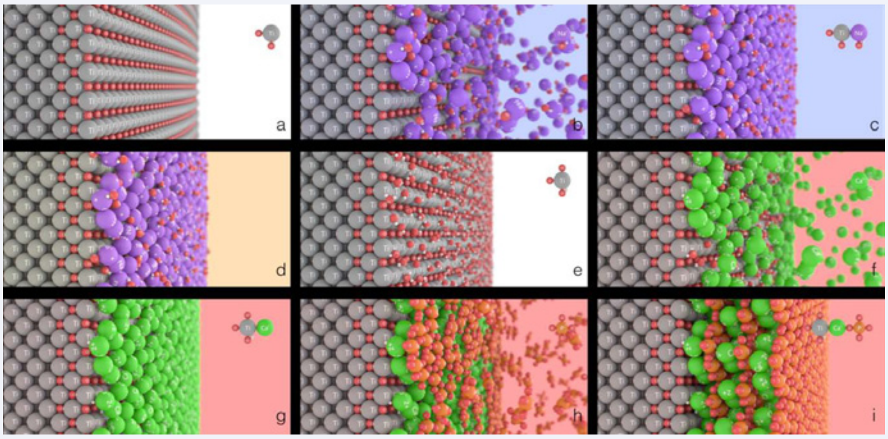

Figure 1 Biochemical sequence of Calcium Phosphate formation on Contac-Tiimplant surface.A) Titanium oxide, b) soaking in NaOH solution, c) formation of sodium titanate hydrogel, d) heating treatment, e) elimination of Na+ ion, f) calcium migration from human plasma, g) calcium adsorption, h) Phosphate migration from human plasma, i) calcium phosphate formation on the surface.

Table 1: Simulated body fluidand blood plasma ions concentration as described by Kokubo et al. (mM).

Ions	***Na+***	***K+***	***Mg2+***	***Ca2+***	***Cl–***	***HC03–***	***HPO24–***	***S024–***
SBF	142	5	1,5	2,5	147,8	4,2	1,0	0,5
BLOOD PLASMA	142	5	1,5	2,5	103	4,2	1,0	0,5

SBF: Simulated Body Fluid

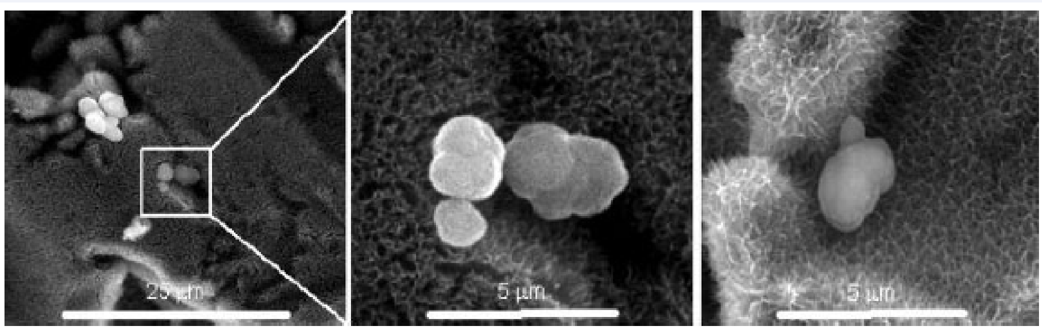

Figure 2 ESEM picture showing nucleation of hydroxyapatite on implant surface after 3 days of soaking in simulated body fluid (SBF).

The bone reacts to implant placement with a healing process that is very similar to intramembranous ossification produced after bone fracture, except that the neo-formed bone is in contact with the surface of an alloplastic material -the implant.

Originally implants had a smooth or minimally rough (Sa <0.5 μm) machined surface, with characteristic repeated irregularities, showing a clear orientation across the implant (anisotropic surface). Over the years new improved surfaces were released with greater roughness to facilitate cell adhesion and thus accelerate implant osseointegration.

Subtraction methods such as aluminum oxide (Al2O3) particle blasting and acid etching provide a surface topography characterized by concavities that form peaks and valleys that increase osteoconduction and, consequently, quicker bone growth with increased bone adhesion force [25].

Many studies have shown a greater ratio of bone surface in contact with the implant surface of rough implant surface compared to machined implant surface, leading to an improved and faster osseointegration [27].

These results may be explained by the apparent different cell response in the early stages of osseointegration. A rough surface will enhance the wet ability and the protein absorption of the implant surface favoring a greater cell migration and adhesion [28].

Davies et al. stated that the more favorable osseointegration of rough surfaces compared to smooth is due to the greater adhesion force of the clot's fibrin scaffold [29]. This scaffold allows osteoblast migration towards the implant surface before these cells start to produce calcium phosphate crystals (hydroxyapatite).

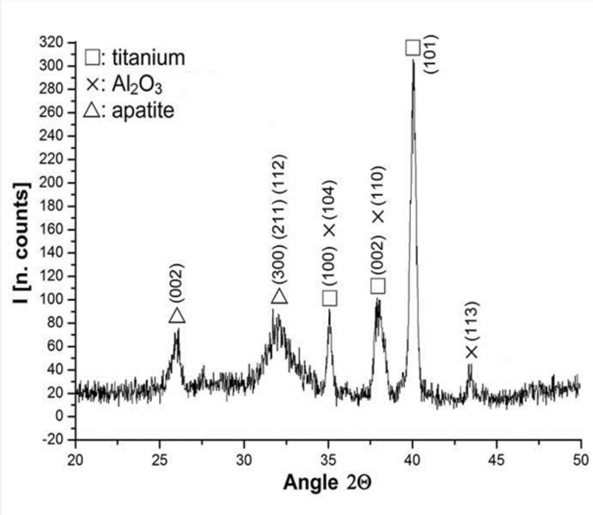

Figure 3 X-ray diffraction (GI-XRD) of2-step method treated titanium after soaking in SBF.

