ORIGINAL RESEARCH

Analysis of the Viability and Morphology of Gingival Cells on Materials Used in Novel Prosthetic Components: *In Vitro* Study

Rafael Cury Cecato[1], Elizabeth Ferreira Martinez[2], Cesar Augusto Magalhães Benfatti[3]

Abstract

Aim: The objective of this *in vitro* study was to evaluate the viability and morphology of human fibroblasts and keratinocytes cells, both grown on stainless steel (steel) (18Cr14Ni2.5Mo), and polyether-ether-ketone (PEEK) surfaces, hypothesizing the use of these surfaces as novel materials for prosthetic components.

Materials and methods: Gingival human keratinocytes and gingival human fibroblasts lines were grown on discs made by steel ($n = 36$), PEEK ($n = 36$), and titanium (Ti) (Ti6A14V) ($n = 36$)—control. For viability assay, cultures were grown at 24 hours (TV1), 48 hours (TV2), and 72 hours (TV3) times and evaluated by the colorimetric tetrazolium assay (MTT). For morphology and cell adhesion assays, after 24 hours (TM1), 48 hours (TM2), and 96 hours (TM3) of cell culture, cells were examined by scanning electron microscopy (SEM) and analyzed at magnifications with 500×, 1,000×, and 2,500×.

Results: Regarding the viability, the keratinocytes did not present statistical difference on the different materials, in TV1 and TV3 times of culture. Their growth rate increased on all materials, being more expressive in steel; the fibroblasts did not present statistical difference on the different materials, in TV2 and TV3 times of culture. The growth rate of these decreased on all materials, being more expressive in PEEK. The morphology analyses show increase in cell numbers, adequate spreading, and adhesion at all cultivation times (TM1, TM2, and TM3) in both cell lines, on all materials.

Conclusion: All materials tested are suitable for use in the manufacture of prosthetic components for implant-supported rehabilitations, considering the limitations of this study.

Clinical significance: This work analyzes the cellular response of cells present in the human gingiva, as a way to simulate the peri-implant tissue response around novel angular prosthetic components made of stainless steel and PEEK.

Keywords: Biocompatible materials, Cytotoxicity, Dental implant abutment, Dental materials, Oral mucosa.

The Journal of Contemporary Dental Practice (2022): 10.5005/jp-journals-10024-3271

Introduction

In the implant oral rehabilitation, the material used to manufacture the prosthetic components should not only provide adequate mechanical behavior to withstand masticatory forces but also biocompatibility for the cellular responses of soft tissues (epithelium and connective tissues) allows predictable functional and esthetic results. The sealing tissue around the prosthetic components intent as a protective seal between the oral environment and the underlying peri-implant bone,[1–3] so the choice of the material should also be based on its ability to promote integration with the connective tissue of peri-implant mucosa.[4–6]

More recently, novel bendable prosthetic components manufactured from stainless steel,[7] and PEEK,[8] are used as permanent and temporary components, respectively.

Polyether-ether-ketone (PEEK) is a semicrystalline polyaromatic linear polymer that shows good combination of strength, stiffness, toughness, and stability.[9,10] Its biocompatibility is proven decades ago, including being tested in implantable devices for trauma, orthopedic, and prostheses for the vertebral column.[10–13]

Stainless steel is used as material for implantable devices for decades in the medical field,[14] especially in orthopedics. Its biocompatibility has already been endorsed and considered adequate for osseointegration to occur, as long as the surgical and healing conditions (including mechanical postoperative requirement) are in agreement with the tissue manipulation boundaries, such as heating during instrumentation and adequate initial stability.[5,6]

The stainless steel alloy currently used in implantology for prosthetic components manufacturing (ASTM F138)[15] is considered

[1,3]Center for Education and Research on Dental Implants, Post Graduation Program in Dentistry, Department of Dentistry, Federal University of Santa Catarina Florianopolis, Santa Catarina, Brazil

[2]Centro de Pesquisa São Leopoldo Mandic, R Dr José Rocha Junqueira, Campinas, São Paulo, Brazil

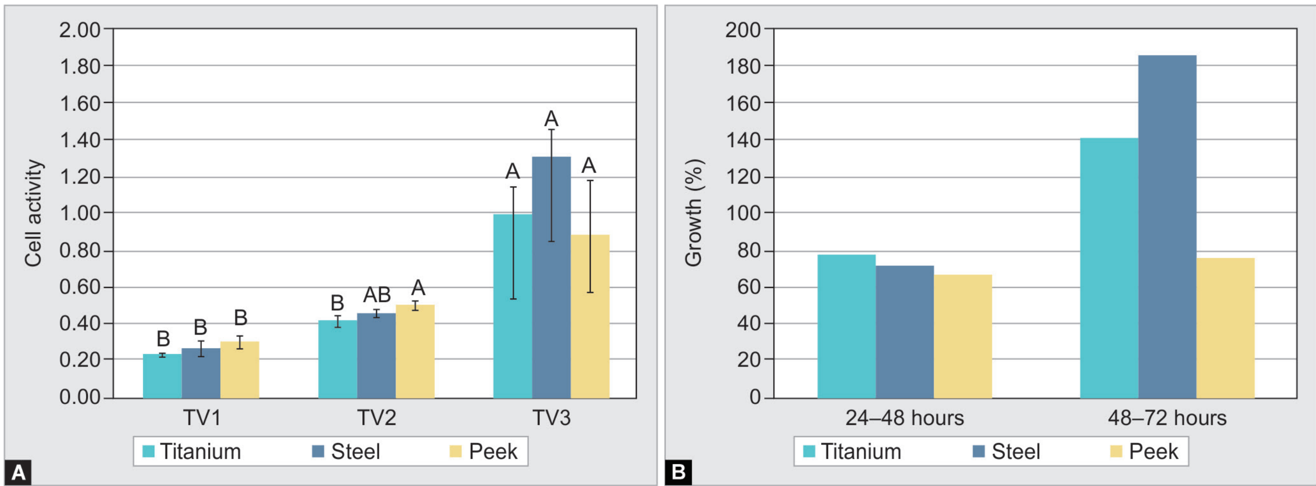
Corresponding Author: Rafael Cury Cecato, Center for Education and Research on Dental Implants, Post Graduation Program in Dentistry, Department of Dentistry, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil, e-mail: rafaelcurycecato@hotmail.com

How to cite this article: Cecato RC, Martinez EF, Benfatti CAM. Analysis of the Viability and Morphology of Gingival Cells on Materials Used in Novel Prosthetic Components: *In Vitro* Study. J Contemp Dent Pract 2022;23(1):22–30.

