# EFFECT OF SURFACE TREATMENTS ON TITANIUM ON THE OSTEOGENESIS AND SIMULATION OF THE MICROSTRESSES IN THE INTERFACE TITANIUM / BONE

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***Abstract.*** *Titanium samples of different roughness ($R_a$) and morphology were prepared using a combination of mechanical with and without chemical attack of hydrofluoric acid based solution treatments. The biological performance of the prepared surfaces was evaluated using human bone marrow osteoblastic cell cultures. Mechanical treated samples presented different $R_a$ values and surface morphology. The hydrofluoric acid based solution (F solution) was effective in smoothing titanium surface and also in eliminating aluminum contamination resulting from the blasting process. Bone marrow cells seeded on the different titanium samples showed a similar pattern of behavior during cell attachment and spreading. Cells proliferated very well on all the titanium surfaces and cell growth was observed during two to three weeks. The samples treated with F solution presented higher ALP activity. Only the blasted samples treated with the acid solutions allowed seeded bone marrow cells to form a mineralized extracellular matrix. The best biological performance was found in the blasted samples treated with the hydrofluoric acid based solution, which could be related to the characteristic microtopography of these samples that presented a homogeneous and smooth roughness. Continuing this work a distribution of tensions at the bone-titanium interface will be simulated with the aid of software ANSYS.*

**Keywords**. Titanium surface treatments, osteogenesis, human bone marrow cell cultures.

## 1. Introduction

As a result of the developments in the past few years a variety of endosseous dental-implant systems have become available with widely different designs, surface textures, and materials of construction [Wennerberg, et al., 1996; Brunski, 1988]. In general, the surface chemistry, surface energy, surface topography and roughness govern the biological response to an implanted material and the events occuring at the bone-implant interface are of major importance to the healing period and osteointegration. The concept of osteointegration is described as a direct bone-to-biomaterial interface (without fibrous tissue) for a functioning implant at the optical microscopy limits of resolution (0,5μm) and a wide range of implant biomaterials and designs have been shown to exhibit this type of interface for functional dental systems [Ratner et al., 1996].

In general there is little scientific evidence for the efficacy of the majority of systems, however, dental implants are usually made from commercially pure titanium because of its biological acceptance in bone. This material has high corrosion resistance when in contact with the body fluids, is lightweight and durable, and can be easily prepared in many different shapes and textures when seeking for the most adequate biocompatibility [Meachim and Pedley, 1981].

The response of cells and tissues at implant interfaces are affect by the surface properties, such a response may involve physical factors as well as chemical factors associated with the surface structure and surface composition. Moreover, several aspects not only related to surface properties, material itself or the esterilization process act altering clinical performance. Each patient exhibits specific answer to a particular implant depending on bone characteristics, patient's health and surgical technique. The extend and the mechanisms of these events are uncertain and much effort goes into developing new or better materials and methods [Smith, 1991; Schwartz and Boyan, 1994; Vezeau et al., 1996].

Surface treatments for titanium dental implants can divided in two major appraches: coating the implants with bioactive substances (calcium phosphate ceramic, glass ceramics, porous layers added to metal) and modifying-surface processes. This last methodology includes treating the titanium with oxide blasting treatments associated, or not, with chemical treatment; claims for generate a suitable topography to allow bone-implant osteointegration. These surface modifications could result in a faster and better osteogenesis and more stable implant-to-bone tissue interface [Wennerberg, et al., 1996; De Groot, 1998].

Biomaterials can be evaluated under *in vitro* conditions to provide rapid and inexpensive data control on biological interaction. Systematic studies performed using cell culture methodology continually provide important information for

predicting how a device performs in humans and concerning the relevance of the surface properties. *In vitro* tests minimize the use of animals in research, a desirable goal [Nakamura et al., 1995; Ratner et al., 1996].

This work describes the effect of acid treatment on surface composition, roughness and morphology parameters of titanium sheets submitted to grinding or blasting. For chemical attack, a hydrofluoric acid solution were employed [Kawahara, 1995]. The solution can alter topography and/or surface composition. In addition, the biological performance of the prepared titanium surfaces was evaluated using human bone marrow osteoblastic cell cultures. Cell morphology, cell viability/proliferation, alkaline phosphatase (ALP) activity and ability to form a mineralized extracellular matrix were monitored as indicators of cellular response. Continuing this work a statistics analysis of the micro morphology of the prepared surfaces for the attainment of its significant dimensions is being carried out. The distribution of tensions at the bone-implant interface will be simulated with the aid of software ANSYS.

## 2. Materials and Methods

### 2.1. Preparation of the titanium samples

The titanium samples used in this study were prepared from titanium sheets (ASTM grade 2). A titanium sheet was divided in three pieces that processed as described below: (1) submitted to grinding with a 600 grit SiC paper (samples $L_0$), (2) blasted with aluminum oxide particles with 65 μm (samples $J65_0$), and (3) blasted with aluminum oxide particles with 250 μm (samples $J250_0$). After these mechanical treatments, the three titanium pieces were then cut in 8 x 8 mm samples and cleaned using a sequence of acetone, absolute ethanol and distilled water in an ultrasonic cleaner.

