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Effects of oxygen plasma treatment on surface properties, osteoblastic response, and bacterial behavior of implant–prosthetic materials

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Abstract

Objective: A major goal in oral implantology is to develop materials that enhance osseointegration while reducing bacterial colonization, thereby preventing peri-implant diseases.

Materials and Methods: This study evaluated the effects of oxygen plasma treatment on materials commonly used in implant–prosthetic systems, including titanium, cobalt–chromium alloy, zirconia, and porcelain. Surface roughness was assessed by confocal microscopy. Plasma treatments were performed at 60 W for 60 s. Hydrophilicity was evaluated by contact angle measurements immediately after treatment, and surface energy along with its dispersive and polar components was determined. Human osteoblastic SaOS-2 cells were cultured on each material, and cell adhesion and mineralization were assessed by alkaline phosphatase and osteocalcin expression after 1, 3, 7, and 21 days. In addition, the metabolic activity of seven common oral biofilm-forming bacteria was evaluated after 3, 7, and 21 days: aerobic species (*Streptococcus gordonii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and anaerobic species (*Streptococcus sanguinis*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*).

Results: No significant differences in surface roughness were observed among the materials. Oxygen plasma treatment induced a marked reduction in contact angle in all materials, with titanium exhibiting superhydrophilic behavior. Surface energy increased significantly, particularly the polar component. Osteoblastic adhesion was minimal at early time points but increased markedly from day 3 onward, with peak alkaline phosphatase and osteocalcin levels observed at day 7, consistent with enhanced mineralization. This response correlated with the increase in surface energy, especially its polar component. While aerobic bacteria showed no significant reduction in colony-forming units or metabolic activity, anaerobic bacteria exhibited a significant decrease, likely due to increased surface oxygen content.

Conclusion: Oxygen plasma treatment appears to be a promising approach for producing osteoconductive surfaces with a bactericidal effect, particularly against anaerobic bacteria, which are the most prevalent and pathogenic microorganisms involved in peri-implantitis.

Clinical relevance: Given the growing clinical impact of peri-implantitis, this surface treatment strategy may play a decisive role in its prevention.

1. Introduction

Dental implants are widely regarded as a highly successful therapeutic option, primarily due to their excellent osseointegration capacity, which enables a stable and durable integration between the implant and the surrounding bone tissue [1]. In addition, implant materials—particularly titanium—exhibit outstanding mechanical properties, high corrosion resistance, and a low incidence of allergic or biocompatibility-related complications [2–3]. Despite these advantages, peri-implantitis remains a major cause of dental implant failure [4]. This condition is estimated to generate treatment-related costs of

approximately USD 2.3 billion in 2024 alone [5–6]. The reported prevalence of peri-implantitis ranges from 12% to 24% of dental implants, corresponding to more than 375,000 affected implants each year [7].

Peri-implantitis has therefore emerged as a significant public health concern in contemporary dentistry. It is well established that peri-implantitis is characterized by an inflammatory lesion of the peri-implant mucosa, accompanied by progressive loss of the supporting bone, primarily as a consequence of bacterial infiltration of the peri-implant space [8–10]. This process leads to bacterial adhesion and biofilm formation on titanium surfaces. Notably, peri-implant pathogens embedded in biofilms exhibit high resistance to antibiotic therapy, while conventional mechanical debridement techniques, such as brushing, are insufficient for effective biofilm removal [11–12].

Although clinical protocols for non-surgical and surgical treatment for peri-implantitis have been recently established [13], predictable peri-implantits resolution still remains unsolved and recurrency of the lesion is high [14]. One commonly employed therapeutic approach for peri-implantitis is implantoplasty, which involves mechanical modification of the implant surface. However, this procedure compromises the mechanical strength and corrosion resistance of the implant and, more critically, promotes the release of titanium particles of varying sizes. Many of these particles are not removed during treatment and may remain within surrounding biological tissues. Several studies have demonstrated the cytotoxic effects of such particles, particularly those in the nanometric range [15–17]. Furthermore, Kotsakis et al. reported that implantoplasty-induced machining of titanium promotes inflammation and generates an anaerobic peri-implant environment, leading to the reduction of titanium oxide to metallic titanium [14]. Subsequent re-oxidation in oxygen-rich conditions results in the formation of non-stoichiometric mixed oxides rather than stable TiO_2, which have been shown to exhibit toxic effects [18–20].

These findings underscore the need for close collaboration between clinicians in implantology and researchers in materials science and engineering to develop a stable passivation layer on titanium surfaces with long-term bacteriostatic and/or bactericidal properties. Such surface modifications are essential to inhibit biofilm formation and, consequently, prevent peri-implantitis. In this context, the present study proposes the development of a nanotextured titanium surface composed of spike-like nanostructures designed to confer bactericidal activity while preserving both the mechanical integrity of the implant and its osseointegration potential.

2. Materials and Methods

2.1. Materials

The materials used were those most commonly used in dental implants and dental prostheses: commercially pure titanium grade 3, zirconia, Cr Co alloy and porcelain. The materials were donated by the Klockner Medical Group (Escaldes Engordany, Andorra). The samples were polished with diamond paste solution (The average size of the diamonds particles was 0.08 ± 0.04 micrometers).

2.2. Determination Roughness

Surface roughness was quantitatively evaluated using a white-light interferometric microscope (Wyko NT1100, Veeco Instruments Inc., USA) coupled with dedicated analysis software (Vision32, Veeco Instruments Inc., USA). Measurements were performed on ten specimens per material. The roughness parameters analyzed included the arithmetic mean surface roughness (Sa), defined as the mean of the absolute height deviations from the reference plane, and the maximum surface height (Sz), calculated as the average peak-to-valley distance.

2.3. Preparation of biomaterial samples.

The specimens were exposed to oxygen plasma using a plasma reactor (Zepto, Diener Electronic, Ebhausen, Germany) operating at a reduced pressure of 10^{-4} atm with high-purity oxygen gas (99.99%). A description of the experimental setup is provided in Fig. 1. A series of preliminary experiments was performed to determine the optimal plasma power and exposure duration; the corresponding protocols and results are reported in the Supplementary Material. Plasma surface modification is known to enhance surface hydrophilicity, a key factor influencing the adsorption of proteins and other organic species that support cell and bacterial attachment and migration [21–25]. For this reason, surface wettability was selected as the primary outcome for optimizing plasma treatment parameters [26–33].

The condition yielding the greatest increase in hydrophilicity was considered optimal. The choice of multiple exposure times was based on the reported variability in plasma residence requirements, as certain materials require extended treatment periods, whereas others rapidly reach oxygen saturation after short exposures, such as 30 s [34–36]. In parallel, plasma power settings were optimized due to the wide range of values described in the literature, although most studies report power levels between 50 and 150 W [35–42]. Wettability was therefore evaluated after treatment durations of 30, 60, and 90 s, which represent clinically relevant time intervals compatible with implant placement procedures and optimal clinical performance.