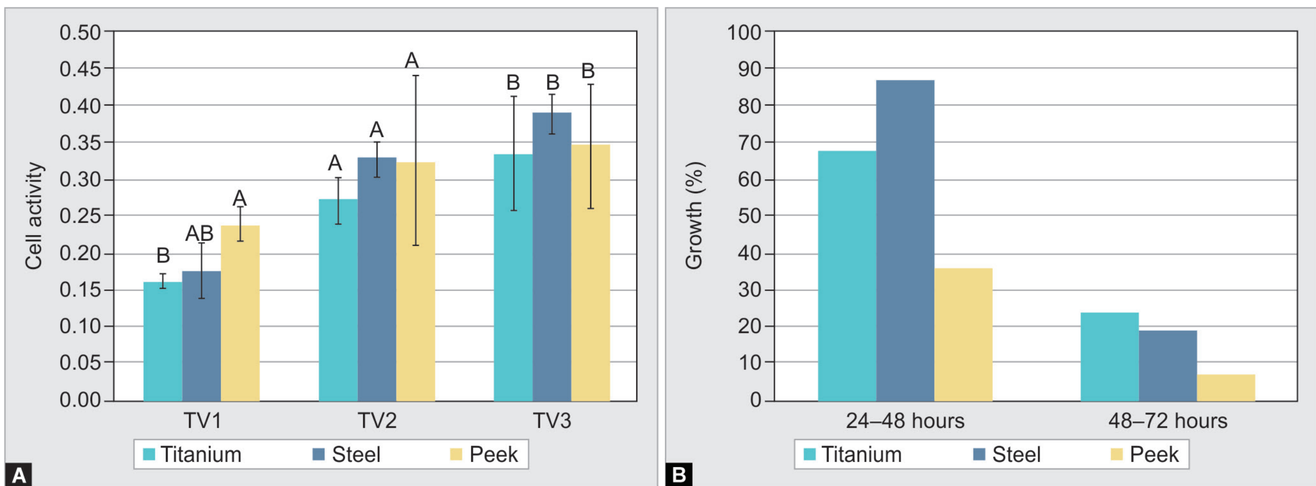
Source of support: Nil

Conflict of interest: None

© The Author(s). 2022 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.



Figs 1A and B: (A) Bar chart showing the keratinocytes cell viability in different materials (titanium, steel, and PEEK) at different culture times (TV1, TV2, and TV3); (B) Bar chart showing the cell growth rate of keratinocytes in different materials (titanium, steel, and PEEK) between culture times (24–48 and 48–72 hours)



Figs 2A and B: (A) Bar chart showing the fibroblasts cell viability in different materials (titanium, steel, and PEEK) at different culture times (TV1, TV2, and TV3); (B) Bar chart showing the cell growth rate of fibroblasts in different materials (titanium, steel, and PEEK) between culture times (24–48 and 48–72 hours)

as a refined derivation of steel AISI 316L (American Iron and Steel Institute), therefore with improved biomechanical results[16,17] and higher corrosion resistance.[17] One of the advantages of using stainless steel alloy compared to titanium alloy (grade V), currently most used material for this purpose, is the mechanical strength.[15,17] Regarding this characteristic, novels prosthetics components were released, for implants with an unfavorable inclination.[7]

The objective of this *in vitro* study was to evaluate the viability and morphology of gingival human keratinocytes and fibroblasts cells, both grown on stainless steel (steel) (18Cr14Ni2.5Mo) and PEEK surfaces, hypothesizing the use of these surfaces as novel prosthetic components.

The null hypothesis is that there is no difference between the novel materials in the viability and morphology of gingival human keratinocytes and fibroblasts cells. The alternative hypothesis is that one novel material performed better than the other in the viability and morphology of gingival human keratinocytes and fibroblasts cells.

Methods

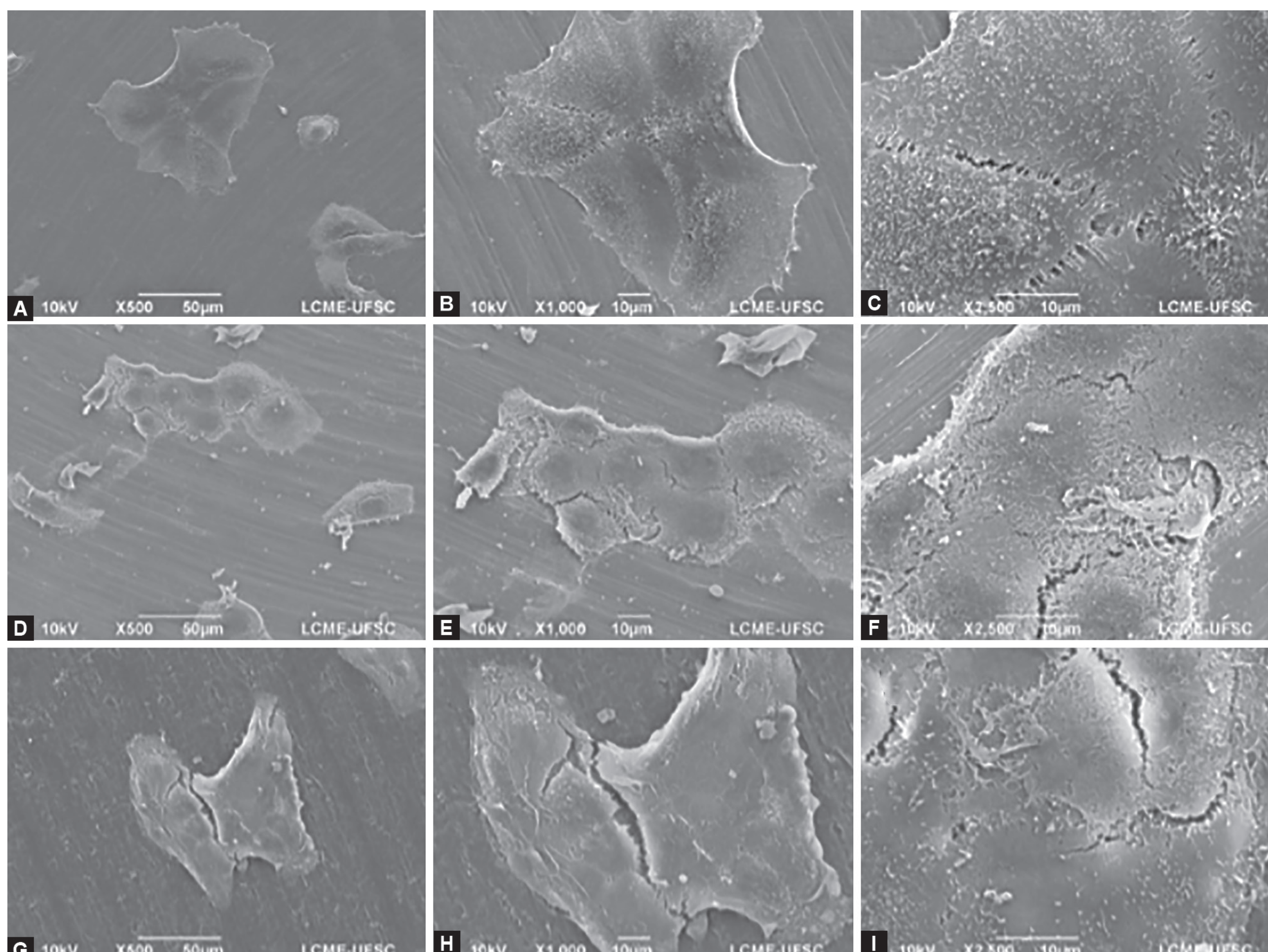
This study was conducted at Federal University of Santa Catarina—Brazil.

