applied sciences

MDPI

Article

Biofilm Adhesion and Micromorphology Analysis after Professional Oral Hygiene Procedures on CAD/CAM Milled and Veneered Zirconia Restorations: In Vitro Study

Mirjana Perić [1], Aleksandra Čairović [1,*], Aleksandra Špadijer Gostović [1], Dragan Stanimirović [2], Marina Kostić [3], Marija Ivanov [3], Ana Ćirić [3], Jasmina Glamočlija [3] and Vladimir B. Pavlović [4]

[1] Department of Prosthodontics, School of Dental Medicine, University of Belgrade, Rankeova 4, 11000 Belgrade, Serbia; mirjana.peric@stomf.bg.ac.rs (M.P.); sanja.dent@gmail.com (A.Š.G.)
[2] Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade, Rankeova 4, 11000 Belgrade, Serbia; dc_dragan@yahoo.com
[3] Department of Plant Physiology, Institute for Biological Research "Siniša Stanković"—National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia; marina.kostic@ibiss.bg.ac.rs (M.K.); marija.smiljkovic@ibiss.bg.ac.rs (M.I.); rancic@ibiss.bg.ac.rs (A.Ć.); jasna@ibiss.bg.ac.rs (J.G.)
[4] Laboratory for Electron Microscopy, Department of Mathematics and Physics, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11000 Belgrade, Serbia; vladimirboskopavlovic@gmail.com
* Correspondence: aleksandra.korona@gmail.com

Abstract: Objective: The purpose of this study was to evaluate the surface texture and biofilm adhesion of veneered or CAD/CAM milled zirconia (partially stabilized with yttrium) after professional oral hygiene procedures. The samples (4 × 4 mm, thickness 2 mm; n = 72) were separated from zirconia blanks (3Y-TZP-LA). One group was veenered with ceramics, and the other group of samples was CAD/CAM milled. Each group had two subgroups: polished and glazed. The samples were subjected to simulated strokes of professional brushing using abrasive paste and ultrasonic scaling. The parameters of surface micromorphology and receptivity to biofilm were calculated before and after simulating the given methods of the professional maintenance of oral hygiene. Scanning electron microscopy (SEM) was used to evaluate zirconia surface properties. Microbial (bacterial/fungal) species (*Staphylococcus aureus*, *Streptococcus sanguinis* and *Candida albicans*) were used and cultured on respective sterilized zirconia surfaces. Colony-forming unit (CFU) counts were used to quantify the amount of biofilm formation on zirconia samples surfaces. Results: The SEM analysis showed the greatest change in surface microtopography after the use of ultrasonic scaling on glazed zirconia samples. Less formation of colonies on the surfaces of CAD/CAM milled zirconia restorations was observed. Conclusion: Routine methods of oral hygiene professional maintenance can damage the surfaces of glazed zirconia restorations.

Keywords: zirconia; CAD/CAM; ultrasonic scaling; SEM; surface microtopography; microbial biofilm

Citation: Perić, M.; Čairović, A.; Špadijer Gostović, A.; Stanimirović, D.; Kostić, M.; Ivanov, M.; Ćirić, A.; Glamočlija, J.; Pavlović, V.B. Biofilm Adhesion and Micromorphology Analysis after Professional Oral Hygiene Procedures on CAD/CAM Milled and Veneered Zirconia Restorations: In Vitro Study. *Appl. Sci.* **2023**, *13*, 8389. https://doi.org/10.3390/app13148389

Academic Editor: Bruno Chrcanovic

Received: 21 June 2023
Revised: 12 July 2023
Accepted: 17 July 2023
Published: 20 July 2023

1. Introduction

In recent decades, we have witnessed the intensive development of dental ceramic materials, especially when it comes to the modern understanding of aesthetics, such as the "Hollywood smile". In particular, zirconia stands out as a biomaterial of great interest.

Due to good mechanical properties (high hardness, strength, high wear resistance, corrosion resistance, modulus of elasticity similar to steel and increased fracture toughness) and aesthetic properties [1], this restorative material is widely used in prosthetic dentistry [2–4].

As dental ceramics develops, so do the methods of its technical production. Significant progress was made with the introduction of the CAD/CAM system, which describes the virtual design and production of dental restorations using machine units [5].