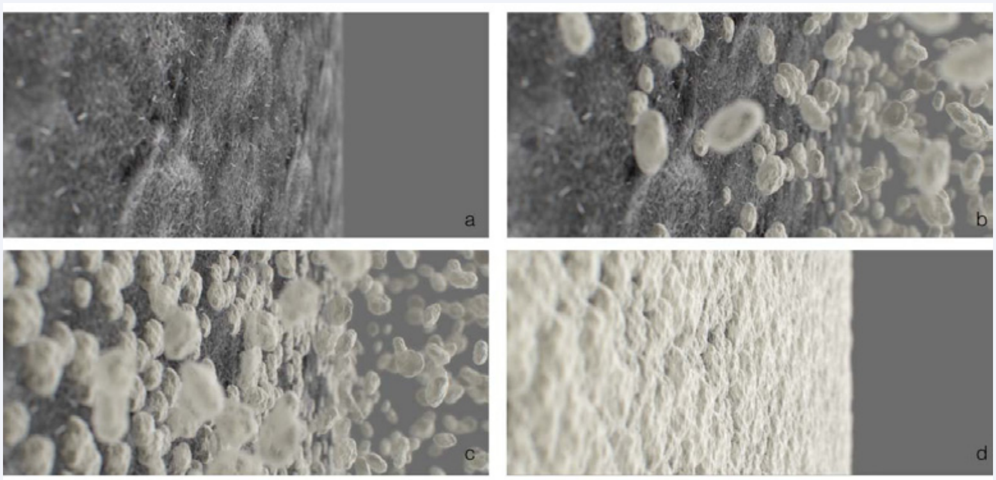

Figure 4 Schematic images of bone formation on the surface.A) Selective adsorption of fibronectin from human plasma, b) migration, adhesion and proliferation of osteoblasts, c) differentiation of osteoblasts and bone apposition on the surface.

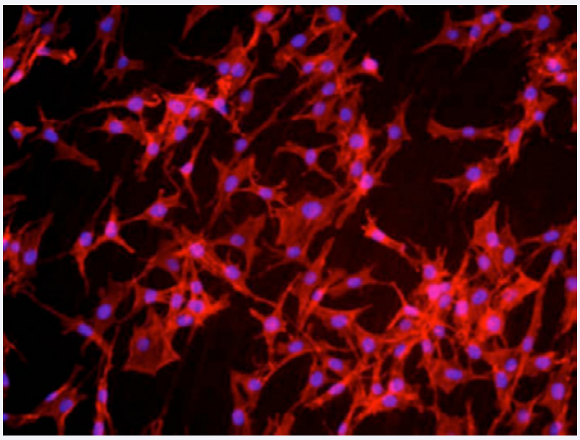

Figure 5 ESEM picture showing human osteoblasts on the thermo-chemically treated implant surface.The shape and distribution of the cells on the surface shows the good differentiation achieved.

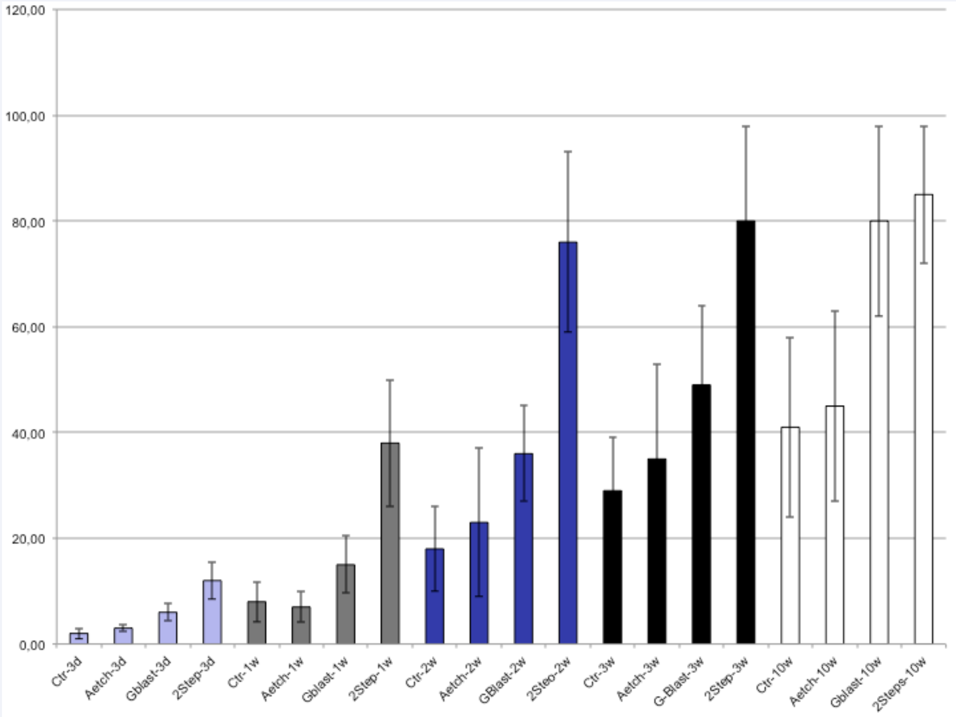

Figure 6 Bone to implant contact (%) at the new surface and controls at 3 days and 1, 2, 3, 10 weeks in a mini-pig model. Ctr: machined surface; AEtch: acid-etched surface; Gblast: grit-blasted surface; 2-Step: grit-blasted, acid-ecthed and thermo-chemical treated surface. Significantly quicker osseointegration occurs at 2-step surface with a BIC greater than 70% at 2 weeks. At 3 weeks osseointegration is completed.

If fibrin's adhesion capacity to implant surface exceeds the threshold, it shall be enough to allow osteoblasts to migrate through the scaffold and get in contact with implant surface. However, in mechanized titanium surfaces, no sufficiently stable bond occurs between it and fibrin so as to withstand the 'weight' of osteoblasts during their migration, thus producing separation between the implant and the fibrin scaffold. In this situation osteoblasts do not reach implant surface and new nuclei of bone formation will be placed closer to implant bed and far from implant surface.

On the contrary, the fibrin scaffold on rough surfaces does not set free from the implant during osteoblast migration due to its tighter surface bond, allowing osteoblasts to reach the surface and start the bone apposition process. Thus, difference can be made between bio-inert surfaces in which 'contact osseointegration' occurs [30], progressive bone apposition from bed periphery to implant surface; and, on the other hand, osseoconductive bioactive surfaces, where the 'bone neoformation' can be observed, bone apposition contemporarily from implant surface and bed [31].

A new concept of implant surface is the bioactive surface; characterized by some bioactive molecules or growth factors that induce bone formation according to different action mechanisms.