2.4. Wettability and surface free energy.

Surface wettability was evaluated by determining the water contact angle (WCA) on the four biomaterial surfaces using the sessile drop technique. Briefly, a 2 µL droplet of ultrapure water (MilliQ) was placed on each surface, and the contact angle was recorded using a goniometer (OCA 11, Dataphysics, Riverside, CA, USA). Measurements were obtained both prior to and following oxygen plasma treatment. For each surface, ten independent measurements were conducted under controlled conditions of 37°C and 100% relative humidity to reproduce physiological environmental conditions. In addition to water, static contact angles of two reference liquids were measured using the same experimental procedure and under identical temperature and humidity conditions.

The total surface free energy (SFE) of the samples was calculated as the sum of the dispersive (London) and polar components. SFE values were determined from the contact angle measurements obtained with three probe liquids, including ultrapure distilled water (MilliQ, Sigma-Aldrich, St. Louis, MO, USA) and diiodomethane. The values obtained were calculated using the Owens and Wendt equations [43–45].

$$\gamma_S = \gamma_{SL} + \gamma_L cos\theta$$

1

$$\gamma_L (1 + \cos\theta) = 2\left(\left(\gamma_L^d \gamma_S^d\right)^{\raisebox{1ex}{1}/\raisebox{-1ex}{2}} + \left(\gamma_L^p \gamma_S^p\right)^{\raisebox{1ex}{1}/\raisebox{-1ex}{2}}\right)$$

2

being γ_S the surface tension of the solid phase (S), γ_L the surface tension of the liquid (L), γ_{SL} the interfacial free energy or SE between L and S, θ the contact angle between L and S, and γ_d and γ_p represent the dispersive and polar components of the SE, respectively. Where is due to dipole-dipole-dipole interactions (London or 'dispersion'), and is the polar component produced by the permanent interaction between dipoles.

2.5. Osteoblastic culture.

For the in vitro experiments, human osteoblast-like cells (Saos-2; ATCC, Manassas, VA, USA) were employed. Cells were maintained in McCoy's modified 5A medium supplemented with 10% fetal bovine serum (FBS; Gibco, New York, NY, USA), 1% penicillin/streptomycin (2 mM; Invitrogen, Carlsbad, CA, USA), and 1% sodium pyruvate (Invitrogen, Carlsbad, CA, USA). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. A total of 25 samples were analyzed for each experimental condition.

Upon reaching confluence, cells were detached by incubation with TrypLE solution (Invitrogen, Carlsbad, CA, USA) for 1 min. Subsequently, 5,000 cells were seeded onto each disc and incubated at 37°C. After incubation periods ranging from 1 to 4 h, samples were gently rinsed with phosphate-buffered saline (PBS) and transferred to new culture plates for metabolic activity assessment using the Alamar Blue assay (Invitrogen–Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's protocol. Briefly, the reagent was prepared and added to fully cover the samples, and the percentage of Alamar Blue reduction was quantified at 37°C, using the Alamar Blue solution as a blank reference. Measurements were performed at five time points: 1, 3, 7, 14, and 21 days.

Osteoblastic differentiation was evaluated by measuring alkaline phosphatase (ALP) activity using the SensoLyte pNPP alkaline phosphatase colorimetric assay (AnaSpec, Fremont, CA, USA). Absorbance was recorded at 495 nm with a standard microplate reader (ELx800, BioTek Instruments, Winooski, VT, USA). In addition, matrix mineralization was assessed by staining extracellular calcium deposits with Alizarin Red S (ARS; Sigma-Aldrich). Cells were cultured for 21 days in osteogenic medium consisting of the basal medium supplemented with 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid, and 100 nM dexamethasone, followed by fixation with 4% (w/v) paraformaldehyde. Titanium discs were then rinsed twice with Milli-Q water and incubated under orbital agitation for 20 min with 500 µL per disc of 40 mM ARS solution (pH 4.2). Excess dye was removed through repeated washing with Milli-Q water. For dye elution, samples were treated with 300 µL per disc of cetylpyridinium chloride (CPC) solution (10% w/v in 10 mM NaH_2PO_4, pH 7) for 30 min. The resulting supernatant was collected, diluted 1:2 with CPC buffer, and 100 µL aliquots were transferred to a microplate for absorbance measurement at 570 nm.

2.6. Bacterial strains and culture conditions.

Seven bacterial strains were used in the study; three aerobics and four anaerobic strains. For aerobics: *Streptococcus gordonii*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and for anaerobics: *Streptococcus sanguinis*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*. These are the most abundant biofilms in peri-implantitis. A total of twelve different titanium surfaces were studied with different materials and each bacteria strain.

Standard reference strains of *Streptococcus sanguinis* obtained from the American Type Culture Collection (ATCC 6249), *Staphylococcus aureus* (ATCC 12600) and *Pseudomonas aeruginosa* (ATCC 9027) and for *Streptococcus gordonii* (ATCC 10588), *Porphyromonas gingivalis* (ATCC49417), *Fusobacterium nucleatum* (ATCC 25586), and *Aggregatibacter actinomycetemcomitans* (ATCC 33384). This species was chosen because it is a primary colonizer of the oral cavity and forms biofilms around dental implants. Cultures were prepared using Trypto-casein Soy Broth (TSB) and Tryptone Soy Agar (TSA). All microbiological experiments were conducted in a Class II laminar flow cabinet (Bio II Advance Plus, Telstar) to ensure aseptic conditions. Twenty-five samples were used for the bacterial culture were used. The analysis was realized at 5 times: 1, 3, 7, 14 and 21 days.

2.7. Bacterial adhesion.

The number of viable bacteria adhered to the disc surfaces was quantified using a colony-forming unit per milliliter (CFU/mL) assay. Initially, the selected bacterial strains were cultured in multiwell plates containing the four different types of biomaterial discs and incubated overnight. Following incubation, the discs were rinsed two to three times with phosphate-buffered saline (PBS) to remove non-adherent bacteria and subsequently transferred to Eppendorf tubes containing 1,000 µL of PBS. To detach the surface-adhered bacteria, the tubes were subjected to vortex agitation at 1,200 rpm for 10 min. Serial dilutions were then prepared by transferring 100 µL of the bacterial suspension into successive tubes containing PBS, resulting in four dilution steps. From the final three dilutions, 10 µL aliquots were plated onto tryptic soy agar (TSA) plates and incubated for 24–48 h. Colony counting was performed using an eCount Colony Counter (manual device), and the initial bacterial concentration was calculated according to Eq. (3).

$$CFU/mL = \frac{nº\ colonies * dilution\ factor}{mL\ seeded\ on\ plate} \quad (3)$$

2.8. atistical Analysis

The results were entered into a database (Microsoft Excel®, Redmond, Washington, USA) and statistically processed using Stata 14 software (version 2.0) (StataCorp®, College Station, USA). The data were

processed with Student's t-analysis, one-way ANOVA, and Tukey's multiple comparisons tests to obtain possible statistical significance. A $p < 0.05$ was assigned as the criterion for statistically significant differences.

