Digital quantification of residual aluminum oxide on sandblasted and acid-etched titanium surfaces

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Abstract. *Aluminum oxide (Al_2O_3) may contaminate the implant surface, affecting the osseointegration. Thus, quantitative analyses of residual Al_2O_3 on sandblasted Ti surfaces may be helpful to evaluate the manufacturing process of dental implants. The aim of this study was to identify the presence of Al_2O_3 particles on Ti surfaces and to quantify its removal when hydrofluoric acid (HF) based solution were subsequently used during different periods of time. A Ti sheet was sandblasted (SB) using Al_2O_3 particles (65 µm) and submitted to a double HF based solution during two distinct periods (SLA1 for 13 s and SLA2 for 60 s). Chemical analyses were performed by Electron Dispersive Spectroscopy to identify Al in the samples. Store images were imported to the Zeiss KS400 program. To avoid the presence of reflections and shadows caused by the rough topography, a minimum area of 20 pixels was set. Digital analyses showed that SB surfaces and SLA1 surfaces had an average area of 5.92% (±0.18) and 0.20% (±0.16) occupied by the Al_2O_3, respectively ($p< 0.001$). Al_2O_3 was not detected on SLA2 surface. The mean diameter of the particles on SB and SLA1 surfaces was similar (9.61 ±4.61 µm vs. 16.35 ±13.09 µm, respectively; $p= 0.142$), indicating that particles bellow of these mean values probably were not detected. Zeiss KS400 program seemed to be a useful tool to quantify Al_2O_3 on sandblasted and acid-etched Ti surfaces, although digital quantification had showed a limitation on rough surface.*

Keywords*: Titanium; Dental implants; Aluminum Oxide; Image Processing, Computer-Assisted.*

1. INTRODUCTION

Endosseous implants are usually manufactured using either commercially pure titanium (cpTi) or a titanium-6-aluminum-4-vanadium (Ti-Al-V) alloy. They are suitable and biologically compatible materials for fabrication of dental implants (Keller et al., 1994).

Biological responses to artificial biomaterials are strongly influenced by the composition and properties of the implant's surface. Thus, the biological response may be modulated by modifying the implant's surface characteristics (Ong et al., 1995). To speed up or to improve the interaction between the bone and the implant, endosseous implants usually receive a surface treatment, which may be done in different ways. A rough surface topography may be obtained by a blasting process with abrasive oxides, which is sometimes followed by chemical treatment using either acid or basic solutions (Diniz et al., 2002; Taborelli et al., 1997; Takeuchi et al., 2003). It has been proposed that superficial roughness is helpful in the initial stages of cellular adhesion, which occurs during the healing process of the osseointegration. An increased surface area may be important to enhance the expression of the osteoblast phenotype (Ong et al., 1997).

Surface cleanliness of the titanium implant is essential for the formation of bone tissue covering the implant. The presence of elements such as iron and aluminum (Al) may contaminate the Ti surface, affecting the healing process and osseointegration. Al contamination may occur as a result of the blasting processes using aluminum oxide (Al_2O_3) (Darvell et al., 1995). Although Al_2O_3 blasting procedure produces effective modifications of the implant microtopography, at low cost, resulting Al residues are difficult to remove (Diniz et al., 2005). Thus, quantitative analyses of residual Al_2O_3 on blasted titanium surfaces may be helpful to evaluate and compare manufacturing process (Diniz et al., 2005).

In the present study, qualitative and quantitative methods were used to identify the presence of Al_2O_3 on sandblasted Ti surfaces. It was also possible to quantify Al_2O_3 removal produced by hydrofluoric acid (HF) based solution at two different periods of time.

2. MATERIAL AND METHODS

2.1. Surface preparation

One sheet of cpTi grade 2 (Ti Brasil, São Paulo, Brazil), with 250 x 250 mm, were used in this study. The sheet was sandblasted using Al_2O_3 particles (average diameter of 65 µm), at a blasting pressure of 414 kPa (indirect), for one minute and were cut into 8 x 8 mm square samples. The samples were divided into three groups. Group 1 was only sandblasted with Al_2O_3 (SB). The two other groups were also blasted with Al_2O_3 and received an additional two step double chemical treatment using a solution of 4% HF for 13 s (Group 2: SLA1) or for 60 s (Group 3: SLA2) followed by a 4% HF/8% H_2O_2 (hydrogen peroxide) for 15 s. Hydrogen Peroxide was only used to promote chemical stability of the surface oxide layer (Kawahara, 1995). After the treatments, all samples were ultrasonically cleaned by a sequential treatment with acetone, 70% alcohol and distilled water for 15 min (each one), and sterilized in an autoclave for 20 min at 120^{o}C.

