Effects of Titanium Surfaces on the Developmental Profile of Monocytes/Macrophages

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Due to the critical role of monocytes/macrophages (Mφ) in bone healing, this study evaluated the effects of bio-anodized, acid-etched, and machined titanium surfaces (Ti) on Mφ behavior. Cells were separated from whole human blood from 10 patients, plated on Ti or polystyrene (control) surfaces, and cultured for 72 h. At 24, 48 and 72 h, cell viability, levels of IL1β, IL10, TNFα, TGFβ1 inflammatory mediators, and nitric oxide (NO) release were analyzed by mitochondrial colorimetric assay (MTT assay) and immunoenzymatic assays, respectively. Real-time PCR was used to verify the expression of TNFα and IL10 at 72 h. The data were subjected to a Kruskal-Wallis analysis. IL1β, TNFα and TGFβ1 release were not significantly different between the Ti surfaces (p>0.05). The presence of NO and IL10 was not detected in the samples. Cell viability did not differ between the samples cultivated on Ti and those cultivated on control surfaces, except at 24 h (p=0.0033). With respect to the mediators evaluated, the surface characteristics did not induce a typical Th1 or Th2 cytokine profile, although the cell morphology and topography were influenced by the Ti surface during the initial period.

Introduction

After implant insertion, the monocyte-derived macrophages are among the first cells to arrive at the implant surface (1,2). These cells are considered to be key regulators in both the initiation and the resolution of inflammatory responses (1), releasing a large array of products into the extracellular environment, including intermediate reactive oxygen and nitrogen species (3), chemokines (1,4,5), cytokines (5,6), and growth factors (7).

The mediators released by monocyte/macrophage cells can be divided into two major categories: pro-inflammatory, such as interleukin-1 beta (IL1β) (1,2,4,5), tumor necrosis factor alpha (TNFα) (1,4-6), and IL6 (1-2,4,5); and anti-inflammatory or modulatory, such as IL10 (4-6,8) and transforming growth factor-beta (TGFβ) (9). This dichotomy established the concept that monocytes/macrophages develop into two major functional types: M1 (classically activated) and M2 (alternatively activated) (10-12).

In general, classically activated M1 macrophages up-regulate pro-inflammatory cytokines, such as TNFα and IL1, inhibit anti-inflammatory cytokines, produce NO, and induce the Th1 cytokine response. In contrast to M1 macrophages, alternatively activated M2 macrophages typically produce low levels of pro-inflammatory cytokines, express anti-inflammatory compounds, such as IL10 and TGFβ, and possess the ability to facilitate tissue repair and regeneration (12,13). Although the M1 and M2 macrophage subsets are commonly referred to as readouts of macrophage functional status, these primary phenotypes can blend into a continuum of secondary phenotypes with distinct biological functions (10-14).

Taking into account that the cytokine profile released by monocytes/macrophages around dental implants is modulated by the surface characteristics of the implant (1,6), it is possible to postulate that the properties of the implant material can influence both cell activity (14,15) and phenotypes (12,13). In other words, the differential wound healing responses elicited by the different biomaterials might be due to the different macrophage functional profiles induced by the materials’ surfaces (12). Although the magnitude of the inflammatory response for an optimal bone response is unknown (6), compelling data have increased interest in understanding macrophage activation in the context of biomaterials (12-16).

Few studies compare the effects of different titanium surfaces on macrophage activation or consider the role of material surface roughness on protein adsorption (9,15,16). Accumulating evidence suggests that the interactions between macrophages and adsorbed proteins on the material surface provide intracellular signals that are essential for regulating cell function (6,12,14). Thus, it seems that the best properties demonstrated on some titanium surfaces might be related to experimental conditions in which the surface-adsorbed proteins were neglected.

Considering that implanted biomaterials are immediately coated with proteins from the blood and that these
adsorbed proteins mediate subsequent cell adhesion (12), studies involving biomaterials should seek to mimic this condition. In this way, additional studies using plasma-coated titanium surfaces to evaluate monocyte/macrophage behavior are helpful for understanding how the microenvironment may modulate the events related to tissue repair. Despite the fact that previous in vitro and in vivo studies have demonstrated the excellent properties of Ca and P in “de novo bone formation” (17,18), the influence of the interaction between plasma proteins and bioactive titanium surfaces on the pattern of macrophage responses as compared to other rough surfaces is not yet known.

