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Research article

Fibroblasts and osteoblasts behavior after contact with different titanium surfaces used as implant abutment: An in vitro experimental study

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ABSTRACT

Background: The goal of this in vitro study was to compare three different surfaces: two types of implant surfaces commercially available ([a] smooth/machined and [b] acid-treated surface) *versus* (c) anodized surface. Discs were manufactured with commercially pure titanium (CP) grade IV, which were subsequently analyzed by scanning microscopy and fibroblastic and osteoblastic cell cultures.

Methods: Ninety-nine discs (5 × 2 mm) were manufactured in titanium grade IV and received different surface treatments: (i) Mach group: machined; (ii) AA group: double acid etch; and (iii) AN group: anodizing treatment. Three discs from each group were analyzed by Scanning Electron Microscopy (SEM) to obtain surface topography images and qualitatively analyzed by EDS. Balb/c 3T3 fibroblasts and pre-osteoblastic cells (MC3T3-E1 lineage) were used to investigate each group's biological response ($n = 10$/cellular type). The data were compared statistically using the ANOVA one-way test, considered as a statistically significant difference $p < 0.05$.

Results: The AA group had numerous micropores with diameters between 5 and 10 μm, while nanopores between 1 and 5 nm were measured in the AN group. The EDX spectrum showed a high titanium concentration in all the analyzed samples. The contact angle and wetting tension were higher in the AA, whereas similar results were observed for the other groups. A lower result was observed for base width in the AA, which was higher in the other two groups. The AN showed the best values in the fibroblast cells, followed by Mach and AA; whereas, in the culture of the MC3T3 cells, the result was precisely the opposite (AA > Mach > AN). There was similar behavior for cell adhesion for the test groups (Mach and AN), with greater adhesion of Balb/c 3T3 fibroblasts

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compared to MC3T3 cells; in the AA group, there was greater adherence for MC3T3 cells compared to Balb/c 3T3 fibroblasts.
Conclusions: The findings suggest that different surface characteristics can produce different biological responses, possibly cell-line dependent. These findings have important implications for the design of implantable medical devices, where the surface characteristics can significantly impact its biocompatibility.

1. Introduction

1.1. Background

The rising geriatric population and the burden of dental diseases have increased the demand for oral rehabilitation. For instance, data updated by the World Health Organization (WHO) in March 2022 shows that estimative oral diseases affect nearly 3.5 billion people worldwide; moreover, severe periodontal disease, which may result in tooth loss, affects around 19.0 % of the global adult population (more than 1 billion cases worldwide) [1]. Dental implants are artificial biomaterials for tooth/teeth loss replacements, surgically placed in the maxilla or mandible. They have one or two major parts, usually manufactured with titanium or zirconium [2]. The Dental Implants Market is growing and is expected to increase from USD 4.58 billion in 2023 to USD 6.67 billion by 2028 [3].

The history of implantology has suffered a significant evolution since the 1980s, when the "root form" endosseous implant was introduced. Initially, the original osseointegrated implants had a moderately smooth machined surface [4] (machined or turned implants). It was the first generation of dental implant surface, described by Brannemark [5]. Even though this surface appears to be relatively smooth, scanning electron microscopy (SEM) showed imperfections, grooves, and ridges created during manufacturing. One disadvantage regarding the morphology of non-threaded or machined implants is that the surface defects resist bone interlocking, which delays the osseointegration process due to osteoblastic growth along the existing surface grooves [6].

Then, more options for surface treatments have been studied, such as mechanical treatments (machining and grit blasting), chemical treatments (acid etching), electrochemical treatments (anodic oxidation), vacuum treatments, thermal treatments, and laser treatments [7]. These surface treatments control osteoblasts' growth and metabolic action, suggesting that the structure of the implant influences the interaction between the metal and the living tissue [8]. Surface roughness has also been shown to influence cytokine and growth factor production by osteoblasts; increased surface roughness allowed transforming growth factor-beta (TGF-β) production, directly increasing osteoblast cell propagation [9].

One of the main reasons for modifying dental implant surfaces is to decrease the healing time for osseointegration, increasing the functional surface area of the implant-bone interface so that stress is effectively transferred. Additionally, the surface coating promotes bone apposition [10]. The surface of a dental implant is the only part in contact with the bio-environment, and the uniqueness of the surface directs the response and affects the mechanical strength of the implant/tissue interface [11]. Several surface textures of titanium implant substrates have been tested to improve osseointegration. The etched surface for dental implants was one of them.