2.2. Surface characterization and digital Al_2O_3 quantification

The average roughness (Ra) was measured using needle profilometer (Mitutoyo SJ 201P, Miyazaki, Japan). Two samples from each group were analyzed. Five horizontal analyses were performed on each of the samples to Ra measurement.

The Ti surfaces were also evaluated by scanning electron microscopy (SEM - Jeol JSM 6301F, MA, USA). Two samples from each group were analyzed. Five fields were randomly chosen from each sample for SEM imaging. The digital SEM images were stored in a 512 X 480 pixels file and imported to a 24-bit uncompressed TIF format. Half-quantitative chemical analyses were performed by Electron Dispersive Spectroscopy (EDS) (Voyager XRMA System, WI, USA) to identify Al in the samples. Store images were imported to the Zeiss KS400 image processing program (Zeiss, Oberkochen, Germany) for digital analysis. A geometric calibration was initially performed (with a 100 x objective one pixel corresponding to 0.125 µm), followed by a densitometric calibration, which related the image to a 0-256 grey scale. A minimum area of 20 pixels was set, allowing the measurement of objects greater than 20 pixels during the analysis. Parameters measured included number of Al_2O_3 particles on the images and their average diameters (feret maximum, which corresponds to the value of the major diagonal connecting the two farthest points at the periphery of the particle), and the area - the sum of all white pixels in the black field - occupied by all the particles. The pre-processing operations on the digitized images were made by an experienced Zeiss KS400 operator (SP) to avoid the alteration of the original information.

2.3. Statistical analysis

The difference between the SLA1 and SB for each Al_2O_3 parameter was analyzed by t test ($p< 0.05$). One way analysis of variance (ANOVA) was performed for Ra comparison of Ti surfaces. When the F-ratios were significant ($p< 0.05$), the data were compared with Duncan's multiple range test ($p< 0.05$).

3. RESULTS

3.1. Surface topography analysis

SEM was able to demonstrate the smooth appearance of cpTi (Fig. 1A). After the sandblasting process, the surface showed irregularities with no predominant direction (Fig. 1B – SB). After the double chemical attack, the surfaces became more homogeneous and more regular than SB surfaces, although maintaining the rough appearance. The sandblasting procedure produced a macroroughness onto with the acid-etch process superimposed a microroughness (Fig. 1C – SLA1, 1D – SLA2).

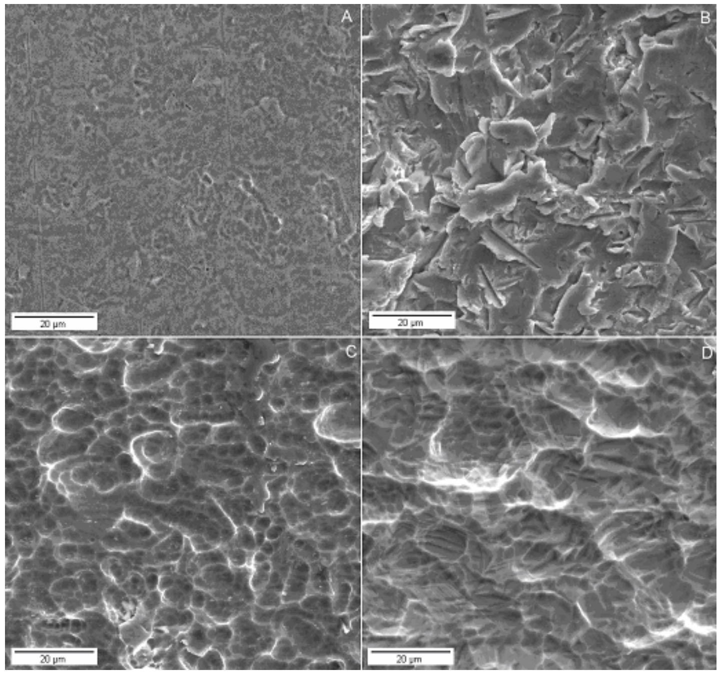

Figure 1**.** Scanning electron micrograph of Ti surfaces (SE mode). (A) Ti-smooth; (B) SB; (C) SLA1; (D) SLA2. Original magnification 1000X.