3. Results

The roughness of the samples studied can be seen in Table 1. As mentioned, the samples were polished with diamond paste and washed with distilled water and dried in hot air flow. As can be seen the differences between the roughness parameters are not statistically significant with a value of $p < 0.05$.

Table 1
Roughness of the different materials studied

Material	Sa (mm)	Sz (mm)
Titanium	0.12 ± 0.08	0.22 ± 0.09
Cr Co	0.10 ± 0.05	0.28 ± 0.10
Zirconia	0.08 ± 0.03	0.21 ± 0.05
Porcelain	0.07 ± 0.03	0.20 ± 0.07

Studies on surface wettability were conducted to determine the optimal treatment conditions for subsequent tests involving bacterial adhesion and viability. Different powers used; times of treatment were tested in order to obtain the best values of wettability. The results of this study with the statistical analysis can be observed in the supplementary material. Analysis of the results has shown that the best treatment for the different materials studied was the plasma treatment with oxygen for 30 seconds at a power of 60W and a measurement time of 60 seconds. In Fig. 2 can be observed the contact angle of different materials used, after the plasma treatment, immediately after of the treatment and after 4 hours of the treatment.

In Figure 3 we can see the surface energy for each of the materials treated by oxygen plasma for 30 seconds at a power of 60W. Measurements were taken at 60 seconds after plasma treatment. It can be seen in all cases an increase in the total surface energy for all materials and especially there is a very important increase in the polar component of the energy due to the adsorption of oxygen on the surface.

These results show the significant increase in total surface energy for the metallic materials: titanium and cobalt-chromium and especially in the polar component. Ceramic materials such as yttria and porcelain also show increases in surface energy and also in the polar component, but the increase is less than for the metals studied. The increase is due to the increase in the polar component of the energy since in all cases the materials treated with plasma have statistically significant differences with respect to the same untreated materials. For the dispersive energy there is a tendency of increase but they do not show statistically significant differences with $p < 0.05$.

Figure 4 shows osteoblast adhesion at different times after culture. Very low values can be observed in the first hour and thereafter in all cases there is a very considerable increase in osteoblast adhesion. It can also be observed how the highest values are for titanium and zirconia, this is one of the reasons because dental implants in general are mainly made of titanium and zirconia..

Figure 5 shows the mineralization of osteoblastic cells by alkaline phosphatase analysis. A greater mineralization can be seen with the treatment time, observing a very important decrease in the values of mineralization in porcelain. This result is not surprising since porcelain is used as a dental prosthesis material and never in contact with bone tissue. The same behavior occurs at very short times of plasma application as in the case of adhesion, obtaining very small values in alkaline phosphatase.

The calcium production by osteoblastic are shown in Fig. 6. The results seem to indicate a positive role for titanium and more moderate mineralization for zircona and chromium cobalt alloys. On the contrary, the extent of mineralization was observed.

Figure 7 shows the metabolic activity of the aerobic strains, where a significant colonization can be seen from the values of 2–3 hours after the plasma treatment, at higher elapsed times the bacterial colonization exceeds the control. This bacterium is aerobic and therefore its bacterial activity may be favored by the presence of oxygen that has been absorbed on the surface of the different materials by the plasma treatment.

Figure 8 shows the metabolic activity of the anaerobic strains, where a lower colonization can be seen with respect to the aerobic bacteria in the previous figure. In this case, the presence of oxygen on the surface of the anaerobic bacteria affects bacterial viability.

4. Discussion

From the results we have seen that the wettability is much higher when plasma treatment is applied but there is no difference between the powers used on our surfaces. Oxygen saturation on our surfaces is highly favored and is reached at very short times and that is why it is not necessary to increase the power to achieve saturation as in other biomaterials, especially polymeric materials [40–44]. However, the time that the treated surface is in the environment does cause a change in surface properties as the hydrophilicity decreases very significantly. This is due to the reaction of the surface-active oxygen with the hydrocarbons and carbon monoxides in the medium which leads to a reduction in wettability. Likewise, it can be observed that the treatment time at a constant value of power causes a slight decrease in wettability but no statistically significant differences.

The results show an increase in the total surface energy of the four materials studied when the material is treated by oxygen plasma. This increase in energy is due to the contribution of the polar component in which the increases are very important especially for metallic materials - Titanium and cobalt-chromium - due to their avidity for oxygen [46–48]. The increase of the polar component is a very important factor for the adsorption of proteins such as fibronectin which plays a very important role for the migration of osteoblastic cells [49].The role of this polarity is not well known for bacterial colonization, but different

authors show that the higher the surface energy the easier the adhesion of bacteria on the surface as well as the bacteria are sensitive to the hydrophilic or hydrophobic properties [50].The role of this polarity is not well known for bacterial colonization, but different authors show that the higher the surface energy the easier the adhesion of bacteria on the surface as well as the bacteria are sensitive to the hydrophilic or hydrophobic properties [51]. From the results we can also observe that the plasma is more effective on metallic materials since metallic materials are more avid for oxygen than ceramic materials since these are constituted by oxides. However, the variation of the contact angle is more damped with time.

Biological results show in osteoblast cultures that at short times after plasma the cell adhesion activity is very low because the surface is so hydrophilic that the surface is in contact with water, but the precursor proteins for osteoblast migration and even the cells are far from the surface and neither the proteins can adsorb nor the cells adhere. As time passes, the superhydrophilia disappears and it is then that the proteins are adsorbed and the cells migrate producing very high values of osteoblastic adhesion. At longer times, it can be observed that adhesion decreases and this is due to the fact that adhesion has reached its maximum since the surface is coated and the cells pass to the mineralization stage. This fact can be observed with the alkaline phosphatase and the calcium content results, which show very high values at times of 7 days for Titanium, CrCo and Zirconia [50–53]. However, porcelain, although it shows good osteoblast adhesion, does not show osteoblast mineralization and therefore there will be no bone tissue formation. These results show that the most favorable materials for osseointegration are titanium and zirconia, since they present high mineralization values, which is why these materials are used for the manufacture of dental implants. CrCo also presents mineralization, although lower than titanium and zirconia, but dental implants are not manufactured with this alloy due to its high thermal conductivity. The ingestion of hot food could cause thermal conductivity at the biological interface causing degradation of cell membranes and inhibiting osseointegration.

The results of the osteoblastic behavior indicate that the oxygen plasma causes a certain bioactivity on the surface of the materials studied, especially metallic materials. This effect is similar to those produced in SLA active Straumann dental implants in which titanium undergoes a shot blasting treatment and then an acid attack on its surface which creates a negative charge (increasing the polar component in the superficial energy). Immediately after the acid etching it is protected by a flow of inert gas and introduced in water (polar di-solvent) to avoid the loss of the negative charge that will be so important for osseointegration. For this reason, for the correct placement of this type of implant in the mouth, it should not be left too long in the air but should be placed quickly to avoid the loss of the negative charge of the implant surface with the gases of the atmosphere [54–55]. The ContacTi Klockner dental implants based on the kokubo treatment are shot blasted and treated with concentrated NaOH to achieve a sodium titanate surface [56–60]. When the dental implant with sodium titanate surface is placed in contact with blood, the sodium dissolves in the physiological medium and the surface becomes negative after the loss of cation. This negative cloud on the surface causes the migration of proteins such as fibronectin which is a precursor of osteoblasts and in turn of Ca^{2+} cations. Calcium cations have an excess of + charge with respect to sodium which is Na^{+} and therefore the substitution of sodium ions by calcium generates an excess of positive charge which is neutralized by phosphates forming apatites that osteoblasts recognize

causing osteoblastic activation and facilitating osseointegration [60–62]. The oxygen plasma presents a mechanism very similar to the above-mentioned dental implants and therefore an osteoconductive capacity of the surface can be assumed.