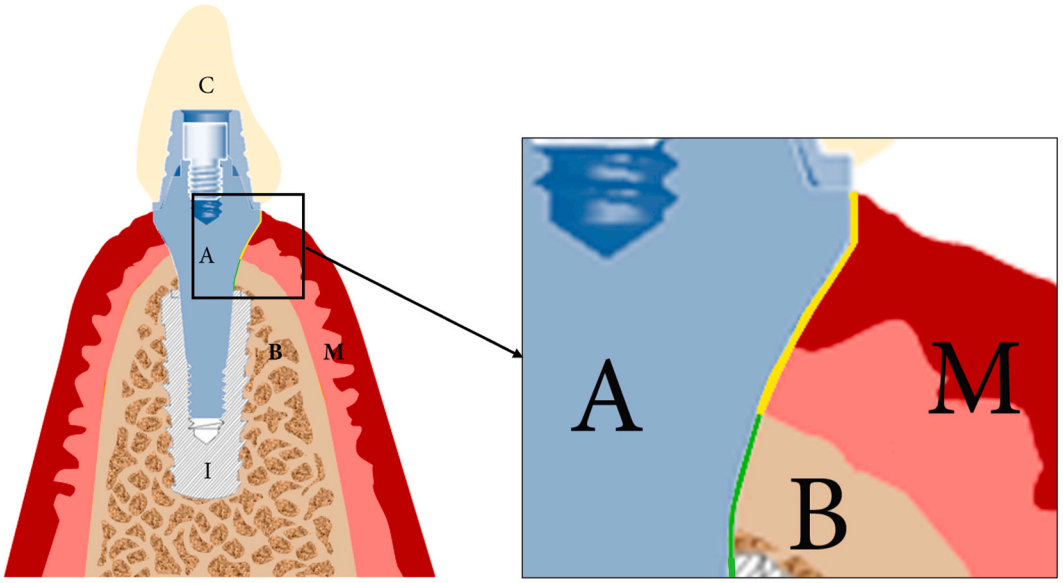


Fig. 1. Schematic image showing the contact relationship of the abutment (A) with the bone tissue (B) and the mucosal tissue (M) in implants installed at the subcrestal bone level. Implant (I) and crown (C).

Etching with strong acids was an alternative to roughen titanium implants. The process allowed for the eradication of the oxide layer and portions of the underlying material of the implant [12], providing equal roughness, an active surface area, and better adhesion [13]. In addition, it improved the viability and cellular adherence, improving the osseointegration for many years [14].

Provenly, the surface treatment of dental implants and abutments, which will contact the peri-implant tissues, can modify cellular responses [15–18]. Currently, many systems indicate the implant placement at the subcrestal bone level, permitting the contact of part of the abutment with the bone and the other with the soft tissue (schematically demonstrated in Fig. 1). As a result, new studies and propositions have emerged on how the surface treatment in the transmucosal abutment portion should be (rough, polished, or anodized) [17,19,20].

Recently, the electrochemical anodizing technique was another proposed modification on the implants' and/or abutments' surfaces. It has been widely studied due to the possibility of creating nanopores on the surface by incorporating other chemical elements (new ions) [21–23]. This fact may facilitate cell adhesion, improving the peripheral sealing-related characteristics in the crestal portion of the implant and abutment (IA) assemblies [24]. Moreover, it would allow the formation of perpendicular gingival fibers in the cervical region, improving soft tissue stability and directly influencing peri-implant health, improving the quality of the "prosthetic-implant cuticle" or biological sealing [25]. Moreover, other authors have shown that roughness close to 0.2 μm is ideal for tissue adhesion to the abutment [26,27].

Differently from a machined surface, the treatment for implant surface with acid etching is to affect the surface roughness and time of osseointegration. It makes micro-roughness of 0.5–3 μm with the formation of irregular different depth pits [28], allowing bone ingrowth. Regarding the anodizing process, recent studies indicate that the diameter and length of the nanopores may have variations, which depend on the density, applied potential, anodizing time process, and concentration and pH of the electrolyte [29,30]. In this sense, the nanopores obtained after electrochemical anodization can favor cell binding and differentiation. Furthermore, these studies emphasize that more data are needed to establish the appropriate diameter and length of the nanopores for cell recognition and adhesion [31,32].

In the culture of osteoblastic and fibroblastic cells on titanium discs, it was observed that the behavior of these cells was directly influenced by the titanium roughness resulting from the surface treatment [33–35]. Roughness between 0.7 and 2.0 μm allowed the union of osteoblasts directly on the implant surface, completely changing the surface treatment perspective, which reduced the osseointegration period and improved its quality. These studies also observed that roughness below 0.7 μm allowed a direct adhesion of fibroblasts [33–35].

1.2. Aim of the study

In this context, the goal of the present in vitro study was to compare three different implant surfaces: two commercially available surfaces (1. smooth/machined; and 2. acid-treated surface) *versus* (3) an anodized surface, in order to verify the viability and behavior of fibroblasts and osteoblasts. The discs were manufactured with commercially pure titanium (CP) grade IV, which were subsequently analyzed by scanning microscopy and fibroblastic and osteoblastic cell cultures.