Samples

Implantable stainless steel (steel) discs (18Cr14Ni2.5Mo, standard ASTM F138,[15] and ABNT NBR ISO 5832-1:2008,[18] $n = 36$), PEEK discs (C_6H_4–O–C_6H_4–O–C_6H_4–CO, $n = 36$), and titanium alloy (Ti) discs (as control) (Ti6Al4V, standard ASTM F136,[19] $n = 36$) were made with 5 mm of diameter and 2 mm of height. Steel and Ti were metallographically prepared. All samples were supplied by the company FGM® (Dentscare/FGM-Brazil). The discs were washed and sterilized by gamma radiation.

Cell Culture

Gingival human keratinocytes HaCaT[20] cells, spontaneously immortalized keratinocyte line, and gingival human fibroblasts



Figs 3A to I: SEM images of keratinocytes growth on titanium, steel, and PEEK at time TM1. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

(third to sixth passage)[21] were used. These cells were used with the approval of the Ethical Committee of the University of São Paulo, Brazil (Protocol# 728/06).

Both cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) (Sigma Chemical Co., St. Louis, Missouri, United States of America) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, California, United States of America), 100 UT/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L glutamine (Gibco, Grand Island, New York, United States of America) in a humidified atmosphere (5%) of CO_2 at 37°C. Subsequently, the cells were cultured at a concentration of 110 cells/mm^2 in all surfaces.

Cell Viability Test

A total of 18 discs of each material were used (steel, PEEK and titanium alloy), 9 for each cell line (keratinocytes and fibroblasts) and 3 discs for each time: 24 hours (TV1), 48 hours (TV2), and 72 hours (TV3).

Cell cultures, in different surfaces, were tested for cell viability using the colorimetric tetrazolium assay (MTT assay).[22]

This assay evaluates the ability of metabolically active cells to reduce MTT converting the yellow tetrazolium salts [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol bromide] to purple formazan crystals and therefore on the ability of viable cells to cleave the tetrazole ring present in MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol bromide] by the action of dehydrogenase enzymes present in active mitochondria, forming formazan crystals.[22]

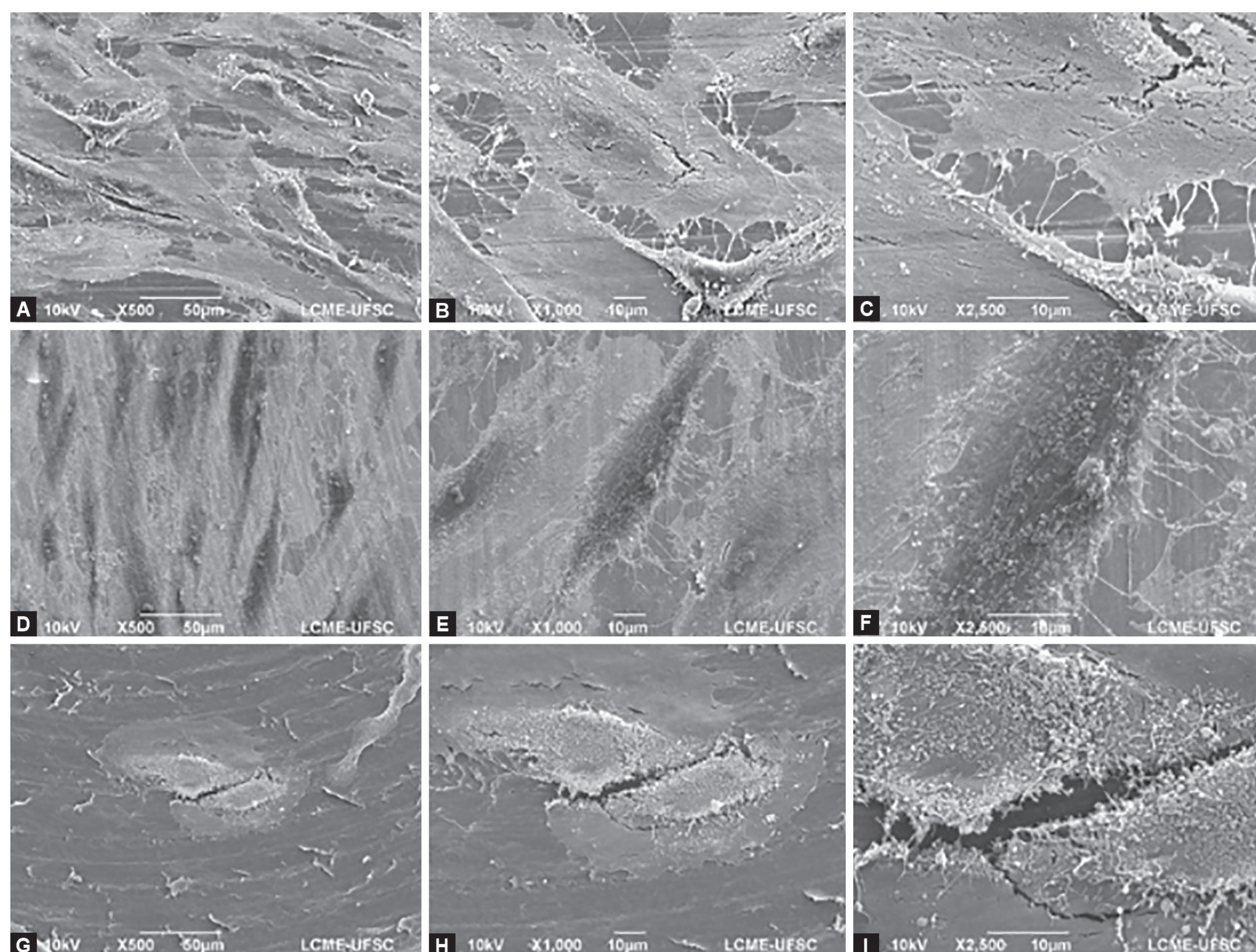
In the cytotoxicity assay, cells were plated at a density of 110 cells/mm^2 on different surfaces. Ten microliter of the MTT solution (5 mg/mL) (Sigma-Aldrich, United States of America), diluted in serum-free DMEM culture medium, were added to the cell cultures, and incubated for 3 hours at 37°C (98.6°F). After this phase, 100 µL of dimethyl sulfoxide (DMSO) (LGC, Brazil) was added and maintained for 15 minutes at room temperature.

After solubilization of the crystals, the measurement was performed in microplate reader ELX800 (Biotek Instruments, United States of America) at 590 nm, according to standard cultivation times.

Cell Morphology

A total of 18 discs of each material were used (steel, PEEK and titanium alloy), 9 for each cell line (keratinocytes and fibroblasts) and 3 discs for each time: 24 hours (TM1), 48 hours (TM2), and 96 hours (TM3).