Ra was affected by mechanical treatment ($p< 0.001$) showing a great roughness on sandblasted surface, but was not affected by HF attack ($p= 0.073$) (Table 1).

Table1. Mean (SD) roughness (Ra) in µm of Ti surfaces.

	Ti-smooth N= 10	SB n= 10	SLA1 n= 10	SLA2 n= 10
Ra	0,25 (0.06)*	1.03 (0,008)	0.95 (0.004)	1.01 (0.10)

*Significant (ANOVA). SB=SLA1=SLA2>Ti smooth

3.2. Digital Al_2O_3 quantification

SEM analysis on backscattered electrons (BSE) mode showed a high number of contaminants (Al), observed as darker regions on SB surface (Fig. 2A), since Al has a lower atomic weight than Ti. After chemical treatment, Al residues were also identified on SLA1 (Fig. 2B), but not on SLA2 surface (Fig. 3). The presence of Al was confirmed by EDS on SB and SLA1 surfaces (Fig. 4A and B, respectively), but not on SLA2 surface (Fig. 4C).

Procedures were followed in order to process and analyze the images: adjusting of the brightness and contrast levels of the images, fulfilling of emptiness and corrections of the grain boundaries of the Al_2O_3 particles. After the identification of the particles (segmentation – Fig. 2C, D) and binarization - a white phase on a black background - of the images (Fig. 2E, 2F), the Al_2O_3 quantification was performed.

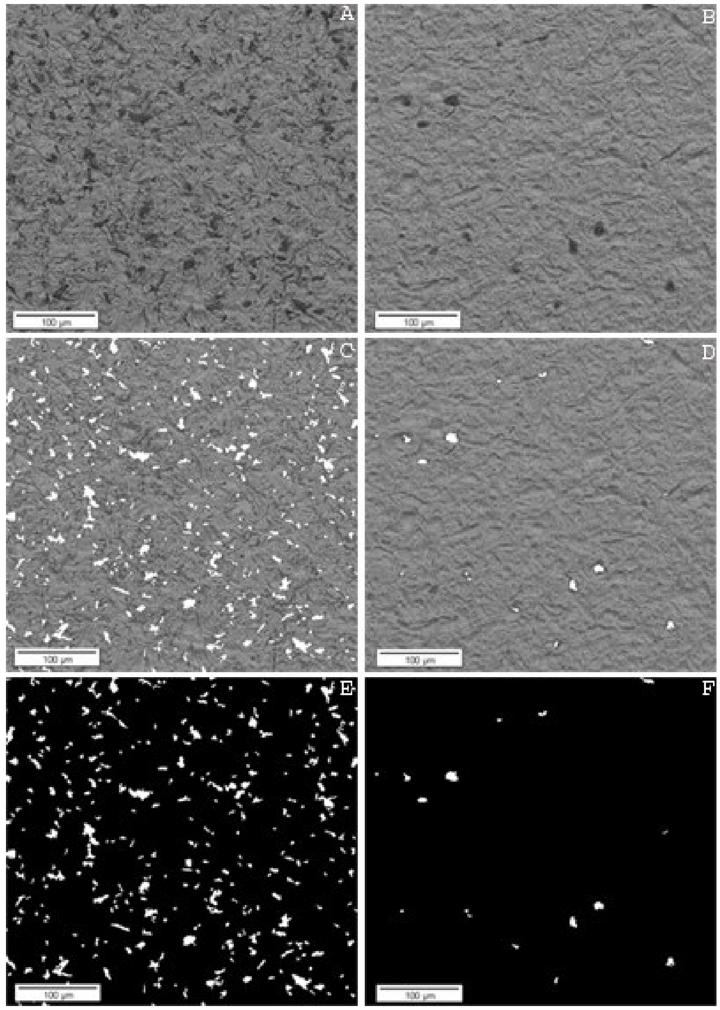

Figure 2. Scanning electron micrograph of Ti surfaces (BSE mode). Initial images: (A) SB and (B) SLA1. (C) SB and (D) SLA1 – process of segmentation of the spots (Al_2O_3). (E) SB and (F) SLA1 - binarization. Original magnification 200X.