Microbiological studies with bacterial strains show that studies with the aerobic *Streptococcus gordonii, Staphylococcus aureus*, and *Pseudomonas aeruginosa* do not show any bactericidal character, on the contrary for some surfaces where this treatment means an increase of bacterial colonization. However, for the anaerobic *Streptococcus sanguinis, Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans* bacteria, a reduction of bacterial colonization is shown with results that are statistically significant with a $p < 0.05$. This fact may be due to the fact that anaerobic bacteria do not process oxygen and therefore can produce bacterial death due to the oxidizing nature of the surface. It must be said that in peri-implant diseases and especially peri-implantitis the most pathogenic bacteria are anaerobic [63–65].

The death of anaerobic bacteria is attributed to the absence or very low levels of oxygen defense systems, such as catalase and superoxide dismutase [66–67]. As a result, these microorganisms are unable to tolerate the oxidative stress generated by oxygen plasma. When anaerobic bacteria come into contact with an oxygen plasma–treated surface, their adhesion is inhibited, their viability is drastically reduced, and many cells die within minutes or even seconds [68].

The cellular damage mechanism involves membrane degradation due to lipid oxidation of membrane constituents, leading to increased permeability and loss of the ionic gradient, which ultimately disrupts metabolic fluxes [69]. In addition, oxygen plasma induces oxidation of key enzymes involved in anaerobic metabolism and causes protein denaturation. DNA damage is also observed, including strand breaks, oxidation of nitrogenous bases, and failures in replication and transcription. These mechanisms also affect anaerobic biofilms, as oxygen plasma disrupts the extracellular matrix and prevents the formation of new biofilms, thereby increasing bacterial susceptibility [70–71].

A marked reduction in bacterial load was observed, remaining constant from the first hour up to 24 hours of evaluation. This effect was consistent across all four studied surfaces, including both metallic and ceramic materials. In contrast, oxygen plasma treatments are less suitable for polymeric materials, as oxygen can break and/or oxidize polymer chains, thereby altering their properties, in addition to the considerable technical difficulty of applying plasma projection processes to polymers [72].

Oxygen plasma treatment appears promising for dental implant and prosthetic materials, as anaerobic bacteria play a central and decisive role in the onset, progression, and severity of peri-implantitis. Anaerobic bacteria are favored by the peri-implant microenvironment, where a low oxidation–reduction potential promotes the formation of deep pockets, oxygen availability is limited, and the rough implant surface facilitates bacterial adhesion [73–74]. Furthermore, peri-implant tissues exhibit lower vascularization compared to the natural periodontium [75]. These conditions are ideal for the proliferation of strict anaerobes [76–78].

The virulence factors of anaerobic bacteria include endotoxins (lipopolysaccharides, LPS), proteases (gingipains), collagenases, and volatile fatty acids, among others. These components trigger several biological responses, such as activation of macrophages and neutrophils, stimulation of pro-inflammatory cytokine release (IL-1β, TNF-α, IL-6), and increased expression of RANKL, which promotes osteoclast activation and ultimately leads to peri-implant bone resorption [79–81].

It is important to distinguish between the presence of molecular oxygen (O_2) and oxygen plasma on a surface. Molecular oxygen is essential for the metabolism of aerobic bacteria, allowing aerobic respiration, but it is not bactericidal [82]. Aerobic bacteria possess catalase and superoxide dismutase, enabling them to adapt without significant damage. In contrast, surfaces treated with oxygen plasma can oxidize bacterial cells; however, in aerobic bacteria, antioxidant defenses may overcome this stress, resulting in minimal membrane damage, infrequent protein denaturation, and limited DNA damage [83]. The results shown in Fig. 8 support the limited damage induced by oxygen plasma on the aerobic bacteria studied, which are able to proliferate again on the surface after a short period.

The presence of molecular oxygen in contact with anaerobic bacteria generates superoxide radicals, and due to the lack of protective enzymes, this leads to moderate growth inhibition or slow bacterial death. Conversely, oxygen plasma treatment induces extreme oxidative stress, causing immediate and multifactorial damage that results in rapid and massive bacterial cell death. In conclusion, it is not the presence of oxygen itself that eliminates anaerobic bacteria, but rather its activation in the form of plasma [81–83].

One of the limitations of the study would be that the plasma treatments be protected with a polar solvent, such as an aqueous solution, to protect the loss of optimal hydrophilic capacity. It is also necessary in future studies to carry out in vivo studies to determine the osseointegrative capacity by determining the Bone Implant Contact (BIC) values that will situate us in the goodness of the treatment. Microbiological studies should be extended to biofilms common in oral infection to confirm these preliminary studies. In this work we wanted to expose the characteristics of oxygen plasma treatment on implant and prosthetic materials and to determine the influence of their osteoblastic and microbiological response with the characteristics of the treatment and the physicochemical properties of the surface.

5. Conclusions

The application of oxygen plasma on titanium, cobalt-chromium, zirconia and porcelain surfaces produces in all cases an increase in hydrophilicity. It could be seen in all cases that the oxygen plasma causes a significant increase in the surface energy values, especially in the polar component. This increase causes an increase in osteoblastic activity over time, especially in the case of titanium and zirconia, which at 7 days there is a high degree of mineralization detected by alkaline phosphatase and by the quantification of calcium. In the microbiological studies when using the aerobic bacterium *Streptococcus gordonii, Staphylococcus aureus* and *Pseudomonas aeruginosa* do not show any bactericidal character, on the contrary for some surfaces where this treatment means an increase of bacterial colonization. However, for the anaerobic strains tested: *Streptococcus sanguinis,*

Porphyromonas gingivalis, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*but an important decrease was observed. This treatment can be taken into account by clinicians for the improvement of the osseointegration capacity, especially of titanium and zirconia, and the decrease of anaerobic bacteria which are the most pathogenic in the mouth.