In order to improve their mechanical properties, tetragonal zirconium polycrystals (TZP) are strengthened by the use of certain elements such as yttrium (zirconium stabilized by yttrium, i.e., Y-TZP). This form of material can be applied to high-strength single crown frames, bridges and implants using CAD/CAM techniques [6].

Zirconia partially stabilized by yttrium (Y-TZP) contains a highly crystalline phase and low translucency, which causes opacity [7]. To exclude the white opaque color of zirconia, transparent porcelain can be used to veneer restorations. However, there raises another problem, the chipping of veneering porcelain [8]. To overcome this clinical limitation, monolithic zirconia, in an anatomical form without veneers, is used.

Monolithic zirconia has the required translucency and excellent mechanical properties without the risk of chipping of the veneering ceramic [9]. Restorations made with this type of zirconia require shorter clinical working time and reduced costs.

Dental plaque causes an inflammatory reaction, which is the main cause of periodontal and peri-implant diseases that can lead to progressive bone loss around the tooth but also around the implant. Early bacterial colonization is a condition and the first step in biofilm formation that leads to infection [10]. Studies have reported a high prevalence of *C. albicans* and *S. aureus* rates in saliva samples from elderly adults and hospitalized patients than healthy persons [11].

Clinical investigations have showed that the presence of *C. albicans* in the peri-implantitis gingival zone was revealed [12]. *C. albicans* is associated with diseases such as peri-implantitis and periodontitis. This opportunistic pathogen is usually the cause of denture-associated stomatitis.

Streptococci, mainly in the supragingival area, are microorganisms of an indigenious oral microbiota. *S. sanguinis* is usually found in healthy individuals, but under certain conditions, it can lead to the settlement of some pathogenic microorganisms, such as *Porphyromonas gingivalis* [13].

Biofilm formation, which is created by the process of bacterial adhesion to the substrate, is related to surface properties, such as surface roughness, hydrophobicity and interaction, between existing microorganisms [14]. Streptococcus is one of the first colonizers of the initial supragingival biofilm. Also, this microorganism is present in greater quantity in the first hours of oral biofilm formation. Microoganisms from the oral cavity tend to adhere to the surface of dental materials. Certain parts of restorations are particularly susceptible to plaque accumulation, because there is poor mechanical cleaning. These are the following surfaces: interface between tooth and restoration [15], the cervical part of the proximal surface and along the gingival margin. This is a particularly sensitive topic, bearing in mind that zirconia is also used to make implant-supported prosthetic restorations, where the implant, gingiva and zirconia are in contact [16].

In order to maintain patients' oral hygiene, dentists use various manual (curettes) and machine instruments (ultrasonic scaling, brushes and pastes). The question arises whether these routines and recommended oral hygiene maintenance procedures damage the surfaces of zirconia restorations and increase surface roughness, thereby creating better conditions for the creation of a biofilm through the adhesion process of various microorganisms [17–19].

The restoration surface roughness is extremely important because of the increased accumulation of plaque, which affects the optical properties of the restoration and the wear of the opposing dentition [20].

On solid surfaces, the ability of microorganisms to aggregate and the environmental conditions are important factors in the formation of an oral biofilm. Certainly, the finishing technique on ceramics significantly affects the surface properties of these materials and the formation of the oral biofilm.

Accordingly, the aim of this study was to determine whether routine dental procedures, such as brushing and ultrasonic scaling, affect the surface microtopography and biofilm adhesion of veneered and CAD/CAM milled zirconia prosthetic restorations.

The null hypothesis of this study was that different methods of professional oral hygiene (brushing and ultrasonic scaling) would not affect the surface microtopography and biofilm adhesion of the tested zirconia samples.

2. Materials and Methods

Sample Preparation

The zirconia sample (n = 72) dimensions 4 × 4 mm and 2 mm thickness were milled from pre-sintered zirconia blanks (DD Bio ZX^2 color—High Translucent (3Y-TZP-LA, Dental Direkt, Dental Direkt GmbH, Spenge, Nordrhein Westfalen, Germany) (Figure 1) by using a 5-axis milling machine (K5, Vhf camfacture, Ammerbuch, Germany). We used burs in the CNC machine (Vhf camfacture, Ammerbuch, Germany), with burs types z200-r3d-40 (milling thickness 2 mm) and z100-r2d-40 (milling thickness 1 mm). The milling speed was 22,000–25,000 revolutions per minute (RPM). Then, the samples were sintered at 1450 °C for 2 h.

