

Keratinocyte Proliferation and Hemidesmosome Formation on Surfaces for Dental Implants: In Vitro Study

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Purpose: To demonstrate the likelihood of the polyetheretherketone (PEEK), zirconia (ZrO₂), and titanium (Ti) disks to support proliferation and hemidesmosome formation of gingival cells. **Materials and Methods:** Water contact angle was performed on each material, and surface roughness (Ra) was measured. Scanning electron microscopy and x-ray photoelectron spectroscopy were used. Later, oral keratinocyte cells were cultured on disks, and metabolic activity and expression of hemidesmosome markers, integrin α6 and β4, in relation to the biomaterial disks at 1, 3, and 5 days of cell culture were quantified. Tissue culture polystyrene was used as the control. Statistical analysis was performed with analysis of variance (ANOVA) with Tukey post hoc comparison test. A *P* value of < .05 was considered statistically significant. **Results:** The water contact angle ranged from 70.2 degrees (Ti) to a maximum of hydrophobicity of 93.3 degrees (PEEK). Ra was highest on ZrO₂, followed by PEEK. Ti showed the most keratinocyte metabolic activity at 1, 3, and 5 culture periods. Contrarily, ZrO₂ and PEEK disks had lower keratinocyte metabolic activity at all observation times, with no statistical differences between both groups. Integrin α6 and β4 expression was highest on TCPS and ZrO₂ compared to Ti and PEEK. **Conclusion:** Keratinocytes proliferated faster on Ti than on ZrO₂ and PEEK substrates, and expression of hemidesmosome formation markers, integrin α6 and β4, were higher on ZrO₂ than either Ti or PEEK. *Int J Oral Maxillofac Implants* 2023;38:496–502. doi: 10.11607/jomi.9894

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Titanium (Ti) is the most commonly used material for dental implants because of its biocompatibility,¹ but it is not exempt of limitations: (1) It may represent an esthetic complication if visible in the oral cavity, especially in thin tissue biotypes and in the esthetic zone; (2) Ti particles have been found in soft and hard tissues surrounding implant and abutment components, and in regional lymph nodes, which may lead to biologic complications^{2–4}; (3) and finally, Ti allergies have been reported by Sicilia et al⁵ at approximately 0.6% in more than 1,500 patients. Therefore, alternatives to Ti have been developed to overcome those potential drawbacks.

Zirconium dioxide (zirconia; ZrO₂) has been suggested as an alternative implant material to Ti. Results for biocompatibility of ZrO₂ implants are similar to those for conventional Ti implants.^{6,7} However, ZrO₂ has been observed to accumulate less plaque,^{7,8} and no metal color can be seen when exposed through the tissue.^{9,10} Furthermore, Depprich et al¹¹ observed that ZrO₂ dental implants displayed a 74% to 98% implant survival rate over 12 to 56 months and 79.6% to 91.6% success rates over 6 to 12 months. Later, according to the meta-analysis results from Elnayef et al,¹² ZrO₂ implants showed a 91.5% survival rate and a 91.6% success rate over a 42.37-month mean follow-up period, reporting that this may be a good alternative to Ti implants, specifically in the anterior esthetic areas with a thin tissue biotype.

Polyetheretherketone (PEEK), a synthetic thermoplastic polymer, is a commonly employed biomaterial in the field of orthopedic surgery¹³ and is increasingly gaining evidence-based applicability in dentistry.¹⁴ This polymer has primarily been used in relation to bone due to its comparable modulus of elasticity (3 to 4 GPa), in addition to high stability and biocompatibility.¹⁵ Aside from PEEK's introduction as a dental implant material,¹⁶ PEEK has been applied as an implant healing abutment.¹⁷

To be considered an alternative material to Ti in the dental implant field, for both implants and transepithelial abutments, a number of characteristics would have

