©Operative Dentistry, 2019, **44-6,** E271-E278

# *Streptococcus mutans* Biofilm Formation and Cell Viability on Polymer-infiltrated Ceramic and Yttria-stabilized Polycrystalline Zirconium Dioxide Ceramic

MA Bottino • SMB Pereira • M Amaral • NVM Milhan
CA Pereira • SEA Camargo • ABG Carvalho • RM Melo

### Clinical Relevance

Y-TZP ceramics are bioinert materials and present good biocompatibility to oral tissues. A polymer-infiltrated ceramic, which is a hybrid material, presents the same biological characteristics as Y-TZP ceramics.

## SUMMARY

**Objective: The aim of this study was to investigate the biofilm formation and cell viability of a polymer-infiltrated ceramic (PIC) and an yttria-stabilized polycrystalline zirconium dioxide ceramic (Y-TZP). The null hypothesis was that there would be no difference in biofilm formation and cell viability between the materials.**

**Methods and Materials: *Streptococcus mutans* biofilm was analyzed with scanning electron microscopy (SEM), confocal laser scanning microscopy, and colony counting (colony-forming units/mL). The cell viability (fibroblasts) of**

Marco Antonio Bottino, PhD, Intitute of Science and Technology, Univ Estadual Paulista UNESP, Dental Materials and Prosthodontics, São José dos Campos, São Paulo, Brazil

Sarina MB Pereira, PhD, Institute of Science and Technology, Univ. Estadual Paulista UNESP, Department of Restorative Dentistry, São José dos Campos, São Paulo, Brazil

Marina Amaral, PhD, University of Taubaté, Dentistry, Taubaté, São Paulo, Brazil

Noala V Milhan, PhD, Institute of Science and Technology, Univ. Estadual Paulista UNESP, São Jose dos Campos, São Paulo, Brazil

Cristiane Aparecida Pereira, PhD, Univ. Estadual Paulista, Department of Biosciences and Oral Diagnosis, São José dos Campos, São Paulo, Brazil

Samira EA Camargo, PhD, Department of Restorative Dental Sciences, Univ. of Florida, College of Dentistry, Gainesville, Florida, USA.

Ana Beatriz G Carvalho, Master student, Intitute of Science and Technology, Univ Estadual Paulista UNESP, Dental Materials and Prosthodontics, São José dos Campos, São Paulo, Brazil

*Renata M Melo, PhD, Intitute of Science and Technology, Univ Estadual Paulista UNESP, Dental Materials and Prosthodontics, São Jose dos Campos, São Paulo, Brazil

*Corresponding author: Av. Engenheiro Francisco José Longo, 777, São Jose dos Campos, São Paulo, 12245000, Brazil; e-mail: renata.marinho@unesp.br

DOI: https://doi.org/10.2341/18-278-L

**both materials was measured with 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium) (MTT) test. Roughness measurements were also performed.**

**Results: The PIC displayed higher roughness but showed similar colony-forming units and biovolume values to those of Y-TZP. SEM showed a higher amount of adhered fibroblasts on the PIC surface on the first day and similar amounts on both materials after seven days. Moreover, the materials were biocompatible with human fibroblasts.**

**Conclusion: PIC and Y-TZP are biocompatible and present the same characteristics for biofilm formation; therefore, they are indicated for indirect restorations and implant abutments.**

## INTRODUCTION

Ideally, lost dental structure should be replaced with materials with properties as close as possible to the natural tissues.[1] Currently, dental ceramics and composites are the materials most employed for restorations in the oral environment.[2,3] Therefore, the microstructure and the surface and mechanical properties, in addition to the interactions of such materials with the oral environment, as well as the capacity of retaining biofilm should all be well known before these materials are used in clinical practice.

A hybrid composite (Vita Enamic, Vita Zahnfabrik, Bad Säckingen, Germany) with about 14% of polymer distributed in the ceramic matrix has recently entered the dental restoration market. According to the manufacturer, this material combines strength with elasticity, allowing its use as implant abutments, where only zirconia was previously indicated. On the other hand, the well-known zirconium oxide–based ceramic (yttria-stabilized polycrystalline zirconium dioxide ceramic [Y-TZP]) is a polymorphous crystal stabilized by oxides (usually yttrium oxide) in the tetragonal phase at room temperature, with high strength and elastic modulus.[4] Both zirconia and hybrid material present optimal esthetic and mechanical properties for dental restorations.[1,2,5,6] However, little is known about the polymer-infiltrated material with regard to bacterial adhesion (ie, biofilm formation) and cell compatibility.