2. Materials and methods

2.1. Groups and surface treatment

Ninety-nine discs with 5 mm diameter and 2 mm thickness were manufactured by Implacil De Bortoli (São Paulo, Brazil) in CP titanium grade IV, specifically for this experimental in vitro study. The discs received different surface treatments and were divided into 3 groups (n = 28/group), as described: (i) Mach group: the disks were machined and received the cleaning and decontamination

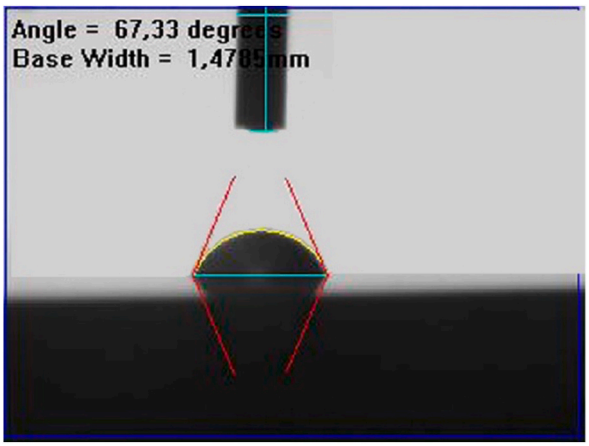


Fig. 2. Representative image of the contact angle measurement.

treatment, without any additional surface treatment to increase roughness; (ii) AA group: the discs received treatment by double acid attack, with immersion in an aqueous solution of hydrofluoric acid and immersion in a mixture of an aqueous solution of sulfuric plus hydrochloric acid and, then, received the cleaning and decontamination treatment; and, (iii) AN group: the discs were subjected to an anodizing treatment, which consisted of using a power of 64V during treatment, having graphite as the cathode. All samples were packaged and submitted to gamma radiation sterilization, following the parameters for marketed implants.

2.2. Surface characterization and analysis

Three discs from each group were analyzed by Scanning Electron Microscopy (SEM) to obtain surface topography images and qualitatively analyzed by (EDS), verifying the chemical elements present in each sample. Both analyses used a Jeol 7100FT microscope (FEG-SEM, Tokyo, Japan) [36]. Five discs from each group were used to measure the roughness parameters: the absolute values of all profile points (Ra) and the root-mean-square of the values of all points (Rq). The measurement used a roughness meter (Prazis Rug-03, Arotec, São Paulo, Brazil). Another five samples were used for the wettability analysis (surface tension), where the contact angle and the base width between the surface and the drop of distilled water (Fig. 2). The contact angle was measured using a goniometer (Ramé-Hart Instrument Co., Succasunna, NJ, USA), and software DSA3 (Krüss GmbH, Hamburg, Germany) was used for this analysis.

2.3. Cell assays

Balb/c 3T3 fibroblasts and pre-osteoblastic cells of the MC3T3-E1 lineage were used to investigate the biological response when in contact with the surface of the discs of each group (n = 10/cellular type of each group). Balb/c 3T3 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, USA) with 10 % fetal bovine serum (FBS, Gibco, Waltham, USA). MC3T3-E1 cells grew in α-MEM medium (Gibco, Waltham, USA) at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. The culture medium was changed at 2-day intervals, and semi-confluent cells were trypsinized and used in all experiments [37]. Cells seeded on glass coverslips were used as a control group. As positive and negative controls, sodium dodecyl sulfate (SDS) 1 mg/mL and DMEM culture medium supplemented with 10 % fetal bovine serum were used, respectively.

The Live and Dead® viability kit (Invitrogen, Waltham, USA) using calcein-AM and ethidium (EthD-1) (Invitrogen, Waltham, USA) was used to quantify the growth of viable cells seeded on the surface of each disc in the 3 proposed groups. Balb/c 3T3 cells and MC3T3-E1 cells, using a density of 5×10^4, were seeded on the surface of each sample for 24 h. Calcein-AM, which was cleaved by cellular esterase, was present in viable cells to form a green fluorescent product that is membrane impermeable. Ethidium homodimer-1 is a red fluorescent marker that binds to nucleic acids and only passes through the compromised membrane of non-viable cells.

After 24 h, the culture media were carefully removed from the wells, and the cells were washed with PBS at pH 7.4. The discs were inverted in a mixture containing calcein-AM (2 mM) and EthD-1 (1 mM) and then incubated for 30 min at 37 °C in a humid atmosphere. Cells were washed 1× PBS, and images were acquired by a fluorescent microscope (Zeiss A1 Observer, Oberkochen, Germany). Live and dead cells adhering to the disc surfaces were then visualized by fluorescence microscopy, observing the number of cells in 3 fields of the same sample at 10× magnification. Calcein AM staining for live cells in green and EthD-1 staining for dead cells in red [38].

2.4. Adhered cell count

The cell adherence on the surfaces was quantified and calculated as a percentage of the residual cells compared to the initial cell count on the same surface after a standardized washing procedure. First, the samples were placed in Karnovsky's solution for cell fixation on the surface of the discs, lavender 3 times with cacodylate buffer, and after fixation with 1 % osmium tetroxide (Sigma-Aldrich, San Luis, USA) [39].

