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Enzymatic and Biofilm Exposures on Surface Degradation and Corrosion Resistance of Dental Crowns

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Abstract

The objectives of the study were to assess the effect of enzymatic and microbial biofilm exposure on the surface roughness and corrosion resistance of dental crown materials commonly used in dentistry in oral environment. Three modern dental crown materials were used: yttria-stabilized tetragonal zirconia polycrystal (Y-TZP), lithium disilicate glass-ceramic, and cobalt–chromium metal-ceramic alloy. Ninety disc-shaped specimens were manufactured and randomized to three experimental groups: control (artificial saliva), enzymatic exposure and combined enzyme–biofilm exposure. Enzymatic degradation was simulated using matrix metalloproteinase-8 (MMP-8), esterase, and proteinase K. Biofilm formation was induced using Streptococcus mutans (ATCC 25175) and Lactobacillus acidophilus under anaerobic incubation. The surface roughness was measured by atomic force microscopy (AFM), and the corrosion resistance was measured by electrochemical impedance spectroscopy (EIS). The measurements were taken at 7, 14 and 28 days. Two-way ANOVA and repeated-measures ANOVA with Bonferroni post hoc testing were used for statistical analysis. All materials investigated showed an increase in surface roughness and loss of corrosion resistance after both the enzymatic and combined enzyme–biofilm treatments. The synergic effect had a greater degradation than the enzymatic effect ($p < 0.05$). The surface roughness values remained as low as zirconia and the impedance values remained as high as zirconia throughout the experimental period. The specimens of metal-ceramic exhibited the lowest corrosion resistance and the highest increase in roughness with time. The surface degradation and corrosion experiments of dental crown materials indicated that both enzymatic and microbial biofilm exposure had great effects on the surface degradation and corrosion behavior of the materials under simulated oral conditions. Bacterially mediated degradation was found to be more resistant for zirconia than for lithium disilicate and metal-ceramic materials.

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Introduction

In restorative dentistry, dental crowns are widely utilized to restore the function, esthetics and structure of damaged teeth. However, their long-term clinical success is dependent not just on their mechanical strength and esthetic qualities, but also on their ability to withstand degradation in the oral environment [1,2].

Oral cavity is a very dynamic biological environment that is constantly exposed to electrochemical changes, pH changes, thermal shifts and microbial biofilms, as well as the presence of salivary enzymes. The environmental factors can cause the materials that are used in the restoration to become degraded over time by ion release, corrosion processes, and structural instability [3-5].

Oral biofilms are complex microorganism populations that can generate acid metabolites, EPS and enzyme by-products which can modify the surface of restorative materials. Streptococcus mutans is recognized as one of the main microorganisms responsible for early biofilm development and acid production while the Lactobacillus species are involved in the maintenance of an acid environment and maturation of the biofilm [5-7].

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As well as microbial activity, material degradation may be caused by enzymes present in the saliva and/or the host. Various matrix metalloproteinases (MMPs), esterases and proteolytic enzymes have been linked to the deterioration of restorative interfaces and hydrolytic degradation of biomaterials [8-11].

The current materials for crowns include zirconia, lithium disilicate ceramics, which have proven to have good mechanical properties and biocompatibility. Zirconia, specifically yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) has high fracture resistance, low plaque affinity and chemical stability. Lithium disilicate ceramics are known for their superior esthetic and translucent properties; however, they can be altered on their surface under acidic conditions. Metal-ceramic restorations are still extensively used due to their strength and durability; however, electrochemical corrosion and ion release are still clinical issues [12-14].

Enzymatic degradation of restorative materials or biofilm associated corrosion have previously been subjected to separate investigations. However, limited studies have investigated the combined influence of enzymatic exposure and microbial biofilm accumulation under a unified experimental model that simulates clinically relevant oral conditions [4,15,16].

Therefore, this study aimed to investigate the effects of enzymatic and microbial biofilm exposure on surface degradation and corrosion resistance of commonly used dental crown materials using controlled in vitro simulation.