Figure 1. Zirconia disc (98 mm diameter, 2 mm thick).

The first group of zirconia samples was veneered with IPS e. max Ceram ceramic (Ivoclar Vivadent, Schaan, Licheinstain) applied in layers. The sintering process was carried out in Programat P500 (Ivoclar Vivadent, Schaan, Licheinstain). This group of samples was divided into two subgroups:

Polished (F1)—the samples were polished with polishing rubber (Edenta AG, Rheintal, St. Galen, Switzerland). The order of rubber use was from the coarsest blue, medium pink and the finest yellow.

Glazed (F2)—the samples were glazed with IPS e.max Ceram Glaze Powder (Ivoclar Vivadent, Schaan, Licheinstain).

The second group, after the CAD/CAM milling process, was divided into two subgroups:

Polished (C1)—the samples were polished with polishing rubber (Edenta AG, Switzerland). The order of rubber use was from the coarsest blue, medium pink and the finest yellow.

Glazed (C2)—the samples were glazed with IPS e.max Ceram Glaze Powder (Ivoclar Vivadent, Schaan, Licheinstain).

The samples of both groups: zirconia veneered and zirconia milled were divided into 3 subgroups:

- **0-** no treatment (control samples).
- **a-** exposed to ultrasonic scaling (with a ultrasonic scaler incorporated in a dental unit) for one minute.
- **b-** exposed to brushing with a professional dental polishing nylon brush (Flat type) and abrasive paste (Super Polish, Kerr) for one minute.

The application of brushing or ultrasonic scaling to each of the samples from the second or third subgroup of the samples lasted 1 min (in 10 rounds), imitating the procedure carried out over a period of 5 years [21]. A description is given in Table 1.

Table 1. Samples used in the study.

	Samples Name	Samples Code
veneered zirconia	Polished	F1
	Polished, treated with ultrasonic scaling	F1a
	Polished, treated with brushing	F1b
	Glazed	F2
	Glazed, treated with ultrasonic scaling	F2a
	Glazed, treated with brushing	F2b
CAD/CAM milled zirconia	Polished	C1
	Polished, treated with ultrasonic scaling	C1a
	Polished, treated with brushing	C1b
	Glazed	C2
	Glazed, treated with ultrasonic scaling	C2a
	Glazed, treated with brushing	C2b

3. Surface Characteristics of Zirconia Surface

Scanning electron microscopy (SEM)

Scanning electron microscope (Scanning Electron Microscope, Model JSM-6390, JEOL, Inc., Tokyo, Japan) was used for the quantative analysis of the surface morphology of different zirconia groups of samples. The samples were gold-sputtered, and the analyzing procedures were carried out at 150× magnification. SEM images were taken of representative zirconia samples of each subgroup in three spots. Sample regions with pronounced surface changes are shown.

Biofilm formation assay

The microbial biofilms of each bacterial/fungal species were formed on surfaces on different zirconia samples. The following strains were used: *Staphylococcus aureus* ATCC 11632, *Streptococcus sanguinis* ATTC 10556 and *Candida albicans* ATCC 10231. Each strain was formed on 16 samples (4 control—untreated material (F1, F2, C1 and C2) and 12 samples treated with ultrasonic scaling (treatment 60 s) and a brush (treatment 60 s) (F1a/b, F2a/b, C1a/b and C2a/b). Following procedure [22], with some modifications, the samples (dimensions 4 × 4 × 2 mm) were placed in 200 µL of medium at 37 °C for 24 h. For *S. aureus* and *S. sanguinis* biofilm formation, triptic soya broth with 2% glucose (Torlak, Belgrade, Serbia) was used, and for the *C. albicans* biofilm, yeast extract pepton dextrose (YPD) medium (HiMedia, Thane West, Thane, Maharashtra, India) was used.

Since only one surface of the samples was treated with ultrasonic scaling or brushing, after incubation, the biofilm was removed mechanically (with alcohol) from all other surfaces. Then, the samples were washed with sterile phosphate-buffered saline (PBS) and placed in sterile plastic tubes containing 500 µL of sterile PBS. Each tube was treated in an ultrasonic bath (40 kHz for 10 min) in order to remove the biofilm from the desired surface. The dilutions were seeded in plate counting agar (PCA) (Neogen, Heywood, UK) and incubated at 37 °C for 24 h. After 24 h, the colonies were counted using a microprocessor colony counter (Supertek, Digital Colony counter, model No. LT-37, Panchkula, Haryana, India), and the results were presented as colony-forming units (CFU)/plate [23].