2.5. Scanning electron microscopy (SEM)

Three discs from each group were randomly selected to be imaged in SEM, verifying the morphology of Balb/c 3T3 and MC3T3-E1 cells adhered to the surface of the disks 24 h after sowing. The procedures for fixing and washing the cells on the surface of the discs were the same as those previously described for counting adhered cells. After these procedures, the samples were dehydrated in increasing concentrations of ethanol (30 %, 50 %, 70 %, 80 %, 90 %, and 100 %), followed by subsequent drying of the critical point, coating with gold, and obtaining images on the Jeol 7100FT microscope (FEG-SEM, Tokyo, Japan) at × 5000 and × 20,000. For cell adherence, SEM had the magnification at×100 and × 1000.

2.6. Statistical analysis

The data were compared statistically using the ANOVA one-way test to verify differences among the 3 groups studied with the 2 types of cells (Balb/c 3T3 fibroblast cells and pre-osteoblastic cells of the MC3T3-E1 lineage). In addition, Bonferroni's multiple comparison tests were used to determine the difference among the 3 groups/cells and to verify the correlation between surface roughness and wettability, roughness and cell adhesion, wettability, and cell adhesion. GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA) was used to analyze the data, considering a statistically significant difference $p < 0.05$.

3. Results

3.1. Surface characterization

Fig. 3 shows the characteristics of the surfaces used in the study obtained by SEM. The surface of the Mach group showed grooves produced by the cutting tool during machining. On the surface of the AA group, numerous micropores with diameters between 5 and 10 μm were observed. Meanwhile, nanopores between 1 and 5 nm were measured on the surface of the AN group. The EDX spectrum showed a high titanium concentration in all the analyzed samples. All groups showed different rugosity values for the parameters analyzed (Table 1).

Table 2 shows the mean values and standard deviation of the contact angle, the base width, and wetting tension between the different surfaces and the water drop deposited. Fig. 4 shows a bar graph with the data distribution and the statistical analysis between the groups. The contact angle and wetting tension were higher in the AA group, whereas similar results were observed for the other groups (MACH and AN). Inversely, a lower result was observed for base width in the AA group, which was higher in the other two groups.

3.2. Cell viability

Three growth curves were performed for two cell lines seeded on the 3 surfaces. After 24 h in the control culture, 95 % of the cells adhered to the plastic. The same cell line grew on the discs, reflecting absorbance values similar to the control (C-). Cell lysis was observed in positive controls with SDS and consequent loss of the cells' morphology (Fig. 5). In the culture of Balb/c 3T3 cells, the best values were shown by the AN, Mach, and AA groups. However, in the culture of the MC3T3 cells, the result was exactly the opposite among the groups (AA > Mach > AN). Figs. 5 and 6 show the viability results of both cells tested on each group.

3.3. Cell adhesion and ultrastructural morphological study

For cell adhesion, there was similar behavior for the test groups (Mach and AN) for both cells tested, with greater adhesion of Balb/c 3T3 cells compared to MC3T3 cells. In the AA group, however, greater adherence was observed for MC3T3 cells compared to the culture of Balb/c 3T3 cells (Fig. 7). The data obtained had the following values for the 3T3 Balb cells: 33.6 ± 2.14 % for the MACH discs, 30.7 ± 2.79 % for the AA discs, 34.3 ± 2.62 % for the AN discs; and for the MC3T3 cells: 32.9 ± 2.85 % for the MACH discs, 37.2 ± 2.98 % for the AA discs, 32.5 ± 2.27 % for the AN discs. Thus, the Balb/c 3T3 cells demonstrated a greater ability to adhere to the AN discs than the other two disc surfaces. In comparison, the MC3T3 cells exhibited a higher ability to adhere to the AA discs than the other two disc surfaces. The bar graph of Fig. 8 shows the comparison between the groups/cells.