Biofilm formation may lead to several negative biological responses in the oral environment,[7] such as peri-implantitis,[8] injury to gingival tissues when colonization is located at the interface of a restoration at the gingival margin,[9] and secondary caries and pulp pathologies when it invades the restoration-tooth interface.[10,11] Biofilm formation on the surface of restorative materials may be evaluated semiquantitatively by counting colony-forming units (CFU)[12,13] and confocal laser scanning microscopes (CLSM),[14,15] as well as qualitatively by scanning electron microscopes (SEMs).[16]

Overall, ceramics are noncytotoxic, but several of them decrease cell proliferation after aging.[17] Therefore, the dual composition of newly engineered materials such as polymer-infiltrated ceramic (PIC) warrants investigations about its effects on cell metabolism.

An *in vitro* cell viability evaluation may be performed by SEM or enzymatic assay. These assays can measure the metabolic activity of the cellular growth in contact with the materials' surface.[18] The 3-(4,5-dimethylthiazol-2-yl) diphenyltetrazolium bromide (MTT) test is based on the activity of enzymes found in viable cells, such as succinyl dehydrogenase, indicating both the number of viable cells in a sample and the level of metabolic activity.[19,20]

Thus, the aim of this study was to evaluate the *in vitro Streptococcus mutans* adhesion and fibroblast viability on the surfaces of two ceramic materials, zirconia and PIC, indicated for dental restorations and implant components. The null hypothesis was there would be no difference regarding biofilm formation and cell viability between the materials.

## METHODS AND MATERIALS

### Sample Manufacturing

Presintered blocks of Y-TZP (Vita In Ceram YZ, VITA Zhanfabrick, Bad Säckingen, Germany) and PIC (VITA Enamic, VITA Zahnfabrik) were sectioned into rectangular pieces (4×4×3 mm) with diamond discs (Extec Corp, Enfield, CT, USA) in a precision saw machine (IsoMet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA), under coolant irrigation. The samples (n=32) were polished with SiC paper of decreasing grit size (#400 through #1200) and cleaned in an ultrasonic bath. After polishing with SiC 1200-grit paper, the Y-TZP was sintered in a Zyrcomat furnace (VITA Zahnfabrik). The final dimensions of the blocks (3×3×2 mm) were checked with a digital caliper.

### Surface Roughness Analysis

Quantitative analysis of surface roughness was performed in a contact profilometer (Mitutoyo SJ 400, Tokyo, Japan). Three measurements were performed with a distance of 1 mm between them

and a measuring length of 3 mm (n=10). The mean roughness Ra (μm; profile roughness parameter) was then recorded.

### Biofilm Adhesion

Ten samples from each material were used to count the CFUs (CFU/mL). Biofilm adhesion was achieved using a modified version of the technique proposed by Anami and others.[1] Standard suspension of *S mutans* (ATCC 35688) containing $10^6$ cells/mL was prepared: the bacteria were plated in a brain-heart infusion agar (Difco, Detroit, MI, USA) and incubated for 24 hours at 37°C in a $CO_2$ chamber. After incubation, the growth was suspended in a sterile physiological solution (0.9% sodium chloride [NaCl]), and the number of suspended cells was counted using a spectrophotometer (B582, Micronal, Sao Paulo, Brazil). The optical density and wavelength parameters used were 0.620 and 398 nm, respectively. These parameters were previously established by means of a standard curve of CFU/mL vs absorbance. Adherence testing was performed in an aseptic environment using a laminar flow chamber. Each specimen was put inside a well of a sterile 24-well polystyrene tissue-culture plate, with 2.0 mL of broth (20 g trypticase, 2 g NaCl, 3 g $K_2HPO_4$, 2 g $KH_2PO_4$, 1 g $K_2CO_3$, 120 mg $MgSO_4$, 15 mg $MnSO_4$, and 50 g $C_6H_8O_7$ dissolved in 1000 mL of distilled water) and 0.1 mL of standardized *S. mutans* suspension. Plates were then sealed and incubated at 37°C for 48 hours in a $CO_2$ chamber.