Statistical Analysis

Data were analysed and presented by a Microsoft program (Excel Spreadsheet, Software Microsoft 365, Excel 2010). The average values and the standard deviations were calculated as colony-forming units (CFU). Statistical analysis was performed by the Student's *t*-test, with * = $p \leq 0.05$. The results of the treated samples were compared with the results of the untreated control samples from the same materials.

4. Results

4.1. SEM Imaging

Scanning electron microscopy (SEM) showed the morphological appearance of zirconia samples surfaces (Figures 2 and 3).

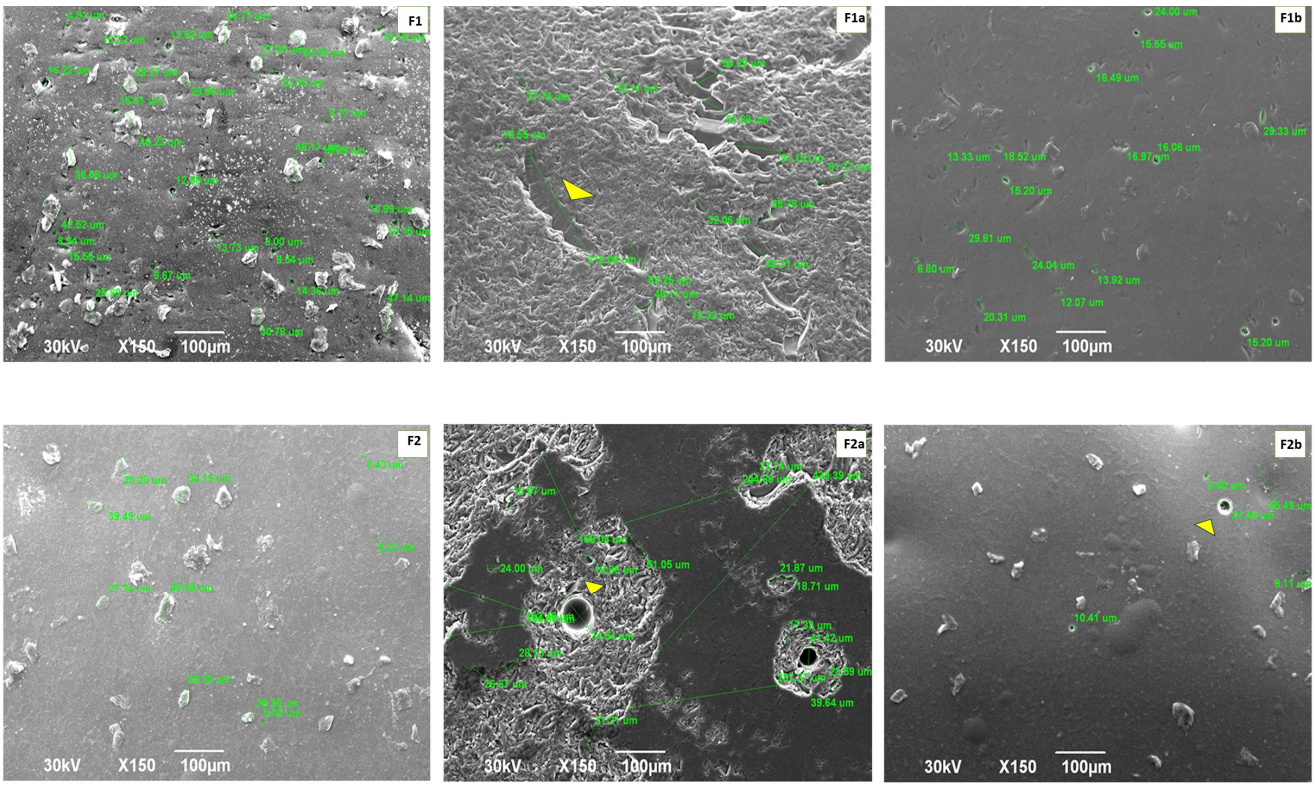

Figure 2. Representative SEM images of the veneered zirconia samples: F1—zirconia veneered and polished samples; F1a—zirconia veneered and polished samples treated with ultrasonic scaling; F1b—zirconia veneered, polished and brushed samples; F2—zirconia veneered and glazed samples; F2a—zirconia veneered and glazed samples treated with ultrasonic scaling and F2b—zirconia veneered, glazed and brushed samples.