In the present study it was hypothesized that the binding of plasma proteins masks the presence of these ions; differences in roughness, rather than chemical differences, mediate the events related to the development of the specific immune profile of human monocytes/macrophages. To address this hypothesis, the levels of nitric oxide (NO), IL1β, TNFα, and IL10, which are chemical mediators related to the diverse biological activity of macrophages, were quantified. The present study also evaluated the cell morphology and viability of human monocytes/macrophages after culture on machined, acid-etched and bio-anodized titanium surfaces, all of which were first coated with autologous plasma.

**Material and Methods**

For this research, commercially pure titanium (cpTi grade 4) disks (10-mm diameter and 4-mm thickness) were manufactured by Conexão Sistema de Próteses, São Paulo, SP, Brazil, with three types of surface treatments: machined, acid-etched, and bio-anodized.

The machined disks were generated from cpTi bars in a turning procedure and did not receive any additional treatment. The acid-etched and bio-anodized surfaces were subjected to different treatments, resulting in modified surfaces when compared to the machined (heterogeneity control) surfaces. The acid-etched dental implants were obtained by immersion in a mixture of HNO3, HCl, and H2SO4, resulting in surfaces with an average surface roughness (Ra) of ~0.51 µm. The bio-anodized samples were prepared using micro-arc oxidation with an electrolyte solution containing Ca and P at high anodic-forming voltages and a galvanostatic current (19), showing an average surface roughness (Ra) of ~0.87 µm. The surface roughness and hydrophobicity of these types of implant surfaces were previously evaluated using scanning electron microscopy, profilometry (20), thin-film X-ray diffraction and X-ray photoelectron spectroscopy (21). A detailed description of the preparation method for these surfaces and their characterization has been previously published (19-21).

**Ethical Considerations**

This research project was approved by the Ethics Committee of the Federal University of Uberlândia, MG, Brazil (Process #246/08), and written informed consent was obtained from all blood donors.

**Cell Culture**

Whole blood from 10 healthy adult volunteers was drawn into heparinized tubes and diluted at 2:1 in Hank's balanced saline solution (HBSS; Cultilab, São Paulo, SP, Brazil) (pH=7.2–7.4). The diluted blood (7 mL) from each donor was carefully layered over 3 mL of gradient separation medium (Percoll™; St. Louis, MO, USA) in a 15 mL conical centrifugation tube (Cultilab) and centrifuged at room temperature for 30 min at 800 g to separate the monocytes and lymphocytes from the remaining blood elements. The interface, which contained the mononuclear cells, was aspirated and transferred to a sterile tube, washed with HBSS and centrifuged at 1000 g for 10 min at 4 ºC. The mononuclear cells were stained with trypan blue, counted, and re-suspended in RPMI 1640 (Sigma Chemical Company; St Louis, MO, USA) containing 5% fetal calf serum (FCS; Cultilab) and 1% penicillin-streptomycin (Gibco-Life Technologies; Grand Island, NY, USA). The plasma from each patient was removed by pipette and filtered through a 0.2-µm filter. The autologous blood plasma fraction was used to incubate the disks for 10 min and was then aspirated away. The mononuclear cells were then plated onto the titanium surfaces at 1x10⁶ cell/mL/well and were incubated for 3 h in a humidified, 5% CO2 incubator at 37 ºC to allow the cells to settle onto the different surfaces. Next, the plates were centrifuged at 400 g for 10 min, and non-adherent cells were removed by aspiration. Fresh RPMI medium was added to each well; the plates were then returned to the incubator and cultivated for 72 h. The samples were analyzed at 24, 48 and 72 h. For each donor in each period, the disks were analyzed in triplicate. Polystyrene-surface (PS) wells were used as a negative control. Therefore, in each analyzed period, 30 independent experiments were analyzed.