### Analysis of the Biofilm Formation Using SEM

Two samples from each material were analyzed for biofilm formation. Samples were fixed for one hour in a 2.5% glutaraldehyde solution and dehydrated in ethanol baths (10%, 25%, 50%, 75%, and 90% for 20 minutes and 100% for one hour). Samples were then fixed in a metallic base with carbon adhesive tape (SPI Supplies, West Chester, PA, USA), sputter coated with a gold-palladium alloy (Polaron SC 7620 Sputter Coater, Quorum Technologies, Newhaven, UK; 130 seconds, 10-15 mA, 130 mTorr vacuum, 3.5 nm/min metallization rate, and 80Å Pd-Au layer [approximate]), and observed using SEM (20 kV, Inspect S50, FEI Company, Brno, Czech Republic). We then performed a descriptive analysis of the biofilm formed on the samples.

### Analysis of Biofilm Biovolume: CLSM

Five samples from each material were analyzed using CLSM (LSM 510-META, Zeiss, Pleasanton, CA, USA) to assess the biovolume ($\mu^3/mm^2$) of the formed biofilm. Samples were removed from incubation and positioned on glass laminate and stained with the Live/Dead Bac Light Bacterial Viability and Counting Kit (Molecular Probes, Eugene, OR, USA). The kit is composed of two fluorescent staining solutions: SYTO 9 in green color, which stains viable cells (penetrates into cells with intact membranes), and red isopropidium iodide, which stains dead cells (penetrates into cells with injured membranes). The number of optical sections varied according to the biofilm's thickness. COMSTAT software (Technical University of Denmark, Lyngby, Denmark) was used for biovolume analysis.

### Cell Viability Evaluation (MTT Test)

Gingival human fibroblasts (FMM-1) were cultured on samples positioned in 24-well polystyrene tissue culture plates. A total of 20,000 fibroblasts were cultured on each sample and maintained in Dulbecco's Modified Eagle Medium (Cultilab, Curitiba, Brazil), supplemented with 10% bovine fetal serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humid atmosphere with 5% $CO_2$ for one, three, and seven days. Next, cellular survival was determined by measuring the succinic dehydrogenase activity that indicates mitochondrial function and may be observed by MTT assay (Sigma-Aldrich, St Louis, MO, USA). The activity was quantified by dissolving MTT in 0.1 N NaOH (6.25 v/v%) in dimethyl sulfoxide (Sigma-Aldrich). Optical density readings for the solution were measured in a spectrophotometer (Bio-Tek, Winooski, VT, USA) at 570 nm. The control group was represented by cells without contact with any of the materials. Spectrophotometric data were expressed in percentages of the control group, which was considered as 100%.

### Data Analysis

Results for roughness (μm), CFUs, and biovolume were assessed using Student *t*-test ($p<0.05$). Cell viability data were assessed using the Z-test, followed by the Tukey test for mean contrast, in which the materials were compared with the control groups (100%), and analysis of variance (ANOVA) for comparisons between the two materials. Images obtained from SEM and CLSM were qualitatively evaluated.

## RESULTS

### Roughness, CFU, and Biovolume

The mean values and standard deviations for the roughness, CFU ($log_{10}$), and biovolume of each material are listed in Table 1. Statistical analysis

Table 1: *Mean Values and Standard Deviations of Roughness, CFU ($log_{10}$), and Biovolume for PIC and Y-TZP*

| | Y-TZP | PIC | *p*-Value* |
|---|---|---|---|
| Roughness (Ra), μm | 0.057 ± 0.012 | 0.132 ± 0.016 | <0.0001* |
| Biofilm adhesion, CFU/mL | 8.311 ± 0.237 | 8.630 ± 0.564 | 0.1342 |
| Biovolume, $\mu m^3/\mu m^2$ | 0.0049 ± 0.007 | 0.0082 ± 0.009 | 0.4569 |

** Indicates a statistically significant difference.*

showed that the PIC presented significantly higher Ra values than the Y-TZP.