Veneered and polished zirconia samples (F1) had a slightly irregular surface, with microcracks and visible particles on the surface. Barely visible horizontal lines might indicate the direction of polishing. However, veneered and polished zirconia after ultrasonic scaling (F1a) showed an uneven surface with pores and grooves of various sizes, cavities and chipping-type defects, which average value was 55.59 µm (highlighted by yellow arrow). On the venneered zirconia samples treated with brushing (F1b), small surface defects and groovelike formations were evident (yellow arrow). On the contrary, venneered and glazed zirconia samples (F2) showed a relatively regular surface texture with whitish particles without voids. The veneered and glazed samples treated with ultrasonic scaling (F2a) showed the most surface irregularities in the form of large defects, with an average value of 88.31 µm. The layered breaking of the material was evident. On the sides of the defects (yellow arrow) remained a porous surface. On the surfaces of the veneered and glazed samples after brushing (F2b), there were visible protrusions and traces of scratches

caused by brushing (yellow arrow). The entire surface was undulated with round-topped particules (Figure 2).

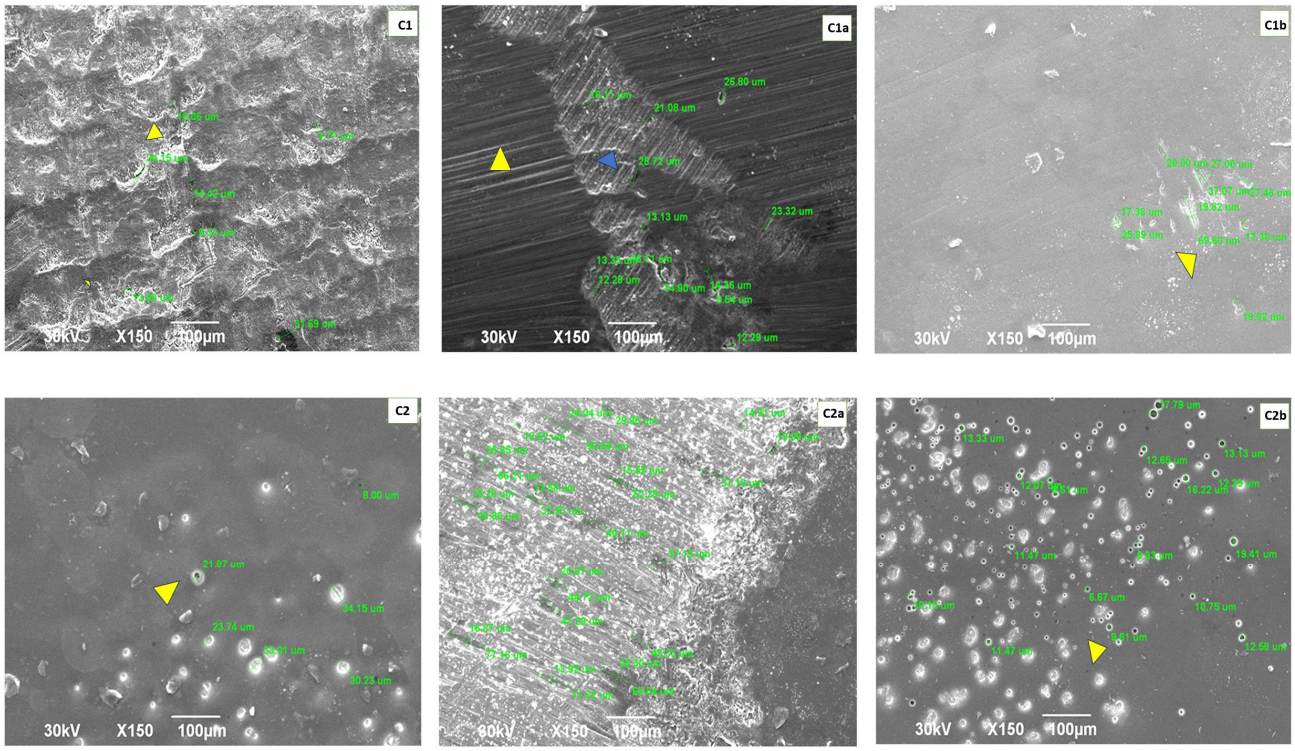

Figure 3. Representative SEM images of the CAD/CAM milled zirconia samples: C1—zirconia milled and polished samples; C1a—zirconia milled and polished samples treated with ultrasonic scaling; C1b—zirconia milled, polished and brushed samples; C2—zirconia milled and glazed samples; C2a—zirconia milled and glazed samples treated with ultrasonic scaling and C2b—zirconia milled, glazed and brushed samples.

Figure 3 shows a SEM examination of the CAD/CAM milled zirconia samples. The CAD/CAM milled and polished zirconia samples (C1) revealed an eroded surface that was created by samples made using the manufacturer's techniques and highlighted by a yellow arrow. These changes were in the forms of two types of traces, one coarser and the other finer. CAD/CAM milled and polished zirconia samples treated with ultrasonic scaling (C1a) showed a rough surface in the form of parallel scratches. Visible irregularities were those created by the effect of ultrasonic scaling (rougher, blue arrow) and