Each of the three types of samples were further divided in two groups: (a) maintained as treated before – samples $L_0$, $J65_0$ and $J250_0$, (b) submitted to chemical attack with a hydrofluoric acid-based solution (solution F) – samples $L_F$, $J65_F$ and $J250_F$. The chemical attack with solution F was performed using the conditions as described as follows: (i) first step: HF (4%), 60s, (ii) second step: HF (4%) plus $H_2O_2$ (8%), 15s. According to the mechanical and chemical treatments described, six different surfaces were prepared, grouped in three families: $L_0$ and $L_F$ ($L_i$ family); $J65_0$ and $J250_0$ ($J65_i$ family); $J250_0$ and $J250_F$ ($J250_i$ family).

### 2.2. Surface characterization of the titanium samples

The $R_a$ was determined using a profilometer SLOAN, model Dektak IIA with vertical precision of 5 Å. For each surface condition, three samples were employed and at least 10 measurements were done on each sample.

X-ray photoelectron spectroscopy (XPS) was carried out in a ESCALAB 200 A - VG SCIENTIFIC operating at 15.0 kV, 399W with the Mg-$K_\alpha$ energy (1253.6eV). This analysis allows for the identification of residual contamination and for the semi-quantification, with an error of order of 10%, of the composition of the external layers of surface.

Scanning electron microscopy (SEM) of the prepared titanium samples was carried out in a ZEISS DSM 940A, operating at 15 kV to characterize surface topography while a LINK energy dispersive X-ray detector (EDX) was used to determine elemental composition of residuals particles.

### 2.3. Cell culture, cell viability/proliferation, ALP activity and ability to form calcium phosphate deposits.

Human bone marrow, obtained from surgery procedures, was cultured in α-Minimal Essential Medium (α-MEM) containing 10% fetal bovine serum, 50 $\mu g ml^{-1}$ gentamicin and 2.5$\mu g ml^{-1}$ amphotericin B. Primary cultures were grown at 37°C in humidified atmosphere of 95% air and 5% $CO_2$ until near confluence (22 days) and, at this stage, adherent cells were enzymatically released (0.04% trypsin and 0.025% collagenase); the cells were automatically counted using a Celtac- NIHON KOHDEN equipment.and seeded in 24-well dishes at density of $4.10^4$ $cells.ml^{-1}$.

Bone marrow cells (first subculture) were cultured for periods of up to 28 days on the surface of the six prepared titanium samples in the same experimental conditions as those used in primary cultures and in presence of ascorbic acid (50 $\mu g ml^{-1}$), β-glycerophosphate (βGP, 10mM) and dexamethasone (10M). Standard plastic tissue culture plates were used as a positive control material (control cultures, absence of material). Culture medium was changed twice a week. Cell cultures were characterization throughout the culture time at defined time intervals. Cell morphology was observed by SEM in the initial stage of the incubation (4, 7 e 24h) in order to assess the cell adhesion process to the material surface and also 3, 7 and 21 days. Cell viability/proliferation was assessed through MTT biochemical assay and cell activity was measured by alkaline phosphatase activity (ALP) were evaluated at 3, 7, 14, 21 and 28 days [Diniz et al., 2002].

For cell culture studies, data are presented from one of two replicates experiments, both of which yielded comparable results. For any given experiment, each data point represents the mean ± standard error of three replicates. Statistical analysis was done by one-way analysis of variance (ANOVA). The statistical differences between the groups were determined by the Bonferroni method. P values $\leq 0.05$ were considered significant. Analysis of variance was also used for the comparison of the surface properties of the prepared titanium samples.

### 2.4. Scanning electron microscopy

SEM was used to examine the morphologic characteristic of cells on the surfaces exposed to cells (seeded titanium samples and control cultures). Materials with cultured cells were fixed with 1.5% glutaraldehyde in 0.14M sodium cacodylate (pH 7.3), then dehydrated in graded alcohols, critical-point dried, sputter-coated with gold and analysed in a JEOL JSM 6301F scanning electron microscope equipped with a X-ray EDS microanalysis capability, Voyager XRMA System, Noran Instruments.

## 3. Results

### 3.1. Surface characterization of the treated samples

Table I shows the $R_a$ parameter (media and standard deviation) for the six prepared titanium samples. The mechanical treatments produced surfaces with significantly different roughness; chemical solution F acts reducing $R_a$, although differences were not significant. The highest and the lowest $R_a$ values were observed respectively in $J250_0$ and $L_F$ samples.

Table I. Roughness of the titanium samples

| Samples | Mechanical treated | After solution F |
|---|---|---|
| $L_i$ | $0{,}47 \pm 0{,}17$ | $0{,}38 \pm 0{,}15$ |
| $J65_i$ | $1{,}00 \pm 0{,}18^*$ | $0{,}80 \pm 0{,}14^*$ |
| $J250_i$ | $3{,}59 \pm 0{,}73^*$ | $3{,}09 \pm 0{,}70^*$ |

*Significantly different from the $J_i$ family samples

The hydrofluoric solution (F solution) was effective in smoothing titanium surface, specially for samples previously blasted with alumina, as can be observed in SEM micrographies showed in Fig.1. The different surfaces show that $L_0$ samples presented a smooth surface with some defects of decreased size and $J65_0$ and $J250_0$ samples an irregular morphology with many craks and pits among flatter-appearing areas of various sizes, characteristics that were more pronounced in $J250_0$ samples. The treatment of $L_0$, $J65_0$ and $J250_0$ with the hydrofluoric acid solution resulted in surfaces with a completely different morphology and the effect was more pronounced in the blasted samples. The $J65_F$ and $J250_F$ surfaces exhibited a homogeneous morphology consisting in uniform craters.