### Qualitative Analysis in SEM and CLSM

The analysis of representative SEM images showed the surface pattern of PIC was rougher than that of Y-TZP (Figure 1), as shown in the Ra values in Table 1. However, both materials showed similar characteristics for *S mutans* adhesion after 48 hours of incubation. The CLSM representative images also demonstrated a similar pattern between the materials with regard to cell viability: viable cells (green) and nonviable cells (red; Figure 2).

An increase in cellular adhesion was observed (Figure 3) depending on the evaluation time, where a larger amount of adhered cells was observed on the PIC surface than on the Y-TZP surface on the first day. However, a similar pattern was observed after 7 days.

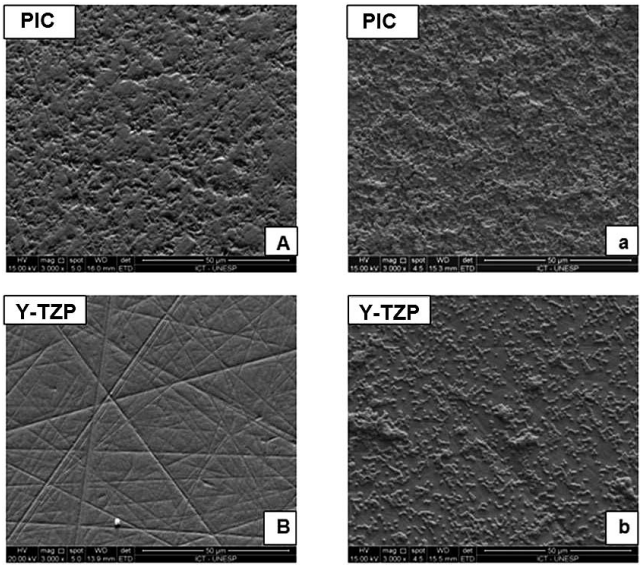


Figure 1. *SEM images of the topography of PIC and Y-TZP (A and B) and the adhesion of* S mutans *on the materials' surfaces (a and b).*

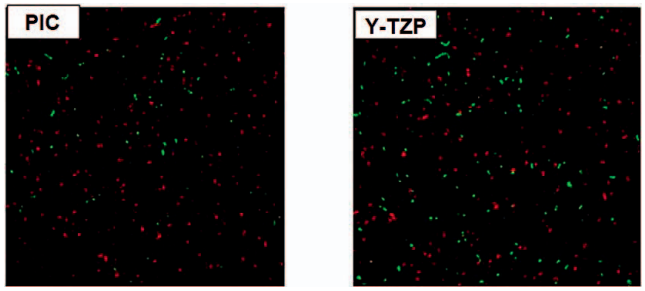


Figure 2. *Confocal laser scanning images used for biovolume and thickness determination of* S mutans *on the surface of the ceramics.*

### Cell Viability

The MTT data indicated the materials cannot be considered cytotoxic since the absorbance percentage, which is related to the amount of viable cells after contact with the two materials, was always higher than 50% of the mean found for the control group (Table 2). A comparison of each material to the control group (Z test) indicated that only Y-TZP was statistically different from the control, meaning that the number of viable cells was significantly lower than the number in the control group but yet noncytotoxic. Tukey post hoc test showed that Y-TZP was different from the control group after one day and seven days (Table 3).

When Y-TZP and PIC were compared, ANOVA indicated that these materials were statistically similar ($p$=0.54). The mean percentages of absorbance after contact with both materials at the evaluation times (one, three, and seven days) are shown in Figure 4.

## DISCUSSION

This study evaluated the biological response of two ceramics indicated for indirect dental restorations. The materials presented similar behavior with respect to biofilm adhesion and cellular viability, thus confirming the anticipated hypothesis.

