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SEM Analysis of the *in situ* Early Bacterial Colonization on Two Novel Feldspathic Ceramics Submitted to Different Types of Glazing

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Abstract

Aim: The aim of this study was to evaluate *in situ*, the early bacterial colonization on feldspar-ceramics submitted to different glazing.

Methods and Materials: Fourteen standardized disc specimens (diameter: 5 mm, thickness: 1.5 mm) of each of two micro-particulate feldspathic ceramics (VM7 and VM13, Vita) were produced according to manufacturers' specifications for a total of 28 specimens (24 for the analysis of biofilm and 4 for topographic analysis analyzing the ceramic surfaces). Specimens from each type of ceramic were submitted to two different glazing methods composing four groups: VM7 glazed using glazing liquid Vita Akzent® 25 (G1) and glaze firing (G2), VM13 glazed using glazing liquid (G3) and glaze firing (G4). Six individuals (n=6) wore oral appliances with four ceramic specimens, fixed on the buccal face of the appliances. After 8 hours, each sample was evaluated for the presence (1) or absence (0) of bacterial colonization under a scanning electron microscope (SEM) on five randomly selected fields. The value for each sample was cumulative of the results observed in the fields. One sample from each group was evaluated under a SEM to verify the topographic pattern.

Results: There was no difference with regard to bacterial colonization between the feldspar-ceramics and between the glazing types (Kruskal-Wallis non-parametric test).

Conclusion: Feldspar-ceramics submitted to firing or glaze firing with Vita Akzent® 25 present a similar condition for in situ bacterial colonization. The similar topographic pattern of the ceramic surfaces seems to have influenced the bacterial colonization.

Keywords: Microscopic analysis, bacterial colonization, acquired pellicle, feldspathic ceramic, glazing

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Introduction

The correlation between biofilm formation and surface roughness has been established in the dental literature. Studies have demonstrated the following:

- Bacterial adhesion begins around irregularities and expands to the entire surface.[1]
- Biofilm maturation occurs more quickly on rough areas.[2,3]
- After cleaning existing bacterial growth, bacterial recolonization occurs rapidly on rough areas.[4]

In a healthy individual an equilibrium exists between the host defense mechanism and bacterial biofilm. A healthy status of the host maintains a stable bacterial community denominated by Gram-positive and facultative anaerobia bacteria. Any modification in this equilibrium (dietary, host defense) can lead to changes in the composition of the biofilm with potential outcomes being the occurrence of dental caries and periodontal disease. Although these two diseases are associated with specific plaque theories, the removal of all bacterial deposits remains essential in their prevention. It is advisable to use esthetic restorative materials that accumulate very few microorganisms on the surface or inhibit adhesion of bacterial colonies while providing good mechanical and esthetic properties. However, little is known regarding the bacterial adherence to newer restorative materials proposed as being materials with enhanced biological and physico-mechanical properties such as micro-particulate ceramics.

The new VitaVM®7 and VitaVM®13 (Vita Zahnfabrik, H. Rauter GmbH & Co. Bad Säckingen, Germany) are micro-particulate two-phase glassy feldspathic ceramics.[5] According to the manufacturer Vita VM®7 is indicated for all-ceramic restorations (inlays, onlays, veneers)

and like veneering material of high-ceramic frameworks such as VITA In-Ceram® Classic Alumina, Spinell, Zirconia, as well as VITA In-Ceram® 2000 AL Cubes (Vita Zahnfabrik, H. Rauter GmbH & Co. Bad Säckingen, Germany). VitaVM®13 is indicated like veneering material of metal substructures. The principal difference between them is the coefficient of thermal expansion.

A surface of a veneering feldspar ceramic of a metal-ceramic or metal-free restoration has to be smoothed using glazing or glaze firing (without the application of liquid glaze) or using finishing/polishing procedures.

Ceramic surface glazing involves the use of a glazing liquid composed of mono-phase glass which is applied on the surface, and then the ceramic material is heated to a temperature recommended by the manufacturer.

The glaze firing technique requires no application of glazing liquid to the surface of the ceramic material. The ceramic is simply fired to a temperature a few degrees lower than the temperature for sintering the feldspar ceramic causing the glass phase of the ceramic to form a glazed surface. Dental ceramic laboratory technicians use both glazing techniques (glazing with glazing liquid or glaze firing).