Chemically modified sand-blasted/acid etching surface is one example of bioactive surface, which promotes a faster bone healing [32,33]. However there's no chemical bonding between titanium and bone due to the fact that commercially pure Titanium is a bio-inert material without bone–bonding ability, thus the interaction between the metal and the hard tissue does not involve a chemical bond [23].

The thermo-chemically-treated surface, as proven by experimental studies [24], provides the implant with a chemically bonded hydroxyapatite layer with the purpose, as other bioactive surfaces, to accelerate bone healing during the critical period for Osseointegration therefore producing better results with advanced clinical procedure as immediate or early loading.

This method, as discussed above, provides the implant with chemically bonded hydroxyapatite layer, which produces a bone healing and mineralization, both from the implant surface and the bone-bed [24]. Once osteoblasts start bone apposition and mineralization by producing and production of calcium phosphate, a chemical bonding between the hydroxyapatite layer of the implant surface and the new osteoblast-produced hydroxyapatite is produced. Several studies have confirmed high bond strength of the hydroxyapatite layer on the implant surface to the implant and also an increased resistance of the implant to the pull-outtest *in-vivo* has been observed [27,34,35].

Therefore, the classical concept of Osseointegration described as 'intimate contact between living well-structured bone and the implant surface', as described by Branemark [1], seems to start changing to a more biomimeticconcept of a 'chemical contact between living well-structured bone and the implant surface'.

The contac-ti surface (2-stepmethod)

Contac-Ti is the evolution of Shot Blasting surface (Klockner Implant System, SOADCO, Andorra), which was based on micro

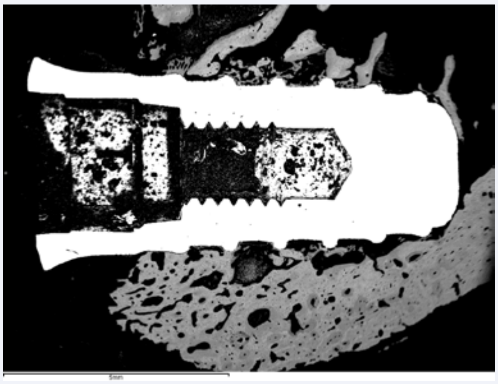

Figure 7a Histologic samples of implants with Contac-Ti surface in rabbit model. Sample at 2 weeks.

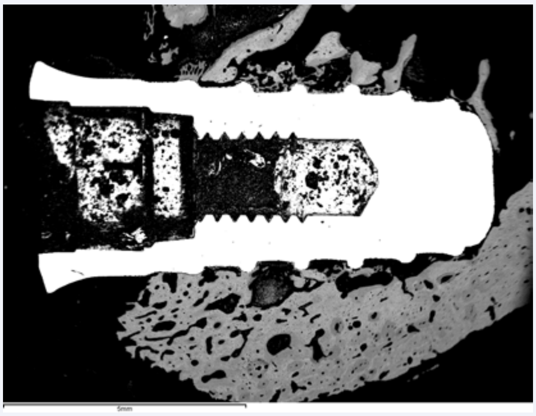

Figure 7b sample at 4 weeks.

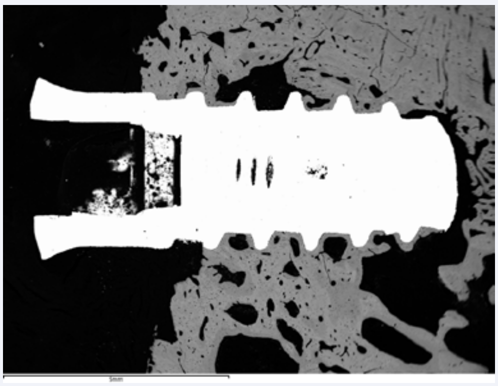

Figure 7c sample at 8 weeks.

roughness obtained by grit-blasting with alumina particles and subsequent acid etching. Excellent clinical results have been demonstrated with the use of this surface by significantly increasing the BIC area as compared to an untreated surface [43,44].

It is well known that moderately rough surfaces (Sa = 1-2 μm) obtained by means of grit-blasting and acid-etching provide a better bone healing [36] and has also been observed that roughness can improve biological response of bioactive titanium surfaces [23].

The new surface is the result of the combination of subtraction procedures to attain a moderately rough surface and a thermo-chemical method based on the principles described by Kokubo et al. [14].

A 2-step procedure, in which first girt-blasting and acid-etching and then a thermo-chemical treatment is performed on machined titanium to obtain the Contac-Ti surface.

The first step: grit blasting/acid etching treatment

Combination of grit blasting and acid etching treatment, which consists of first bombarding a surface with a myriad of small abrasive biologically-inert ceramic particles and then soaking the implant in an corrosive-acid solution, is one of the most frequently used treatments for obtaining a rough surface of dental implants [37].

There is a consensus in the literature about the improvement of osteoblastic response provided by grit-blasting/acid-etching treatment [38,39]. Moreover, a better long-term in-vivo response is achieved when the surface roughness increases since the percentage of implant in direct contact with bone increases as well as loads and torques for extracting implant from bone [40].

Improvements in fibronectin adsorption at implants which received grit blasting treatment with a specific size of alumina (A6) has been demonstrated by in-vitro studies [28,41] as well as a better osteoblasts response in terms of integrin expression at implants with grit blasted/acid-etched surfaces [42].

Commercially-pure grade IV titanium (according to ASTM F67) is used as substratum to obtain the Contac-Ti surface (Klockner Implant System, SOADCO, Andorra) and particles smaller than the ones used for the Shot Blasting since lower roughness value was pursued. During the grit-blasting treatment, 300 μm Aluminium oxide particle size is used, in a second stage acid-etching procedure with HCl is performed to attain a 1,6 Ra value of the implant surface.

The second step: thermo/chemical treatment

The second step to attain Contact-Ti is the thermo-chemical treatment for rough titanium surfaces [45,46].

It consists in submerging the metal in a NaOH solution at 60 ºC for 24 hours, then rinsed with distilled water and dried at 40 ºC for 24 hours and finally it is submitted to a thermal treatment in a tubular furnace at 600ºC for an hour and finally subjected to a cooling process. After completing the surface treatments, all implants are ultrasonically cleaned in soap and distilled water for 10 min, dried with nitrogen gas, and sterilized in ethylene oxide at 37 ºC and 760 mbar for 5 hours.