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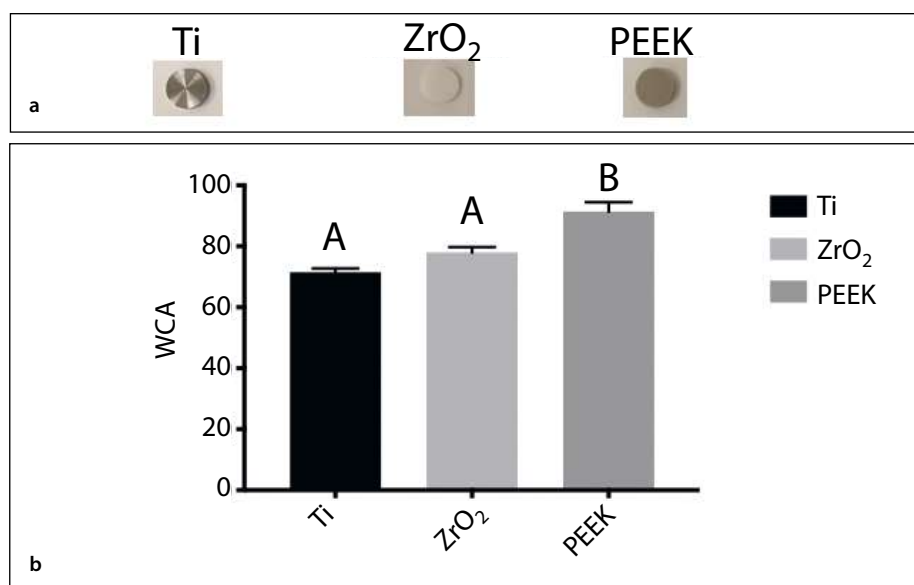
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Fig 1 (a) Disks made of the three different materials tested and (b) their water contact angles (WCA). Groups with different letters had statistically significant differences.



to be considered, namely, biocompatibility, adequate biomechanical effects on the surrounding anatomy, superior esthetic outcomes, fracture resistance, and a well-adapted seal at the implant-abutment junction.

Considering that the junctional epithelium is a specialized epithelial structure that seals off the supporting tissues of the tooth and dental implant and represents the first line of defense against periodontal diseases,¹⁸ the interaction of the biomaterial surface with the junctional epithelium would be of great interest. This interaction is mediated by hemidesmosomes, cell adhesion complexes initiated by integrin $\alpha 6 \beta 4$ binding to extracellular laminin 332.¹⁹ The coronal soft tissue integration success of dental implants and their transepithelial components may be due to earlier attachment of gingival epithelial cells, via hemidesmosomes,²⁰ and consequently providing a better soft tissue sealing and potentially preventing peri-implant diseases.¹⁸ However, *in vitro* comparisons between PEEK, Ti, and ZrO₂ show mixed results when comparing epithelial cell proliferation and hemidesmosome formation. For example, Kimura et al²¹ showed no differences between Ti and ZrO₂, while Atsuta et al²² showed significantly higher hemidesmosome formation on titanium compared to zirconia. Indeed, a systematic review by Corvino et al²³ recently noted “extreme heterogeneity” in the literature for ideal material properties for epithelial sealing, and a recent meta-analysis by Canullo et al²⁴ could not draw definitive conclusions about transmucosal implant components affecting soft tissue conditions.

The aim of this *in vitro*, preliminary investigation

was to demonstrate the likelihood of PEEK, ZrO₂, and Ti materials supporting proliferation of gingival cells and hemidesmosome formation, related to the potential epithelial seal that may be established to these commonly used biomaterials for dental and orthopedic implants.

MATERIALS AND METHODS

Overview of Experimental Plan

PEEK, Ti, and ZrO₂ disks were used to imitate the application of these materials as transepithelial implant abutments. Physical-chemical and topographic properties of the surfaces of these materials were assessed, and oral epithelial cells were cultured to be placed onto the implant materials. The cellular parameters measured included the quantification of epithelial cell metabolic activity and expression of hemidesmosome formation markers on the biomaterial disks.

Experimental Specimens

Experimental disks.

The specimens employed in this investigation were non-surface-modified, laboratory-milled disks of different biomaterials. The dimensions of each disk were fit to a single well in a standard 12-well Clear TC-treated Multiple Well Plate (Corning), at 1-cm diameter and 2-mm thickness. The disks were made of Ti, ZrO₂, and PEEK (Fig 1a).

Study Design

Sessile drop water contact angle (WCA) was performed in each disk material using a contact angle analyzer (DM-CE1, Kyowa Interface Science). Deionized water was used as the wetting liquid with a drop volume of 2 μ L. Three angles were obtained per material. A white-light confocal laser microscope (HS200, Hyphenated Systems) was used to quantify surface roughness (Ra; arithmetic mean of height deviations) at $\times 50$ magnification. Scans were processed with a Gaussian waviness filter ($\lambda c = 20$) and smoothed using a circular Hann window (0.70- μ m diameter). Two scans per sample were obtained from three samples.