Keratinocytes and fibroblasts plated on different surfaces under the same conditions described above were determined after programmed cultivation time (TM1, TM2, and TM3) with



Figs 4A to I: SEM images of fibroblasts growth on titanium, steel, and PEEK at time TM1. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

glutaraldehyde solution v/v to 2.5% in 0.1 M cacodylate buffer (pH 7.2) for 1 hour at 4°C (39.2°F). After this time, they were washed in the same buffer solution at 0.05 M, followed by dehydration with increasing concentrations of ethyl alcohol. The samples were submitted to final drying with critical point (EM CPD 030—LEICA, Germany), to that there was no effect of the forces occurring on surface tension and consequently sensitive shape changes. They were then assembled on aluminum brackets (stubs) and placed on a metallizer using a cathodic spray coater (208HR—Cressington Company, England) attached to thickness controller (MTM-20 Cressington High Resolution Thickness Controller, England) to receive gold-palladium coating (80/20%) with 15 nm thick then examined in a Scanning Electron Microscope (SEM) (JEOL JSM-6390LV, Japan) and analyzed qualitatively for cell adhesion, morphology, spreading, and confluence, at 500×, 1,000×, and 2,500× magnifications, respectively.

Statistical Analyses

Quantitative data were tabulated and statistically analyzed in an one-way ANOVA followed by Tukey post-test using a significance level of 5% (Minitab 17) (Minitab Inc., United States of America). The numerical distribution of the values from the samples seems to be better represented by their means, not the medians. ANOVA was used because it compares the means of the groups, while nonparametric tests will check differences between medians. The methodology was reviewed by an independent statistician.

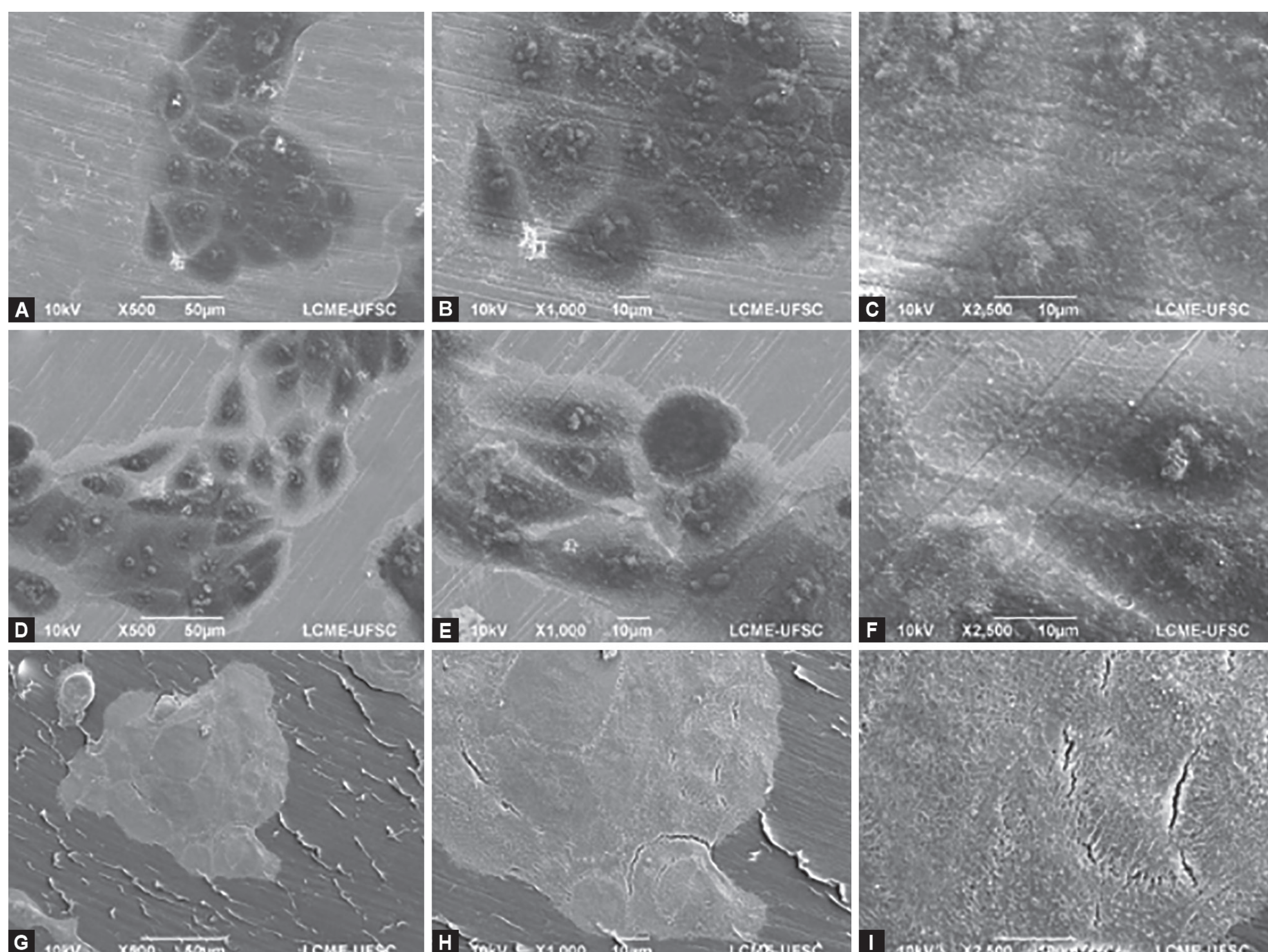
Results

Cell Viability

The viability assays showed increased mitochondrial activity, consequently cell activity growth, in both cell lines on all the materials tested, between TV1, TV2, and TV3 times (Figs 1 and 2).

In relation to the keratinocytes in first and last times (TV1 and TV3), there was no statistically significant difference in all materials tested. In medium time (TV2), PEEK viability showed a statistically significant difference for more in relation to the Ti (control) (Fig. 1A).

The growth rate of keratinocytes between culture times was also analyzed and showed an increase between the initial (24–48 hours) and final (48–72 hours) times. On average, there was similarity between materials between the initial times (24–48 hours) (titanium 78.0%, steel 72.1%, PEEK 67.1%), but between the final times, the steel (187.3%) presented a higher rate than the others, followed by titanium and PEEK (140.6% and 75%, respectively). In relation, the growth rate to the comparison between the last (72 hours) and



Figs 5A to I: SEM images of keratinocytes growth on titanium, steel, and PEEK at time TM2. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

the first time (24 hours), steel presented the highest growth rate (394.4%), followed by titanium (328.4%) and peek (193.4%) (Fig. 1B).

Regarding fibroblasts, PEEK viability showed a statistically significant difference for more in relation to Ti (control) at the first time (TV1). At the second and third measurement, the response equalized, with no statistically significant difference between groups (Fig. 2A).

