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Evaluation of biofilm formation on titanium and zirconia implant surfaces: An *in vitro* comparative study

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Abstract:
Bacterial biofilm formation on implant surfaces is a major problem because it contributes to peri-implant mucosal inflammation and subsequent peri-implant bone loss. Therefore, it is of interest to compare the early multispecies oral biofilm formation on polished titanium, sandblasted and acid-etched titanium, polished zirconia and roughened zirconia discs. A total of 120 sterile discs were exposed to salivary-pellicle mixed bacterial suspension and incubated for 24, 48 and 72 hours, followed by assessment of optical density, colony-forming units, confocal biofilm thickness and scanning electron microscopic surface coverage. At 72 hours, rough titanium showed the highest biofilm biomass, viable count and surface coverage, whereas polished zirconia showed the lowest values, with statistically significant differences among groups ($p<0.001$). Thus, polished zirconia showed reduced biofilm formation compared with titanium, suggesting its potential usefulness as a biologically favorable implant-abutment material in patients at risk of plaque accumulation.

Keywords: Biofilm; titanium implants; zirconia implants; peri-implantitis; surface roughness; bacterial adhesion; *in vitro* study

Background:
The dental implant has revolutionized oral rehabilitation by offering predictable functional and esthetic replacement of missing teeth. But, success is not only based on osseointegration, as the transmucosal and exposed parts of implant systems are still susceptible to microbial colonization. After bacterial adhesion, an acquired biofilm can develop into a complex polymicrobial structure that is resistant to salivary clearance, host defense and antimicrobial intervention. Peri-implant mucositis and peri-implantitis are now known to be frequent biological complications that start with the accumulation of biofilm and worsen when the microflora-host response relationship is disturbed **[1, 2]**. The implant surface is a living surface. It affects the behavior of bone cells, protein pellicle adsorption, early bacterial attachment and its chemistry, roughness, wettability and surface free energy. The material most commonly used for implants is titanium, which is biocompatible, has a stable oxide layer, is strong and has good clinical documentation. However, surfaces modified to promote osseointegration can also create micro-retentive areas for plaque to form after being exposed to the oral environment. A rough surface may provide protection from shear forces and help retain the extracellular polymeric matrix, especially when oral hygiene is poor or peri-implant soft-tissue breakdown occurs **[3, 4]**. Zirconia has become an alternative implant and abutment material due to its tooth-like color, corrosion resistance, low thermal conductivity, soft-tissue appearance and good biocompatibility. Zirconia has been the focus of interest from a microbiological point of view because some studies indicate that the surface of zirconia may have a lower propensity for early bacterial adhesion than the surface of titanium, when the same smoothness and salivary conditioning are present **[5]**. This difference is attributed not only to the material itself, but also to surface free energy, wettability, charge characteristics and pellicle composition **[6]**. The process of biofilm formation around implants is complicated. Early adhesion is often mediated by salivary proteins and early colonizers and later growth is by coaggregation, anaerobic expansion, production of matrices and metabolic cooperation among organisms. Multispecies laboratory models may then be helpful for comparing implant materials under controlled conditions, but cannot mimic all features of the oral cavity. Experimental studies have yielded conflicting results regarding the adhesion of bacteria on zirconia versus titanium, some reporting decreased bacterial adhesion on zirconia, others reporting similar mature biofilms and some reporting that surface roughness may be more important than the bulk material **[7, 8]**. This is a clinical problem because different surface types are frequently used in implant systems. Endosseous surfaces are intentionally roughened to promote osseointegration while collar, abutment and prosthetic surfaces are expected to reduce plaque retention. If the soft tissue around the implant has receded, exposing rough implant threads or collars, the bacteria can become more difficult to retain and to clean up professionally. Hence, the knowledge of the difference in the accumulation of biofilm on the surface of titanium and zirconia under controlled conditions can be useful in selecting

material and preventive maintenance procedures **[9, 10]**. Therefore, it is of interest to assess and compare the ability of titanium and zirconia implant surfaces to form biofilm with a standardized multispecies oral biofilm model, both with a polished and a roughened topography.