To further analyze the implant material surface, scanning electron microscopy (SEM) was used to observe implant material surface morphology. Samples were imaged with a desktop SEM (TM3000, Hitachi) at $\times 200$ magnification at an acceleration voltage of 15 kV. Finally, x-ray photoelectron spectroscopy (XPS; PHI 5000 VersaProbe III, ULVAC) was used to confirm surface elemental composition of implant materials using a monochromatic Al K α x-ray source (45 degrees, 1,486.6 eV, 50 W, and a sampling area: 200- μ m diameter) at a pass energy of 280 eV and step size of 1.0 eV with charge compensation. Spectra were calibrated to the C 1s signal (284.8 eV) for analysis in MultiPak (ULVAC Inc). Representative spectra are shown from around three scans per material.

Immortalized gingival keratinocytes ([TERT2/OKF6; RRID:CVCL_L225]²⁵ BWH Cell Culture and Microscopy Core) were cultured in defined keratinocyte serum-free medium supplemented with epidermal growth factor, bovine pituitary extract, and 1% penicillin/streptomycin (Thermo Fisher Scientific). Cells were provided by scientist Prof James Rheinwald in 2015 after original isolation, previously authenticated, stained positive for keratinocyte marker cytokeratin 14, tested negative for mycoplasma, and were not previously noted as a commonly misidentified cell (International Cell Line Authentication Committee). Medium was routinely changed every 3 days as the cells grew at 37°C in a humidified, 5% CO₂ atmosphere.

Prior to the study, [substrates $\phi = 1$ cm] were disinfected with absolute ethanol for 30 minutes, extensively washed in phosphate-buffered saline (PBS), and exposed to UV for 20 minutes per side for final sterilization. Keratinocytes at passage 11 were seeded in medium at 76.4 cells/mm² onto Ti, ZrO₂, and PEEK disks of identical diameter and volume, and tissue-culture polystyrene (TCPS) disks were used as the control. Three disks were used per group.

At 1, 3, and 5 days, metabolic activity was measured with Cell Counting Kit-8 (CCK8, Dojindo Molecular Technologies). Medium was changed at days 2 and 4. Disks were washed three times in PBS, transferred to a new well-plate, and CCK8 solution added at 1:10 dilution in medium. Absorbance was read following 2.5 hours of incubation on a plate reader (Synergy, BioTek) and controlled for background. Data are expressed as mean optical density (OD) $\pm$ SD of three technical replicates.

Enzyme-linked immunosorbent assays (ELISAs) were used to quantify integrin $\alpha 6$ (OKCD07282 Aviva Systems Biology) and integrin $\beta 4$ (OKCD07398 Aviva Systems Biology) expression. Gingival keratinocytes were cultured as described, and ELISAs were performed at 1, 3, and 5 days of culture per the manufacturer's suggestions (lysed with ice-cold 9803S, Cell Signaling Technology, and 1% v/v protease inhibitor [78429, Thermo Fisher Scientific] and cleared [12,000 g, 10 minutes]). Expression was then normalized to total protein with a micro bicinchoinic acid assay following the manufacturer's instructions (23235, Thermo Fisher Scientific). Data are expressed as mean protein concentration (ng/mg of total protein) $\pm$ SD of three technical replicates.

To examine keratinocyte proliferation after 18 hours of culture, one disk from each material was fixed in 4% paraformaldehyde at room temperature for 10 minutes, permeabilized with 1% Triton X-100 at room temperature for 10 minutes, and then blocked in 5% bovine serum albumin for 30 minutes at room temperature. Samples were then stained and counterstained with 4',6-diamidino-2-phenylindole (DAPI; D9542, Thermo Fisher Scientific) for 10 minutes at room temperature. Samples were washed three times in PBS between each step. Visualization was performed on a Leica DM6 B upright fluorescent microscope at $\times 10$ magnification and visualized in ImageJ (National Institutes of Health).

Statistical Analysis

Statistical analysis was performed with two-way analysis of variance (ANOVA; factors: time and material) with Tukey post hoc comparison test (GraphPad Prism 7.0e) on the OD values obtained with CCK8 and on integrin expression values. One-way ANOVA (factor: material) with Tukey post hoc comparison test was used to compare WCAs and roughness of each implant material. The normality of the data was confirmed by Anderson-Darling and Shapiro-Wilk tests. A *P* value of $< .05$ was considered statistically significant. Mean and SD are reported.

Fig 2 (a) Surface roughness (Ra) of the three different materials tested and (b) SEM micrograph of surface morphology. Scale is 500 μm . Groups with different letters had statistically significant differences.