The purpose of this *in situ* study was to evaluate the surface preparation of micro-particle feldspar-ceramics with different clinical indications for early dental biofilm formation. The null hypothesis was glazing techniques and the types of feldspathic ceramic do not influence the biofilm formation.

Methods and Materials

Two micro-particulate feldspar-ceramics were selected for the study. The first was VitaVM®7 which is designed for use in all-ceramic restorations (inlays, onlays, veneers) and for the veneering material that is fused to high-ceramic frameworks such as VITA In-Ceram® Classic Alumina, Spinell, Zirconia, and VITA In-Ceram®2000 AL Cubes. The second was Vita VM®13 which is designed as a veneering material that is fused to metal frameworks.

Specimen Preparation

Fourteen standardized disc specimens (diameter: 5 mm, thickness: 1.5 mm) of each ceramic material (28 total) were produced according to manufacturers' specifications. The specimens were produced using a metallic plate with calibrated holes of 5.3 mm in diameter to compensate for the sintering shrinkage (nearly 12%) of the ceramics. The powder and liquid components of each ceramic were mixed and placed into a hole in the metallic plate. The specimens were sintered in a Vacumat® furnace (Vita Zahnfabrik) and regularized with a silicon-carbide abrasive paper #1000 (3M, St. Paul, MN, USA). The specimens were ultrasonically cleaned in distilled water and randomly divided into four groups (N=6), according to the surface type:

- **G1:** VM7 feldspar-ceramic glazed using Vita Akzent 25 (Vita Zahnfabrik) glazing liquid.
- **G2:** VM7 feldspar-ceramic glazed using glaze firing.
- **G3:** VM13 feldspar-ceramic glazed using Vita Akzent 25 glazing liquid.
- **G4:** VM13 feldspar-ceramic glazed using glaze firing.

Analysis of Surface Topography

One sample from each group was evaluated under a scanning electron microscope (SEM) (Zeiss DSM-962, Jena, Germany) to verify the topographic pattern.

Assessment of Early Dental Biofilm

SEM Evaluation: Six dental students (n=6) without signs of caries or periodontal diseases, and who had not used antibiotics for three months prior to the onset of the study were selected. Informed consent was conveyed from all participants.

Oral appliances were made for each participant that covered the crowns of the maxillary premolars and molars. The ceramic specimens were mechanically fixed to the buccal face of the appliances. Prior to the experiment participants cleaned their teeth using only a toothbrush without toothpaste or dental floss. No food or beverages were consumed during the intraoral exposure of the specimens. After eight hours, the specimens were removed for a SEM evaluation. A total area of 100 x 125 µm was examined for each specimen. This area was composed of five randomly selected fields (20 x 25 µm). For each field, the presence (=1) or absence (=0) of bacterial colonization was analyzed. The values quoted for the total area were cumulative of those values observed in the fields.[3]

Statistical Analysis: The Kruskal-Wallis non-parametric test was used to compare the cumulative values of the total area analyzed in each specimen. Any difference of $p<0.05$ was considered statistically significant.

Results

No statistical differences were observed for the presence of bacteria observed among the groups after eight hours (P=.4030). There was also no significant difference between the feldspar ceramics or between the glazing methods used (Table 1).

Table 1. Scores verified considering the two main factors (glazing and ceramic material).

Feldspar-Ceramic	Surface Treatments	
	Glaze-firing	Glaze
VM7	14	12
VM13	24	19

‡ Similar statistically (P=.4030).

When comparing the ceramic surface topography, similar patterns were noted (Figure 1).

Micrographs of the bacterial colonization are shown in Figure 2.

Discussion

The formation of dental biofilm can occur on natural (enamel, dentin, and root surface) or restorative material surfaces in the oral environment.[6] *In vivo* and *in vitro* studies have been performed demonstrating differences in the biofilm formed on different restorative materials.[7,8] These variations can be the outcome of the material properties, such as surface free-energy and surface roughness.[9,10,11] A reduction in roughness will result in the reduction of biofilm formation and maturation. A reduction in surface free-energy will result in a decrease in the biofilm retention capacity. However, the surface roughness is considered more important for the adhesion process than the surface free-energy.[2] Roughness favors adhesion because it increases the available area and promotes niches were bacteria are protected from the shear forces.[11] As noted in (Figure 1), the topographic patterns of the four ceramic surfaces are similar which might explain why no significant differences were found for the presence of bacteria between the four test groups in the current study.