Blasted samples ($J65_0$ and $J250_0$) presented aluminum-rich particles. Those particles with 20μm or less were incrusted in the titanium surface as a consequence of the blasting process and were easily identified using backscattered image as aluminum-rich particles (with low atomic number), as show in Fig. 2 for $J250_0$ sample. It seems that solution F removes all aluminum contamination as evident by observation of $J65_F$ and $J250_F$ samples.

XPS spectra for $L_i$ samples show the presence of the nitrogen, carbon and oxigen peaks, as might be expected as those elements are commonly adsorbed on titanium surface. A layer of titanium oxide, $TiO_2$, was present on the surface of all the prepared samples and its stoichiometry could be confirmed through the identification of an oxygen peak with binding energy of 531 eV.

Fig. 3 shows the XPS spectra for $J65_i$ family samples; similar spectra were observed for $J250_i$ family samples. The results of XPS quantification are show on Table II. Sample $J65_0$ shows the presence of an aluminum peak with low intensity corresponding to 10.5%; the oxygen around 532 eV can be associated to aluminum oxide while the oxygen peak around 531 eV is related to $TiO_2$. XPS analysis shows that treatment with solution F remove aluminum contamination occurred during the blasting process; the oxygen peak related to the aluminum oxide was eliminated (Fig. 3 and Table II).

### 3.2. Biological practice of the titanium surfaces

#### 3.2.1. Control cultures

Control cultures grow on the standard plastic culture plates proliferated during the two weeks; maximum values for the MTT assay were observed around day 14, decreasing after that. ALP activity increased especially during the second week, attained a stationary phase during the third week and decreased afterwards. SEM observation of 21-day cultures showed the presence of abundant mineral deposits that contained Ca and P as shown by X-ray microanalysis [Diniz et al., 2002].

Table II. Surface composition obtained from XPS spectra (at %) for $L_i$, $J65_i$ and $J250_i$ family materials.

| | C | Al | Ti | O | N |
|---|---|---|---|---|---|
| $L_0$ | 41,79 | — | 13,53 | 42,93 | 1,75 |
| $L_F$ | 42,82 | — | 13,75 | 41,57 | 1,86 |
| $J65_0$ | 44,95 | 10,53 | 6,11 | 36,74 | 1,67 |
| $J65_F$ | 41,1 | — | 14,53 | 43,06 | 1,31 |
| $J250_0$ | 45,89 | 7,59 | 7,71 | 37,99 | 0,82 |
| $J250_0$ | 42,26 | — | 15,01 | 42,73 | — |