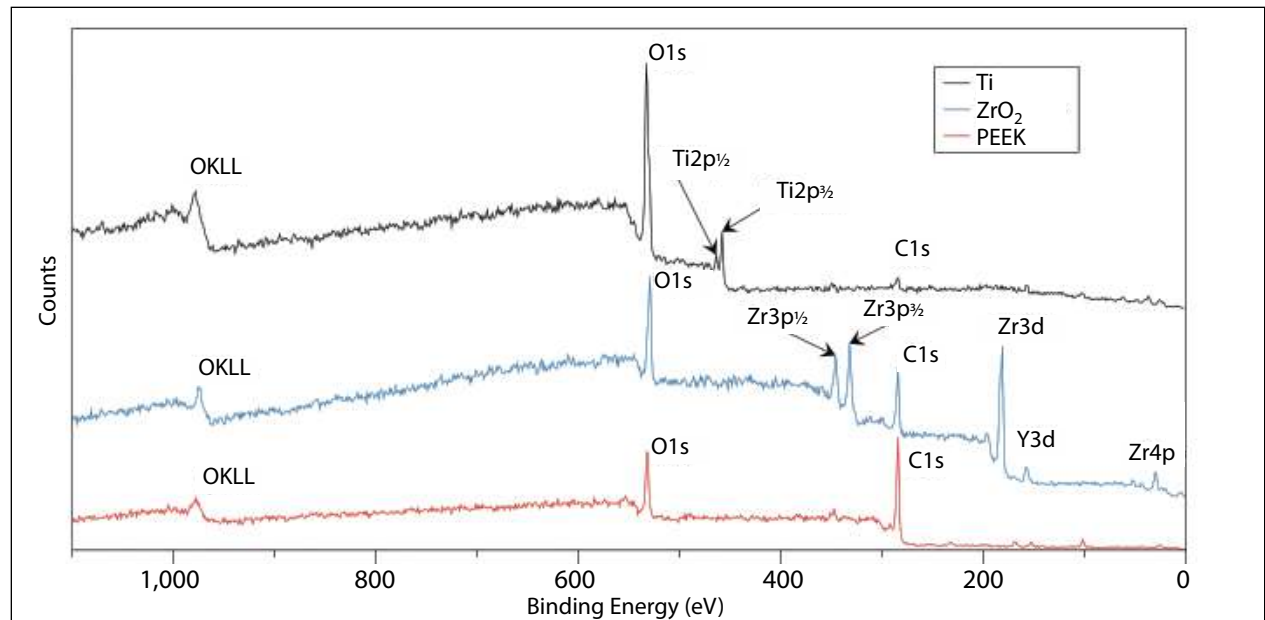
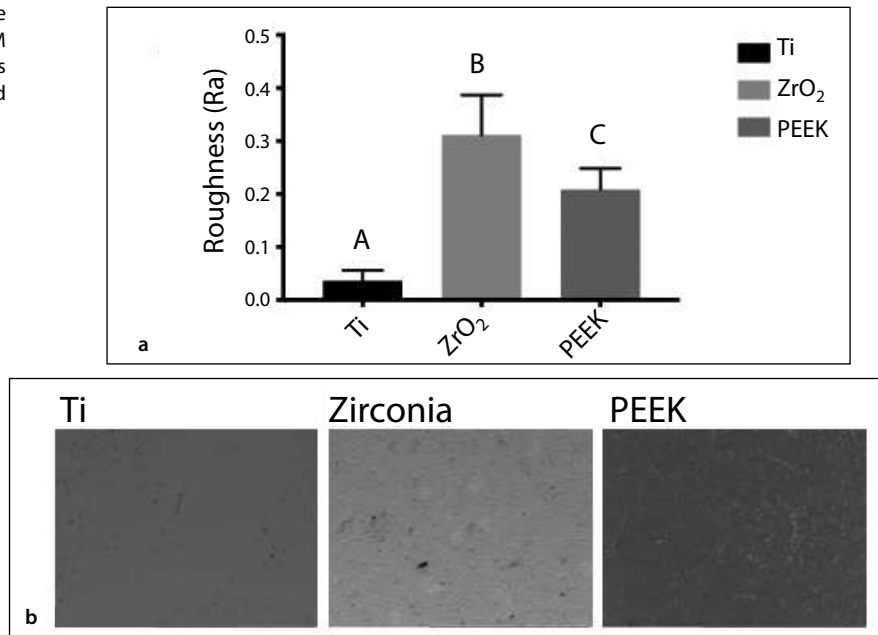


Fig 3 XPS full survey spectra of the three different materials tested. Individual peaks of interest are denoted.