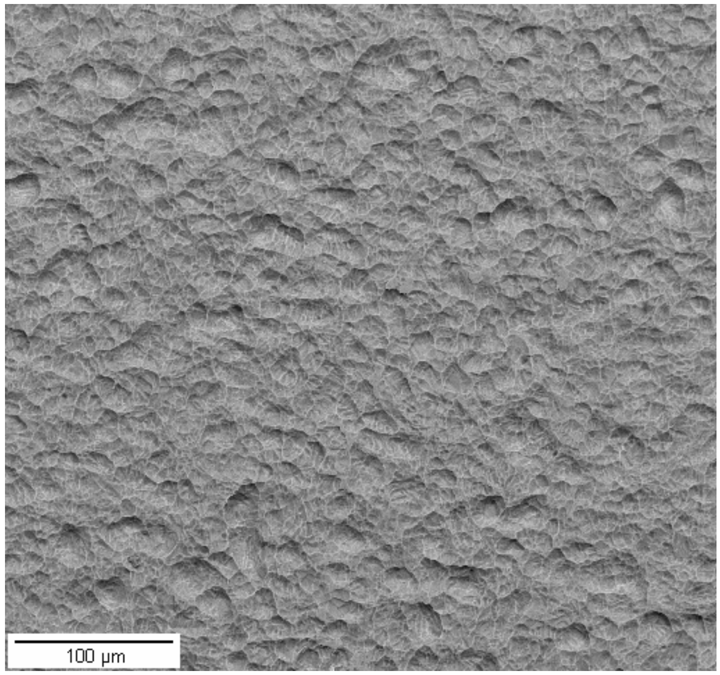

Figure 3. Scanning electron micrograph of SLA2 surface (BSE mode). Original magnification 200X.

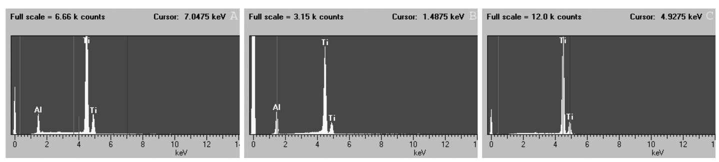

Figure 4. EDS spectra showing Al on SB (A) and on SLA1 (B) surfaces, but not on SLA2 (C) surface.

Table 2 shows the Al_2O_3 parameters in five analyzed images of two different prepared samples of each group. The Al_2O_3 particles occupied a higher mean area on SB surface as compared to SLA1 surface ($p<0.001$). SB surface also presented the highest mean number of Al_2O_3 regions ($p<0.001$). However, the mean diameter of the particles was similar between the two surfaces ($p= 0.142$).

Table 2. Mean (SD) values of area (%), diameter of particles (µm) and number of regions occupied by Al_2O_3 on different surfaces.

	Area* n= 10	Diameter n= 10	Number of Regions* n= 10
Ti-smooth	ND	ND	ND
SB	5.92 (0.18)	9.61 (4.61)	344.40 (17.32)
SLA1	0.20 (0.16)	16.35 (13.09)	7.80 (4.10)
SLA2	ND	ND	ND

ND: not detected by EDS. *Significant (t test); $p< 0.05$.

4. DISCUSSION

Modifications of the surface morphology and roughness have been used to influence cell and tissue responses to implants. Due to mechanical interlocking, bone ingrowth would increase fixation and stability of the implant (Puleo and Nanci, 1995; Wennerberg et al., 1996). Another important observation is that procedures used to increase implant surface areas in direct contact with bone may reduce failure due to overload (Esposito et al., 1998). Therefore, to improve the bone integration of Ti implants, surface treatments such as surface machining, acid-etching, sand blasting or plasma spraying may be undertaken (Anselme et al., 2000). However, these surface treatments are known to induce chemical modifications of the implant, associated with modifications of surface topography (Caruntu, 2002). The results of the present study showed that sandblasting process produced surfaces with higher roughness, but Al_2O_3 residues could also be identified. Accordingly, Schuh et al. (2000)

recently observed that grit blasting with Al_2O_3 particles resulted in rough titanium surfaces contaminated with Al_2O_3 at an unexpected high percentage. In the present study, Al_2O_3 residues were quantified by Zeiss KS400, a versatile image processing program designed to support demanding professional applications (Caruntu, 2002). The processing of images is based on mathematical operations capable of modifying the values of the images' pixels, to identify image attributes that may be related either to the manufacturing processes or to the material characteristics (Diniz et al., 2005; Malkusch, 2000). To avoid the presence of reflections and shadows caused by the rough topography, a minimum area of 20 pixels was set, after previous tests conducted by a "blind" examiner on Zeiss KS400 using different sizes (10, 20 or 30 pixels), allowing the measurement of objects greater than 20 pixels during the analysis. Digital analyses showed that SB and SLA1 surfaces had an average area covered by Al_2O_3 particles of 5.92% (±0.18) and 0.20% (±0.16), respectively. Al_2O_3 particles were not detected on SLA2 surface. The mean diameter of the particles was similar in both SB and SLA1 surfaces (9.61 ±4.61 µm vs. 16.35 ±13.09 µm, respectively), indicating that particles bellow this average value probably were not detected. These results showed that the digital method was not able to detect particles within phagocytosable size (less than 3 µm diameter). Particle phagocytosis is a central event in the pathogenesis of periprosthetic osteolysis (Vermes et al., 2001). Osteoblasts phagocytose particulate wear debris, and this process induces interleukin-6 production and suppresses type-I collagen synthesis (Vermes et al., 2001). Therefore, another method should be used to detect particles smaller than those that were found on our study.