**Cell Adhesion and Viability**

The qualitative analysis of cell adhesion was determined at 24 h using fluorescence labeling. The cells were fixed for 10 min at room temperature with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.2. The cells were permeabilized with 0.5% Triton X-100 in PB for 10 min, followed by blocking with 5% skim milk in PB for 30 min. The samples were incubated with Alexa-Fluor 488-phalloidin (1:200 in PB; Molecular Probes, Eugene, OR, USA) for 50 min for cytoskeleton visualization. The disks were then washed three times in PB and stained with 300 nM of
nuclei. The titanium disks were placed face-up on glass slides and mounted with a 10-mm round glass coverslip using an anti-fade mounting medium (Molecular Probes). Replacement of the phalloidin with PB was used as control. The samples were examined under epifluorescence using a Nikon Eclipse E200 microscope (Nikon Instruments Inc., New York, NY, USA) connected to an Evolution MP Color Camera (Media Cybernetics Inc., Bethesda, MD, USA). The camera used Image-Pro Plus 7.0 software (Media Cybernetics Inc.), and the acquired digital images were processed with ImageJ software (National Institutes of Health, Bethesda, MD, USA) for qualitative analysis.

Cell viability was estimated at 24, 48 and 72 h using a mitochondrial colorimetric assay (MTT assay). Briefly, in each well, 600 µL of culture medium was aspirated, leaving 400 µL to which 40 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, Sigma Chemical Company) was added. The cells were incubated at 37 °C and 5% CO₂ for 4 h. The upper medium was removed carefully, and the intracellular formazan was solubilized by adding 400 µL of dimethylsulfoxide to each well (Sigma Chemical Company). Then, the contents of the wells were mixed thoroughly using a pipette. Two hundred microliters from each well were transferred to a separate well on a 96-well ELISA plate (Corning Costar, Lowell, MA, USA). The absorbance was measured at 570 nm. The results, expressed as optical density (OD), were obtained for three different experiments from each surface modification.

**Measurement of Nitric Oxide (NO)**

The concentration of nitrates in the supernatants of the cells was taken as a measure of NO production. Nitrite levels were determined by measuring the accumulated level of nitrite in the culture supernatant using a colorimetric reaction with Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% H₃PO₄). In each well, equal volumes of the culture supernatant and the Griess reagent were mixed and incubated in the dark for 10 min at room temperature; the absorbance was measured with a microplate reader at 540 nm. The concentration of nitrite in the samples was determined using a sodium nitrite standard curve.

**Cytokine Levels**

IL1β, TNFα, IL10 and TGFβ1 levels were measured with sandwich enzyme-linked immunosorbsent assays (ELISAs) according to the manufacturer’s instructions (Elisa Ready-Set Go; e-Bioscience, San Diego, CA, USA). The optical density of each well was determined using a microplate reader set to 450 nm and corrected at 570 nm. The sample values were determined by comparison to a standard curve. All of the samples were assayed in triplicate.

**Cytokine Expression**

Total RNA at 72 h was isolated using TRIZOL. Five micrograms of total RNA was used as a template for first strand cDNA synthesis in a 20-µL reaction using Superscript™ III Reverse Transcriptase (Invitrogen life Technologies, Carlsbad, CA, USA). The following procedure was used in all cases: 5 µg total RNA, 0.5 µg oligo (dT), 1 µL dNTP mix, and sterile distilled water up to a total volume of 13 µL were added to a microcentrifuge tube and mixed. The tubes were incubated for 5 min at 65 °C before being quickly chilled on ice. Then, 1 µL 0.1 M DTT, 1 µL RNaseOUT, 4 µL 5X First Strand Buffer, and 1 µL (200 U) of Superscript™ III Reverse Transcriptase were added to the mix and incubated, first at 25 °C for 5 min, followed by 55 °C for 60 min. The samples were heated at 70 °C for 15 min and inactivated at 4 °C. The cDNA was stored at −20 °C until required. Real time polymerase chain reaction (RT-PCR) amplification was performed with 25 µL of master mix (10 mM dNTP, 2.5 µL 10x PCR buffer, 10 µM of each primer (sense and antisense from each analyzed cytokine, TNFα 5’-CTGTTAATAGCAACTCTATCTGG-3’ and 5’-TGTTAGTGTCTATCTGG-3’, IL10), 18.05 µL water, 5 U Taq polymerase, and 50 mM MgCl₂ (Invitrogen life Technologies). For each reaction, 2 µL of cDNA was added to the master mixture. The reactions were run in a thermocycler (Applied Biosystems) programmed to 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C (denaturation), 30 s at 55 °C (annealing), and 1 min at 72 °C (extension). These cycles were followed by 10 min at 72 °C (final extension) and inactivation at 4 °C. The PCR products were analyzed by electrophoresis in a 6% acrylamide gel in 0.05 M Tris-borate-EDTA buffer, pH 8.0, containing 0.4 µg/mL of ethidium bromide. GAPDH was used as an internal control. The expected sizes of the PCR products were 409 bp for TNFα, 781 bp for IL10 and 593 bp for GAPDH. The images were digitally captured and evaluated using ImageJ.