The main difference between this treatment and the one previously described [14] is that the conditions of reagent concentrations, temperatures changes and heat treatment times have been optimized for moderately rough titanium surfaces as well as heating and cooling rates.

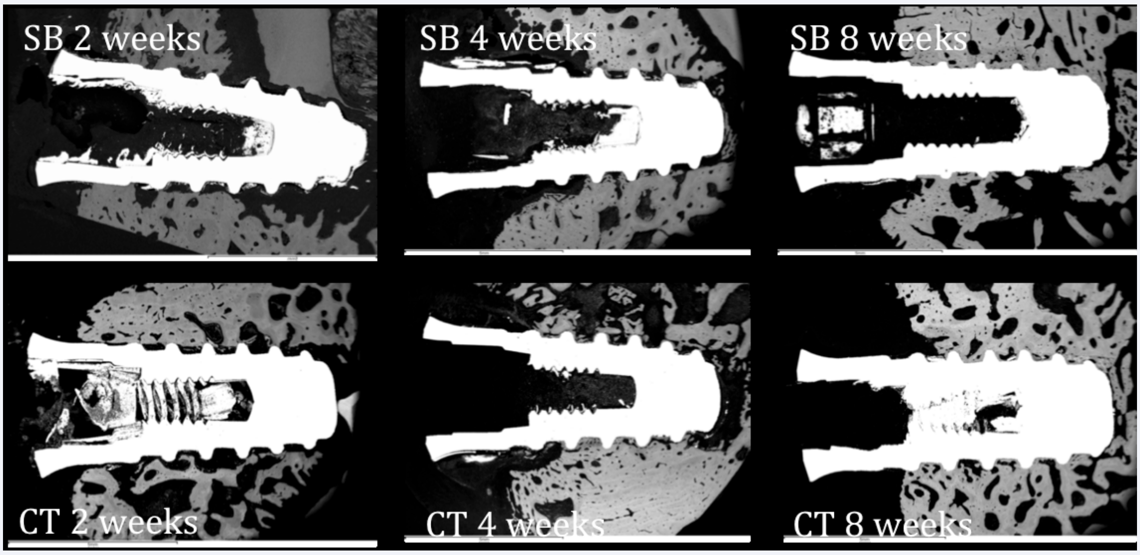

Figure 8 Histologic samples of implants with Contac-Ti surface and sand-blasted implants controls in rabbit model.
SB: Sand-blasted implants controls, CT: Contact-Ti implants.

Microscopical characterization

Surface roughness: Surface characterization of Contact-Ti compared with machine titanium has been recently analyzed by our research group using an optical profiling system device (Optical Profiling System, Wyko NT9300, Veeco Instruments, EEUU) and data analysis means of Wyko Vision 232TM software (Veeco). 10 measurements have been performed and Sa, Sq, Sz and S area index topographic parameters have been used to describe surface characterization. Values of a 1,74 Sa, 2,20 Sq, 16,74 Sz and 1,03 S area index have been obtained from the analysis as shown in Table (2).

Grit-blasting and acid-etching procedure as described above produces a moderately rough surface with good homogeneity as described by Albrektsson [3], and the additional thermo-chemical treatment seems not to alter surface topography [27].

Surface hydrophily: Implant surface can be defined as hydrophilic when it's characterized by a high wettability which is the process by which a drop of liquid spreads over the surface as a result of the interaction of adhesive forces, between liquid and substrate, and internal cohesive forces of the liquid. "The contact angle (CA) is a technique used to determine the wettability of materials and, as the name suggests involves determining the angle between a drops of liquid in contact with the surface a solid. This value depends on the relationship between the adhesive forces between the liquid and the solid and liquid cohesive forces. When the adhesive forces with the solid surface are greater than the cohesive, the contact angle is less than 90 degrees, so that the liquid wets the surface.

Our research group performed an analysis of the wettability of Contac-Ti compared with machined and other rough surfaces by measuring the contact angles so that information above hydrophilic and hydrophobic characteristics could be obtained.

A device for contact angle (CA) measurements and drop dispenser (DATAPHYSICS OCA 15 model) has been used to obtain CA values, 5 measurements for each surface were made and a drop of 1 µl of pure water (Milli-Q, Merck Millipore) was used. A constant of 3 seconds was the time the water droplaidon the surfaces before measurements were performed, results are shown in Table (3,4).

CA higher than 90 have been registered on the machined titanium showing the hydrophobic behavior of this surface, while all the other surfaces have hydrophilic characteristics. However, results show the new surface have the lower value of contact angle, therefore the highest wettability. These hydrophilic characteristics are able to promote protein adsorption and cells adhesion, which contribute to accelerate osseointegration [28,36].

DISCUSSION AND CONCLUSION

Influence of the 2-step treatment on mechanical properties of titanium

The lack of osseointegration due to several factors in the early stages after implantation is the most common form of implant failure whilst peri-implantitis and implant fracture represents the most common causes of implant loss in the long term [47]. Therefore, fatigue is a very important aspect to be taken into account when considering the long-term behavior of dental implants. Fatigue of a material is closely related to the surface structure, meaning that all these surface modification methods conducted to promote a better osseointegration may affect the fatigue performance of the implant. Furthermore, it has to be considered that post-thermal processes may alter the microstructure of the implant material.

Gil et al. [48], carried out an in-vitro study where mechanical properties of 2-step-treated-implants were assessed. Fatigue test were carried out at 37 ºC on 500 dental implants, residual stresses and fatigue-crack nucleation were analyzed comparing machined, grit-blasted and 2-step surfaces. Although a minimal

Table 2: Roughness values of Contac-Ti.

SURFACE	*Sa (µm)*		*Sq (µm)*		*Sz (µm)*		*S area index*	
	Mean	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**
Machined	0,15	0,01	0,19	0,02	3,47	1,53	1,04	0,01
Contac-Ti	1,74	0,07	2,20	0,09	16,74	1,11	1,03	0,01

Machined: machined titanium, Contac-Ti: surface attained after the 2-step treatment, S_a: average surface roughness, S_q: quadratic mean surface roughness, S_z: maximum peak/valley surface, S area index: index between surfaces, homogeneity of the surface.S.d.: standard deviation.
S.D.: standard deviation. *Statistically significant difference (p 0.005).
from Aparicio et al., 2011.