The role of surface contamination in implant failures is not yet well understood (Piatelli et al., 2003). It has been suggested that the presence of inorganic contaminants could lead to lack of clinical success of the dental implants (Esposito et al., 1998). Al ions are suspected to impair bone formation by a possible competitive action with calcium (Bushinsky et al., 1995). Ti implant casting associated with tissue breakdown revealed embedded particles of alumina (Darvell et al., 1995). The presence and accumulation of Al in bone tissues has been implicated in the development of aplastic bone lesions and osteomalacia (Bushinsky et al., 1995). Histomorphometric data from animal studies and human biopsies suggest that these problems result at least in part from an inhibitory effect of Al on bone mineralization (Bellows et al., 1999). Furthermore, these corrosive byproducts may also spread systemically (Puleo and Nanci, 1999). Therefore, the potential harmful effects of slow and continuous release of trace metals cannot be ignored (Darvell et al., 1995).

Specific surface chemical treatments have been used for removing contaminants originating from either the implant's manufacturing processes or the clinical/surgical preparation. These treatments involve the use of solutions such as HF, sulfuric acid, nitric acid, phosphoric acid and organic acids, as well as methods using anodic oxidation with electric current in acid environment and nitretation techniques (Kawahara, 1995). In the present study, we used HF because it was more effective than the sulphuric acid in smoothing Ti surface and in removing surface contamination resulting from the sandblasting procedure (Diniz et al., 2002). The double etching (4% HF for 60 s + 4% HF/8% H_2O_2 for 15 s) treatment was effective to modify the surface morphology, creating two levels of roughness. It has been suggested that this surface (SLA) promotes greater osseous contact at earlier points (Cochran et al., 1998). Furthermore, the double etching removes the Al from the blasting procedure, with no statistical change in mean roughness.

Avoidance of Al_2O_3 sandblasting is not recommended because rougher microtopographies seem to allow better biological response (Diniz et al., 2002). Also, there is little evidence to support any toxic effects due to wear particles or metal ion release on implant failure (Esposito et al., 1998). However, while more studies addressing the biological effects of Al_2O_3 contamination are not available, a 60 s 4% HF treatment followed by a 4% HF/8% H_2O_2 treatment for 15 s, after Al_2O_3 blasting, may be used to eliminate contamination of roughened surfaces, maintaining sufficient roughness for osseointegration.

5. CONCLUSION

Although the conditioning with HF for 13 s was able to remove Al_2O_3 particles, residual large particles of such contaminant could still be identified on SLA1 surfaces. Only when the sandblasted surfaces were attacked with HF for 60 s (SLA2), all residual Al_2O_3 particles were removed. Zeiss KS400 program seemed to be a useful tool to quantify Al_2O_3 on sandblasted and acid-etched Ti surfaces, although digital quantification had showed a limitation on rough surface.

6. REFERENCES

Anselme, K., Line, P., Bigerelle, M,. Le Maguer, D., Le Marger, A., Hardouin, P., Hildebrand, H.F., Iost, A. and Leroy, J.M., 2000, "The relative influence of topography and chemistry of TiAl6V4 surfaces on osteoblastic cell behaviour", Biomaterials, Vol. 21, pp. 1567-1577.

Bellows, C.G., Heersche, J.N.M. and Aubin, J.E., 1999, "Aluminum accelerates osteoblastic differentiation but is cytotoxic in long-term rat calvaria cell cultures", Calcif. Tissue. Int., Vol. 65, pp. 59-65.

Bushinsky, D.A., Sprague, S.M., Hallegot, P., Girod, C., Chabala, J.M. and Levi-Setti, R., 1995, "Effects of aluminum on bone surface ion composition", J. Bone Miner. Res., Vol. 10, pp. 1988-1997.