**Statistical Analysis**

Data normality was evaluated using the D’Agostino and Pearson omnibus normality test. A Kruskal-Wallis test was performed to determine significance. Then, Dunn’s Multiple Comparison test was performed for the cytokine measurements and the cell viability assays at 24, 48 and 72 h. Values of p<0.05 were considered significant.

**Results**

**Cell Adhesion and Viability**

The analysis of the cell morphology confirmed that monocytes/macrophages adhered to the titanium surfaces.
Effects of titanium surfaces on monocytes/macrophages profile

Cells attached to the machined (Fig. 1A) or acid-etched (Fig 1B) surfaces exhibited a typical macrophage-like morphology, indicated by the presence of surface ruffles, while the cells attached to the bio-anodized surfaces showed a shrinkage morphology (Fig. 1C).

The results of the viability assay, expressed as absorbance levels, are presented in Fig. 2. At 24 h after cell seeding (Fig. 2A), a statistically significant difference was observed on the titanium surfaces compared to the control group (p=0.003), except on the bio-anodized surface (p=0.20). The highest absorbance values were found in the control and bio-anodized groups. However, when comparing the 3 titanium surfaces (machined, acid-etched and bio-anodized), no statistical difference was detected during this period (p=0.0921).

At 48 h, mononuclear cell viability did not show differences when cultivated on cpTi disks or in PS (control) wells, independent of the titanium surface characteristics (p=0.0728, Fig. 2B). Similarly, no significant difference was observed among the titanium surfaces at this time point (p=0.4568).

Cell viability also was maintained without statistically significant differences at 72 h, when comparing the titanium surfaces and the control group (p=0.0735, Fig. 2C) as well as when comparing the titanium surfaces to each other (p=0.0758).

In general, the absorbance levels decreased over time on the titanium surfaces or in the PS (control) group. On the machined and bio-anodized surfaces, a significant reduction in cell viability was observed from 24 h to 72 h (p=0.0008 and p=0.0005, respectively). However, at 48 h and at 72 h, the absorbance values on the bio-anodized surface were maintained at the same level without statistical differences (p>0.05). In the control group, a behavior similar to that of the bio-anodized surface was observed - there were no significant differences from 48 h to 72 h (p>0.05). In the acid-etched group, a statistically significant reduction was found in cell viability between 24 and 72 h (p=0.001).

Nitric Oxide and Cytokine Levels

NO was produced in non-detectable amounts by mononuclear cells cultured on smooth and rough surfaces at 72 h. Similarly, TNFα and IL-10 concentrations in culture supernatants were also non-detectable. For this reason, the samples were not subjected to statistical analysis.

After contact with the different titanium surfaces or with the PS (control), human monocytes released IL1β and TGFβ1. IL1β released by mononuclear cells that had adhered onto rough surfaces (acid-etched group or bio-anodized group) did not show statistically significant differences compared to cells cultured on a smooth surface (machined group) or the PS (control group). At all analyzed times (24 h, 48 h and 72 h), the cell cultures secreted small but detectable levels of IL1β into the media (24 h: p=0.3830; 48 h: p=0.0804; 72 h: p=0.6063, Fig. 3). The values measured at 24, 48 and 72 h were approximately equal (Figs. 4A, B, and C, respectively) except for the PS (control group, p=0.0174). As observed for IL1β, no differences were found in TGFβ1 levels detected in media from cultures grown on treated titanium surfaces (etched acid group or bio-anodized group), machined surfaces or the PS (24 h: p=0.4938; 48 h: p=0.9825; 72 h: p=0.6213, Fig. 4).

RT-PCR

The surface topography did not affect the expression of TNFα in adherent cells at 72 h. In all of the analyzed samples, TNFα mRNA was detected, although no statistically significant difference was detected (p=0.276). IL-10 had no detectable expression in the samples utilized in this study (data not shown). Glyceraldehyde-3-phosphate dehydrogenase was used as a housekeeping reference gene for normalization purposes and as an internal control for the reactions.