The growth rate of the fibroblasts between the culture times was also analyzed and shows that, unlike keratinocytes, there was a decrease between the initial (24–48 hours) and final (48–72 hours) times. At initial analyses (24–48 hours), steel (86.1%) presented a higher rate than the Ti (control) (67.4%) and PEEK (35.2%). However, between the final times (48–72 hours), Ti (control) presented a higher rate than the others (23.4%), following by steel (18.9%) with PEEK again with the lowest rate among the others (6.6%). In relation, the growth rate to the comparison between the last (72 hours) and the first time (24 hours), steel again presented the highest growth rate (121.2%), followed by titanium (106.4%) and PEEK (44.2%) (Fig. 2B).

Cell Morphology

The morphology and cell adhesion analyses show both cell number increase and adequate spreading at first and second cultivation times (TM1 and TM2) in both cell lines on all materials, highlighting their adhesion on all surfaces tested. It can be also seen in the images, appropriate union between the cells and consequently a positive growth response (Figs 3 to 6).

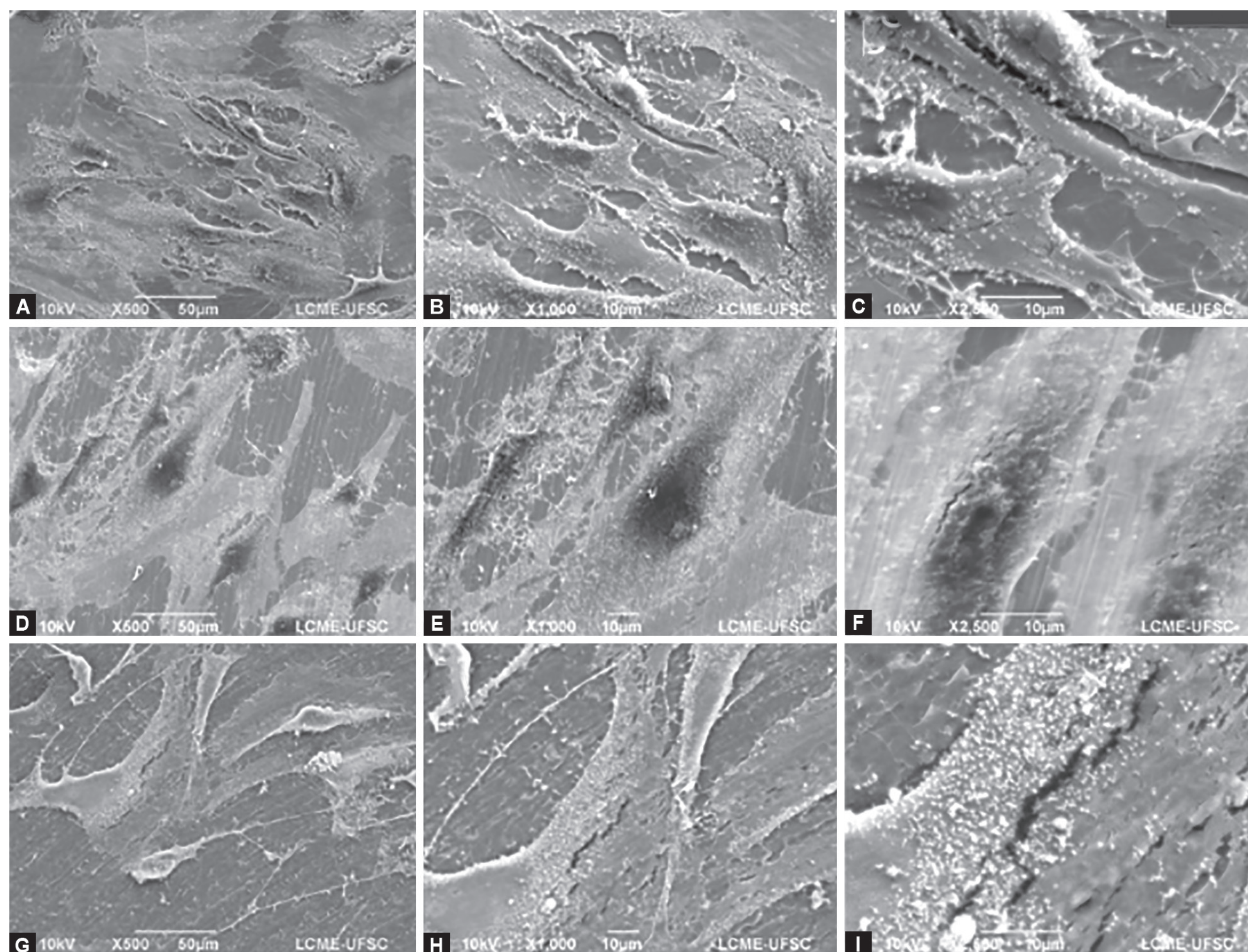
At third cultivation times (TM3), all samples showed increase in the number of cells (Figs 7 and 8).

Regarding cell viability and morphology of gingival human keratinocytes and fibroblasts, the null hypothesis was accepted.

Discussion

It is commonly accepted that the peri-implant soft tissues have similarities with periodontal soft tissues,[3,23,24] including the inflammatory response to the presence of biofilm.[25] However, regardless of the similarity of the gingiva and the peri-implant mucosa, there are still differences, being the underlying connective tissue structure the most striking.[3,24]

The gingival epithelial tissue is classified as stratified squamous, being the keratinocyte of its main cell.[26] Keratinocyte cultures can be classified as immature, mature, or senescent, according to their morphology, being this variation dependent on the culture time or even the age of the donator.[27] The keratinocytes in the qualitative analysis at all times (TM1, TM2, and TM3) and on all surfaces (titanium, steel, and PEEK) show regular and evident morphology and spreading. However, in TM2 and TM3 times, the morphology



Figs 6A to I: SEM images of fibroblasts growth on titanium, steel, and PEEK at time TM2. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

becomes more characteristic. This may be an indication that this cell adhesion of all tested materials is already well accepted in the first 24 hours, and it is confirmed in the following hours. It is evident the union and intimacy between the cells, through desmosomes, at all evaluated times, inherent characteristic of keratinocyte from epithelial basal layer. In some areas, this union is so evident that it is difficult to define the limits of plasma membranes, especially in the more advanced culture (TM3). In addition, the increase in concentration clearly thrives as culture time increases as well as intercellular adhesion.