It is important to clarify bacterial colonization on the glazed ceramic surface was evaluated; however, the micro-morphology of the ceramic surface can be modified if some chair side finishing-polishing of the ceramic surface is done. Considering only recent veneering feldspathic ceramics were evaluated in this current study, future investigations are recommended using altered ceramic surfaces.

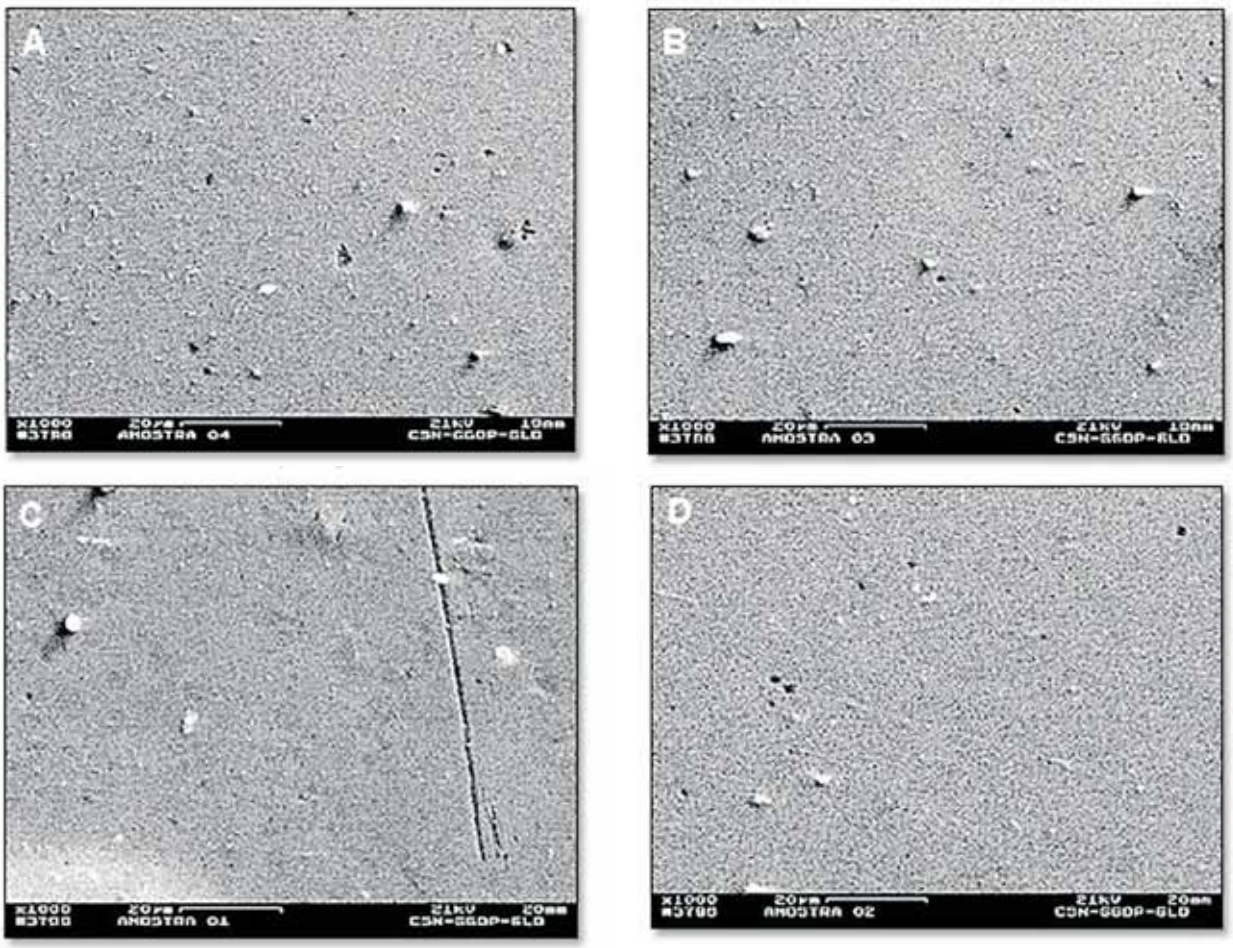

Figure 1. Representative micrographs (x1000) from the two ceramics and the two types of glazing. **A.** VM7 plus glaze. **B.** VM7 plus glaze firing. **C.** VM13 plus glaze. **D.** VM13 plus firing glaze. Similar topographic patterns can be noted.