Materials and Methodos

This study was designed as a controlled in vitro experimental investigation to evaluate the influence of enzymatic and microbial biofilm exposure on surface roughness and corrosion resistance of dental crown materials over time.

Sample size estimation was performed using G*Power software version 3.1. Based on a statistical power of 90%, significance level of 0.05, and estimated effect size of 0.40, a minimum of nine specimens per subgroup was required. To improve reliability and compensate for possible variability during experimental procedures, ten specimens were included in each subgroup.

Three commonly used dental crown materials representing ceramic, glass-ceramic, and metal-based restorative systems were investigated:

1. Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) (Ivoclar Vivadent)
2. Lithium disilicate glass-ceramic (IPS e.max)
3. Cobalt–chromium metal-ceramic alloy (BEGO)

A total of 90 disc-shaped specimens were fabricated with standardized dimensions of 10 mm diameter and 2 mm thickness.

All specimens underwent sequential polishing using 600-, 1200-, and 2000-grit silicon carbide abrasive papers under continuous water irrigation to standardize surface finishing. Specimens were ultrasonically cleaned in distilled water for 10 minutes and sterilized using ultraviolet irradiation for 30 minutes prior to experimentation.

Specimens from each material were randomly allocated into three exposure conditions:

Group 1 - Control: Specimens immersed in artificial saliva only.

Group 2 - Enzymatic Exposure: Specimens exposed to multi-enzyme solution.

Group 3 - Combined Enzyme–Biofilm Exposure: Specimens exposed to both enzymatic solution and bacterial biofilm.

Artificial saliva was prepared using NaCl (0.4 g/L), KCl (0.4 g/L), CaCl2 (0.795 g/L), and urea (1.0 g/L). The pH was adjusted to 6.8.

An enzymatic degradation model was established to simulate the biological activity of the oral environment. The enzymatic solution was prepared with the matrix metalloproteinase-8 (MMP-8) of 100 ng/mL, esterase of 50 U/mL and proteinase K of 20 µg/mL. All the specimens were cultivated in the enzymatic medium at 37°C during the experimental period with the change of the medium every 48 hours to ensure the enzymatic stability and activity level.

Streptococcus mutans (ATCC 25175) and Lactobacillus acidophilus were cultured in Brain Heart Infusion (BHI) broth under anaerobic conditions at 37°C for biofilm development.

The culture media was changed every 48 hours to keep the bacteria alive to avoid any fluctuation in the development of the biofilm.

The surface roughness was measured by using atomic force microscopy (AFM, FlexAFM, Nanosurf AG, Switzerland). Surface roughness measurements (Ra (µm)) were taken for all specimens.

It was conducted with electrochemical impedance spectroscopy (EIS) using a potentiostat system at frequencies between 10-2 and 105 Hz. The corrosion resistance values were reported in the units of impedance ($k\Omega \cdot cm^2$).

Seven, 14, and 28 days of exposure were recorded to assess the changes in surface roughness and corrosion resistance with time under different experimental conditions.

The data was analyzed with SPSS software version 26. Shapiro–Wilk test was used to assess normality. Comparisons between groups were made using two-way ANOVA and repeated measures ANOVA, followed by Bonferroni post hoc tests. A p value < 0.05 was defined as statistical significance.

Results

The results of the experiments showed clear differences between the tested crown materials when they were exposed to the enzymes as well as to the combined enzyme/biofilm exposure. In general, zirconia exhibited the least degradation on its surface and electrochemical behavior, whereas the metal-ceramic specimens exhibited the highest susceptibility to degradation.

The surface roughness was found to be increased in a progressive manner with the enzymatic and combined enzyme–biofilm exposure of all the materials. The increase in the roughness was the highest in metal-ceramic specimens, while the specimens with zirconia had the lowest Ra values throughout the experimental period.

Repeated-measures ANOVA revealed statistically significant differences ($p < 0.001$) for the effects of material type, exposure condition and time interval on surface roughness values. Under the combined enzyme-biofilm exposure conditions the surface roughness increased gradually with time as shown in Figure 1.