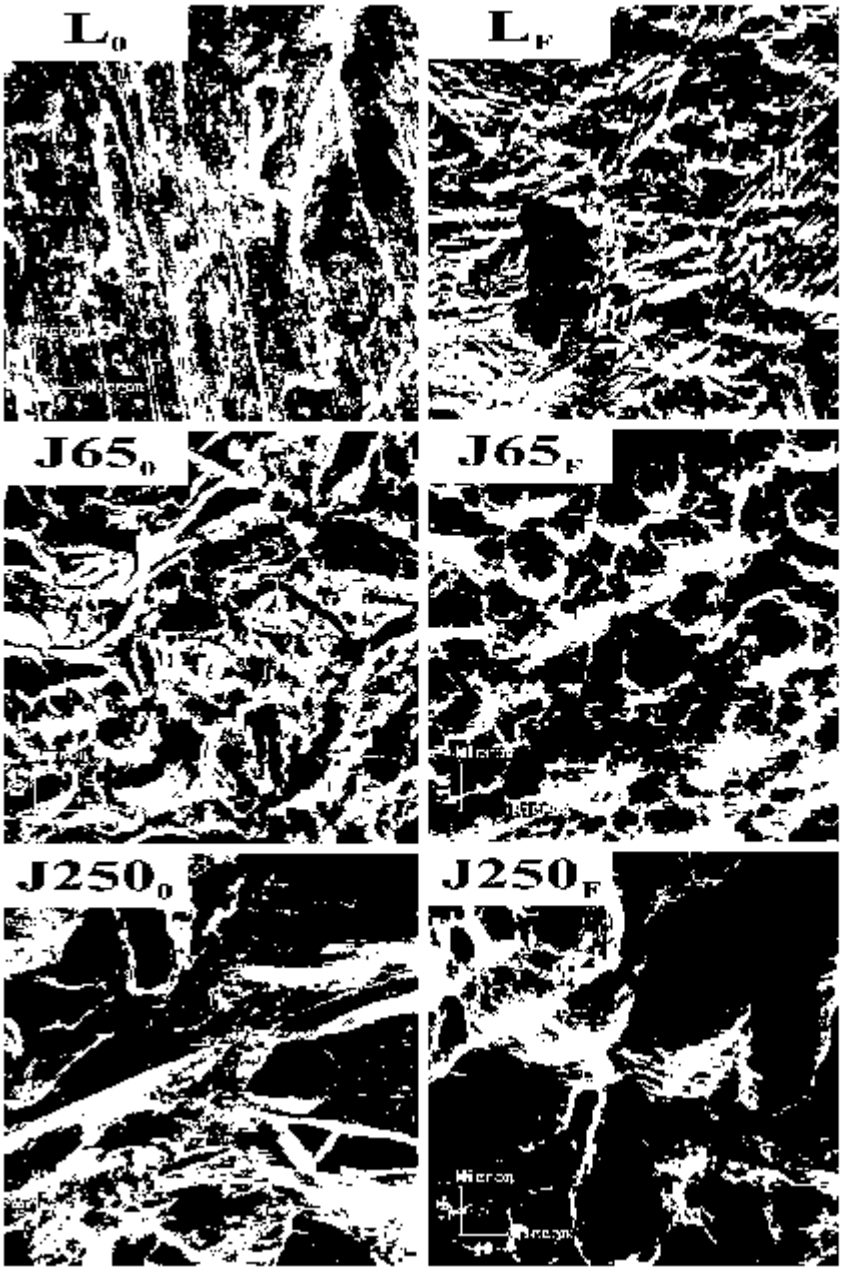



Figure 1. SEM micrographs (secondary image, x1000) of the prepared titanium samples

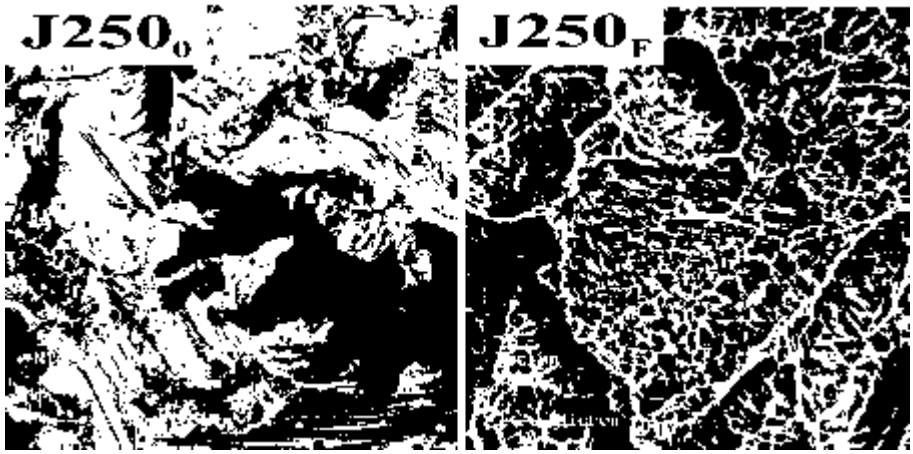



Figure 2. SEM micrographs of J250$_i$ family samples (backscattered image, x1000)