Table 3: Angle contact measurements expressed in degrees of the analysed surfaces.

	Contact angle (°)		**Contact angle (°)**
Machined	83.1	**Ra 2,5**	80.6
	86.8		80.8
	94.3		80.6
	97.8		82.4
	92.4		80.2
Contact-Ti Ra 1,5	79.0	**Ra 3,5**	86.2
	79.2		89.8
	81.3		88.4
	75.1		88.9
	73.9		86.3

Machined: machined titanium, Contac-Ti Ra 1,5: Contact-Ti surface, Ra 2,5: higly-rough surface, Ra 3,5: extremely-rough surface.

Table 4: Mean and standard deviation (S.d.) of contact angle measurements of the analysed surfaces.

Surface	*Machined*	*Contac-Ti Ra 1,5*	*Ra 2,5*	*Ra 3,5*
Mean	90.88	**77.70**	80.92	87.94
S.d.	5.90	3.09	0.85	1.62

Machined: machined titanium, Contac-Ti Ra 1,5: Contact-Ti surface, Ra 2,5: higly-rough surface, Ra 3,5: extremely-rough surface.

Table 5: Mean adhesion force values of the different samples with apatite layers†.

Samples	*Force adhesion* ± S.D. (mN)
Ti-2-steps	451±124
Ti-PS	160±56*
AL6-2-steps	501±90
AL6-PS	190±65*
SI6-2-steps	-
SI6-PS	178±66*

Ti-PS: machined (lathe cut) commercially pure titanium surface + Plasma-spray treatment; AL6-2-step: titanium grit-blasted with Al2O3 particles with a mean diameter of 425-600 µm at a pressure of 2.5 MPa + thermo-chemical treatment; AL6-PS: titanium grit-blasted with Al2O3 particles with a mean diameter of 425-600 µm at a pressure of 2.5 MPa + Plasma-spray treatment; SI6-2-step: titanium grit-blasted with SiC particles with a mean diameter of 425–600 µm at a pressure of 2.5 MPa + thermo-chemical treatment; SI6-PS: titanium grit-blasted with SiC particles with a mean diameter of 425–600 µm at a pressure of 2.5 MPa + Plasma-spray treatment.

decrease (10%) in fatigue life of 2-step implants in comparison with grit-blasted was registered, a high fatigue limit of 315 N was registered and all of the implants showed fractures at 15 106 cycles. The slight decrease was due to the oxygen diffusion inside the titanium of the dental implant with thermo-chemical treatment, which significantly reduced the ductility of the alloy.

According to previous works that compared apatite coatings obtained by different methods like plasma spray, laser ablation, the coatings did not last longer than 106 cycles in any of the cases, being the rapid propagation of the crack either in the coatings or at the interface with the metal implant the main cause of failure [49,50].

The 2-step procedure, obtained by grit-blasting and thermo-chemical treatment reaches a 10 times higher fatigue life in comparison with classical plasma-spray apatite coating. This encouraging result, which has to be confirmed by clinical studies, make implants treated with this new technology allows a great balance in an excellent between enhanced osseointegration and long-term fatigue life.

Adhesive properties of the hydroxyapatite coating: Hydroxyapatite coating is a highly osteoconductive material and allows a predictable osseointegration of dental implants in a short period of time. Nevertheless one of the most critical considerations of hydroxyapatite-coated implants is the adhesion of the apatite layer to the titanium. Plasma-spray was used in the past to provide the apatite layer over the implant surface, however only a scarcely-adhered to titanium amorphous calcium phosphate was produced with this technology leading to a progressive loss of osseointegration due to a bacterial micro-leakage between titanium and apatite coating [12].

The thermo-chemical treatment, as discussed previously, provides the implant with a chemically bonded hydroxyapatite layer by means of a chemical reaction of precipitation of calcium phosphate from ions-saturated human plasma. Adhesion force between implant titanium and the hydroxyapatite layer attained by the 2-step treatment have been investigated in the last years by several authors.

Aparicio et al. [51], assessed the adhesion strength of the apatite-coating layer attained by plasma-spray and by the 2-step procedure with different grinding agents after immersion in SBF. The adhesion strength for the plasma-sprayed apatite layers was around 170 mN with a mean thickness of 20 μm, which were statistically lower than those measured for the 2-step samples, with mean values of 470 mN and a mean thickness of the apatite layer of 15 μm (Table 5).

Similar results have been attained by other authors [16,34] which demonstrate that the bonding strength of apatite layers formed after immersion in SBF of thermo-chemically-treated samples is significantly higher than those of plasma-sprayed hydroxyapatite layers. These results confirm the thermo-chemical treatment provides a chemical bonding between titanium and hydroxyapatite layer.

Biological behavior

Cellular response to the surface: Osteblasts are the cells responsible for bone apposition and mineralization, thus the main cells implicated in the osseointegration process. The assessment of human osteoblasts response (proliferation, differentiation, and cell morphology) to implant surfaces is on one of the most used in-vitro methods to investigate the potential of osseointegration of dental implants.

Aparicio et al., in 2002 [45] investigated in-vitro biological response as proliferation, differentiation -ALP (alkaline phosphatase) activity- and cell morphology by means of environmentalscanningelectronmicroscopyofhumanosteoblasts on machined, grit-blasted and 2-step-treatment titanium. Cells response was assessed by the cell count (proliferation), the analysis of alkaline phosphatase activity (differentiation) and the observation of cell morphology with environmental scanning electron microscopy (ESEM). An increased cell proliferation after 1 day was registered on 2-step-treated surface compared with machined and grit-blasted ones showing the new bioactive surface to provide better cell adhesion probably due to an augmented initial protein adsorption. No statistically significant difference at 3 and 7 days between the samples was registered and a lower proliferation of 2-step surface was shown at 7 and 14 days confirming the good behavior and the higher differentiation of the cells, which –as described by other authors- is reciprocally related to the late proliferation process [52].

ALP-activity was always higher (statistically significant) in the thermo-chemical treated surfaces, indicating stimulation of human-osteoblasts differentiation because of the bioactive surfaces and this result confirms the conclusions of other authors [53,54] (Figure 5).