Caruntu, I.D., 2002, “Highly effective techniques in computerized dental tissue morphometry”, J. Cell Mol. Med., Vol. 6, pp. 631-642.
Cochran, D.L., Schenk, R.K., Lussi, A., Higginbottom, F.L. and Buser, D., 1998, “Bone response to unloaded and loaded titanium implants with sandblasted and acid-etched surface: a histometric study in the canine mandible”, J. Biomed. Mater. Res., Vol. 40, pp. 1-11.
Darvell, B.W., Samman, N., Luk, W.K., Clark, R.K.F. and Tideman, H., 1995, “Contamination of titanium castings by aluminum oxide blasting”, J. Dent., Vol. 23, pp. 319-322.
Diniz, M.G., Pinheiro, M.A.S., Andrade Junior, A.C.C. and Fischer, R.G., 2005, “Characterization of titanium surfaces for dental implants with inorganic contaminant”, Braz. Oral Res., Vol. 19, pp. 106-111.
Diniz, M.G., Soares, G.A., Coelho, M.J. and Fernandes, M.H., 2002, “Surface topography modulates the osteogenesis in human bone marrow cell cultures grown on titanium samples prepared by a combination of mechanical and acid treatments”, J. Mater. Sci. Mater. Med., Vol. 13, pp. 421-432.
Esposito, M., Hirsch, J-M., Lekholm, U. and Thomsen, P., 1998, “Biological factors contributing to failures of osseointegrated oral implants (II). Etiopathogenesis”, Eur. J. Oral Sci., Vol. 106, pp. 721-764.
Kawahara, H., 1995, Biomaterials for Dental Implants. In: Wise, D.L. (ed). “Encyclopedic Handbook of Biomaterials and Bioengineering”, 2nd ed. Ed. Marcel Dekker, New York, pp.1469-1524.
Keller, J.C., Stanford, C.M., Wightman, J.P., Draughn, R.A. and Zaharias, R., 1994, “Characterizations of titanium implant surfaces”, J. Biomed. Mater. Res., Vol. 28, pp. 939-946.
Malkusch, W., 2000, “Fundamentals and problem-solving: sequences of quantitative image analysis”, Lab. Focus, Vol. 4, pp. 15-17.
Ong, J.L., Carnes, D.L., Cardenas, H.L. and Cavin, R., 1997, “Surface roughness of titanium on bone morphogenetic protein-2 treated osteoblast cells in vitro”, Implant. Dent., Vol. 6, pp. 19-24.
Ong, J.L., Prince, C.W. and Lucas, L.C., 1995, “Cellular response to wellcharacterized calcium phosphate coatings and titanium surfaces in vitro”, J. Biomed. Mater. Res., Vol. 29, pp. 165-172.
Piattelli, A., Degidi, M., Paolantonio, M., Mangano, C. and Scarano, A., 2003, “Residual aluminum oxide on the surface of titanium implants has no effect on osseointegration”, Biomaterials, Vol. 24, pp. 4081-4089.
Puleo, D.A. and Nanci, A., 1999, “Understanding and controlling the bone-implant interface”, Biomaterials, Vol. 20, pp. 2311-2321.
Schuh, A., Holzwarth, U., Kachler, W., Goske, J. and Zeiler, G, 2004, “Surface characterization of Al2O3-blasted titanium implants in total hip arthroplasty”, Orthopade, Vol. 33, pp. 905-910.
Taborelli, M., Jobin, M. and François, P., 1997, “Influence of surface treatments developed for oral implants on the physical and biological properties of titanium. (I) Surface Characterization”, Clin. Oral Implants Res., Vol. 8, pp. 208-216.
Takeuchi, M., Abe, Y., Yoshida, Y., Nakayama, Y., Okazaki, M. and Akagawa, Y., 2003, “Acid pretreatment of titanium implants”, Biomaterials, Vol. 24, pp. 1821-1827.
Vermes, C., Chandrasekaran, R., Jacobs, J.J., Galante, J.O., Roebuck, K.A. and Glant, T.T., 2001, “The effects of particulate wear debris, cytokines, and growth factors on the functions of MG-63 osteoblasts”, J. Bone Joint. Surg. Am., Vol. 83, pp. 201-211.
Wennerberg, A., Albrektsson, T., Johansson, C. and Andersson, B., 1996, “Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography”, Biomaterials, Vol. 17, pp. 15-22.

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