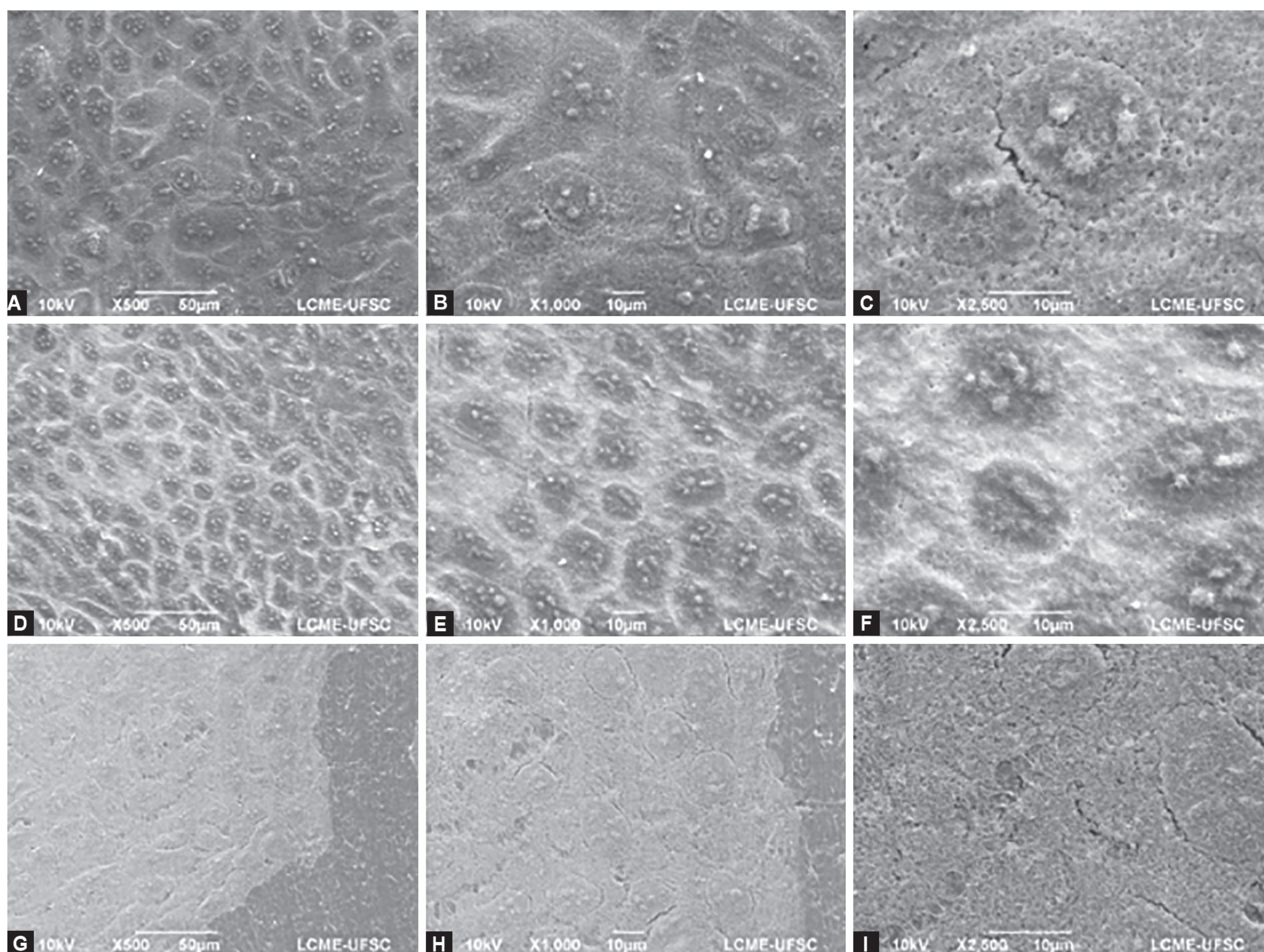
Cell viability (quantitative) of keratinocytes was evaluated by the MTT assay indicated to measure cytotoxicity, proliferation, or cell activation by reading mitochondrial activity.[22] This corroborates with the qualitative analysis (SEM), where despite the statistical difference between PEEK and the control at TV2, there was homogeneous growth of cell lines, with no statistical difference in the first (TV1) and last (TV3) times. In addition, such cell activity increased proportionally with the time of cultivation, indicating increased concentration of cell numbers.

The increase in the growth rate of keratinocytes was observed on all materials, and their behavior is consistent with epithelial cells, where there is a vertiginous growth at the beginning of the cultures, decreasing in the following times (rapid turnover—high proliferation capacity for protective function). But the growth on the steel stood out in relation to the others, which demonstrates the effectiveness of this cell line on this material.

Regarding fibroplasts, in the first 24 hours (TM1), it is possible to notice the formation of "filopodia" on all surfaces, but on the PEEK, these extensions become more numerous and longer only in TM2 and TM3 times. Such observation possibly means the need for longer cell adhesion time in this material in comparison to the others. It is also noticed that its morphology keeps up its template, which means elongated and scattered in all analyzed times (TM1, TM2, and TM3), on all tested materials, also indicating increased concentration of cell numbers, as in keratinocytes.

An important aspect noticed in the fibroblast lineage was the response to the disc surfaces topographic characteristics, not clearly noticed in the keratinocytes. An expected response of the fibroblasts regarding topography is that they have orientation aligned with the grooves of the substrate, with up to 10° of angular variation, when the grooves are smaller than 4.0 μm.[28] In this study, this analysis corroborates such proposed, but only in TM3 these characteristics are well noticed in all materials, probably by cell maturation. This finding is interesting for future guidelines in the manufacture of implant devices, so that the structural orientation of adjacent tissues is better managed.

Also, its own morphology seems to have been influenced by the grooves of the surfaces of the substrates, since in respecting



Figs 7A to I: SEM images of keratinocytes growth on titanium, steel, and PEEK at time TM3. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

this direction, they have been elongated at all times (TM1, TM2, and TM3). Characteristic is intensified as the growing time increases, indicating cell maturation.

From the images, we can notice that there was a similar growth in the concentration of fibroblasts between titanium and steel, whereas in PEEK, we noticed a lower concentration of fibroblasts at all times. These data are different from that found in the quantitative analysis, since the activity of these cells was significantly higher in TV1 time, and became equivalent in other times (TV2 and TV3). This fact exposes an important limitation of the MTT test, where mitochondrial activity is measured, but even existing, does not necessarily match the increase in the concentration of cell numbers in the culture.[29]