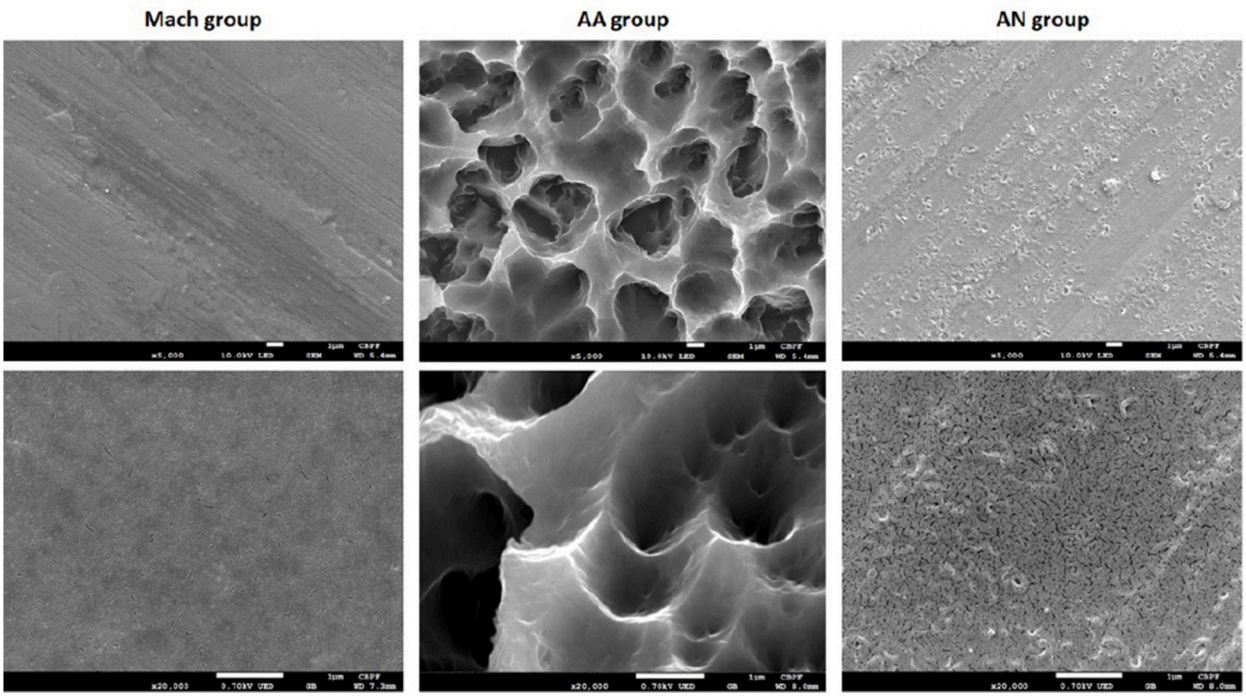


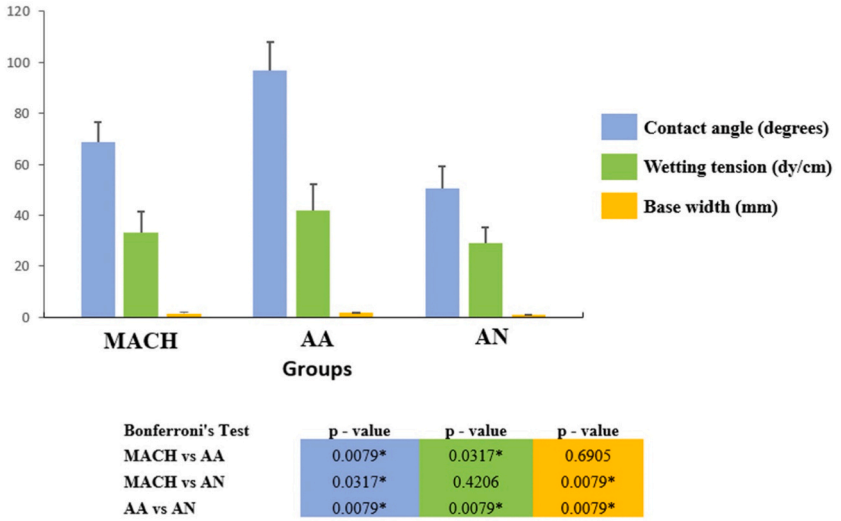
Fig. 3. SEM images for the three groups with magnifications of×5000 and × 20,000.

Table 1
The analysis of surface roughness.

Group	Ra (SD)	Rq (SD)
Mach	0.32 (0.04) μm	0.35 (0.03) μm
AA	0.80 (0.07) μm	0.97 (0.07) μm
AN	0.52 (0.04) μm	0.63 (0.03) μm

Table 2
Mean values and standard deviation of the contact angle, the base width, and wetting tension.

Groups	Contact angle (degrees)	Base width (mm)	Wetting tension (dy/cm)
MACH	68.7 ± 8.08	1.51 ± 0.31	27.0 ± 13.82
AA	118.9 ± 8.76	0.96 ± 0.16	41.8 ± 18.65
AN	66.6 ± 5.46	1.59 ± 0.13	28.9 ± 6.40



Bonferroni's Test	p - value	p - value	p - value
MACH vs AA	0.0079*	0.0317*	0.6905
MACH vs AN	0.0317*	0.4206	0.0079*
AA vs AN	0.0079*	0.0079*	0.0079*

Fig. 4. Bar graph with the data distribution of the parameters measured and the statistical analysis between the groups. *Statistically significant difference ($p < 0.05$).