Nisho et al. [53], investigated the behavior of rat bone marrow cells on commercially pure titanium (Cp Ti), thermo-chemical treatment (Tc Ti) and thermo-chemical treatment incubated in a simulated body fluid (SBF) to deposit crystalline hydroxyapatite on the surface (Tc AP Ti). The alkaline phosphatase (ALP) activity of the cells cultured on Tc AP Ti was significantly higher at day 7 and day 14 than the ALP activity observed for the other titanium surfaces. At day 14, the ALP activity on Tc Ti was significantly increased compared with the ALP activity on Cp Ti. Northern blot analysis of alpha1(I) collagen mRNA was assessed revealing that expression of osteocalcin and alpha1(I) collagen mRNA was higher in the cells cultured on Tc AP Ti than the cells cultured on Tc Ti at day 14 and the cells cultured on Cp Ti showed the lowest mRNA levels. This study confirms that the thermo-chemical treatment provides the most favorable conditions for differentiation of bone marrow cells. The rough and bioactive surface obtained by a grit-blasting thermo-chemical treatment provided enhanced adhesion and differentiation of human osteoblast cells. This fact may play an important role in a rapid formation of the extracellular matrix and, consequently, in an accelerated short-term osseointegration.

Similar results have recently been reported by Quan et al., on bio-activated zirconia implants [55]. Zirconia implant disks were submerged in SBF for 1, 4, 7, and 14 days and statistically significant differences of ALP activity of cultured osteoblasts was observed between treated and non-treated samples at 9 days; cell attachment, proliferation, and differentiation of SBF-treated zirconia disks was superior to that of non-treated disks.

In-vivo results histological studies

Several animal studies which investigate bone healing around implants with the novel 2-steps treatment have been carried out in the last years and the encouraging results attained by previous in-vitro studies have been confirmed.

The first histological study on implants coated with Kokubo method was conducted by Nagano et al., in 1996 [56] where coated and non-coated polyethersulfone (PSE) discs were implanted in rabbit tibia. Mechanical analysis by means detachment test and histological measurements were obtained after sacrificing the animals. Differences in failure loads were statistically significant between coated samples and uncoated ones, with values at 8,16 and 30 weeks of 1.7 ± 0.35, 2.36 ± 0.53, 1.45 ± 0,48 kg in the first ones and 0.08 ± 0.06, 0.04 ± 0.03, and 0.023 ± 0.038 kg, in the second ones. Examination at SEM (scanning electron microscope) showed differences between the two groups of samples with a direct contact of bone to the plate at coated whilst areas of soft tissues were observed at uncoated. Author's claims apatite layer after 30 weeks seemed to have been incorporated to the bone after an osteoclasts-mediated resorption.

These results are in line with others from animal studies carried out by Fujibayashi [57] and Nishiguchi et al. [58, 59], where machined, porous and porous–apatite-coated cylinders were implanted in rabbit tibia and pull-out and histological analysis were assessed. Statistically significant differences were obtained after pull-out test between apatite-coated cylinders and machined ones; no apatite layer detachment was registered at histological examination.

In 2011 Aparicio et al., [27] conducted a study, in mini-pigs, comparing the new 2-steps treatment to a grit-blasted and acid etched surface, with a machined surface as control. Histological and histomorphometric analysis was performed at 2, 4, 6 and

10 weeks' time points, showing a new mineralized bone growth around the 2-steps implants at only 2 weeks. The investigated surface reached the highest values of BIC (bone-to-implant contact) compared to the other samples, with 22% at 2 weeks, 55% at 4 weeks, 65% at 6 weeks and 52% at 10 weeks. The differences between the last three values and the first values were statically significant.

A similar study was recently undertaken by Gil et al. [23], in which three hundred twenty implants were used in a mini-pig model assessing the BIC %, surface composition, topography and wettability in a mini-pig animal experimental model, comparing the 4 surfaces previously described at 3 days, 1, 2, 3 and 10 weeks. Low BIC values for the acid-etched surface and the machined surface were obtained, while the results for the bioactive surface were significantly higher than all the other surfaces for all time points with exception to the alumina blasted surface at the 10 weeks' time point, where there was no statically significant difference (Figure 6).

The surface presented surprisingly high osseointegration values in early healing stages after placement in this animal model, being around 75% and 80% 2 and 3 weeks, respectively, and 85% of BIC was achieved at 10 weeks. The 2-steps surface was the only one that clearly showed extensive areas of bone neo-formation in direct contact with the implant after only one week after implantation (Figure 7).

Van Oirschot et al. [60], have recently investigated the influence of a bioactive hydroxyapatite and composite hydroxyapatite/bioactive glass coatings on the iliac crest of 8 goats. A total of 96 implants were placed and removal torque test and histomorphometrical evaluation were carried out after 4 weeks. Significant higher bone area attached to the implants and BIC% was registered for bioactive implants compared to grit-blasted/acid-etched ones showing the bioactive surface treatments enhanced the bone healing.

Caparrós et al., in 2016 [61] also found significant differences in terms of BIC% between thermo-chemically treated and non-treated porous titanium implants. In vivo results demonstrated that the bioactive titanium achieved over 75 % tissue colonization compared to the 40 % value for the untreated titanium.

Up to the present, very encouraging results have been attained with this surface; nevertheless, randomized-controlled clinical trials are needed in order to validate them in humans under functional loading conditions. According to the biologic bone response of the surface emerged from in-vivo studies, early and immediate loading protocols have been proposed for human-clinical trials, which are currently being carried out by our research group.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Klockner-UPC Chair for their financial support.