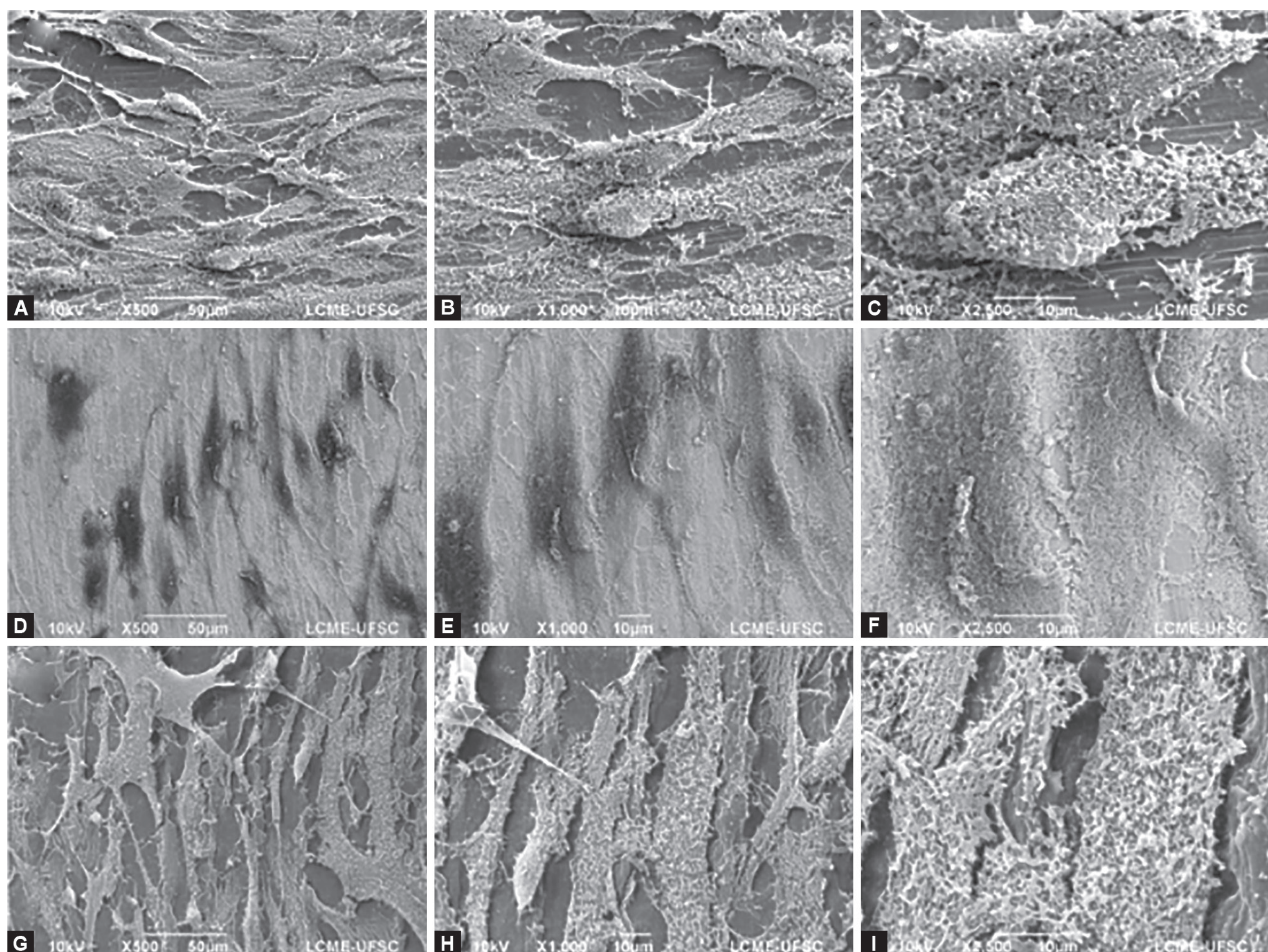
The fibroblast growth rate decreased over all materials. This characteristic is expected in fibroblasts, where the proliferation is lower at the beginning of the culture and grows at the end time, unlike what occurs with keratinocytes. Growth on PEEK showed the greatest decrease, reflecting the higher cellular activity on this material in the first culture time (TV1).

Titanium, including titanium alloy grade V (Ti4Al6V),[19] has been used for decades as an implantable material, and currently, it is chosen as a "gold standard" in dental implants[30,31] and widely used as a prosthetic component. Shah et al.[30] concluded that the bone tissue presents an adequate biomechanical response to the titanium alloy and that no difference could be demonstrated in relation to the commercially pure titanium (cp-Ti). Velasco-Ortega et al.[32] investigated the cytotoxicity with human fibroblasts of Titanium alloy grade V and confirmed the biocompatibility.

However, in a recent systematic review to assess the cytotoxicity of titanium alloy grade V on gingival fibroblasts, Willis et al.[33] concluded that the release of ions (i.e., vanadium) may occur in the medium and consequently increase cytotoxicity.

The stainless steel (ASTM F138),[15] though used for decades in the medical field,[5,6,14] only recently had its use indicated for novel bendable prosthetic components[7] with undefined function of time, that is, to support final prosthetic restorations on implant rehabilitation. Its mechanical performance is superior to titanium, therefore, with predicates that support a greater range of clinical indications, such as angled components. However, probably because of the uniqueness of the indication, cell viability in peri-implant mucosa lineage had not been certified under conditions that simulate prosthetic intermediate (abutment). In both qualitative and quantitative analyses, keratinocytes as well as gingival fibroblasts showed no alterations and/or abnormalities during growth and maturation. An interesting finding is that the viability of both cell lines (keratinocytes and fibroblasts) was higher on this material compared to titanium.

JAYPEE



Figs 8A to I: SEM images of fibroblasts growth on titanium, steel, and PEEK at time TM3. (A to C) Titanium; (D to F) Steel; (G to I) PEEK. Magnification of 500×, 1,000×, and 2,500×, respectively

Polyether-ether-ketone (PEEK) is a thermoplastic material and as such can be converted into a variety of shapes and sizes of components by the full spectrum of manufacturing technologies such as machining or injection molding.[9] Its cell cytotoxicity has previously been evaluated[34,35] with appropriate results, confirming the results in this study. Its viability did not show significant statistical difference in relation to the materials tested in keratinocyte cultures in first (TV1) and last times (TV3), that is, equivalence between them. In fibroblast cultures, statistically significant difference was greater in TV1 time, that is, greater viability in the first 24 hours of culture and with no statistically significant difference in TV2 and TV3 times. Although it shows absence of cytotoxicity for the tested cell lines in the morphological evaluation, lower cellular concentration was observed, mainly in the first times (TM1 and TM2) in relation to the other materials.

Through the viability and morphological analyses executed, all the evaluated materials are consistent with the growth of cells of the tested cell lines, according to their indications, as options for medical devices manufacture. However, as an important limitation of this study, a qualitative analysis of the morphology only shows a two-dimensional image, and a proposal of cellular adhesion, without evidences of important cell characteristics on the surfaces under other angles. Also, there were no tests performed on cell adhesion markers, which are important to evaluate more accurately the effectiveness of adherence to each surface. Another point is, as previously stated, the viability assay (MTT) only quantifies the mitochondrial activity,[29] without showing the actual concentration and/or cell adhesion. Therefore, new assays with such analyses are required for a better understanding of these properties.

Conclusions

The cell viability assays of human gingival keratinocytes and fibroblasts showed viability at the times evaluated, on all surfaces tested.

The evaluation of the morphology of both cell lines was considered within normality at established culture times, on all surfaces tested.

The null hypothesis was accepted. Considering the limitations of this study, all materials tested are suitable for use in the manufacture of prosthetic components for implant-supported rehabilitations.

Acknowledgments

Gabriel Westphal—Independent Statistician—Automotive Engineer—Federal University of Santa Catarina (UFSC), Florianópolis/Santa Catarina, 88040-900, Brazil.