others created by the effect of milling (mild, yellow arrow). As for the CAD/CAM milled and polished zirconia samples after brushing (C1b), there were slight traces, which were probably the result of the action of the brush (yellow arrow). CAD/CAM milled and glazed zirconia samples (C2) showed a slightly smoother surface layer with pale dimples and poured glaze drops (yellow arrow). On the surface of CAD/CAM milled and glazed zirconia after ultrasonic scaling (C2a), extreme irregularities in the forms of scratches and defects were visible, with a mean value of 45.38 µm. CAD/CAM milled and glazed zirconia samples after brushing (C2b) showed a porous surface with black and white holes. The black holes (blue arrow) had uneven bottoms and were not covered with glaze, as the glaze remained only on the edges. It could be assumed that the tip of the bristles of the brush and the applied abrasive polishing paste broke off parts of the glaze.

The values of the surface defects of the various groups of samples are shown in Table 2. The highest defect mean value was observed for the zirconia glazed samples treated with ultrasonic scaling, groups F2a (88.31 µm) and C2a (45.38) samples.

Table 2. Defects mean values of different zirconia samples.

Samples	Mean Values (μm)
F1	19.73
F1a	55.59
F1b	17.97
F2	25.78
F2a	88.31
F2b	21.92
C1	17.57
C1a	19.07
C1b	27.74
C2	23.5
C2a	45.38
C2b	12.25

4.2. Biofilm Formation on Different Zirconia Samples

This study determined the potential of *S. aureus*, *S. sanguinis* and *C. albicans* to form biofilms on different zirconia samples. The results of the microbial biofilms formed on the control and test samples are presented in Figures 4–6. The ones with the lowest obtained CFUs were the zirconia veneered and polished samples (F1, control) with *S. aureus* biofilm. Comparing *S. aureus* biofilm formation between the zirconia veneered and zirconia milled samples showed that the CFUs of *S. aureus* were higher on the veneered samples (Figure 4). Among the veneered samples on the glazed ones treated with brush and abrasive paste (F2b), a significantly lower (* = $p \leq 0.05$) number of *S. aureus* colonies was detected, while, among the zirconia milled samples, the glazed ones treated with ultrasonic scaling (C2a) had the lowest CFUs.