Declarations

Conflict of Interest and source funding statement

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Author Contribution

All authors conceived and planned the experiments. JG carried out the wettability and surface energy experiments. JG, AT, RP and MA carried out the cellular and microbiological analysis. JN. and JG. carried out the microscopy analysis and mechanical studies. AT, and JG. obtention of the different surfaces and passivation treatments and materials characterization, JG. and MS. performed the statistical analyses. JN, JG and MA. contributed to the interpretation of the results. JG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Figures



Figure 1

Oxygen plasma used in this research

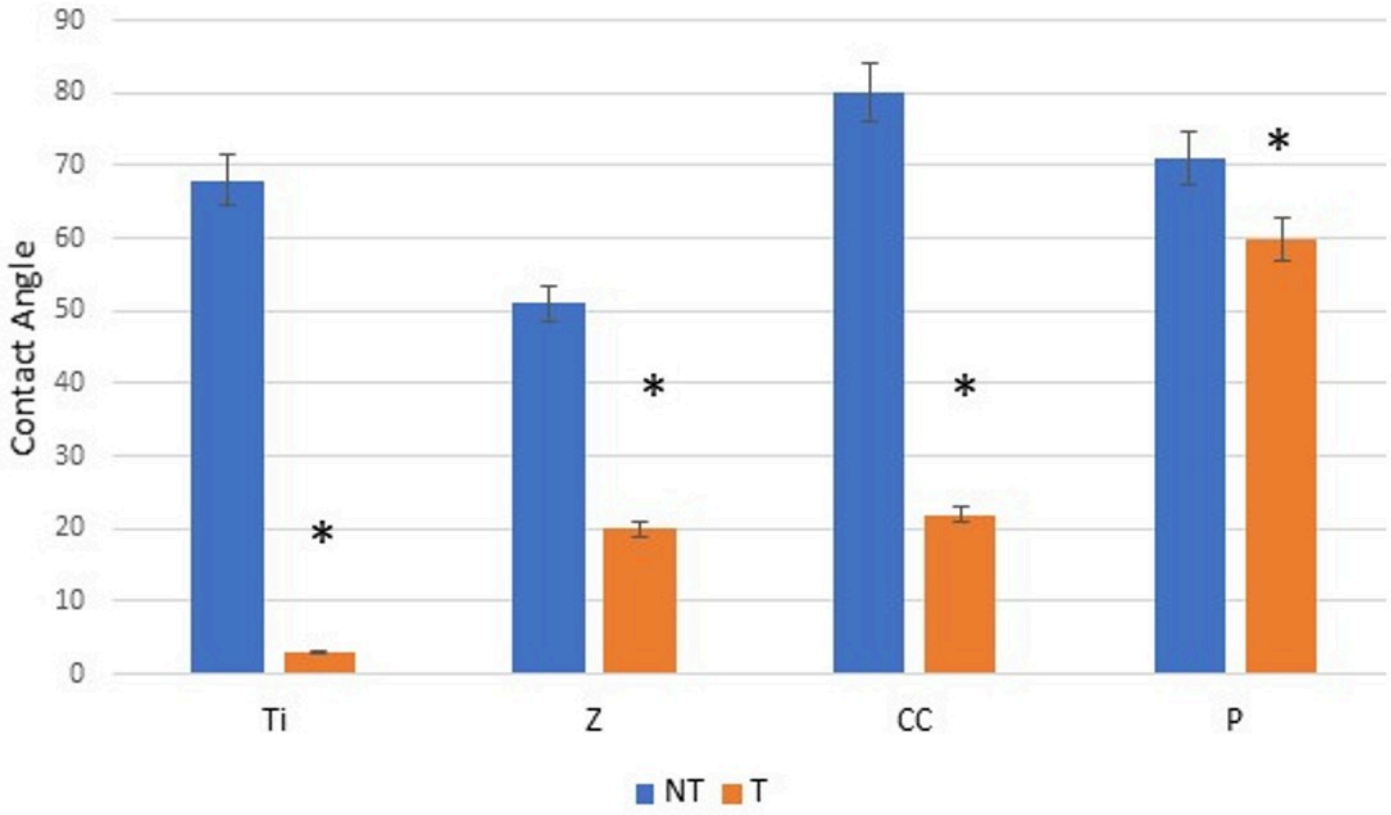


Figure 2

Contact angles of different materials tested. NT: without plasma treatment, T: treatment of plasma with oxygen for 30 seconds and wettability determined at 60 s after the treatment. Asterisk indicates the statistical differences significance with $p<0.05$.

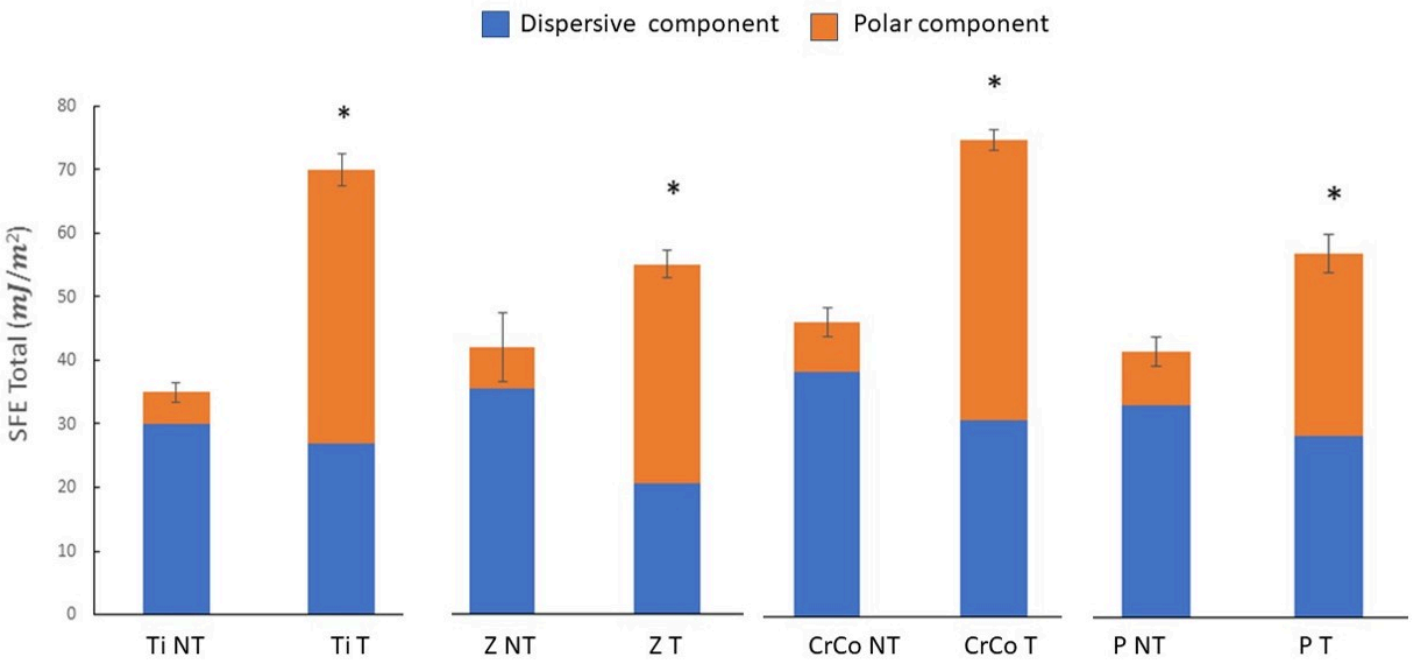


Figure 3

Surface free energy of the different samples Ti: titanium, Z: zirconia, CrCo: cobalt-chromium, P: porcelain. NT: no plasma treatment T: treated with 60W plasma for 30 seconds. Measurements were performed 60 seconds after plasma treatment. The asterisks indicate statistically significant differences with respect to the untreated sample and the difference is only for the polar component since in the dispersive component no statistically significant differences were found between any of the treated and untreated samples. The statistical criterion was $p<0.05$.