REFERENCES

1. Brånemark PI, Hansson BO, Adell R, Breine U, Lindström J, Hallén O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl. 1977; 16: 1-132.
2. Adell R, Lekholm U, Rockler B, Brånemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg. 1981; 10: 387-416.
3. Albrektsson T, Wennerberg A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and *in vivo* responses to them. Int J Prosthodont. 2004; 17: 536-43.
4. Kim HS, Kim YJ, Jang JH, Park JW. Surface Engineering of Nanostructured Titanium Implants with Bioactive Ions. J Dent Res. 2016; 95: 558-65.
5. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. Clin Oral Implants Res. 2003; 14: 251-262.
6. Felice P, Grusovin MG, Barausse C, Grandi G, Esposito M. Safety and effectiveness of early loaded maxillary titanium implants with a novel nanostructured calcium-incorporated surface (Xpeed): 3-year results from a pilot multicenter randomised controlled trial. Eur J Oral Implantol. 2015; 8: 245-254.
7. Wallkamm B, Ciocco M, Ettlin D, Syfrig B, Abbott W, Listrom R, et al. Three-year outcomes of Straumann Bone Level SLActive dental implants in daily dental practice: a prospective non-interventional study. Quintessence Int. 2015; 46: 591-602.
8. Mertens C, Steveling HG. Early and immediate loading of titanium implants with fluoride-modified surfaces: results of 5-year prospective study. Clin Oral Implants Res. 2011; 22: 1354-1360.
9. van Oirschot BA, Bronkhorst EM, van den Beucken JJ, Meijer GJ, Jansen JA, Junker R. A systematic review on the long-term success of calcium phosphate plasma-spray-coated dental implants. Odontology. 2016; 104: 347-356.
10. Mistry S, Roy R, Kundu B, Datta S, Kumar M, Chanda A, et al. Clinical Outcome of Hydroxyapatite Coated, Bioactive Glass Coated, and Machined Ti6Al4V Threaded Dental Implant in Human Jaws: A Short-Term Comparative Study. Implant Dent. 2016; 25: 252-260.
11. Cannizzaro G, Felice P, Minciarelli AF, Leone M, Viola P, Esposito M. Early implant loading in the atrophic posterior maxilla: 1-stage lateral versus crestal sinus lift and 8 mm hydroxyapatite-coated implants. A 5-year randomised controlled trial. Eur J Oral Implantol. 2013; 6: 13-25.
12. Yang Y, Kim KH, Ong JL. A review on calcium phosphate coatings produced using a sputtering process--an alternative to plasma spraying. Biomaterials. 2005; 26: 327-337.
13. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T. Solutions able to reproduce *in vivo* surface-structure changes in bioactive glass-ceramic A-W. J Biomed Mater Res. 1990; 24: 721-734.
14. Kim HM, Miyaji F, Kokubo T, Nakamura T. Preparation of bioactive Ti and its alloys via simple chemical surface treatment. J Biomed Mater Res. 1996; 32: 409-417.
15. Aparicio C, Gil FJ, Planell JA, Engel E. Human-osteoblast proliferation and differentiation on grit-blasted and bioactive titanium for dental applications. J Mater Sci Mater Med. 2002; 13: 1105-1111.
16. Nogueras-Bayona J, Gil FJ, Salsench J, Martinez-Gomis J. Roughness and bonding strength of bioactive apatite layer on dental implants. Implant Dent. 2004; 13: 185-189.
17. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res. 2009; 20: 172-184.
18. Liu Y, de Groot K, Hunziker EB. Osteoinductive implants: the mise-en-scène for drug-bearing biomimetic coatings. Ann Biomed Eng. 2004; 32: 398-406.
19. Kim H, Camata RP, Lee S, Rohrer GS, Rollett AD, Vohra YK.

Crystallographic texture in pulsed laser deposited hydroxyapatite bioceramic coatings. Acta Mater. 2007; 55: 131-139.

20. Kokubo T, Kim HM, Kawashita M, Nakamura T. Bioactive metals: preparation and properties. J Mater Sci Mater Med. 2004; 15: 99-107.

21. Kokubo T, Takadama H. How useful is SBF in predicting *in vivo* bone bioactivity? Biomaterials. 2006; 27: 2907-2915.

22. Pattanayak DK, Yamaguchi S, Matsushita T, Kokubo T. Nanostructured positively charged bioactive TiO2 layer formed on Ti metal by NaOH, acid and heat treatments. J Mater Sci Mater Med. 2011; 22: 1803-1812.

23. Aparicio C, Manero JM, Conde F, Pegueroles M, Planell JA, Gil FJ, et al. Acceleration of apatite nucleation on microrough bioactive titanium for bone-replacing implants. J Biomed Mater Res. 2007; 82: 521-529.

24. Gil FJ, Manzanares N, Badet A, Aparicio C, Ginebra MP. Biomimetic treatment on dental implants for short-term bone regeneration. Clin Oral Investig. 2014; 18: 59-66.

25. Herrero-Climent M, Lázaro P, Vicente Rios J, Lluch S, Marqués M, Guillem-Martí J, et al. Influence of acid-etching after grit-blasted on osseointegration of titanium dental implants: *in vitro* and *in vivo* studies. J Mater Sci Mater Med. 2013; 24: 2047-2055.

26. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: a 1-year follow-up of removal torque of titanium implants. Int J Oral Maxillofac Implants. 1987; 2: 69-75.

27. Aparicio C, Padrós A, Gil FJ. *In vivo* evaluation of micro-rough and bioactive titanium dental implants using histometry and pull-out tests. J Mech Behav Biomed Mater. 2011; 4: 1672-1682.

28. Pegueroles M, Tonda-Turo C, Planell JA, Gil FJ, Aparicio C. Adsorption of fibronectin, fibrinogen, and albumin on TiO2: time- resolved kinetics, structural changes, and competition study. Biointerphases. 2012; 7: 48.

29. Davies JE. Mechanisms of endosseous integration. Int J Prosthodont. 1998; 11: 391-401.

30. Osborn JF, Newesely H. The material science of calcium phosphate ceramics. Biomaterials. 1980; 1: 108-111.

31. Davies JE. *In vitro* modeling of the bone/implant interface. Anat Rec. 1996; 245: 426-445.

32. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res. 2004; 83: 529-533.

33. Schwarz F, Herten M, Sager M, Wieland M, Dard M, Becker J. Bone regeneration in dehiscence-type defects at chemically modified (SLActive) and conventional titanium implants: a pilot study in dogs. J ClinPeriodontol. 2007; 34: 78-86.

34. Miyazaki T, Kim HM, Kokubo T, Ohtsuki C, Kato H, Nakamura T. Enhancement of bonding strength by graded structure at interface between apatite layer and bioactive tantalum metal. J Mater Sci Mater Med. 2002; 13: 651-655.