All the materials tested showed an increase in surface roughness over time. The lowest roughness was observed consistently in zirconia specimens especially under control conditions. However, combined enzyme–biofilm exposures resulted in the highest Ra values at day 28 which suggests more surface degradation. Enzymatic exposure alone did not cause as much roughness changes as the combined exposure.

All materials showed that their impedance value decreased with time, implying the decrease in corrosion resistance after the biological exposure.

The most significant decrease in impedance values with time was observed for metal-ceramic specimens while zirconia exhibited significantly high corrosion resistance under all experimental conditions.

The electrochemical impedance for all of the material groups was seen to decrease with time, which indicated a decrease in corrosion resistance after the biological exposure. Metal-ceramic specimens exhibited the highest reduction in impedance values, particularly when combined enzyme and biofilm exposure occurred at day 28, while zirconia had the highest values throughout the experiment.

Two-way ANOVA showed that the material type, exposure to enzymes, exposure to biofilms, and time interval had significant effects on surface degradation and corrosion resistance. The interaction effect was also statistically significant, suggesting that the biological exposure and time interacted to affect material behavior.

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In summary, the findings suggest that both enzymatic and microbial exposure to biofilms resulted in material-related degradation over time. The most resistant to changes caused by biological agents was zirconia, while the most sensitive to deterioration due to surface roughening and corrosion was metal-ceramic materials.

Discussion

The aim of the present study was to assess the effect of enzymatic and microbial biofilm exposure on surface degradation and corrosion resistance of modern dental crown materials under simulated oral environment. The results showed that all the materials studied were significantly affected by surface characteristics and electrochemical stability after biological exposure [3,4].

The increase in surface roughness was progressive after being exposed to enzymes and combined enzyme–biofilm. The joint exposure condition also could lead to more degradation than enzymatic exposure alone, indicating that microbial biofilm and enzymatic activity may be a combination that further increases the rate of material degradation [7,15,16].

There are several co-occurring mechanisms that may explain biofilm-associated degradation. Acidic microbial metabolites may affect local electrochemical conditions and lead to instability on restorative surfaces, while extracellular polymeric substances may lead to extended adhesion of bacterial by-products on restorative surfaces. These processes can improve the ion exchange and promote surface degradation [5,6].

Hydrolytic changes and surface destabilization can also be caused by enzymatic degradation. Previous studies have shown that matrix metalloproteinases and esterase enzymes are known to be related to degradation of restorative interfaces and biomaterial surfaces following extended biological exposure [8-10].

Zirconia had the highest resistance to surface alteration and corrosion of all materials studied, including lithium disilicate and metal-ceramic materials. Zirconia is highly crystalline which may have a positive effect on the microbial adhesion and lower surface energy properties may have a positive effect on the electrochemical stability [1,2,12].

The time-durability was the most deteriorated for the metal-ceramic specimens. Under acidic biofilm conditions, the electrochemical changes and ion release of metallic materials might be more pronounced, especially when the surface integrity is altered by a prolonged biological exposure [4,13,14].

The intermediate degradation was observed for lithium disilicate. While the material was still resistant under controlled conditions, there was a progressive increase in surface roughness and a decrease in impedance values with combined enzymatic and microbial exposure.

The correlation between the surface roughness and the corrosion resistance is very negative, indicating that the further surface changes, the greater the level of electrochemical instability and microbial retention. The roughness may create favorable niche conditions that can promote the formation of biofilm, which can promote the biologically mediated degradation process [15,16].

The results of the present study confirm the need to consider biological degradation when selecting restorative materials. Mechanical strength, esthetics, and resistance to enzymatic and microbial exposure are other properties that can be related to long-term clinical performance [1,2,4].

This study involved attempting to simulate conditions in the mouth, but there are some limitations. The investigation was carried out in in vitro conditions that may not reflect the complexity of intraoral environment. Furthermore, only some bacteria species and enzymes were incorporated in the experimental model. Further research is warranted to include the use of multispecies biofilm systems, longer observation period.