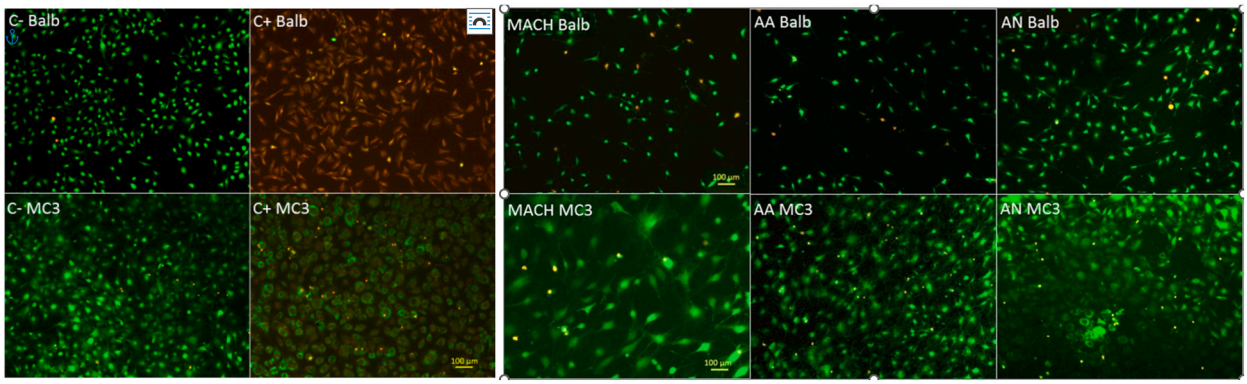


Fig. 5. Images of the positive and negative control tests (left side); Image of the fluorescent cells present in each group tested with both types of cells (right side).

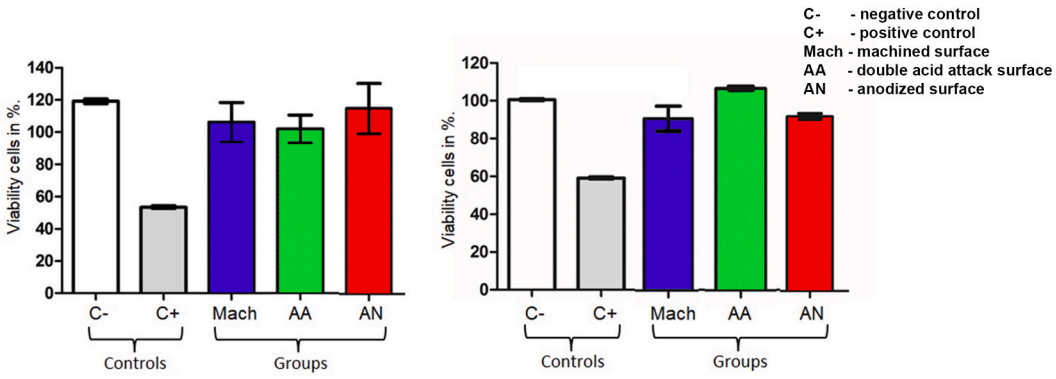


Fig. 6. Results for the viability of both cells tested on each surface (group). 3T3 Balb cells (left) and MC3T3 cells (right).

4. Discussion

Biomaterials are very important tools in Regenerative Medicine, and evaluations must precede their clinical application to anticipate possible impacts on the biological medium [40] and avoid adverse outcomes. This assumption implies the need for in vitro biocompatibility testing of biomaterials. Within this context, chemical modifications on the implant surface have been investigated as a potential modulation for tissue reactions, enhancing the cells spreading, signaling, and differentiation [41] and significantly benefiting bone formation [42,43]. Several chemical modifications have been performed on titanium surfaces, increasing the roughness and improving the osseointegration. Even after decades of research, the influences of roughness and other surface parameters on biological outcomes remain a complex topic. The literature shows how surface parameters reliably and definitely predict osteoblast cell behavior on titanium implant surfaces [44,45]. Furthermore, authors demonstrated that surface treatment can positively help the osteoinductive potential of surfaces of dental implants and the biological components [46] and, other authors have demonstrated the importance of the structure and the molecular features of dental implant titanium alloy [47]. The most common modifications/treatments reported in the literature and currently used are acid-etched treatment, anodic oxidation, crystalline deposition, and ultraviolet treatment/photofunctionalization [48–50]. Then, the aim of this in vitro study was to compare machined, acid-treated surface, and anodized surfaces of discs manufactured with CP titanium grade IV, which were analyzed by SEM and in cells cultures (Balb/c 3T3 fibroblast cells and osteoblastic cells [MC3T3 cells]).

Surface wettability refers to the ability of a material to attract or repel liquids. A hydrophilic surface attracts water, while a hydrophobic surface repels water. In implantable materials, surface wettability is essential because it can affect how the material interacts with biological fluids such as blood and tissue [51]. Surface wettability (hydrophilic or hydrophobic) is a feature that can lead implantable materials to achieve these goals more efficiently [52], as they promote the initial interactions between the surface and the blood clot, being relevant to the healing process [53]. The initial interactions between the surface of the material and these fluids are critical to the healing process. The physical-chemical characteristics of the surface, such as the presence or not of roughness on the surface of the implant components, can alter the interaction of the tissues [51]. However, the results obtained in our study showed a different behavior between the evaluated cell types (fibroblasts and osteoblasts) concerning the type of surface. The fibroblastic [3T3 Balb cells] performed better in discs from the AN group, which showed greater hydrophilicity than the other 2 groups (MACh and AA). These findings corroborate results presented in other similar studies [54]. On the other hand, the osteoblastic cells (MC3T3 cells) performed better in the AA group, which showed greater roughness and less hydrophilicity compared to the MACH and AN groups. Regarding these results, there is a great deal of discussion in the literature, with studies showing that greater surface roughness presents better outcomes for the growth of fibroblastic cells [55] and others showing that surfaces with lower roughness values can present a superior performance [56].