Materials and Methods:

Study design and setting:

It was an *in vitro* comparative study carried out in the department of periodontology, oral microbiology and dental materials science of a tertiary dental teaching institute. The experiment assessed the ability of biofilm to form on standardised titanium and zirconia implant-surface discs following exposure to a multispecies oral biofilm model. The study was a four-group comparison with repeated observations at 24, 48 and 72 hours.

Sample size:

Sample size was determined based on the difference in optical density of crystal violet between polished zirconia and rough titanium from pilot testing. With an effect size of 0.42, alpha error of 5%, power of 80% and experimental loss, 30 discs were assigned to each group. So, 120 discs were added. At each time point, 10 discs were tested from each group.

Specimen grouping:

The specimens were grouped into four categories: Group I, polished commercially pure titanium discs; Group II, sand blasted and acid etched titanium discs; Group III, polished yttria-stabilized tetragonal zirconia polycrystal discs; and Group IV, airborne-particle-abraded roughened zirconia discs. The thickness of all discs was 2 mm and the diameter was 8 mm. Samples that had visible defects, cracks, edge chipping, surface contamination, or dimensional irregularity was rejected.

Surface preparation and characterization:

Grade IV commercially pure titanium rods were used to prepare the titanium discs. Pre-sintered zirconia blanks were milled into zirconia discs and sintered following the manufacturer's recommendations. Polished groups were sequentially finished with silicon carbide papers and diamond polishing paste to achieve smooth surface. Rough titanium discs were sand blasted and acid etched with alumina particles. The rough zirconia discs were abraded in the air with alumina particles under the standard pressure and distance. All discs were ultrasonically cleaned in distilled water, 70% ethanol and phosphate-buffered saline and then sterilized by autoclaving or dry heat depending on the material. The surface roughness was measured using a contact profilometer and the static water contact angle was measured by the sessile drop method.

Biofilm inoculum:

Stimulated saliva was collected from five systemically healthy adult volunteers, who were not taking antibiotics or antimicrobial mouthrinses in the last three months. For two hours prior to sample collection, donors were requested to refrain from eating and oral hygiene measures. A mixed oral microbial inoculum was prepared by diluting saliva in sterile brain-heart infusion broth containing hemin, vitamin K and sucrose. For the purpose of simulating acquired pellicle formation, sterile discs were pre-conditioned in sterile filtered saliva for two hours prior to inoculation.

Biofilm growth protocol:

The discs were each put into a separate well of a sterile 24-well culture plate. Each well was then inoculated with a standardized bacterial suspension at a concentration of ~10^7 CFU/mL and incubated at 37°C under microaerophilic conditions. The medium was changed every 24 hours, without disturbing the adherent biofilm. Processed discs were gently rinsed with phosphate buffered saline (PBS) to remove loosely adherent cells at 24, 48 and 72 hours and then processed for analysis.

Assessment of biofilm biomass:

Total biofilm biomass was determined by the crystal violet assay. Discs were fixed in methanol, stained with 0.1% crystal violet, washed and air dried. The bound dye was extracted with ethanol-acetone solution and absorbance was read at 590 nm on a microplate reader. The higher the OD, the higher the total amount of biofilm biomass.

Viable bacterial count:

Adherent biofilm was removed from each disc by overtaxing and mild sonication in sterile phosphate buffered saline for the estimation of colony forming units. Enriched blood agar was used for serial dilutions and incubated anaerobically. The colony count was transformed to log10 (CFU/disc) for statistical analysis. Biofilm viability and thickness were determined by live/dead fluorescent staining and confocal laser scanning microscopy. Biofilm thickness was measured at 10 randomly selected standardized fields and the mean value was calculated. Selected discs were fixed in glutaraldehyde, dehydrated in a series of ethanol, sputter coated and then examined at a fixed magnification for SEM. Surface coverage was determined by image-analysis software as the percentage of the disc area covered by microbial aggregates and extracellular matrix.