35. Kato H, Nishiguchi S, Furukawa T, Neo M, Kawanabe K, Saito K, et al. Bone bonding in sintered hydroxyapatite combined with a new synthesized agent, TAK-778. J Biomed Mater Res. 2001; 54: 619-629.

36. Albertini M, Fernandez-Yague M, Lázaro P, Herrero-Climent M, Rios-Santos JV, Bullon P, et al. Advances in surfaces and osseointegration in implantology. Biomimetic surfaces. Med Oral Patol Oral Cir Bucal. 2015; 20: 316-325.

37. Jones FH. Teeth and bones: Applications of surface science to dental materials and related biomaterials. Surf Sci Rep. 2001; 42: 75-205.

38. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials. 2000; 21: 667-681.

39. Boyan BD, Lohmann CH, Dean DD, Sylvia VL, Cochran DL, Schwartz Z. Mechanisms involved in osteoblast response to implant surface morphology. Annu Rev Mater Res. 2001; 31: 357-371.

40. Buser D. Titanium for dental applications (II): Implant with roughened surfaces. In: Brunette DM, Tengvall P, Textor M, Thomsen P, editors. Titanium in Medicine: Material Science, Surface Science, Engineering, Biological Responses and Medical Applications. Berlin: Springer-Verlag. 2001; 875-885.

41. Pegueroles M, Aparicio C, Bosio M, Engel E, Gil FJ, Planell JA, et al. Spatial organization of osteoblast fibronectin matrix on titanium surfaces: effects of roughness, chemical heterogeneity and surface energy. ActaBiomater. 2010; 6: 291-301.

42. Ramaglia L, Postiglione L, Di Spigna G, Capece G, Salzano S, Rossi G. Sandblasted-acid-etched titanium surface influences *in vitro* the biological behavior of SaOS-2 human osteoblast-like cells. Dent Mater J. 2011; 30: 183-192.

43. Aparicio C, Gil FJ, Fonseca C, Barbosa M, Planell JA. Corrosion behaviour of commercially pure titanium shot blasted with different materials and sizes of shot blasted with different materials and sizes of shot particles for dental implant applications. Biomaterials. 2003; 24: 263-273.

44. Gil FJ, Planell JA, Padrós A. Fracture and fatigue behavior of shot-blasted titanium dental implants. Implant Dent. 2002; 11: 28-32.

45. Aparicio C, Gil FJ, Planell JA, Engel E. Human-osteoblast proliferation and differentiation on grit-blasted and bioactive titanium for dental applications. J Mater Sci Mater Med. 2002; 13: 1105-1111.

46. Nogueras-Bayona J, Gil FJ, Salsench J, Martinez-Gomis J. Roughness and bonding strength of bioactive apatite layer on dental implants. Implant Dent. 2004; 13: 185-189.

47. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J ClinPeriodontol. 2002; 29: 197-212.

48. Gil FJ, Espinar E, Llamas JM, Sevilla P. Fatigue life of bioactive titanium dental implants treated by means of grit-blasting and thermo-chemical treatment. Clin Implant Dent Relat Res. 2014; 16: 273-281.

49. Chang YL, Lew D, Park JB, Keller JC. Biomechanical and morphometric analysis of hydroxyapatite-coated implants with varying crystallinity. J Oral Maxillofac Surg. 1999; 57: 1096-1108.

50. Geesink RG, de Groot K, Klein CP. Chemical implant fixation using hydroxyl-apatite coatings. The development of a human total hip prosthesis for chemical fixation to bone using hydr. Clin Orthop Relat Res. 1987; 147-170.

51. Aparicio C, Rodriguez B, Gil FJ. Variation of roughness and adhesion strength of deposited apatite layers on titanium dental implants. Mater. Sci. Eng. 2011; 31: 320-324.

52. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials. 2000; 21: 667-681.

53. Nishio K, Neo M, Akiyama H, Nishiguchi S, Kim HM, Kokubo T, et al. The effect of alkali- and heat-treated titanium and apatite-formed titanium on osteoblastic differentiation of bone marrow cells. J Biomed Mater Res. 2000; 52: 652-661.

54. Sandrini E, Giordano C, Busini V, Signorelli E, Cigada A. Apatite formation and cellular response of a novel bioactive titanium. J Mater Sci Mater Med. 2007; 18: 1225-1237.

55. Quan H, Park YK, Kim SK, Heo SJ, Koak JY, Han JS, et al. Surface Characterization and Human Stem Cell Behaviors of Zirconia Implant Disks Biomimetic-Treated in Simulated Body Fluid. Int J Oral Maxillofac Implants. 2016; 31: 928-938.

56. Nagano M, Kitsugi T, Nakamura T, Kokubo T, Tanahashi M. Bone bonding ability of an apatite-coated polymer produced using a biomimetic method: a mechanical and histological study *in vivo*. J Biomed Mater Res. 1996; 31: 487-494.

57. Fujibayashi S, Nakamura T, Nishiguchi S, Tamura J, Uchida M, Kim HM, et al. Bioactive titanium: effect of sodium removal on the bone-bonding ability of bioactive titanium prepared by alkali and heat treatment. J Biomed Mater Res. 2001; 56: 562-570.

58. Nishiguchi S, Kato H, Neo M, Oka M, Kim HM, Kokubo T, et al. Alkali- and heat-treated porous titanium for orthopedic implants. J Biomed Mater Res. 2001; 54: 198-208.

59. Nishiguchi S, Fujibayashi S, Kim HM, Kokubo T, Nakamura T. Biology of alkali- and heat-treated titanium implants. J Biomed Mater Res A. 2003; 67: 26-35.

60. van Oirschot BA, Meijer GJ, Bronkhorst EM, Närhi T, Jansen JA. Comparison of different surface modifications for titanium implants installed into the goat iliac crest. Clin Oral Implants Res. 2016; 27: 57-67.

61. Caparrós C, Ortiz-Hernandez M, Molmeneu M, Punset M, Calero JA, Aparicio C, et al. Bioactive macroporous titanium implants highly interconnected. J Mater Sci Mater Med. 2016; 27: 151.

Cite this article

Albertini M, Herrero-Climent M, Nart J, Falcao C, Gil FJ (2016) A Biomimetic Surface for Immediate and Early Loading of Dental Implants Surface Characterization and Results from Histological Studies. JSM Dent Surg 1(1): 1008.