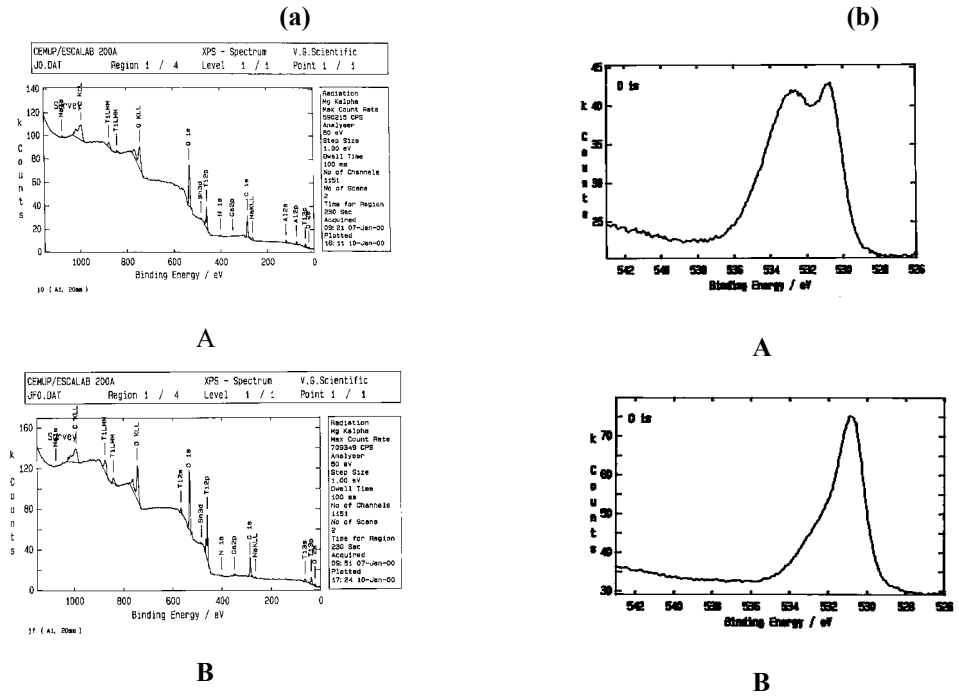


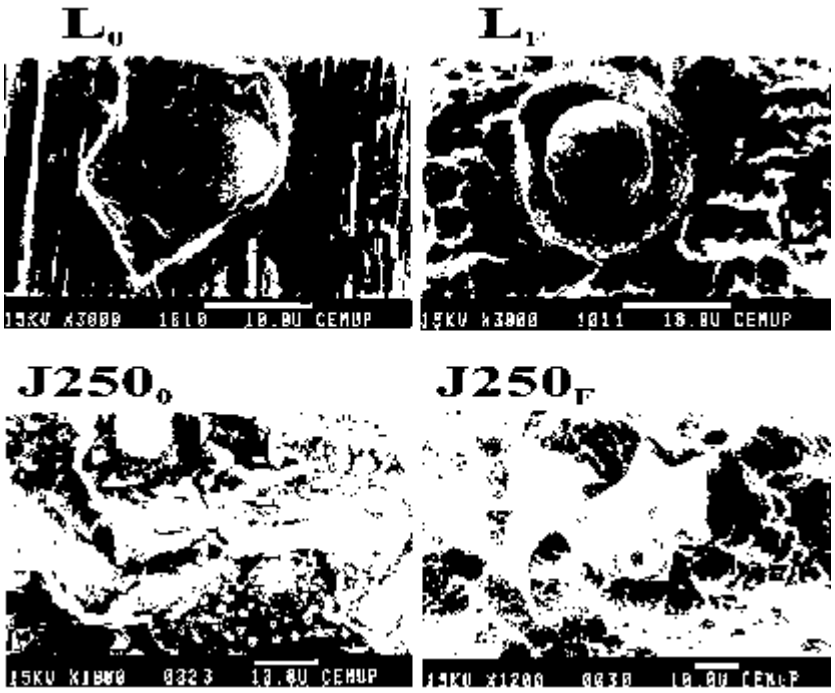

Figure 3. XPS spectra of J65$_0$ (A) and J65$_F$ (B) samples; (a) general view, (b) detail near oxygen peak.

### 3.2.2. Seeded Titanium samples

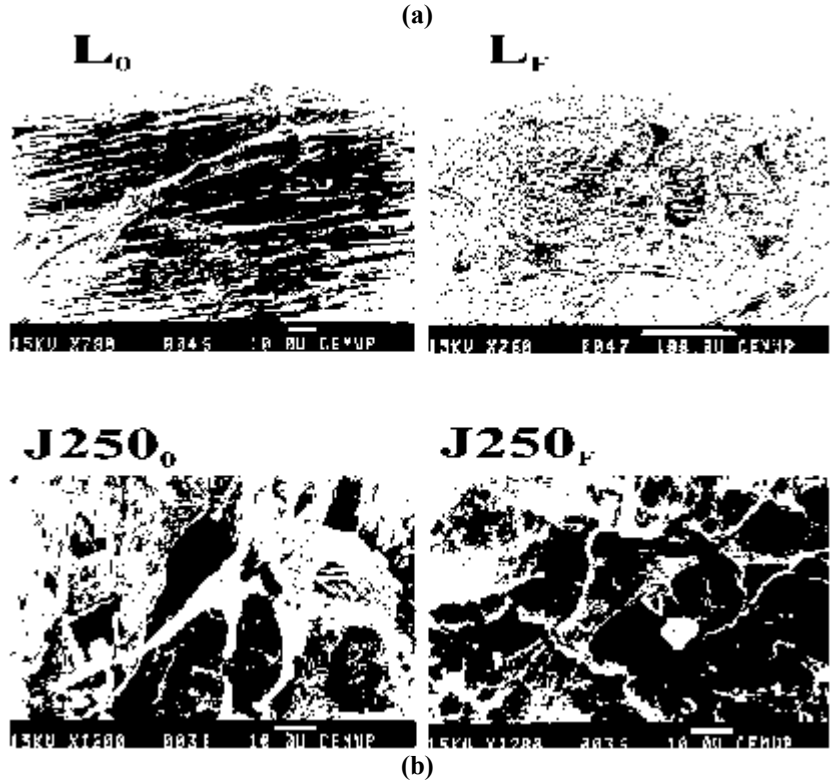
*Cell adhesion to the material surface*: Bone marrow cells began to adhere and spread on the material surface within minutes after being seeded. Observation of the cultures at time intervals during the first 24 h showed that expansion of the cytoplasm began to accur after 30 min and 4 h, cells presented a typical morphology of a central spherical body with the cytoplasm extending away from the central area in all directions and adhering to the adjacent titanium surface. As this process went on, the cells appeared to flatten and spread and, after 24 h, they were completely spread, presenting an extended morphology (similar to that observed at three days of culture). This pattern of behaviour was similar in all the samples and is exemplified in Fig. 4 for L$_i$ and J250$_i$ family samples. In L$_0$ samples, cells appeared to be oriented by shape of the surface but in the other surfaces they were randomly distribuited. On the blasted materials, most of the cells remained on the surface with filopofial-like extensions adapted to the irregular rough material but some of them took on the morphology of the underlying substrate and intimately conform to the interstices of the irregular surface (Fig. 4).