Conclusion

Within the limitations of this in vitro investigation, enzymatic and microbial biofilm exposure significantly influenced surface roughness and corrosion resistance of dental crown materials. Combined enzyme–biofilm exposure produced greater degradation compared with enzymatic exposure alone. Zirconia demonstrated the greatest resistance to biologically mediated degradation, whereas metal-ceramic materials showed the highest susceptibility to surface alteration and corrosion.

Study Limitations

The present study was conducted under controlled laboratory conditions that may not fully represent the complexity of the oral environment. Factors such as salivary flow, thermal cycling, mechanical loading, dietary variations, and host immune response were not incorporated into the experimental model. In addition, the biofilm model was limited to selected bacterial species and did not reproduce the diversity of the natural oral microbiome. Future studies involving long-term clinical simulation and broader microbiological models are recommended.

Declarations

Conflict of Interest: The authors declare no conflict of interest.

Funding: No external funding was received for this study.

Ethical Approval: Not applicable for in vitro experimental investigations.

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Table 1. Characteristics of dental crown materials used in the study.

Material	Type	Manufacturer	Surface Characteristics	Clinical Application
Zirconia (Y-TZP)	Ceramic	Ivoclar Vivadent	High crystalline stability and low surface energy	Posterior and anterior crowns
Lithium Disilicate	Glass-ceramic	IPS e.max	High translucency and smooth glazed surface	Esthetic crowns and veneers
Co–Cr Alloy	Metal-ceramic	BEGO	Metallic structure with corrosion susceptibility	Fixed prosthodontic restorations

Table 2. Experimental conditions and exposure protocols.

Group	Experimental Condition	Biological Components	Exposure Environment	Duration
G1	Control	Artificial saliva only	pH 6.8	28 days
G2	Enzymatic Exposure	MMP-8, esterase, proteinase K	Simulated enzymatic degradation	28 days
G3	Enzyme + Biofilm	Enzymes + bacterial biofilm	Anaerobic incubation	28 days

Table 3. Surface roughness values (Ra µm) at different time intervals.

Material	Condition	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 28 Mean ± SD	Percentage Increase
Zirconia	Control	0.13 ± 0.03	0.14 ± 0.04	0.15 ± 0.04	15%
Zirconia	Enzyme	0.21 ± 0.05	0.25 ± 0.06	0.31 ± 0.07	47%
Zirconia	Enzyme + Biofilm	0.28 ± 0.06	0.34 ± 0.08	0.39 ± 0.09	39%
Lithium Disilicate	Control	0.17 ± 0.04	0.18 ± 0.05	0.19 ± 0.05	11%
Lithium Disilicate	Enzyme	0.28 ± 0.07	0.34 ± 0.08	0.40 ± 0.09	43%
Lithium Disilicate	Enzyme + Biofilm	0.33 ± 0.08	0.41 ± 0.09	0.48 ± 0.1	45%
Metal-Ceramic	Control	0.22 ± 0.05	0.23 ± 0.05	0.24 ± 0.06	9%
Metal-Ceramic	Enzyme	0.37 ± 0.08	0.45 ± 0.09	0.52 ± 0.11	41%
Metal-Ceramic	Enzyme + Biofilm	0.42 ± 0.09	0.54 ± 0.11	0.63 ± 0.13	50%