Restorative materials are subjected to biofilm adhesion when placed in the oral environment. The

Table 2: *Mean (%), Standard Deviation, and* p*-Value of Data Obtained for Cellular Viability When Comparing PIC and Y-TZP With the Control Group (100% Absorbance)*

| Cytotoxicity | PIC | Y-TZP |
|---|---|---|
| Mean | 95.06 | 90.53 |
| Standard deviation | 20.30 | 16.00 |
| *p*-value | 0.39 | 0.04* |

** Indicates a statistically significant difference.*

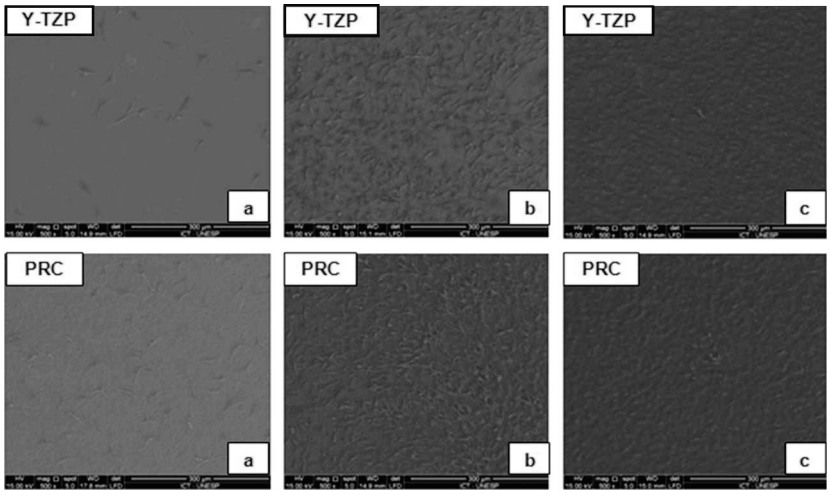


Figure 3. *Representative images of adhered cells on Y-TZP and PIC after one (a), three (b), and seven (c) days. The shape of the cells (narrow and with several extensions) was unchanged until day 3 but could no longer be distinguished on day 7 because of the high number of cells.*

amount of biofilm varies according to the nature of the material[13] and properties such as surface energy and roughness.[21,22] Rough surfaces are more prone to biofilm accumulation than smooth surfaces because the former provide niches where bacteria adhere and grow.[23,24] In general, ceramics are reported to present less bacterial adhesion than other restorative materials.[25] The roughness parameter (Ra) value in this study was significantly higher for the PIC than for the Y-TZP (Table 1). However, the CFU and biovolume values were similar. Thus, according to these quantitative parameters, the roughness did not influence biofilm accumulation, a fact also seen in previous studies.[24,26] On the other hand, rougher ceramic surfaces are more prone to cell adhesion and proliferation.[27,28] Therefore, PIC presented a more favorable surface for cell attachment than zirconia.

Obtaining images via SEM required fixation and dehydration of the biofilm. This procedure can alter the biofilm characteristics but is a well-accepted method for bacteria identification and adherence.[16,21] Using CLSM makes it possible to obtain quantitative data about the formation of biofilm, mean thickness,[29,30] and biovolume (Table 1). This method is considered noninvasive and nondestructive and represents the main tool for evaluation of *in situ* biofilm.[31] The thickness and biovolume parameters, respectively, morphological characteristics of the biofilm and the extracellular material not covalently attached to the cell membrane, were equally expressed in PIC and Y-TZP. Therefore, neither materials affected the structure and capacity of cells to produce the extracellular matrix.

The interpretation of SEM images was difficult, as the topography of PIC was rougher than that of Y-TZP, with the main differences being between microorganism adherence in these materials (Figures 1 and 2). Qualitative CLSM images were important to confirm the SEM findings and showed similarity between the materials in both the amount and the spread of bacteria (Figure 2).

The analysis of the cytotoxic potential of both materials revealed that neither PIC nor Y-TZP were

Table 3: *Mean (%), Standard Deviation, and* p*-Value of Data for Y-TZP of the Cellular Viability Test*

| Cytotoxicity | 1 Day | 3 Days | 7 Days |
|---|---|---|---|
| Mean | 85.77 | 109.50 | 76.31 |
| Standard deviation | 8.05 | 6.82 | 6.90 |
| *p*-value | 0.03* | 0.06 | 0.006* |