RESULTS

Implant Surface Characteristics

The WCA of the implant materials (Fig 1a) ranged from 70.2 degrees (Ti disk) to a maximum of 93.3 degrees (PEEK disk; Fig 1b). The comparison between groups was significant between Ti or ZrO₂ compared to PEEK disks ($P \leq .05$). In contrast, the comparison between Ti and ZrO₂ disks marginally lacked statistical significance ($P = .065$). Surface Ra was significantly higher on ZrO₂

than both Ti and PEEK; PEEK Ra was significantly higher than Ti (Fig 2a). SEM micrographs revealed (Fig 2b) a relatively smooth Ti surface, a slightly rougher PEEK surface, and a rather rough ZrO₂ surface, with grooves from milling visible. XPS analysis (Fig 3) revealed expected characteristic peaks for each material; for example, Ti2p for Ti, Zr3p_{1/2} for ZrO₂, and C1s and O1s for PEEK. Surfaces did not show the presence of contaminants.

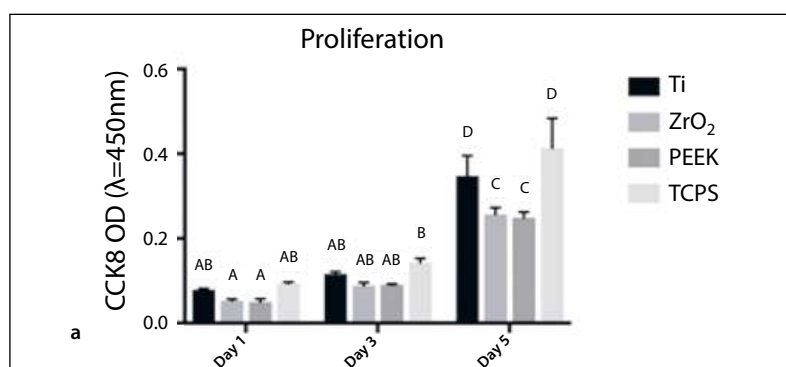


Fig 4 Proliferation of human keratinocytes on materials tested. (a) CCK8 metabolic activity up to 5 days in culture. (b) DAPI fluorescent staining of the nuclei of the cells at 18 hours. Groups with different letters had statistically significant differences. Scale bar is 100 μ m.

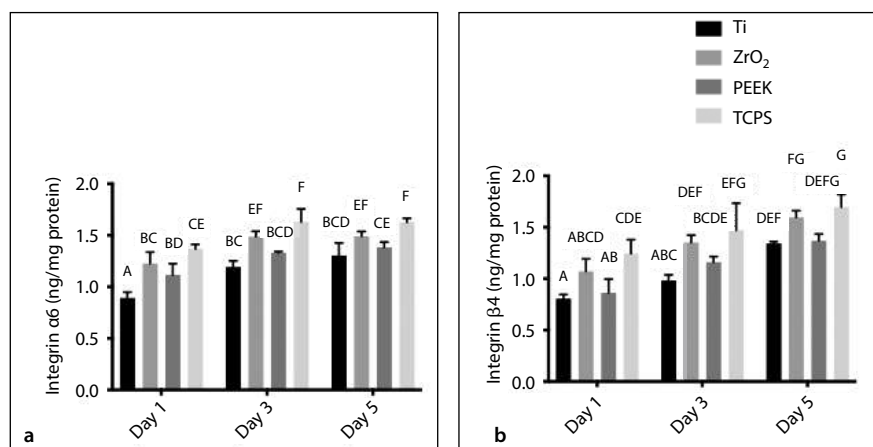
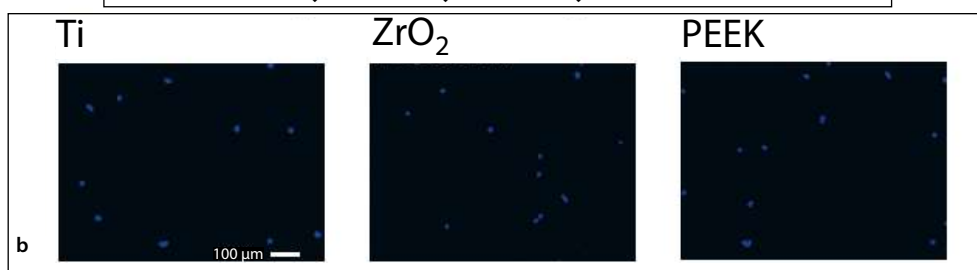


Fig 5 Hemidesmosome integrin α 6 and β 4 expression. (a) Integrin α 6 and (b) integrin β 4 expression up to 5 days in culture. Groups with different letters had statistically significant differences.