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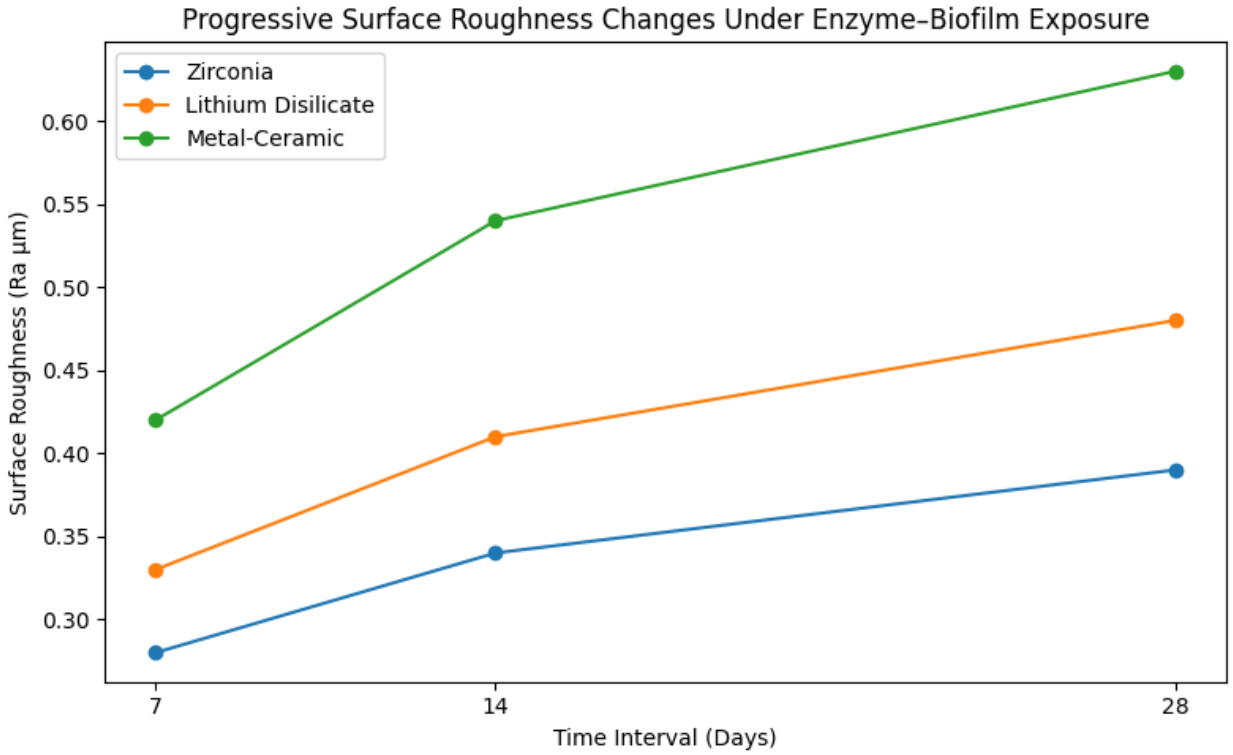

Figure 1. Changes in surface roughness (Ra µm) of the investigated dental crown materials after 28 days of exposure to both enzymes and biofilms. It was observed that the surface roughness of metal-ceramic specimens had the highest value while the surface roughness of zirconia remained the lowest during the experimental period.

Table 4. ECV (kΩ·cm²) of investigated materials.

Material	Condition	Day 7 Mean ± SD	Day 14 Mean ± SD	Day 28 Mean ± SD
Zirconia	Control	128 ± 15	124 ± 14	119 ± 13
Zirconia	Enzyme	117 ± 14	104 ± 12	92 ± 11
Zirconia	Enzyme + Biofilm	109 ± 13	95 ± 11	83 ± 10
Lithium Disilicate	Control	111 ± 13	106 ± 12	101 ± 11
Lithium Disilicate	Enzyme	98 ± 12	84 ± 10	71 ± 9
Lithium Disilicate	Enzyme + Biofilm	89 ± 11	72 ± 9	60 ± 8
Metal-Ceramic	Control	87 ± 11	81 ± 10	75 ± 9
Metal-Ceramic	Enzyme	76 ± 10	61 ± 8	49 ± 7

Table 5. This study shows the surface degradation and corrosion resistance by the help of Two-Way ANOVA analysis. This study reveals surface degradation and corrosion resistance with Two-Way ANOVA Analysis.

Factor	F-value	p-value	Partial Eta Squared
Material Type	18.64	<0.001	0.39
Enzymatic Exposure	20.11	<0.001	0.41
Biofilm Exposure	24.32	<0.001	0.46
Time Interval	17.25	<0.001	0.35
Interaction Effect	11.08	0.002	0.31