Usually, the TiO_2 layer of an unmodified titanium implant is 17–200 nm thick on the surface, whereas an anodic oxidation surface can expand the TiO_2 layer to between 600 and 1000 nm [50]. Moreover, many porosities are present in an anodic oxidation TiO_2 layer, favoring osteoblasts and fibroblast deposition, adhesion, and proliferation [57,58], characterizing it as a cell-conductive surface [57]. In our study, analyzing cell viability in 3T3 Balb cells culture, the following sequence was found: AN group > Mach group > AA group, whereas an opposite result was verified for MC3T3 cells culture: AA > Mach > AN. Similar viability was observed for adhesion results, which had a significantly higher attachment in the test surfaces. This fact shows that anodic treatment (AN) had an enhanced attraction for fibroblast cells, favoring its use in an intermediate abutment; otherwise, acid-etched (AA) treatment had a preference for osteoblasts. Therefore, many studies found increased osteoblast differentiation with a higher microroughness surface, contradicting our results when investigating machined or polished titanium surfaces and comparing them with different surface treatments [59–63].

The anodized surface, 16 weeks after implantation in a type IV bone (monkeys), displayed 74 % bone-to-implant contact [64]; on the other hand, our study had low osteoblasts adhesion (33 %). In our in vitro results, for anodized surface (AN group), similar adhesion was observed between machined and AN surfaces. Contrastingly, other authors reported clinical findings that suggested a 10 % higher success rate for anodized implants than machined implants after immediate loading [65]. In addition, studies showed that anodization of titanium surfaces significantly reduced the number of adhered bacteria [66], which might be interesting in preventing