Metabolic Activity and Proliferation of Human Keratinocytes

TCPS disks (control) showed the most keratinocyte metabolic activity (used as a proxy for proliferation), followed by the Ti disks at 1, 3, and 5 days of culture (Fig 4a). No statistical difference between Ti and TCPS was found, except during the 3 days of observation where TCPS revealed statistically significant differences with respect to Ti disks. Contrarily, ZrO₂ and PEEK disks had the lowest keratinocyte metabolic activity at all observation times, with no statistical differences between both groups. When comparing Ti or TCPS groups with ZrO₂ or PEEK groups, the differences were always statistically significant with the exception of the day 3 observation, where no difference were found between

Ti, ZrO₂, and PEEK groups. These findings are shown in Fig 4a. In support of 1-day results, similarity was observed when analyzing the fluorescent staining of the nuclei of the cells in contact with the Ti, ZrO₂, and PEEK disks at 18 hours (Fig 4b).

Expression of Hemidesmosome Integrin α 6 and β 4 by Human Keratinocytes

Keratinocyte expression of integrin α 6 was generally higher on ZrO₂ and TCPS than Ti and PEEK and slightly increased from days 1 to 3 to 5 (Fig 5a). Similarly, expression of integrin β 4 was higher on ZrO₂ and TCPS compared to Ti and PEEK, again increasing from days 1 to 3 to 5 (Fig 5b). Statistical significance between groups is detailed in Figs 5a and 5b.

DISCUSSION

Metal-free restorations have gradually generated interest in the field of implant therapy for a number of reasons, such as superior esthetic outcomes in thin gingival biotype,¹⁶ increased elasticity and therefore resistance to cyclic loading related to PEEK abutments,²⁶ and growing fear of allergic reactions to the presence of Ti particles in the peri-implant area, the effects and implications of which have not been thoroughly investigated.¹⁶

While PEEK has been suggested in the aforementioned literature as an orthopedic implant,²⁷ a dental implant¹⁶ and a healing/provisional abutment,¹⁷ it is yet to be deemed a reliable option as a definitive implant abutment. Meanwhile, ZrO₂ has already been proposed with enough scientific evidence for a dental implant alternative to Ti,¹² and as a definitive prosthetic abutment or for a definitive nonmetal restoration.^{9,28}

Innovation in the dental implantology field has experienced huge advances in multiple areas. These innovations involve, but are not limited to, new materials, advances in prosthetic components, and an enormous advance in understanding the importance of the long-term bone and soft tissue implant seal in relation to preventing marginal bone loss and peri-implantitis. Unfortunately, peri-implantitis has become the most frequent complication related to dental implantology, affecting approximately one-third of patients and one-fifth of all implants.²⁹

Epithelial and connective tissue sealing to dental implant components is critical to preventing peri-implantitis and simultaneously improving long-term implant success.³⁰ The junctional epithelium adheres to the tooth and to the implant components and seals off the supporting tissues from the aggressive oral environment. The junctional epithelium represents the first line of defense against periodontal and peri-implant diseases.¹⁸

In this investigation, keratinocytes showed lower metabolic activity on PEEK and ZrO₂ disks compared to Ti substrates. Thereby, based on the results of the present study, it seems that PEEK and ZrO₂ surfaces do not support proliferation of epithelial cells as well as Ti surfaces. However, ZrO₂ supported higher expression of hemidesmosome components integrin $\alpha 6$ and $\beta 4$ than Ti and PEEK. These results would be in line with those that validated less plaque accumulation on PEEK abutments compared to Ti abutments. Koutouzis et al³¹ showed a statistically significant difference regarding plaque accumulation between PEEK and Ti abutment groups (20.5% vs 40.9%) in a prospective randomized controlled clinical trial during 3 months of follow-up. Accordingly, ZrO₂ implants also presented lower bacterial

attachment compared with Ti implants,³² which may lead to less inflammation and ultimately to a lower prevalence of mucositis and peri-implantitis. Less epithelial cell attachment to Ti and PEEK surfaces may be related to a lower surface roughness of Ti and low wettability of PEEK,³³ respectively, combining the potential advantage of lower biofilm formation with a lower capacity for a better soft tissue seal. The present results suggest that higher wettability (lower WCA) associated with Ti favors keratinocyte colonization of the implant surface and that higher roughness, as is the case of the ZrO₂ surfaces that were tested, support formation of hemidesmosomes.