** Indicates a statistically significant difference.*

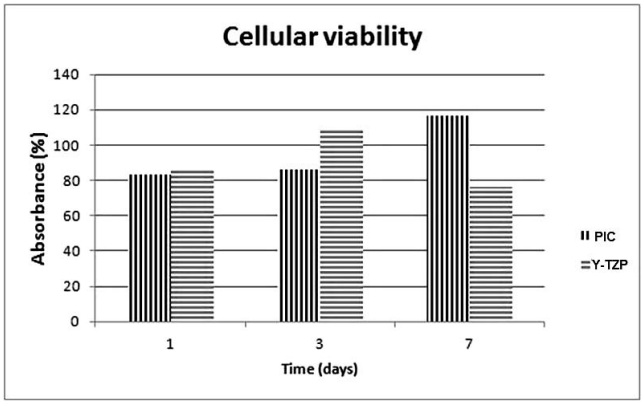


Figure 4. *Representative graph of the mean percentage of absorbance obtained by the MTT test after contact of cells with PIC and Y-TZP at days 1, 3, and 7.*

detrimental to fibroblasts, since both presented cellular viability higher than 50% of the control group (ie, cells not submitted to any material). The number of viable FMM-1 was significantly lower for the Y-TZP group than for the control group (Table 2) at days 1 and 7 (Table 3), but the material was not considered cytotoxic for the cells (90% of cellular viability). Figure 3 indicates that the cellular adhesion was apparently higher on the first day for the PIC material. This was probably a result of time-dependent factors that occur after implantation of the first fibroblasts. One important event is protein adsorption occurring before cell adhesion, as cells adhere and spread quickly on the first days, while the response of upcoming cell layers will be controlled by the protein film.[32] SEM analysis showed homogeneity in cell spreading and intimate contact with the materials after the seven-day analysis, indicating the materials were biocompatible and allowed a high proliferation rate per day.[28]

In long-term clinical studies, zirconia infrastructures were gentle to the periodontium and presented an overall good biological response.[33,34] Our findings suggest that the attachment of fibroblasts to Y-TZP and PIC *in vivo* occurs normally, and the materials themselves should not cause inflammation and bone loss around the peri-implant.[35] These results contradict those of Grenade and others,[36] who differentiated two groups of materials in terms of fibroblasts adhesion, including a Ti-Zi group (more biocompatible) and an eM-PICN group (less biocompatible), with the latter being represented by a PIC. However, the authors call attention to the fact that the hybrid material is not the same commercial brand used herein, and this could partly explain the differences in the results.

Furthermore, PIC contains methacrylate (urethane dimethacrylate and triethylene glycol dimethacrylate) in its composition, which has a significant cytotoxic effect in its uncured form.[37] Although the resin portion of PIC seems highly polymerized, examining the degree of polymer conversion and the effects of monomer elution on cell viability is a must for future studies. Moreover, the biofilm adhesion scenario is more complex *in situ*,[38,39] and additional clinical studies are warranted.

## CONCLUSION

Both zirconia and PIC, which are indicated for indirect dental restorations and implant components, were noncytotoxic and presented similar capacity for biofilm adhesion.

### Acknowledgement

This study was supported by São Paulo State Research Foundation (FAPESP), under grant 2014/19357-9.

### Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institute of Science and Technology at São Jose dos Campos, UNESP.

### Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

*(Accepted 19 March 2019)*

### REFERENCES

1. Anami LC, Pereira CA, Guerra E, Assunção e Souza RO, Jorge AO, & Bottino MA (2012) Morphology and bacterial colonisation of tooth/ceramic restoration interface after different cement excess removal techniques *Journal of Dentistry* **40(9)** 742-749. doi:10.1016/j.jdent.2012.05.005
2. Auschill TM, Arweiler NB, Netuschil L, Brecx M, Reich E, Sculean A, & Artweiler NB (2001) Spatial distribution of vital and dead microorganisms in dental biofilms *Archives of Oral Biology* **46(5)** 471-476.
3. Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, & Ozkan S (2010) Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion *Journal of Prosthetic Dentistry* **103(4)** 221-227. doi:10.1016/S0022-3913(10)60034-0
4. Azevedo SM, Kantorski KZ, Valandro LF, Bottino MA, & Pavanelli CA (2012) Effect of brushing with conventional versus whitening dentifrices on surface roughness and biofilm formation of dental ceramics *General Dentistry* **60(3)** e123-e130.
5. Bin CV, Valera MC, Camargo SE, Rabelo SB, Silva GO, Balducci I, & Camargo CH (2012) Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate *Journal of Endodontics* **38(4)** 495-500. doi:10.1016/j.joen. 2011.11.003
6. Bottino MA, Campos F, Ramos NC, Rippe MP, Valandro LF, & Melo RM (2015) Inlays made from a hybrid material: adaptation and bond strengths *Operative Dentistry* **40(3)** E83-E91. doi:10.2341/13-343-L
7. Bouillaguet S, Wataha JC, Lockwood PE, Galgano C, Golay A, & Krejci I (2004) Cytotoxicity and sealing properties of four classes of endodontic sealers evaluated by succinic dehydrogenase activity and confocal laser scanning microscopy *European Journal of Oral Science* **112(2)** 182-187. doi:10.1111/j.1600-0722.2004.00115.x
8. Bremer F, Grade S, Kohorst P, & Stiesch M (2011) *In vivo* biofilm formation on different dental ceramics *Quintessence International* **42(7)** 565-574.
9. Brentel AS, Kantorski KZ, Valandro LF, Fúcio SB, Puppin-Rontani RM, & Bottino MA (2011) Confocal laser