References

1. Lavelle CL. Mucosal seal around endosseous dental implants. J Oral Implantol 1981;9(3):357–371. PMID: 6942173.
2. Gould TRL. Clinical implications of the attachment of oral tissue to permucosal implants. Tissue integration in oral and maxillo-facial reconstruction. In: Proc an Int Congr Brussels Excerpta Medica. 1985. p. 253–270.
3. Narula IS, Chaubey KK, Arora VK, et al. Implanto-gingival complex: an indispensable junctional complex for the clinical success of an implant. J Dent Implant 2012;2(2):110–114. DOI: 10.4103/0974-6781.102225.
4. Welander M, Abrahamsson I, Berglundh T. The mucosal barrier at implant abutments of different materials. Clin Oral Implants Res 2008;19(7):635–641. DOI: 10.1111/j.1600-0501.2008.01543.x.
5. Linder L. Osseointegration of metallic implants. I. Light microscopy in the rabbit. Acta Orthop Scand 1989;60(2):129–134. DOI: 10.3109/17453678909149239.
6. Linder L, Obrant K, Boivin G, et al. Osseointegration of metallic implants. II. Transmission electron microscopy in the rabbit. Acta Orthop Scand 1989;60(2):135–139. DOI: 10.3109/17453678909149240.
7. Carpena ALM de M, Kinalski M de A, Bergoli CD, et al. Novel bendable abutments as a solution to correct unfavorable implant inclination. A clinical report. J Esthet Restor Dent 2020;32(8):757–762. DOI: 10.1111/jerd.12654.
8. Ruales-Carrera E, Pauletto P, Apaza-Bedoya K, et al. Peri-implant tissue management after immediate implant placement using a customized healing abutment. J Esthet Restor Dent 2019;31(6):533–541. DOI: 10.1111/jerd.12512.
9. Jones DP, Leach DC, Moore DR. Mechanical properties of poly (ether-ether-ketone) for engineering applications. Polymer (Guildf) 1985;26:1385–1393. DOI: 10.1016/0032-3861(85)90316-7.
10. Williams D, McNamara A, Turner R. Potential of polyetheretherketone (PEEK) and carbon fibre reinforced PEEK in medical applications. J Mater 1987;18:267. https://doi.org/10.1007/BF01728981.
11. Marya K, Dua JS, Chawla S, et al. Polyetheretherketone (PEEK) dental implants : a case for immediate loading. Int J Oral Implantol Clin Res 2011;2(2):97–103. DOI: 10.5005/jp-journals-10012-1043.
12. Kurtz SM, Devine JN. PEEK biomaterials in trauma, orthopedic, and spinal implants. Biomaterials 2007;28(32):4845–4869. DOI: 10.1016/j.biomaterials.2007.07.013.
13. Passoni BB, Venâncio F, Formiga M de C, et al. Implante imediato com provisionalização imediata através de cicatrizador multifuncional de PEEK. Implant News Perio 2017;2(4):885–892. ID: biblio-877294.
14. Blackwood DJ, Pereira BP. No corrosion of 304 stainless steel implant after 40 years of service. J Mater Sci Mater Med 2004;15(7):755–758. DOI: 10.1023/b:jmsm.0000032814.20695.3c.
15. American Society for Testing and Materials (ASTM). F138–13a-standard specification for wrought 18chromium-14nickel-2.5molybdenum stainless steel bar and wire for surgical implants (UNS S31673). 2013.
16. Buss GAM, Donath KS, Vicente MG. Utilização de aços inoxidáveis em implantes. Bol Inf Tecnovigilância 2011. p. 1–6.
17. Donachie M. Biomedical alloys. Adv Mater Process 1998;154:63. ISSN: 0882-7958.
18. Associação Brasileira de Normas Técnicas. Implantes cirúrgicos–Materiais metálicos Parte 1: Aço inoxidável conformado ABNT NBR ISO 5832-1:2008. 2010.
19. American Society for Testing and Materials (ASTM). F136–12a-standard specification for wrought titanium-6aluminum-4vanadium eli (extra low interstitial) alloy for surgical implant applications (UNS R56401). 2013.
20. Huacho PMM, Nogueira MNM, Basso FG, et al. Analyses of biofilm on implant abutment surfaces coating with diamond-like carbon and biocompatibility. Braz Dent J 2017;28(3):317–323. DOI: 10.1590/0103-6440201601136.
21. Martinez EF, Araújo VC, Sousa SOM, et al. TGF-β1 enhances the expression of α-smooth muscle actin in cultured human pulpal fibroblasts: immunochemical and ultrastructural analyses. J Endod 2007;33(11):1313–1318. DOI: 10.1016/j.joen.2007.07.040.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65(1-2):55–63. DOI: 10.1016/0022-1759(83)90303-4.
23. Berglundh T, Lindhe J, Ericsson I, et al. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991;2(2):81–90. DOI: 10.1034/j.1600-0501.1991.020206.x.
24. Atsuta I, Ayukawa Y, Kondo R, et al. Soft tissue sealing around dental implants based on histological interpretation. J Prosthodont Res 2016;60(1):3–11. DOI: 10.1016/j.jpor.2015.07.001.
25. Berglundh T, Lindhe J, Marinell C, et al. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res 1992;3(1):1–8. DOI: 10.1034/j.1600-0501.1992.030101.x.
26. Newman MG, Takey HH, Klollevold PR, et al. Clinical periodontology. 11th ed. Elsevier/Saunders; 2012.
27. Merrick P, Meyer AA, Herzog S, et al. Scanning electron microscopy of cultured human keratinocytes. J Burn Care Rehabil 1990;11(3):228–236. DOI: 10.1097/00004630-199005000-00009.
28. Den Braber ET, De Ruijter JE, Ginsel LA, et al. Quantitative analysis of fibroblast morphology on microgrooved surfaces with various groove and ridge dimensions. Biomaterials 1996;17(21):2037–2044. DOI: 10.1016/0142-9612(96)00032-4.
29. Van Tonder A, Joubert AM, Cromarty A. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes 2015;8:47. DOI: 10.1186/s13104-015-1000-8.
30. Shah FA, Trobos M, Thomsen P, et al. Commercially pure titanium (cp-Ti) versus titanium alloy (Ti6Al4V) materials as bone anchored implants–is one truly better than the other? Mater Sci Eng C 2016;62:960–966. DOI: 10.1016/j.msec.2016.01.032.
31. Steinemann SG. Titanium–the material of choice? Periodontol 2000 1998;17:7–21. DOI: 10.1111/j.1600-0757.1998.tb00119.x.
32. Velasco-Ortega E, Jos A, Cameán AM, et al. In vitro evaluation of cytotoxicity and genotoxicity of a commercial titanium alloy for dental implantology. Mutat Res Genet Toxicol Environ Mutagen 2010;702(1):17–23. DOI: 10.1016/j.mrgentox.2010.06.013.
33. Willis J, Li S, Crean SJ, et al. Is titanium alloy Ti-6Al-4 V cytotoxic to gingival fibroblasts–a systematic review. Clin Exp Dent Res 2021;7(6):1037–1044. DOI: 10.1002/cre2.444.
34. Wenz LM, Memitt K, Brown SA, et al. In vitro biocompatibility of polyetheretherketone and polysulfone composites. J Biomed Mater Res 1990;24(2):207–215. DOI: 10.1002/jbm.820240207.
35. Hunter A, Archer CW, Walker PS, et al. Attachment and proliferation of osteoblasts and fibroblasts on biomaterials for orthopaedic use. Biomaterials 1995;16(4):287–295. DOI: 10.1016/0142-9612(95)93256-d.

JAYPEE