# *In vitro* evaluation of osteoblastic cell adhesion on machined osseointegrated implants

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**Abstract:** At present the major consideration in planning an implant design is to seek biocompatible surfaces that promote a favorable response from both cells and host tissues. Different treatments of implant surfaces may modulate the adhesion, proliferation and phenotypic expression of osteoblastic cells. For this reason, the aim of the present study was to evaluate the biocompatibility of an implant surface, observing adhesion, cell morphology and proliferation of osteoblast-like cells cultivated on a commercially available titanium dental implant (Titamax Liso®, Neodent, Curitiba, PR, Brazil). The implant samples were immersed into an osteoblast-like cell (Osteo-1) suspension for a period of 24, 48 and 72 hours. After seeding the cells, the samples were prepared for analyses through scanning electron microscopy. Based on the surface analysis, the osteoblastic cells adhered to the machined surface after 24 hours in culture. In 48 hours, the cells spread over the implant surface, and after 72 hours a proliferation of cells with large and flat bodies was observed over the machined implant surface. These results demonstrate that the machined titanium surface studied is biocompatible since it allowed adhesion and proliferation of the osteoblast-like cells, in addition to preserving cell integrity and the morphologic characteristics of cells during the studied period.

**Descriptors:** Titanium; Dental implants; Osteoblasts; Osseointegration; Scanning electron microscopy.

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## Introduction

Studies in the Implant Dentistry area have expanded rapidly over the last 40 years. The recognition that oral implants could attain high success rates provided the basis for this large number of studies.[1-5]

The finding that titanium is a biocompatible material led some authors[6,7] to study its surface properties, such as chemical composition, micro- and macrostructure, contamination, cleanliness and surface properties of interaction with biomolecules.

As a biocompatible material, the applicability of titanium was confirmed mainly because of two factors: (1) its excellent resistance to corrosion, limiting the quantity of titanium ions released into the tissues; (2) its biological inactivity, in which signs of the presence of the metal appear not to influence the tissues.[8,9]

With regard to titanium biocompatibility, the surface of the implant must be chemically and mechanically cleaned to remove any strange particle or contaminative material to preserve the integrity of the receptor bed cells.[10] Irrespective of the material chosen for making the implants, it is the material's surface that comes into contact with the bone, and, in the case of titanium, the surface is covered with titanium oxide, which is formed as soon as the implant comes into contact with the oxygen molecules in the environment. Titanium can form numerous oxides, titanium dioxide being of major relevance because of its high dielectric constant.[11]

Titanium biocompatibility, as regards cellular adhesion and proliferation, has been assessed in various *in vitro* studies that have shown that osteoblasts appear to adhere more rapidly to titanium surfaces with a rougher microtopography.[12-17]

Some studies have also demonstrated the capacity of machined titanium surfaces to promote cellular adhesion and proliferation.[18-20] In addition, rough surfaces enhance osteoblastic phenotype differentiation and the capacity of osteoblasts to synthesize bone matrix.[21-23]

With regard to cellular morphology related to surfaces with different textures, the related literature has shown that cells spread themselves over a larger area on machined surfaces than they do on rough surfaces. On machined surfaces, cells present a compact and flattened morphology, and must make a big lateral effort to spread. According to an author,[24] this could be because they do not find a three dimensional structure as they do on rough substrates, which allows cellular accommodation and a larger contact surface to accommodate themselves. The cells on rough substrates present a more polygonal shape.[25,26]

Some studies have shown that, in implants with machined surfaces, cells have a tendency to follow an orientation parallel to the scratches arising from the machining of the titanium surface (anisotropic characteristic) as opposed to rough surfaces, where the cells spread throughout the entire extent of the surface (isotropic characteristic).[27-29]

In view of the foregoing explanations, the aim of the present *in vitro* study was to analyze the biocompatibility of an implant surface, observing the adhesion, cell morphology and proliferation of an osteoblastic cell lineage[30] cultivated on a commercially available dental implant (Titamax Liso®, Neodent, Curitiba, PR, Brazil) through scanning electron microscopy (SEM).

## Material and Methods

For this experiment Titamax Liso® implants (Neodent, Curitiba, PR, Brazil) were used, measuring 3.75 mm in diameter and 13 mm in length. These implants have a cylindrical design with pyramidal threads and pitch varying with implant diameter (macro surface). They have a smooth surface, resulting from machining in an automatic CNC lathe (TNL 12, Index Traub, Esslingen, Baden-Württemberg, Germany). The raw material used for manufacturing the implants was Grade II Titanium (ASTMF67). The cellular lineage used was originated from the parietal bone tissue of newborn rats (Osteo-1).[15] The cells were stored in liquid nitrogen, protected by dimethyl-sulphoxide, and defrosted in a *bain-marie* at 37°C for 2 minutes. The cells in suspension were transferred to culture plates containing 15 ml of a fresh culture with Dulbecco's Modified Eagle Medium (DMEM – Sigma Chemical Co., St. Louis, MO, USA), pH 7.4, 1% of an antibiotic/antimycotic solution and supplemented with 10%