microscopic analysis of biofilm on newer feldspar ceramic *Operative Dentistry* **36(1)** 43-51. doi:10.2341/10-093-LR

10. Busscher HJ, Rinastiti M, Siswomihardjo W, & van der Mei HC (2010) Biofilm formation on dental restorative and implant materials *Journal of Dental Research* **89(7)** 657-665. doi:10.1177/0022034510368644
11. Camilleri J & Pitt Ford TR (2006) Mineral trioxide aggregate: a review of the constituents and biological properties of the material *International Endodontic Journal* **39(10)** 747-754. doi:10.1111/j.1365-2591.2006.01135.x
12. Cenci MS, Lund RG, Pereira CL, de Carvalho RM, & Demarco FF (2006) *In vivo* and *in vitro* evaluation of Class II composite resin restorations with different matrix systems *Journal of Adhesive Dentistry* **8(2)** 127-132.
13. Chen X, Li Y, & Aparicio C (2013) *Thin Film and Coatings in Biology* Springer, Montreal.
14. Collins CJ, Bryant RW, & Hodge KL (1998) A clinical evaluation of posterior composite resin restorations: 8-year findings *Journal of Dentistry* **26(4)** 311-317.
15. Della Bona A, Corazza PH, & Zhang Y (2014) Characterization of a polymer-infiltrated ceramic-network material *Dental Materials* **30(5)** 564-569. doi:10.1016/j.dental.2014.02.019
16. Coldea A, Swain MV, & Thiel N (2013) Mechanical properties of polymer-infiltrated-ceramic-network materials *Dental Materials* **29(4)** 419-426. doi:10.1016/j.dental.2013.01.002
17. Denry I & Kelly JR (2008) State of the art of zirconia for dental applications *Dental Materials* **24(3)** 299-307. doi:10.1016/j.dental.2007.05.007
18. Eick S, Glockmann E, Brandl B, & Pfister W (2004) Adherence of *Streptococcus mutans* to various restorative materials in a continuous flow system *Journal of Oral Rehabilitation* **31(3)** 278-285. doi:10.1046/j.0305-182X.2003.01233.x
19. Falconi M, Teti G, Zago M, Pelotti S, Breschi L, & Mazzotti G (2007) Effects of HEMA on type I collagen protein in human gingival fibroblasts *Cell Biology and Toxicology* **23(5)** 313-322. doi:10.1007/s10565-006-0148-3
20. Grössner-Schreiber B, Teichmann J, Hannig M, Dörfer C, Wenderoth DF, & Ott SJ (2009) Modified implant surfaces show different biofilm compositions under *in vivo* conditions *Clinical Oral Implants Research* **20(8)** 817-826. doi:10.1111/j.1600-0501.2009.01729.x
21. Hobbert TA (2013) *Adsorbed Proteins on Biomaterials* Elsevier, Philadelphia PA.
22. Kantorski KZ, Scotti R, Valandro LF, Bottino MA, Koga-Ito CY, & Jorge AO (2008) Adherence of *Streptococcus mutans* to uncoated and saliva-coated glass-ceramics and composites *General Dentistry* **56(7)** 740-747.
23. Kawai K, Urano M, & Ebisu S (2000) Effect of surface roughness of porcelain on adhesion of bacteria and their synthesizing glucans *Journal of Prosthetic Dentistry* **83(6)** 664-667.
24. Kilic K, Kesim B, Sumer Z, Polat Z, & Kesim S (2013) *In vitro* cytotoxicity of all-ceramic sub structural materials after aging *Journal of Dental Science* **8(3)** 231-238.
25. Kim DJ, Lee MH, Lee DY, & Han JS (2000) Mechanical properties, phase stability, and biocompatibility of (Y, Nb)-TZP/Al(2)O(3) composite abutments for dental implant *Journal of Biomedical Materials Research* **53(4)** 438-443.