*Cell viability/proliferation and ALP activity*: Bone marrow cells proliferated very well on all the titanium surfaces and cell growth was significantly higher than that observed in control cultures. Cell growth was observed during approximately three weeks, decreasing after that [Diniz et al., 2002].

A comparison of results concerning the samples submitted to the three mechanical treatments – L$_0$, J65$_0$ and J250$_0$, showed that the blasted samples presented increased cell growth during the third week as compared with L$_0$ samples, especially J65$_0$ samples (around 30% at day 21). However, ALP activity was significantly higher in L$_0$.




(a)




(b)

Figure 4. SEM morphology of human bone marrow cells cultured on the $L_i$ and $J250_i$ family samples after 4h (a) and 24h (b).

Treatment of $L_0$ samples with the acid solution F resulted in an evident increased in cell proliferation during the third week. However, treatment of $J65_0$ surfaces resulted in a small decreased in cell growth rate. The chemical treatment of $J250_0$ samples did not affect cell proliferation [Diniz et al., 2002].

Determination of ALP activity in the seeded titanium samples showed that the levels of the enzyme were higher in the $L_i$ family samples. Treatment of $L_0$, $J65_0$ and $J250_0$ with the hydrofluoric acid solution did affect significantly ALP activity; an evident increased on the levels of the enzyme was observed. As compared with cultures performed in the standard plastic cultures plates, cultures grow on the titanium surfaces presented a significantly lower ALP activity [Diniz et al., 2002].

*Formation of calcium phosphate deposits*: Fig. 5 shows the SEM appearance of 21-day seeded titanium samples. $L_i$ family samples presented the surface covered by cell layers with a homogeneous appearance but no clear evidence of the presence of calcium phosphate deposits was found. $J65_0$ and $J250_0$ also presented most of the surface covered by

cells with numerous extending processes that adapted firmly to the irregular surface, however, mineral deposition did not seem to occur. By contrast, blasted samples submitted to the acid treatment F presented clear evidence of the formation of calcium phosphate deposits. $J65_F$ and $J250_F$ showed a similar appearance with abundant extracellular matrix and numerous globular mineral deposits.

## 4. Discussion and Conclusions

*In vivo* and *in vitro* data indicate that the microarchiture of the implant surface influences the structure and function of cells and the bone formation at the interface, including recruitment of osteoprogenitor cells, cell adhesion, cell migration, cell activation, cell proliferation and differentiation into functional osteoblasts that are able to produce a mineralized extracellular collagenous matrix [Brunette, 1988; Curtis and Clark, 1990; De Leonardis et al., Brunette and Chehroudi, 1999].During the different phases in the healing process around an implant it is also quite possible that the types of proteins adsorbed to the surface, and thereby the reactivity of the surface, may change as the result of a local production by the cells adherent to the surface or present in the vicinity of the surface. Several of the products released from the cells may also cause an alteration of the structure and physiochemical properties of the surface [Kasemo and Lausmaa, 1986]. Methods of implant surface preparation can significantly affect the resultant properties of the surface and, subsequently, the biological response of the several kinds of cells, including osteoblastic cells, as described in a number of studies [Boyan et al., 1996; Verrier et al., 1996; Ahmad et al., 1999; Cooper et al., 1999; Lohmann et al., 1999; Anselme et al., 2000; Krause et al., 2000].

Where, a combination of mechanical and chemical treatment was used to produce titanium surfaces of different roughness and morphology. Biological performance of the prepared samples was evaluated using osteoblastic cell cultures obtained from human bone marrow, the biological compartment with which the bone substitution material is confronted *in vivo* situation.

Bone marrow cells were cultured in the presence of ascorbic acid, necessary for the formation and maturation of the collagen (base for the cellular room) [Martin et al., 1993], βGP, a potential source of phosphate ions for the mineralization process [Bellows et al., 1992], and the dezamethasone, a compound that induces the proliferation and/or differentiation of osteoblastic cells [Martin et al., 1993, Cheng et al., 1994]. These experimental conditions allow the complete expression of the osteoblast phenotype, that is, the formation of a collagenous mineralized matrix in cultures maintained for long periods (3 – 4 weeks), as described in previous studies [Coelho and Fernandes, 2000; Ferraz et al., 2001].

Results showed that samples submitted to the three mechanical treatments ($L_0$, $J65_0$ and $J250_0$) presented different roughness and morphology. $L_0$ samples showed a smooth appearance and the blasted samples a very irregular morphology. In each material family, the acid treatment reduced the average rugosity $R_a$, although the differences observed were not significant (Table I). The hydrofluoric acid was effective in smoothing titanium surface, especially for samples previously blasted with alumina; $J65_F$ and $J250_F$ samples presented a homogenous topography. Aluminum-rich particles were identified on samples just blasted ($J65_0$ and $J250_0$; Fig.2 and Table II). Treatment with F solution seems to be an efficient way to dissolve aluminum oxide as in $J65_0$ and $J250_0$ samples aluminum content dropped to zero and the oxygen peak related to the aluminum oxide disappeared (Fig.3 and Table II). XPS results were in agreement with the SEM observation of the samples. As expected, all the samples presented a layer of titanium oxide on their surface.