Discussion

The present in vitro study investigated the influence of commercially available dental implant surfaces previously coated with blood plasma on the response of
human monocytes/macrophages. The microrough surfaces evaluated in the current study were produced by means of acid etching or bio-anodization and differed in roughness and chemical composition (19-21). The bio-anodized surface possesses Ca and P ions, incorporated from the electrolytic solution, which probably improves its biological properties (19,20). High levels of anti-inflammatory compounds, such as IL10 and TGFβ, are associated with low levels of pro-inflammatory mediators, such as NO and IL1β. TNFα is related to the activation of the M2 macrophage.

Figure 2. Comparison of cell viability for each surface. A = 24 h; B = 48 h; C = 72 h. Values of p<0.05 were considered significant.

Figure 3. Comparison of IL1β levels for each surface. A = 24 h; B = 48 h; C = 72 h. Values of p<0.05 were considered significant.
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subset (11,12,14), which is associated with host healing and regeneration (12,13). Thus, it would be reasonable to hypothesize that bio-anodized surfaces induce a Th2 profile. From data obtained in the literature using other cell culture models (7,9), one could infer that the association between surface roughness and the chemical composition of bio-anodized surfaces will induce the release of high amounts of IL10 and TGFβ. However, we do not assume this hypothesis in the present study because the proteins adsorbed by the implant surface may change the way in which the cells adhere onto the biomaterial surface and the way in which the cells are activated (12,14). Indeed, there was no difference between the levels of mediators measured for the acid-etched and bio-anodized groups. According to Anderson et al. (14), the conformation of surface-adsorbed proteins is dependent on biomaterial surface properties and dictates the inflammatory response. Although the current research did not assess the type, number, or conformation of proteins adsorbed onto titanium surfaces, it is possible that titanium roughness characteristics promote an interaction between macrophages, integrins and the protein layer that masks the effect of Ca and P ions on the cell’s response.

However, an intriguing finding needs to be addressed: the machined surface, obtained by a turning procedure without special treatment, and the control group (PS) presented similar results compared to both of the microrough surfaces – acid-etched and bio-anodized. These results indicate that the three titanium surfaces were bio-inert, as the mediators were also secreted at basal levels in the control group.

Regarding the specific mediators evaluated, the current study did not find detectable levels of IL10 and TNFα in the supernatant, which motivated the use of semiquantitative RT-PCR to evaluate the expression of both cytokines, which are related to macrophage modulation or its inflammatory role, respectively (4). The inability to detect TNFα and IL10 was not due to deficiencies of the cytokine immunoassay kits because we have previously used these assays to quantify these cytokines (data not published). The actual secreted TNFα and IL10 concentrations might simply be too low to be detectable in the culture supernatant. However, IL10 was also not detected by RT-PCR in the samples. The high biocompatibility of titanium, independent of the surface treatment, probably influenced the results. IL10 is a modulatory cytokine that is expressed in response to inflammatory mediators (8). The absence of high levels of these mediators, such as IL1β, was not able to stimulate the production of IL10.

In contrast, TNFα was expressed on all of the analyzed surfaces, including the control group. This cytokine has been related to aseptic loosening, increases in the osteoclast survival rate and osteoclast maturation (4). A possible explanation for the difference between the levels of TNFα expression and the release that was observed in the current study could be related to the effects of other cytokines on TNFα, blocking the intrinsic pathways necessary for its release. Another possibility is that TNFα might be expressed and released at basal levels, without the expected potential for inflammation. This assumption is consistent with the statement that a single cytokine has

Figure 4. Comparison of TGFβ1 levels for each surface. A = 24 h; B = 48 h; C = 72 h. Values of p<0.05 were considered significant.
many different effects, not only on different cell types, but even on the same cell type (1). Furthermore, TNFα is not always related to deleterious phenomena around implants. The first 72 h after a surgical procedure correspond to the inflammatory phase of wound healing, in which the detection of TNFα in both surfaces would be implicated indirectly in angiogenesis stimulation and would regulate osteoblast proliferation (22).

In the current study, the low levels of IL1β identified are in agreement with previous research (5,9) that observed little response from unstimulated macrophages cultivated on rough surfaces. Thus, the release of IL1β by monocytes/macrophage cells detected in the present study probably would not influence implant osseointegration once this cytokine was produced on the control group and titanium surfaces.