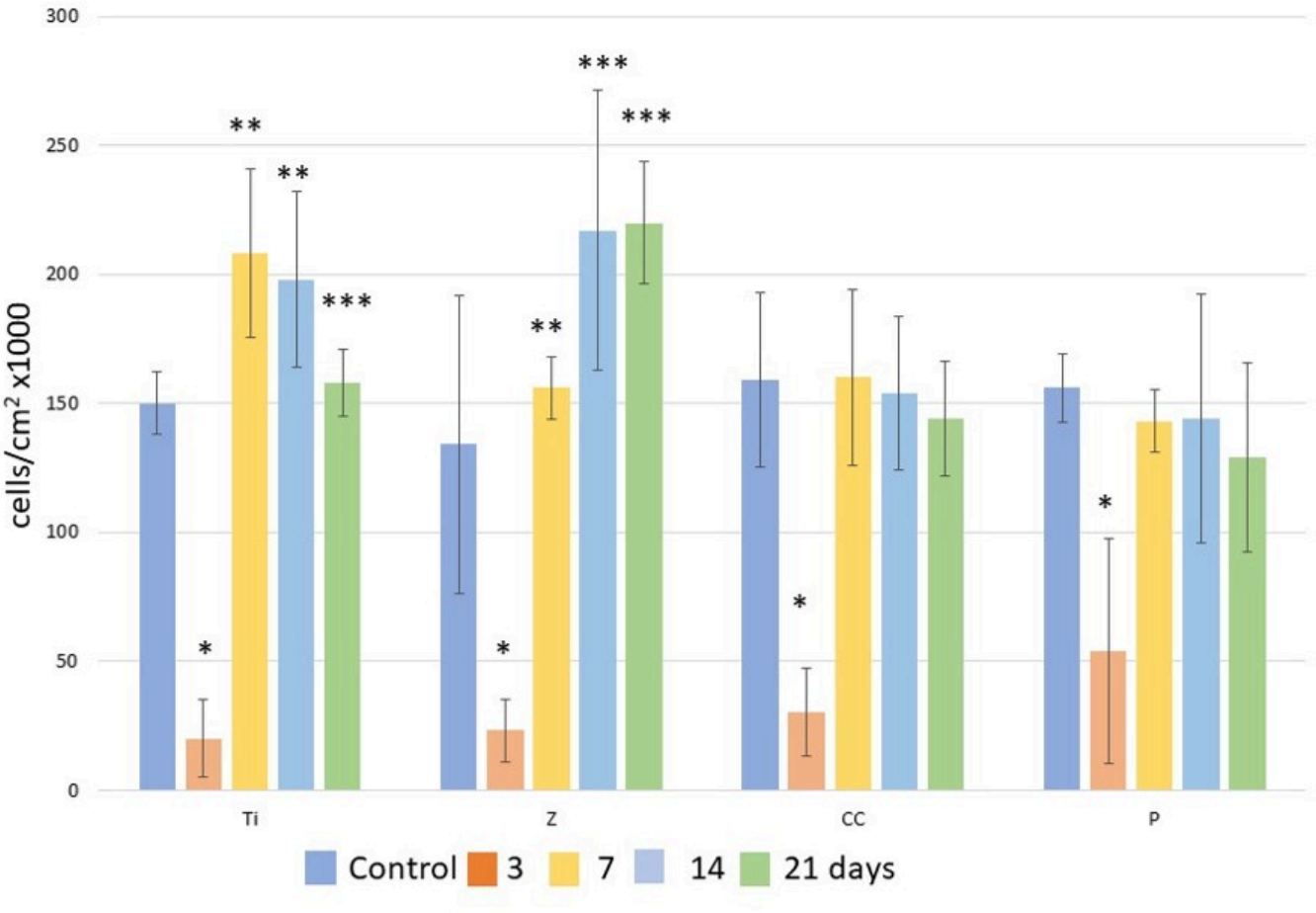


Figure 4

Osteoblastic adhesion at different times after culture in relation to the control (0h). The samples were Ti: titanium, Z: zirconia, CrCo: cobalt-chromium, P: porcelain. Values marked with an asterisk (*) indicate statistically significant differences ($p < 0.05$) compared to those without an asterisk. When the asterisk is double (**) it means that the results have statistically significant differences with respect to those without an asterisk (*) or those with only one asterisk, with a $p < 0.05$.