bovine fetal serum (Cultilab Ltda., Campinas, SP, Brazil). The cells were maintained in an incubator at 37°C, in a humidified 5% $CO_2$ atmosphere. Cell culture development was assessed under an inverted phase microscope. After the surface was colonized, the culture medium was removed, the plates were washed in PBS and the cells were enzymatically released. The enzyme was inactivated with culture medium and the cells in suspension were centrifuged at 3,000 rpm for five minutes. After the supernatant had been aspired, aliquots were distributed onto new plates. After cultivation, a cell suspension of $10^9$ cells per $mm^2$ was prepared, and this suspension was plated onto three samples of the implant. The receptacles with the samples were incubated at 37°C in a humidified atmosphere with 5% of $CO_2$. After 24, 48 and 72 hours of plating, the samples were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer and post fixed in 1% osmium tetroxide in the same buffer. The samples were then dehydrated in 100% ethanol and submitted to chemical drying in hexamethyldisilane (HMDS – Electron Microscopy Sciences, Fort Washington, PA, USA). The samples were then sputter-coated with gold (Sputtering SCD 020, Bal-Tec, Balzers, Liechtenstein) and studied by means of scanning electron microscopy (Leo 430 SEM, Leo Ldta., Cambridge, UK).

## Results

A surface topography of clean titanium was observed, without the presence of foreign particles and with scratches arising from the machining process (Figure 1).

After 24 hours of plating (Figure 2), cells adhered on part of the machine surface of the implant were observed. The cells had an anisotropic orientation, with affinity for the scratches of the implant machining process.

After 48 hours, when analyzing a photomicrograph at 400 X magnification (Figure 3), it could be observed that the cells presented a more defined morphologic characteristic, with a large and flat body (flattened) and with short cytoplasmatic prolongations.

Analysis after 72 hours (Figures 4 and 5) also revealed a favorable cell behavior response by spreading and proliferation of cells over the machined titanium surface, with large and flat cells with short cytoplasmatic prolongations accompanying the implant machining scratches.

## Discussion

In the related literature, many publications reporting a superiority of rough surfaces submitted to different treatment procedures in comparison with machined surfaces as regards osseointegration in terms of cellular adhesion and proliferation can be found.[12,14,15,17,19] However, the present *in vitro* experiment demonstrated that the machined surfaces promoted a favorable cell behavior response, as is also shown in the related literature.[12,14,18,20]