Observation of the seeded titanium samples by SEM during the first hours of incubation showed that surface topography did not appear to inhibit cellular spreading following initial attachment to the material. In addition, a similar pattern of behaviour was observed and, after 24 h, cells have flattened on the surfaces and were completely spread. Morphological changes occurring during the attachment and spreading of the cells correspond to the reorganization of the cytoskeleton, a structure that plays important role in the control of the cell shape and behaviour [Gwynn, 1994]. In this work, the cell layer organization was influenced by the topography of the underlying substrata, results that are in agreement with other studies [Martin et al., 1995; Verrier et al., 1996; Boyan et al., 1998; Brunette and Chehroudi, 1999; Anselme et al., 2000]. On $L_0$ samples, cells seem to be oriented according to the shape of the material, occurring a species of cellular preferential orientation and for later culture times they were organized in a parallel order, covering the all surface (Fig. 5). On the other surfaces, cells were randomly distributed and appeared to successfully adapt to the irregular rough surfaces.

Results concerning the cell proliferation evaluated by the MTT assay showed that cell growth was observed until later on the blasted samples, as compared to the $L_0$ samples [Diniz et al., 2002]. These results suggest that on the rougher surfaces cell growth was enhanced, observation that in agreement with others studies that suggest that surface roughness directly affects cell proliferation [Groessner-Schreiber and Tuan, 1992]. However, it is also possible that the greater surface area of the blasted samples allowed for a long period of cell growth. By contrast, ALP activity was higher on $L_0$ samples than on $B65_0$ and $B250_0$ surfaces {DINIZ et al, 2002], suggesting a lower expression of this marker. This observation may be related with the increased proliferation rate observed in the blasted samples, as a reciprocal relationship between proliferation and differentiation has been described in the development of the osteoblast phenotype [Stein et al., 1996].

$L_F$ surface allowed for longer period of cell growth as compared to $L_0$ samples [Diniz et al., 2002]. This is most probably due to surface morphology modifications and/or increased surface area induced by the acid treatment as both parameters influence cell growth. Chemical treatment of the blasted samples did not result in relevant effects in the cell

proliferation. Concerning ALP activity, it is interesting to note that, for each material family, the samples treated with the F solution presented higher ALP activity [Diniz et al., 2002], an important osteoblastic differentiation marker [Aubin and Liu, 1996].

The mineralization of the extracellular matrix is an unknown event of cellular lines, only known in primary cellular cultures. Biological performance of the titanium samples was also evaluated concerning their ability to allow for the formation of a mineralized extracellular matrix, the last event of the osteoblastic differentiation [Aubin and Liu, 1996] and essential to the osteointegration of the implant, that is, the bone formation at the bone/material interface. $L_i$ family samples did not show the formation of mineral deposits and he same behavior was found on $J65_0$ and $J250_0$ surfaces. Only the blasted samples treated with the acid solution allowed seeded bone marrow cells to form mineral deposits. These results showed that the blasted samples treated with the acid solution F presented a better biological performance concerning the ability to form a mineralized matrix than the $L_i$ samples submitted to the same chemical treatments. Samples submitted to the grinding (Li samples) or the blasting process ($J65_i$ and $J250_i$ samples) presented different surface roughness and several studies suggest that increasing surface roughness enhances *in vitro* osteoblastic differentiation [Groessner-Schreiber and Tuan, 1992; Links et al., 1998] and that are evidences that osteoblast tend to exhibit a more mature phenotype when grown on rougher surfaces [Kieswetter et al., 1996]. The $J65_F$ and $J250_F$ samples, despite of the differences in $R_a$, presented a similar behavior with the formation of an exuberant mineralized extracellular matrix. Also, $J65_0$ and $J250_0$, with the similar roughness to, respectively, $J65_F$ and $J250_F$ did not show evidence for the presence of mineral deposits. These results suggest that, in a way similar to that observed in other studies, other surface properties such as surface texture, a combination of topography and roughness, appear to be an important variable in the biological performance of the materials [Schwartz and Boyan, 1994; Kieswetter et al., 1996]. The lack of mineralization observed on the seeded $L_i$ family samples and the blasted surfaces ($J65_0$ and $J250_0$ samples) may be related to surface characteristics that did not allow for the formation of an extracellular matrix in quantity and/or quality to support the mineralization process [Stein et al., 1996].

Aluminum contamination presented on the surface of $J65_0$ and $J250_0$ did not appear to significantly effect cell behavior or, at least, be a factor as important as the surface topography [Diniz et al., 2002]. This observation is suggested by a similar results concerning cell response observed in titanium samples with and without aluminum contamination on the surface.

For a global analysis of the gotten results, the best biological performance was found in the blasted samples treated with the hydrofluoric acid solution (that presented an increased in ALP activity and in ability to form mineralized deposits). This behaviour could be correlated to the characteristic microtopography of these samples as the hydrofluoric acid-based solution produced a homogeneous and smooth roughness. These samples may provide a more appropriate surface for the reactions that occur as the material surface is conditioned by the culture medium. The initial interaction results in the adsorption of biologically active molecules to the material surface, including cell-attachment proteins that play a critical role in the adhesion and spreading of bone cells and, also, cell growth and function [Thomas et al., 1997]. The surface properties (chemistry, energy, topography, roughness and differences in the oxide layer) would also influence the type, amount and conformation of the adsorbed proteins and may further influence the secretion of the extracellular matrix proteins [Steel et al., 1992; Moller et al., 1994]. These are important aspects because there is a general agreement that growth enhancing surface properties of the material are a prerequisite for sufficient long-term performance of an implant.