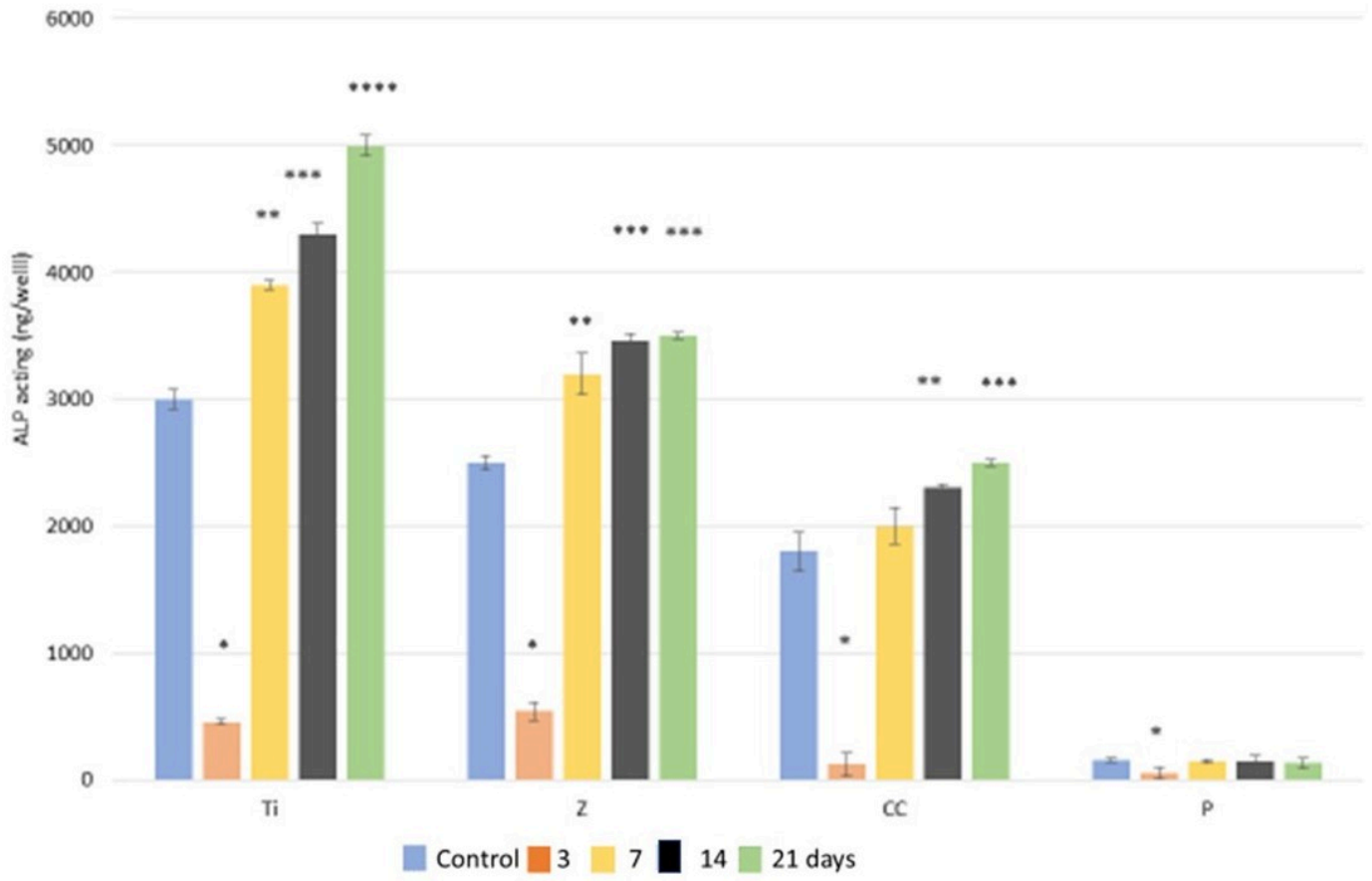


Figure 5

Phosphatase alkaline as mineralization indicator at different times after culture in relation to the control (0h). The samples were Ti: titanium, Z: zirconia, CrCo: cobalt-chromium, P: porcelain. Values marked with an asterisk (*) indicate statistically significant differences ($p < 0.05$) compared to those without an asterisk. When the asterisk is double (**) it means that the results have statistically significant differences with respect to those without an asterisk (*) or those with only one asterisk, with a $p < 0.05$.

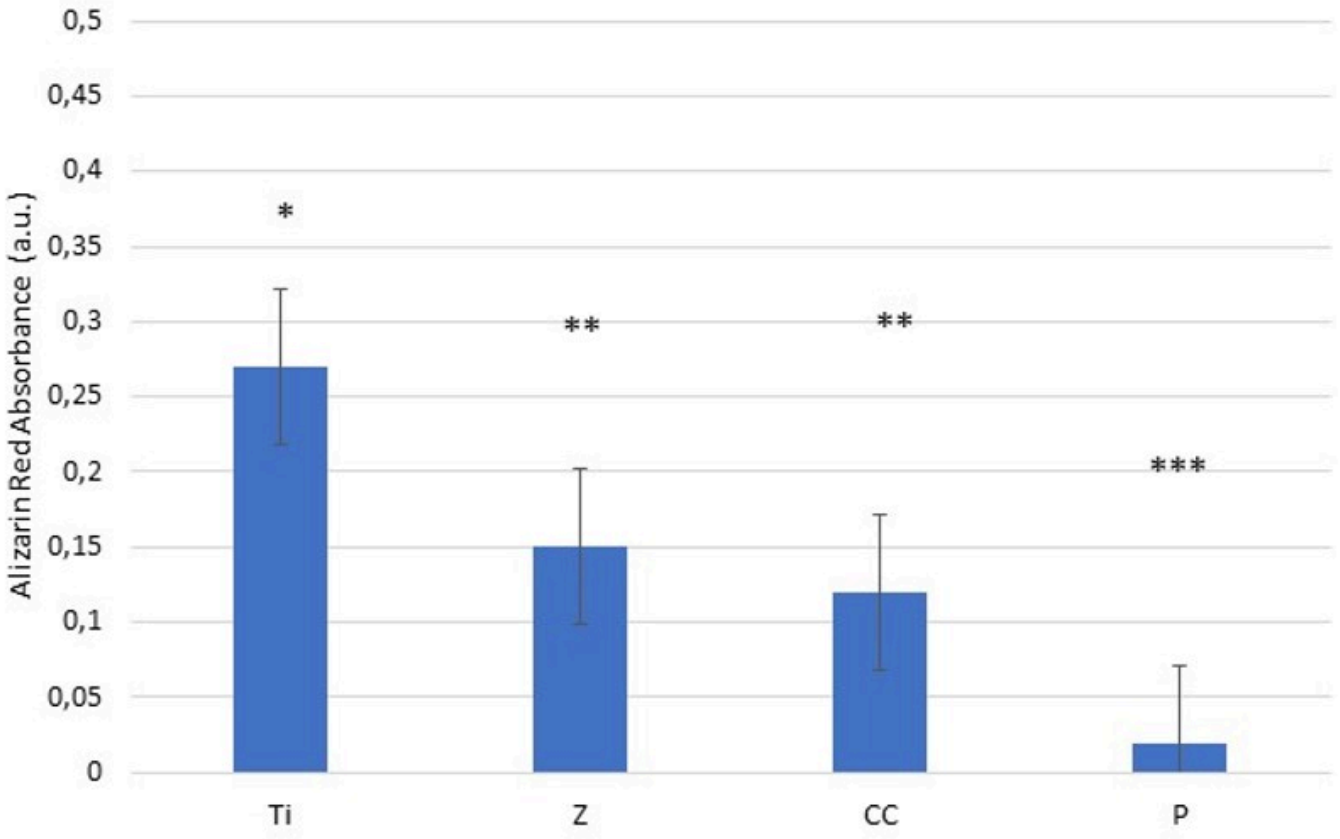


Figure 6

Quantification of calcium production by Saos-2 cells after 21 days of incubation. Distinct asterisks denote statistically significant differences ($p < 0.05$) between groups.