Data analysis:

Data was entered into a spreadsheet and analyzed with the use of statistical software. The Shapiro-Wilk test was used to determine normality. Data for continuous variables were presented as mean ± SD. One-way analysis of variance (ANOVA) with Tukey post hoc test was used to compare intergroup at each time point. The chi-square test was used to compare categorical SEM distribution scores. Pearson correlation was used to explore associations between surface roughness and biofilm parameters. A p-value < 0.05 was taken as statistically significant.

Results:

No specimen losses during incubation or processing, 120 discs were analyzed. The mean surface roughness (Ra) of the rough titanium was the highest at 1.48 ± 0.18 μm, followed by rough

zirconia at 1.21 ± 0.16 µm, polished titanium at 0.22 ± 0.05 µm and polished zirconia at 0.17 ± 0.04 µm. The contact angle values indicated that rough titanium was more hydrophilic and comparatively less hydrophilic for polished zirconia. There were significant differences in surface roughness and contact angle between groups (p<0.001). In all groups, the amount of biofilm biomass increased with time from 24 to 72 hours. The optical density of rough titanium was the highest and that of polished zirconia was the lowest at each time point. At 72 hours, mean OD590 values were 1.02 ± 0.16 for polished titanium, 1.34 ± 0.18 for rough titanium, 0.76 ± 0.14 for polished zirconia and 0.93 ± 0.15 for rough zirconia. The difference between the intergroups was statistically significant at 24, 48 and 72 hours (p<0.001). The viable bacterial counts were similar. At 72 hours, rough titanium recorded the highest viable count (7.21 ± 0.32 log10 CFU/disc), followed by polished titanium (6.82 ± 0.29), rough zirconia (6.55 ± 0.31) and polished zirconia (6.18 ± 0.27). The thickness of the biofilm was significantly greater on the rough titanium than on the other surfaces as observed by confocal microscopy. The SEM results revealed that the rough titanium had dense confluent microbial clusters, the polished titanium and rough zirconia had moderate patchy biofilm and the polished zirconia had sparse discontinuous biofilm. Surface roughness showed a positive correlation with OD590 (r=0.68, p<0.001), viable counts (r=0.61, p<0.001) and SEM coverage (r=0.65, p<0.001) **(Table 1-3).**

Table 1: Surface characteristics of titanium and zirconia implant discs

Parameter	Polished titanium	Rough titanium	Polished zirconia	Rough zirconia	p-value
Ra (µm)	0.22 ± 0.05	1.48 ± 0.18	0.17 ± 0.04	1.21 ± 0.16	<0.001
Rz (µm)	1.36 ± 0.28	8.74 ± 1.12	1.02 ± 0.24	7.11 ± 0.96	<0.001
Contact angle (degrees)	68.4 ± 5.9	43.8 ± 6.7	78.6 ± 7.2	59.3 ± 6.4	<0.001
Surface free energy (mN/m)	42.1 ± 4.8	58.7 ± 5.2	36.8 ± 4.5	48.3 ± 5.0	<0.001

Table 2: Biofilm biomass and viable bacterial counts at different incubation periods

	Group	OD590 biomass	Viable count (log10 CFU/disc)	p-value
24 h	Polished titanium	0.46 ± 0.09	5.72 ± 0.24	<0.001
24 h	Rough titanium	0.62 ± 0.11	6.08 ± 0.26	
24 h	Polished zirconia	0.31 ± 0.07	5.22 ± 0.21	
24 h	Rough zirconia	0.39 ± 0.08	5.51 ± 0.23	
48 h	Polished titanium	0.76 ± 0.13	6.31 ± 0.27	<0.001
48 h	Rough titanium	1.05 ± 0.17	6.78 ± 0.30	
48 h	Polished zirconia	0.55 ± 0.10	5.81 ± 0.25	
48 h	Rough zirconia	0.67 ± 0.12	6.12 ± 0.26	
72 h	Polished titanium	1.02 ± 0.16	6.82 ± 0.29	<0.001
72 h	Rough titanium	1.34 ± 0.18	7.21 ± 0.32	
72 h	Polished zirconia	0.76 ± 0.14	6.18 ± 0.27	
72 h	Rough zirconia	0.93 ± 0.15	6.55 ± 0.31	