Continuing this work a FEM-Simulation (FEM: finite element method) will be used as a well-estimated research tool for the prediction of regional stress and stress-compatibility, in the contact area between the titanium implant and the bone tissue. The titanium surfaces $J65_F$ will be submitted to the techniques of profilometry and digital image processing for attainment of the representative significant dimensions of the micro topography, such as morphology, the depth and the average diameter of the crater created by chemical treatment with the hydrofluoric acid-based solution. These parameters will be part of the input data to be introduced in the Ansys program, version 5.6, to solve the problem of the implant under pressure coming from the bone tissue. This procedure will allow analyzing the stress distribution in the implant material close to its contact area with the bone tissue, besides to study the initial micro topography changing under different loading conditions. Primarily, we decided to work on small specimens, experimentally tested, and investigate to decide the degree of detail being necessary for analyzing the microstructure with respect to macroscopic relevant information. The model will be considered as a massive solid, built with two diferent matterials, using tetrahedral elements. Close to the contact area, the most interesting area, it will be used a refined mesh elements.

## 5. Acknowledgements

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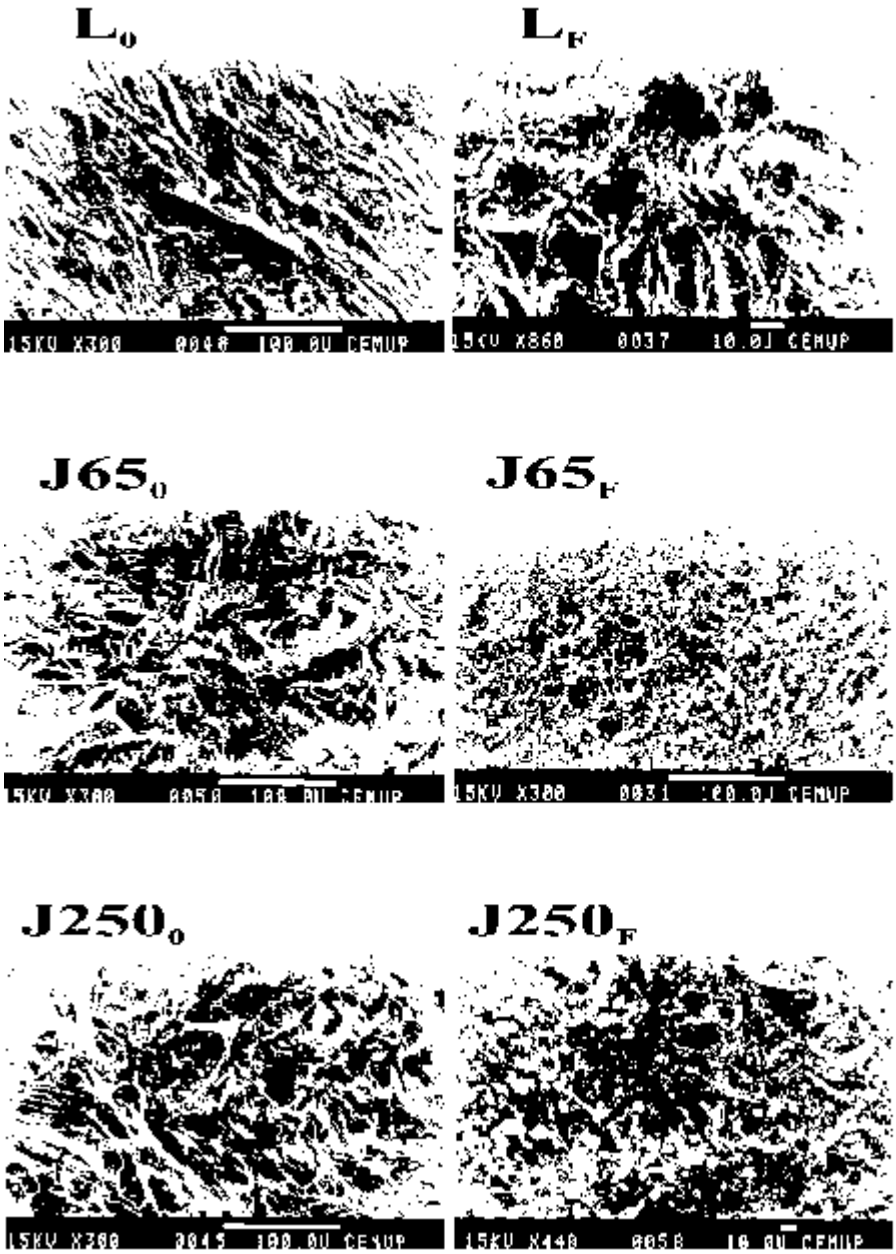


Figure 5. SEM appearance of 21-day seeded titanium samples.

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