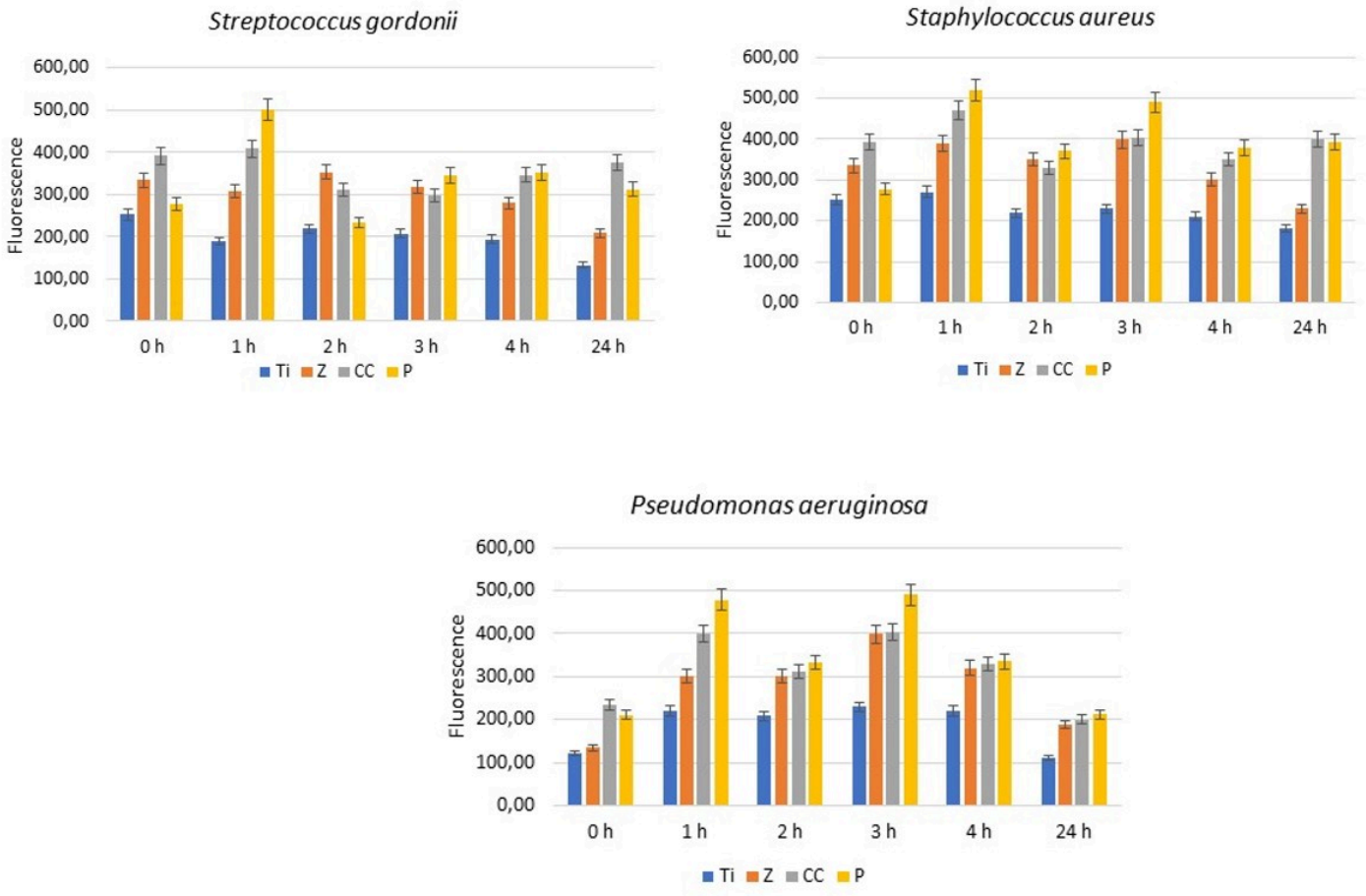


Figure 7

Metabolic activity of the aerobic strains at different times of bacteria culture and for each material studies. The samples were Ti: titanium, Z: zirconia, CrCo: cobalt-chromium, P: porcelain.

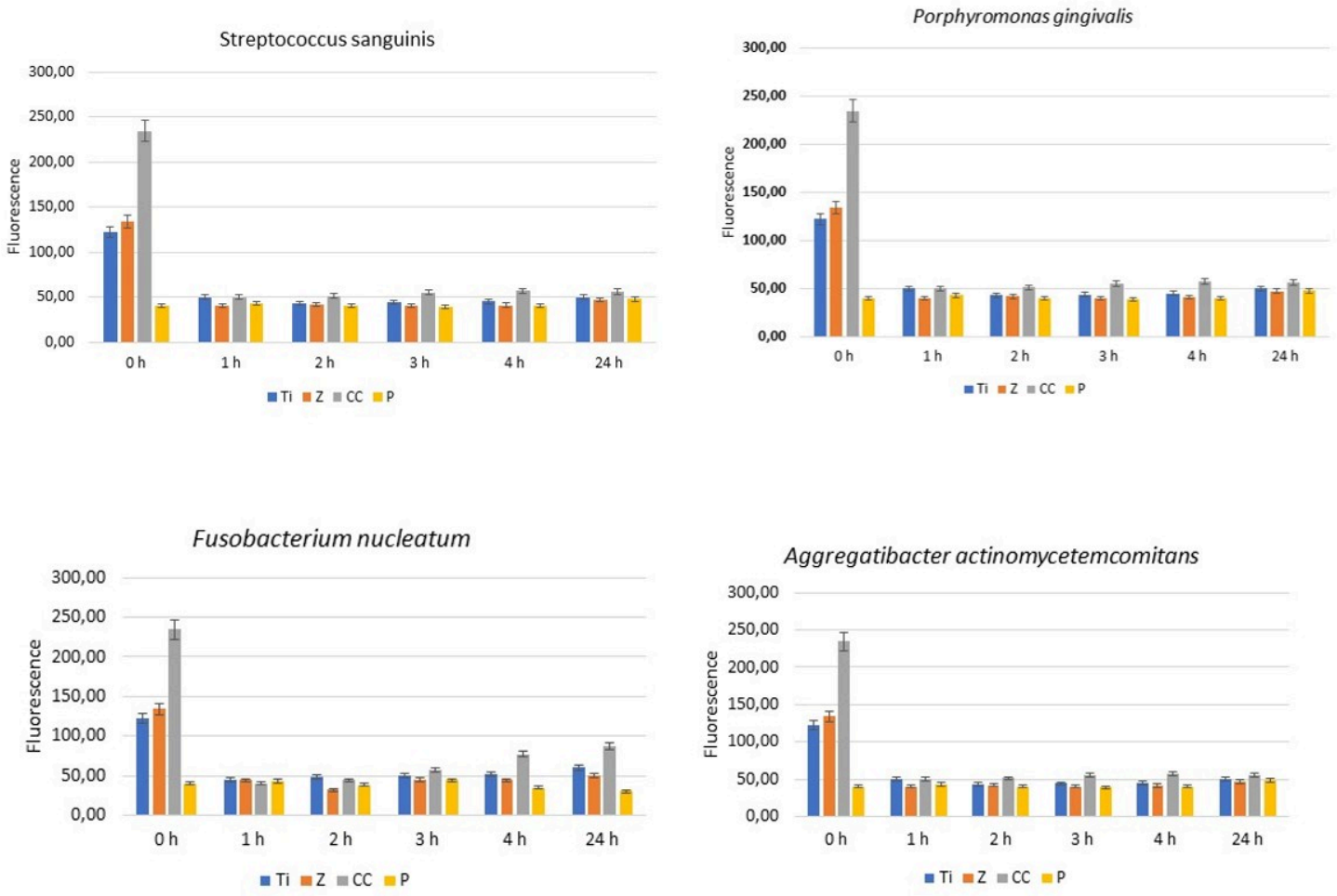


Figure 8

Metabolic activity of the anaerobic strains at different times of bacteria culture and for each material studies. The samples were Ti: titanium, Z: zirconia, CrCo: cobalt-chromium, P: porcelain.

Supplementary Files

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