Table 3: Confocal and SEM-based evaluation of 72-hour biofilm formation

Parameter	Polished titanium	Rough titanium	Polished zirconia	Rough zirconia	p-value
Mean biofilm thickness (µm)	21.4 ± 4.3	34.8 ± 5.7	14.6 ± 3.9	19.8 ± 4.1	<0.001
Live bacterial proportion (%)	72.3 ± 6.5	78.9 ± 5.8	64.7 ± 7.1	69.2 ± 6.8	<0.001
SEM surface coverage (%)	55.8 ± 8.1	72.6 ± 8.4	38.2 ± 7.6	49.5 ± 8.0	<0.001
Dense confluent biofilm, n (%)	4 (40.0)	8 (80.0)	1 (10.0)	3 (30.0)	0.006
Sparse or discontinuous biofilm, n (%)	2 (20.0)	0 (0.0)	6 (60.0)	3 (30.0)	0.011

Discussion:

The present *in vitro* study showed that the surface topography and material of the implant had a significant effect on the formation of multispecies biofilms. The surface with the highest biofilm biomass, viable bacterial load, biofilm thickness and microscopic surface coverage was rough titanium, while the lowest surface coverage of biofilm was consistently observed on polished zirconia. The results show that the null hypothesis is rejected and that the interaction between roughness and material composition is important when it comes to microbial behavior at the implant surface. The pattern seen in this study is biologically feasible. The initial phase of oral biofilm formation involves the adsorption of salivary glycoproteins, peptides and other conditioning molecules to the implant surface. Bacteria then attach via physicochemical and receptor-mediated attachment and extracellular polymeric substances consolidate the biofilm. Surface irregularities provide more area for adhesion and provide protected microenvironments that minimize the effects of rinsing and shear stress. The correlation between the roughness and the biofilm parameters in this study is positive and it confirms the idea that surface texture is one of the most important factors in the retention of plaque on hard oral materials **[11]**. The highest biofilm values were obtained for rough titanium during the experimental period. The microtopography is often used to promote bone anchorage, but can be detrimental to the oral cavity. In the clinical situation, if the soft tissue has receded exposing rough implant threads or collars, this can lead to an increase in plaque retention and make decontamination more difficult. It does not detract from the use of rough endosseous titanium for osseointegration, but rather emphasizes the need for a proper peri-implant soft-tissue seal and proper plaque control around transmucosal components **[12]**. In this study, the least microbial accumulation was observed with polished zirconia. This could be associated with its smoother surface, lower surface free energy and less wettability than rough titanium. The results are in agreement with the experimental observations, which indicate that zirconia may exhibit reduced bacterial adhesion compared to titanium under similar conditions **[13]**. However, the results of the present study also indicate that roughened zirconia had greater biofilm accumulation than polished zirconia, suggesting that zirconia is not necessarily anti-biofilm in all circumstances.