In summary, this study revealed that ZrO₂ enhanced hemidesmosome formation by keratinocytes compared to Ti and PEEK. In addition, the keratinocytes' metabolic activity was lower on ZrO₂ and PEEK than on Ti, which might have been driven by their higher hydrophobicity and is in line with studies reporting less plaque accumulation. Both findings supported that, among the materials tested, ZrO₂ has the most favorable properties for potential enhancement of peri-implant soft tissue attachment and thus, soft tissue implant seal to hinder bacterial access and biofilm accumulation at the implant surfaces, a critical challenge for preventing future peri-implantitis.

Further investigations will be imperative to better clarify the relationship between the lower epithelial cell proliferation/metabolic activity, the lower surface roughness and related wettability combined to lower plaque accumulation, and the soft tissue seal quality and the influence on development or prevention of peri-implantitis. One possible mechanism is surface characteristics affecting extracellular matrix glycoprotein laminin 332, which is critical to hemidesmosome formation. Future work is necessary to elucidate this. Other work should define methods to upregulate both keratinocyte proliferation and hemidesmosome formation simultaneously.

However, the results of the present investigation should be considered with caution; thereby, some limitations should be stated, in view of cell behavior variability in comparison to live and multicellular hard and soft tissues. Additionally, other relevant capacities of the keratinocytes, mainly the hemidesmosome formation in relation to the capacity to create new connections with the foreign materials and to achieve a new seal, have not been evaluated here. Other work could include comparison of different oral keratinocyte sources. Furthermore, all the study materials employed in this investigation were relatively smooth surfaces, which might vary the cell interaction in comparison with microrough and other modified surfaces that are popular for osseointegration.

CONCLUSIONS

Expression of hemidesmosomal integrins $\alpha 6$ and $\beta 4$ was higher in ZrO_2 than in PEEK and Ti. Thus, ZrO_2 surfaces for implants and transepithelial abutment materials might enhance hemidesmosome formation by keratinocytes. Selecting suitable materials for optimizing soft tissue attachment may affect implant success.