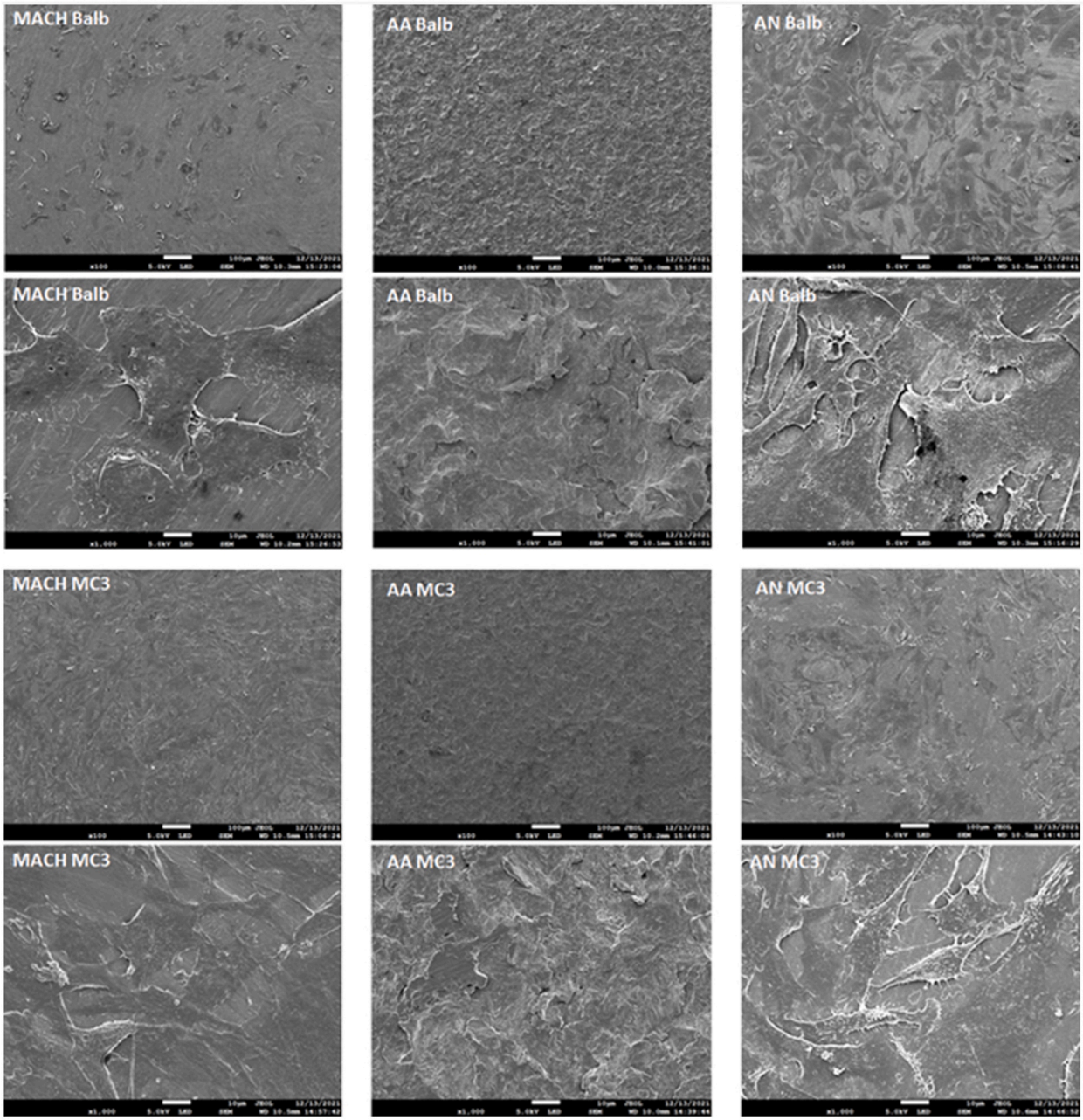


Fig. 7. Cells adhesion demonstration spreading and growing. Mach group, left; AA group, middle; and AN group, right. 3T3 Balb cells (2 rows at the top); MC3T3 cells (2 rows at the bottom). Magnification at×100 and × 1000.

infection and possible peri-implant diseases [67]. Nevertheless, this fact cannot overcome bacterial accumulation challenges, needing patient cooperation or attention if the patient has a compromised immune system.

The cell culture experiments demonstrated that all surfaces tested were biocompatible, regardless of the topography and chemical composition. This fact was shown due to the cell attachment, proliferation, and a high proportion of viability found. Initial interaction between the surface and cells produces a layer of macromolecules that mediate cell attachment and spread [68]. It agrees with other studies on the biocompatibility of titanium [69,70].

4.1. Limitations of the study

This study had limitations. It was an in vitro study, which has a reduced level of complexity compared to in vivo studies. Also, this study did not include all types the surfaces existent, such as sandblasted, sandblasted plus acid etch or addition methods with

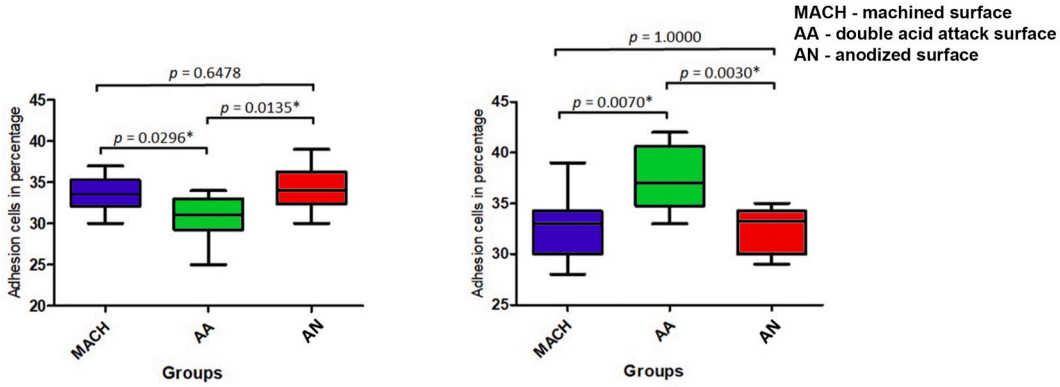


Fig. 8. Statistical analysis for the cell count in 3T3 Balb cells (left) and MC3T3 cells (right).

hydroxyapatite, staying limited to machined, anodized, and acid-etch surfaces. In this sense, Velasco-Ortega and Collaborators [xx] recently published that higher values of alkaline phosphatase were observed for surfaces treated with sandblasting with and without acid etching compared to smooth surfaces and only treated by acid etching, indicating greater activity in osteoblastic differentiation. However, another study showed that these surfaces showed low bacterial proliferation [71]. Finally, it is important to highlight that other materials as an alternative to titanium [72], such as zirconia, available on the market, were not tested in the present study.

5. Conclusions

Our results permitted us to observe that all surfaces were biocompatible and non-cytotoxic. Therefore, anodized treatment had significantly greater adhesion for Balb/c 3T3 fibroblast cells, whereas acid-etched presented more significant attraction for MC3T3. In conclusion, this study's results demonstrate that titanium discs' surface characteristics, such as cell viability and adhesion, can significantly impact cell behavior. The findings suggest that different surface characteristics can result in different biological responses, which may be cell-line dependent. These findings have important implications for the design of implantable medical devices, where the device's surface characteristics can significantly impact its biocompatibility. Future studies evaluating other surfaces and alternative materials to titanium should be developed to verify their behavior.

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CRediT authorship contribution statement

José Henrique Cavalcanti de Lima: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Patricia Cristina Matos Bobbs:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elena Mavropoulos Oliveira Tude:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Piedad N. De Aza:** Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Eleani Maria da Costa:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Antonio Scarano:** Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Juan Carlos Prados-Frutos:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Gustavo Vicentis Oliveira Fernandes:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis. **Sergio Alexandre Gehrke:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interestsSergio Alexandre Gehrke reports was provided by Ministry of Science Technology and Innovations. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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