Surface finish is still an important consideration even if the bulk material is ceramic. The results also corroborate the hypothesis that early and mature biofilm outcomes can be different. The intergroup differences at 24 hours were due to initial adhesion, while at 72 hours the difference between the smooth and rough surfaces was due to matrix maturation and bacterial multiplication. Some studies suggest that early biofilms can be more distinguishable between titanium and zirconia, whereas older biofilms can be more similar after a mature microbial matrix has formed **[14]**. In the present model, zirconia had lower values even at 72 hours and the difference between the polished titanium and rough zirconia was smaller than the difference between the polished zirconia and rough titanium. The viable count results are clinically relevant because optical density cannot distinguish between living bacteria, extracellular matrix, dead cells and absorbed stain. The crystal violet assay, CFU estimation, confocal microscopy and SEM were used in combination to give a more comprehensive understanding of biofilm behavior. The live/dead staining results indicated that rough titanium not only had more biomass but also had a higher percentage of living organisms. This could be due to the nutrient retention in surface micro depressions and increased protection of bacterial aggregates in the biofilm structure **[15]**. These observations have implications from a periodontal and implant maintenance point of view. The surface finish of implant-abutment surfaces, healing abutments, temporary cylinders and prosthetic emergence profiles should ideally be designed to resist plaque retention and be compatible with soft tissues. In patients with thin gingival phenotype, high smile line, metal display concerns, or recurrent peri-implant mucosal inflammation, esthetic and biomechanical conditions may be favorable for the use of polished zirconia abutments, which may provide a favorable microbiological profile. The selection of the material, however, should not be limited to the biofilm behavior, as fracture resistance, connection design, occlusal load, prosthetic space and tissue thickness are also important **[16]**. There are some limitations in the present study. First, it was an *in vitro* study and was not able to simulate the dynamic conditions of the oral cavity, such as salivary flow, dietary changes, host immunity, professional cleaning and patient-specific plaque ecology. Second, the mixed saliva-derived biofilm was biologically relevant but contained inherent microbial variability. Third, the observation period was restricted to 72 hours and the long-term maturation of the biofilm, mineralization and repeated cleaning cycles were not assessed. Fourth, the tested discs were standard material surfaces and not the full geometry of threaded implants or abutment connections. Long-term multispecies biofilm models, dynamic flow models, peri-implantitis-associated microbial communities and surface decontamination models should be incorporated into future research **[17, 18]**. In these constraints, the study highlights the role of both material and surface finish on bacterial adhesion. The highly polished zirconia exhibited the lowest level of biofilm formation, whereas rough titanium exhibited the highest level of microbial colonization. The results of this study highlight the significance of careful surface design and plaque-control measures in implant dentistry.

Conclusion:

We show that biofilm formation differed significantly between implant surfaces, with rough titanium showing the highest and polished zirconia the lowest microbial accumulation. Surface roughness was strongly associated with biofilm retention, indicating that topography is a major determinant of bacterial colonization. Thus, polished zirconia may offer a microbiologically favorable transmucosal surface, although clinical selection should consider mechanical and patient-specific factors.

References:

[1] https://pubmed.ncbi.nlm.nih.gov/18724855/
[2] Berglundh T *et al. J Clin Periodontol.* 2018 **45:**S286 [PMID: 29926491]
[3] Heitz-Mayfield LJA & Salvi GE. *J Clin Periodontol*. 2018 **45:**S237 [PMID: 29926488]
[4] Schwarz F *et al. J Clin Periodontol.* 2018 **45:**S246 [PMID: 29926484]
[5] Al-Radha ASD *et al. J Dent.* 2012 **40:**146 [PMID: 22182466]
[6] Roehling S *et al. J Periodontol*. 2017 **88:**298 [PMID: 27712464]
[7] Sanchez MC *et al. Dent Mater*. 2014 **30:**1161 [PMID: 25110288]
[8] Wassmann T *et al. Int J Implant Dent.* 2017 **3:**32 [PMID: 28714053]
[9] Kniha K *et al. Int J Oral Maxillofac Surg.* 2021 **50:**1605 [PMID: 33618967]
[10] Belloni A *et al. J Transl Med.* 2024 **22:**120. [PMID: 38297308]
[11] do Nascimento C *et al. Microsc Res Tech*. 2013 **76:**126 [PMID: 23109001]
[12] Chiou LL *et al. J Dent.* 2023 **132:**104477 [PMID: 37096222]
[13] Jaeggi M *et al. Clin Oral Investig.* 2022 **26:**4167 [PMID: 35137461]
[14] Cionca N *et al. Periodontol* 2000. 2017 **73:**241 [PMID: 28000266]
[15] Souza JGS *et al. iScience.* 2021 **24:**102391 [PMID: 31762055]
[16] Vernon JJ *et al. J Oral Microbiol*. 2025 **18**:2607199. [PMID: 41509039]
[17] Anitua E *et al. J Oral Microbiol*. 2025 **17:**2459922 [PMID: 39916977]
[18] Dipalma G *et al. Bioengineering (Basel)*. 2026 **13:**299 [PMID: 41899830]