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REFERENCES

- Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater* 2007;23:844–854.
- Solar RJ, Pollack SR, Korostoff E. In vitro corrosion testing of titanium surgical implant alloys: An approach to understanding titanium release from implants. *J Biomed Mater Res* 1979;13:217–250.
- Wilson TG Jr, Valderrama P, Burbano M, et al. Foreign bodies associated with peri-implantitis human biopsies. *J Periodontol* 2015;86:9–15.
- Weingart D, Steinemann S, Schilli W, et al. Titanium deposition in regional lymph nodes after insertion of titanium screw implants in maxillofacial region. *Int J Oral Maxillofac Surgery* 1994;23:450–452.
- Sicilia A, Cuesta S, Coma G, et al. Titanium allergy in dental implant patients: A clinical study on 1500 consecutive patients. *Clin Oral Implants Res* 2008;19:823–835.
- Kohal RJ, Weng D, Bächle M, Strub JR. Loaded custom-made zirconia and titanium implants show similar osseointegration: An animal experiment. *J Periodontol* 2004;75:1262–1268.
- Teté S, Mastrangelo F, Bianchi A, Zizzari V, Scarano A. Collagen fiber orientation around machined titanium and zirconia dental implant necks: An animal study. *Int J Oral Maxillofac Implants* 2009;24:52–58.
- Manzano G, Herrero LR, Montero J. Comparison of clinical performance of zirconia implants and titanium implants in animal models: A systematic review. *Int J Oral Maxillofac Implants* 2014;29:311–320.
- van Brakel R, Noordmans HJ, Frenken J, de Rooze R, de Wit GC, Cune MS. The effect of zirconia and titanium implant abutments on light reflection of the supporting soft tissues. *Clin Oral Implants Res* 2011;22:1172–1178.
- Andreietelli M, Wenz HJ, Kohal RJ. Are ceramic implants a viable alternative to titanium implants? A systematic literature review. *Clin Oral Implants Res* 2009;20(suppl 4):32–47.
- Depprich R, Naujoks C, Ommerborn M, Schwarz F, Kübler NR, Hand-schel J. Current findings regarding zirconia implants. *Clin Implant Dent Relat Res* 2014;16:124–137.
- Elnayef B, Lázaro A, Suárez-López del Amo F, et al. Zirconia implants as an alternative to titanium: A systematic review and meta-analysis. *Int J Oral Maxillofac Implants* 2017;32:e125–e134.
- Stratton-Powell AA, Pasko KM, Brockett CL, Tipper JL. The biologic response to polyetheretherketone (PEEK) wear particles in total joint replacement: A systematic review. *Clin Orthop Relat Res* 2016;474:2394–2404.
- Schwitalla AD, Abou-Emara M, Zimmermann T, et al. The applicability of PEEK-based abutment screws. *J Mech Behav Biomed Mater* 2016;63:244–251.
- Schwitalla AD, Spintig T, Kallage I, Müller WD. Pressure behavior of different PEEK materials for dental implants. *J Mech Behav Biomed Mater* 2016;54:295–304.
- Schwitalla A, Müller WD. PEEK dental implants: A review of the literature. *J Oral Implantol* 2013;39:743–749.
- Najeeb S, Zafar MS, Khurshid Z, Siddiqui F. Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics. *J Prosthodont Res* 2016;60:12–19.
- Pöllänen MT, Salonen JI, Uitto VJ. Structure and function of the tooth-epithelial interface in health and disease. *Periodontol* 2000 2003;31:12–31.
- Walko G, Castañón MJ, Wiche G. Molecular architecture and function of the hemidesmosome. *Cell Tissue Res* 2015;360: 363–378.
- Pendegrass CJ, Lancashire HT, Fontaine C, Chan G, Hosseini P, Blunn GW. Intraosseous transcutaneous amputation prostheses versus dental implants: A comparison between keratinocyte and gingival epithelial cell adhesion in vitro. *Eur Cell Mater* 2015;29:237–249.
- Kimura Y, Matsuzaka K, Yoshinari M, Inoue T. Initial attachment of human oral keratinocytes cultured on zirconia or titanium. *Dent Mater J* 2012;31:346–353.
- Atsuta I, Ayukawa Y, Furuhashi A, et al. Epithelial sealing effectiveness against titanium or zirconia implants surface. *J Biomed Mater Res A* 2019;107:1379–1385.
- Corvino E, Pesce P, Mura R, Marcano E, Canullo L. Influence of modified titanium abutment surface on peri-implant soft tissue behavior: A systematic review of in vitro studies. *Int J Oral Maxillofac Implants* 2020;35:503–519.
- Canullo L, Annunziata M, Pesce P, Tommasato G, Nastri L, Guida L. Influence of abutment material and modifications on peri-implant soft-tissue attachment: A systematic review and meta-analysis of histological animal studies. *J Prosthet Dent* 2021;125:426–436.
- Dickson MA, Hahn WC, Ito Y, et al. Human keratinocytes that express hTERT and also bypass a p16(INK4a)-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol Cell Biol* 2000;20:1436–1447.
- Schwitalla AD, Zimmermann T, Spintig T, Kallage I, Müller WD. Fatigue limits of different PEEK materials for dental implants. *J Mech Behav Biomed Mater* 2017;69:163–168.
- Xie Y, Li H, Yuan J, Fu L, Yang J, Zhang P. A prospective randomized comparison of PEEK cage containing calcium sulphate or demineralized bone matrix with autograft in anterior cervical interbody fusion. *Int Orthop* 2015;39:1129–1136.
- van Brakel R, Meijer GJ, Verhoeven JW, Jansen J, de Putter C, Cune MS. Soft tissue response to zirconia and titanium implant abutments: An in vivo within-subject comparison. *J Clin Periodontol* 2012;39:995–1001.
- Kordbacheh Changi K, Finkelstein J, Papapanou PN. Peri-implantitis prevalence, incidence rate, and risk factors: A study of electronic health records at a U.S. dental school. *Clin Oral Implants Res* 2019;30:306–314.
- Abdallah MN, Badran Z, Ciobanu O, Hamdan N, Tamimi F. Strategies for optimizing the soft tissue seal around osseointegrated implants. *Adv Healthc Mater* 2017. Epub ahead of print Sept 27.
- Koutouzis T, Richardson J, Lundgren T. Comparative soft and hard tissue responses to titanium and polymer healing abutments. *J Oral Implantol* 2011;37(spec no):174–182.
- Rimondini L, Cerroni L, Carrassi A, Torricelli P. Bacterial colonization of zirconia ceramic surfaces: An in vitro and in vivo study. *Int J Oral Maxillofac Implants* 2002;17:793–798.
- Hahnel S, Wieser A, Lang R, Rosentritt M. Biofilm formation on the surface of modern implant abutment materials. *Clin Oral Implants Res* 2015;26:1297–1301.

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