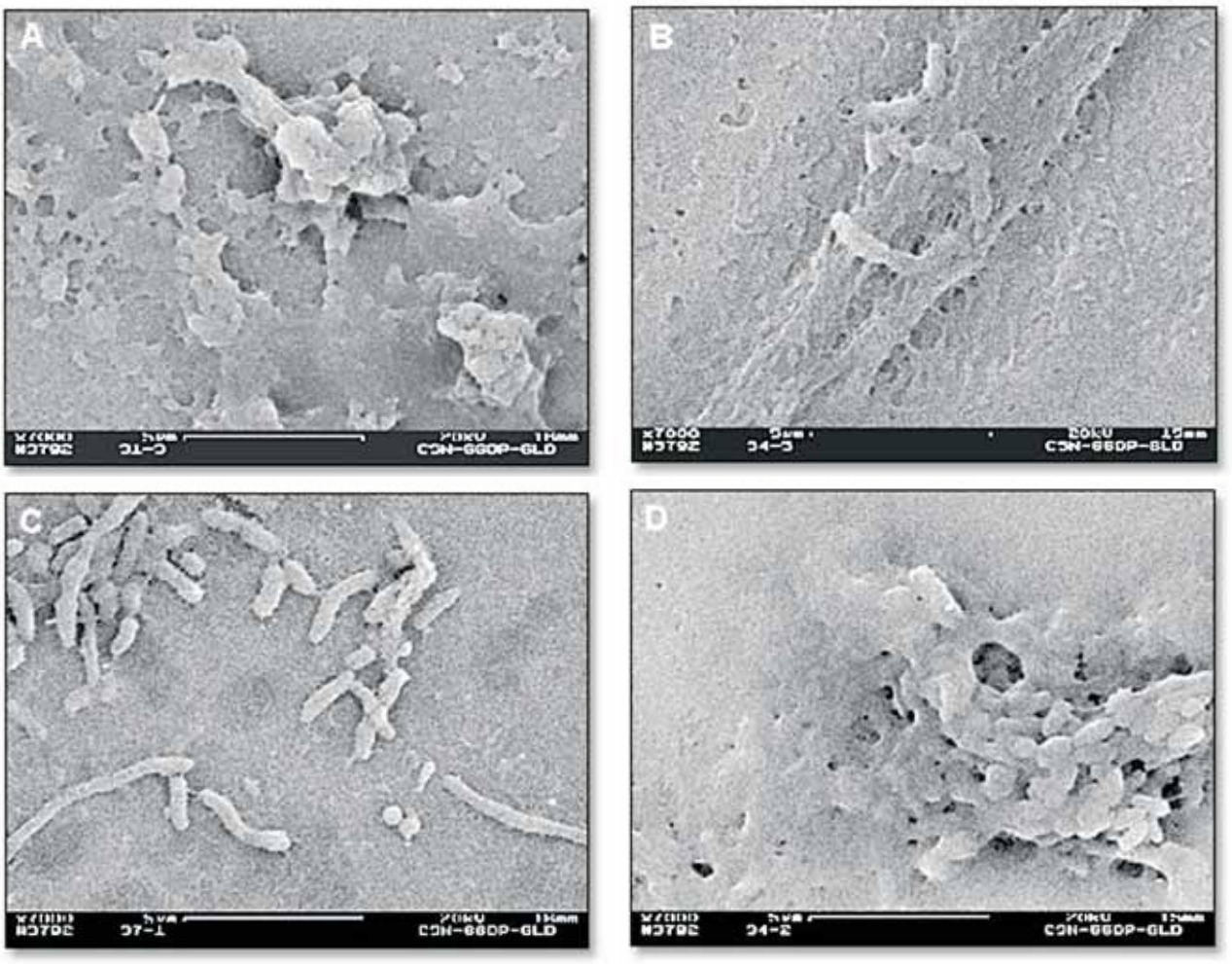

Figure 2. Representative micrographs (x7000) of the bacterial colonization. **A.** VM7 plus glaze firing: A cellular material coated cocci. **B.** VM13 plus Glaze: short rods are observed. **C.** VM13 plus glaze firing: Presence of the short and long rod aggregates. **D.** VM13 plus Glaze: Multi-layers of short rods are visualized.

Conclusions

In the eight hour period, the two micro-particle feldspathic-ceramics submitted to the glaze firing or glaze with Vita Akzent® 25 were similar regarding the *in situ* bacterial colonization.

The similar topographic patterns of the ceramic surfaces of the materials tested appears to have influenced the findings with regard to bacterial colonization.

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