Irrespective of the type of surface treatment to

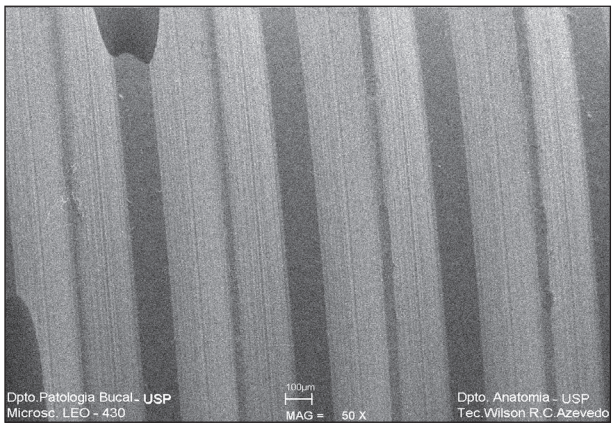


**Figure 1 -** The titanium surface topography (50 X).

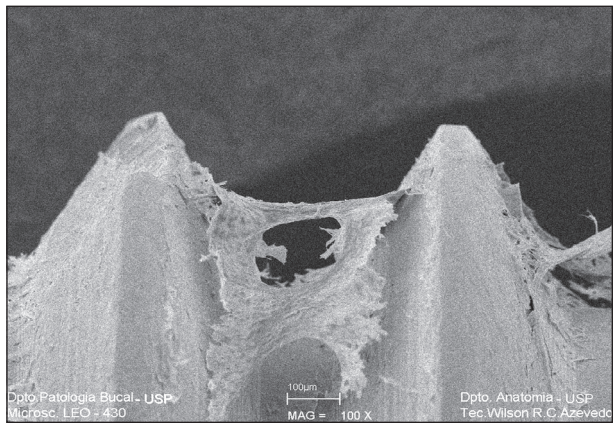


**Figure 2 -** SEM - 24 hours - Cell adhesion to the machined dental implant (100 X).

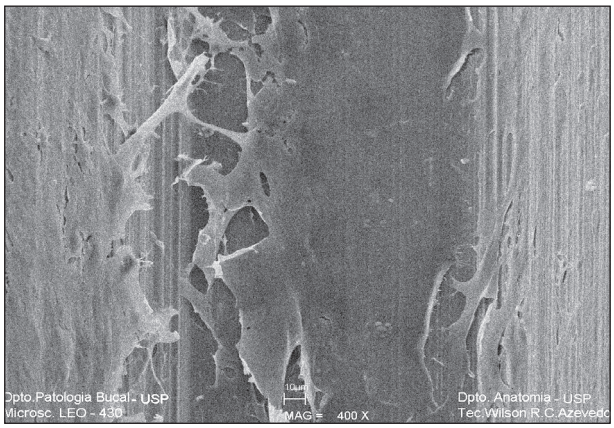


**Figure 3 -** SEM - 48 hours - Morphologic aspect of the Osteo-1 cells (400 X).

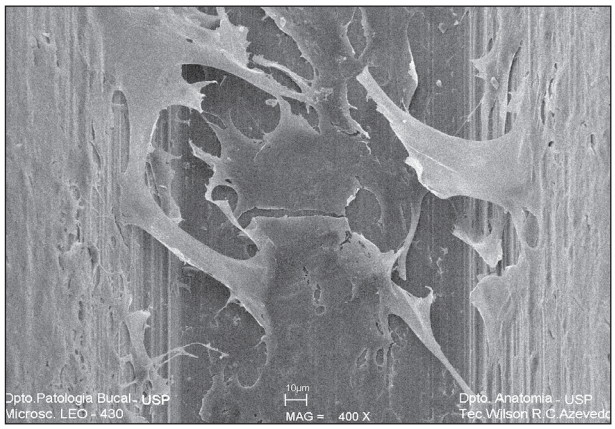


**Figure 4 -** SEM - 72 hours - Cells with large flat bodies, spread over the machined titanium surface (400 X).

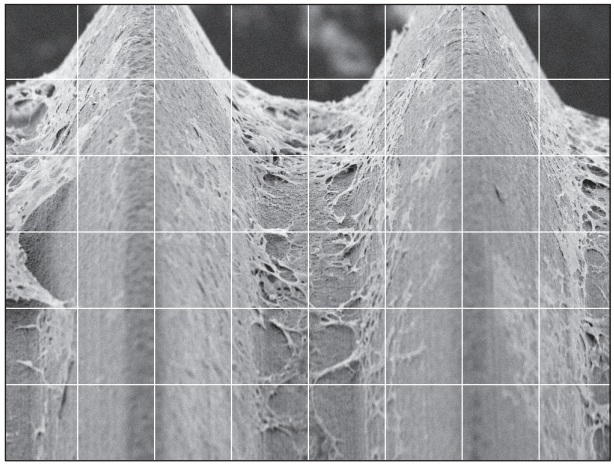

**Figure 5 -** SEM - 72 hours - Cellular proliferation over the machined titanium surface (100 X).

which an implant is submitted, in the case of titanium implants, it is their superficial layer of oxide which comes into contact with bone and provides the basis for their exceptional biocompatibility.[11] Moreover, in order for a material to be biocompatible, it must not be cytotoxic.[9,10] In the present study, the samples used and analyzed by SEM were shown to be biocompatible for their capacity of preserving the integrity of the Osteo-1 cells for a period of 24 to 72 hours.

Our observations allowed us to conclude that the machined surfaces analyzed promoted cell adhesion observed by SEM at 24 hours, and that, at 48 hours, the cells were adhered to the implant surface, with morphologic characteristics already defined. After 72 hours, our SEM observation revealed spreading and proliferation of the Osteo-1 cells over the analyzed substrate.

As regards the morphologic characteristics of the cells submitted to culture, the related literature has shown some differences with regard to cellular shape among surfaces with different textures. This could be due to the three dimensional structure of rough substrates, where cells conforming to the surface roughness attained a contact surface without the need for a greater lateral effort to spread out. In the present experiment, when analyzed by SEM, the cells were shown to have a large flattened body with short cellular prolongations, in agreement with what has been observed in the related literature with respect to cellular interaction and smooth surfaces[21] In contrast, cells on rough surfaces present a more elongated or polygonal shape, and longer cellular extensions.[25,26]

We also observed that the cells analyzed followed a direction parallel to the machining scratches. The literature relates this tendency in machined implants as opposed to rough surfaces, where the cells proliferate over the entire surface without any predominant direction.[27-29]

In the present experiment, and in agreement with the related literature, it was possible to observe that adhesion and an initial interaction between cell and substrate occurred irrespective of the treatment used on the titanium surface.

## Conclusion

Based on the findings of the present study, we concluded that the studied machined implant surface is biocompatible since it preserved the integrity of the cultivated osteoblast-like cells (Osteo-1) for a period of 24 to 72 hours, allowing their adhesion and proliferation, and maintaining their morphologic characteristics.

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