26. Lourenço BN, Marchioli G, Song W, Reis RL, van Blitterswijk CA, Karperien M, van Apeldoorn A, & Mano JF (2012) Wettability influences cell behavior on superhydrophobic surfaces with different topographies biointerphases **7(1-4)** 46. doi:10.1007/s13758-012-0046-6
27. Palmer RJ & Sternberg C (1999) Modern microscopy in biofilm research: confocal microscopy and other approaches *Current Opinion in Biotechnology* **10(3)** 263-268. doi:10.1016/S0958-1669(99)80046-9
28. Pashley DH (1990) Clinical considerations of microleakage *Journal of Endodontics* **16(2)** 70-77. doi:10.1016/S0099-2399(06)81567-0
29. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL, & van Steenberghe D (1989) The influence of surface free-energy on planimetric plaque growth in man *Journal of Dental Research* **68(5)** 796-799.
30. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, & van Steenberghe D (1990) The influence of surface free energy and surface roughness on early plaque formation: an *in vivo* study in man *Journal of Clinical Periodontology* **17(3)** 138-144.
31. Rimondini L, Cerroni L, Carrassi A, & Torricelli P (2002) Bacterial colonization of zirconia ceramic surfaces: an *in vitro* and *in vivo* study *International Journal of Oral and Maxillofacial Implants* **17(6)** 793-798.
32. Rimondini L, Farè S, Brambilla E, Felloni A, Consonni C, Brossa F, & Carrassi A (1997) The effect of surface roughness on early *in vivo* plaque colonization on titanium *Journal of Periodontology* **68(6)** 556-562. doi:10.1902/jop.1997.68.6.556
33. Scotti R, Kantorski KZ, Monaco C, Valandro LF, Ciocca L, & Bottino MA (2007) SEM evaluation of *in situ* early bacterial colonization on a Y-TZP ceramic: a pilot study *International Journal of Prosthodontics* **20(4)** 419-422.
34. Sorrentino R, De Simone G, Tetè S, Russo S, & Zarone F (2012) Five-year prospective clinical study of posterior three-unit zirconia-based fixed dental prostheses *Clinical Oral Investigations* **16(3)** 977-985. doi:10.1007/s00784-011-0575-2
35. Staudt C, Horn H, Hempel DC, & Neu TR (2004) Volumetric measurements of bacterial cells and extracellular polymeric substance glycoconjugates in biofilms *Biotechnology and Bioengineering* **88(5)** 585-592. doi:10.1002/bit.20241
36. Grenade C, De Pauw-Gillet MC, Gailly P, Vanheusden A, & Mainjot A (2016) Biocompatibility of polymer-infiltrated-ceramic-network (PICN) materials with human gingival fibroblasts (HGFs) *Dental Materials* **32(9)** 1152-1164. doi:10.1016/j.dental.2016.06.020
37. Strietzel R & Lahl C (2009) Einfuhrung in die CAD/CAM-Systeme *Teil V Dental Labor* **10** 1400-1407.

38. Tetè S, Zizzari VL, Borelli B, De Colli M, Zara S, Sorrentino R, Scarano A, Gherlone E, Cataldi A, & Zarone F (2014) Proliferation and adhesion capability of human gingival fibroblasts onto zirconia, lithium disilicate and feldspathic veneering ceramic *in vitro Dental Materials Journal* **33(1)** 7-15.

39. Zembic A, Bösch A, Jung RE, Hämmerle CH, & Sailer I (2013) Five-year results of a randomized controlled clinical trial comparing zirconia and titanium abutments supporting single-implant crowns in canine and posterior regions *Clinical Oral Implants Research* **24(4)** 384-390. doi:10.1111/clr.12044