The other cytokine studied, TGFβ1, is one of the most important growth factors (9) of human bone, stimulating the proliferation and differentiation of pre-osteoblasts (23) and modulating wound healing while promoting angiogenesis and collagen synthesis (23). High levels of TGFβ1 were detected on all of the analyzed surfaces, including the PS, without statistically significant differences. It was previously postulated that, in the absence of inflammatory stimuli, macrophages might contribute pro-osteogenic stimuli, such as TGFβ release (24).

Therefore, with respect to the mediators evaluated, the surface characteristics did not induce either a typical Th1 or Th2 cytokine profile. Additionally, the protein layer on the titanium surfaces did not modulate macrophage polarization into the M1 or M2 subset. The absence of NO in all of the samples indicates that the cells were not activated by external agents, such as bacteria, and did not develop an M1 profile (10,11). Furthermore, it is important to recognize that the current study utilized the material surface itself as the stimulus, without the confounding influence of other exogenous stimuli, such as lipopolysaccharide or growth factors.

Such findings cannot be directly compared with data from the literature because differences in experimental models and biomaterials may have produced the discrepancies in the results (13,16). However, it is important to point out that primary cells obtained from peripheral blood are closer to clinical conditions than animal cells or cell lines (25). Furthermore, to avoid conclusions related to individual variations, we used ten patients to obtain the samples.

Regarding the other parameters evaluated, cell morphology and viability, some considerations need to be highlighted. The cell adhesion onto the titanium surfaces was confirmed by double-staining with phalloidin and DAPI. Interestingly, the cells attached to the bio-anodized surface showed shrinkage morphology compared to the cells attached to the machined or acid-etched surfaces. These morphological findings differ from the findings of previous studies in which monocytes/macrophages cultured in contact with calcium and phosphate (22) or hydroxyapatite (7) presented spread morphology. It is possible that the particle size influences cell morphology (12) or that the protein layer adsorbed on the bio-anodized surface changes the morphological pattern when compared to the machined group. No images were acquired from the control group because imaging would require the use of coverslips, which were not used during the other experimental steps.

Regarding cell viability, the treatment used to obtain the rough surfaces, as well as the resulting surface topography, did not influence this parameter until 72 h, except for the bio-anodized surface. The higher absorbance levels observed on the bio-anodized group at 24 h were similar to the control, which indicates that the chemical composition and associated roughness characteristics reduced cell death during the initial periods. The reduction observed in cell viability during this period for both titanium surfaces and the control group can be explained by the intrinsic characteristic of these cell cultures. Because primary and non-stimulated cell cultures were used and because monocytes are comprehensively differentiated cells, these results were predicted. These findings are in agreement with Goransson et al. (6), who observed a decrease in the total number of viable cells on all titanium surfaces over time.

Despite the limitations of this study, the current data demonstrate that the bio-anodized surface coated with plasma proteins promotes changes in cell morphology and viability at 24 h. However, these features are not maintained over time and are not related to cytokine levels. Therefore, the present preliminary report suggests that all of the analyzed surfaces demonstrated behavior similar to that observed in the control group (PS), which was also pre-coated with plasma proteins. These results indicate that the evaluated titanium surfaces are inert to monocytes/macrophages and do not alter the cell response patterns.

Resumo
Devido ao papel crítico dos monócitos / macrófagos (Mφ) na cicatrização óssea, este estudo avaliou os efeitos de superfícies de titânio (Ti) bio-anodizada, ataque ácido e usinada sobre o comportamento Mφ. As células foram separadas a partir de sangue humano de 10 pacientes, placadas em Ti ou superfícies de poliestireno (controle), e cultivadas durante 72 h. Às 24, 48 e 72 h, a viabilidade celular e IL1β, IL10, TNFα, TGFβ1 e liberação de óxido nítrico foram analisados por ensaio colorimétrico mitocondrial (MTT) e ensaios imunoenzimáticos, respectivamente. PCR em tempo real foi utilizado para verificar a expressão de TNFα e IL-10 às 72 h. Os dados foram submetidos a uma análise de Kruskal-Wallis, IL1β autorização, TNFα e TGFβ1, não foram significativamente diferentes entre as superfícies de Ti (p>0,05). A presença de NO e de IL-10 não foi detectada nas amostras. A viabilidade celular não diferiu entre as amostras cultivadas em Ti e aquelas
cultivated in different cultures, except for 24 h (p=0.0033). No other differences were found in the rest of the culture times.

References


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