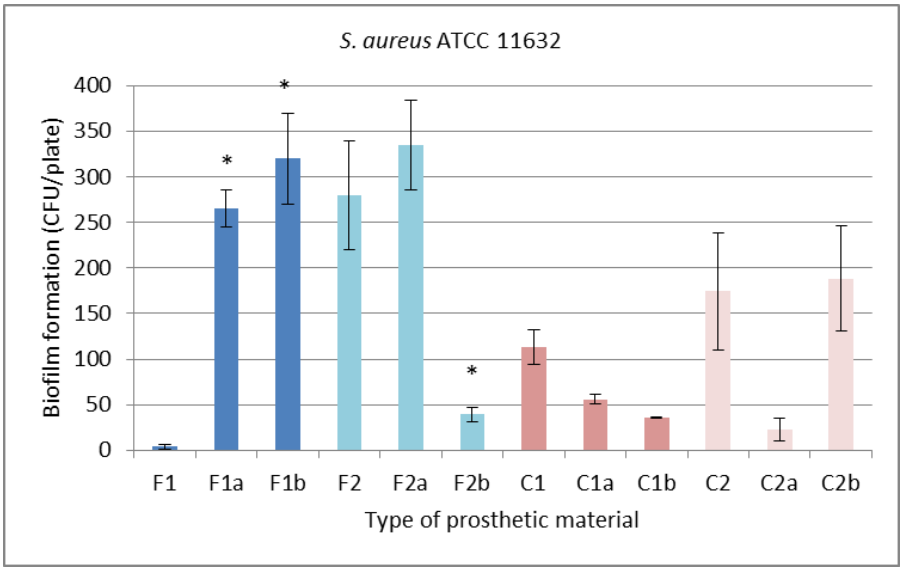

Figure 4. Biofilm formation of *Staphylococcus aureus* ATCC 11632 on different zirconia samples. The error bars indicate standard deviations. The data were presented as the mean ± SD of two replicates. * = $p \leq 0.05$. F1—zirconia veneered and polished samples; F1a—zirconia veneered and polished samples treated with ultrasonic scaling; F1b—zirconia veneered, polished and brushed samples; F2—zirconia veneered and glazed samples; F2a—zirconia veneered and glazed samples treated with ultrasonic scaling; F2b—zirconia veneered, glazed and brushed samples; C1—zirconia milled and polished samples; C1a—zirconia milled and polished samples treated with ultrasonic scaling; C1b—zirconia milled, polished and brushed samples; C2—zirconia milled and glazed samples; C2a—zirconia milled and glazed samples treated with ultrasonic scaling and C2b—zirconia milled, glazed and brushed samples.

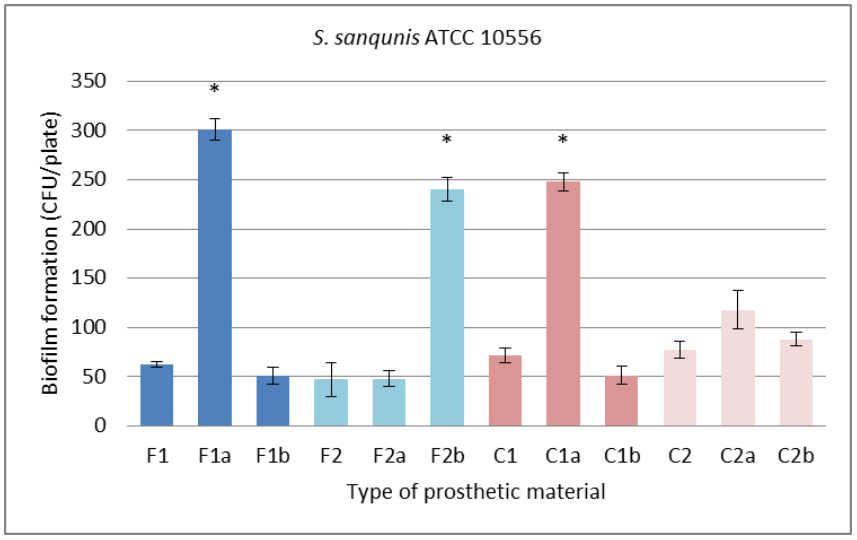

Figure 5. Biofilm formation of *Streptococcus sanguinis* ATCC 10556 on different zirconia samples. The error bars indicate standard deviations. The data were presented as the mean ± SD of two replicates. * = $p \leq 0.05$. F1—zirconia veneered and polished samples; F1a—zirconia veneered and polished samples treated with ultrasonic scaling; F1b—zirconia veneered, polished and brushed samples; F2—zirconia veneered and glazed samples; F2a—zirconia veneered and glazed samples treated with ultrasonic scaling; F2b—zirconia veneered, glazed and brushed samples; C1—zirconia milled and polished samples; C1a—zirconia milled and polished samples treated with ultrasonic scaling; C1b—zirconia milled, polished and brushed samples; C2—zirconia milled and glazed samples; C2a—zirconia milled and glazed samples treated with ultrasonic scaling and C2b—zirconia milled, glazed and brushed samples.

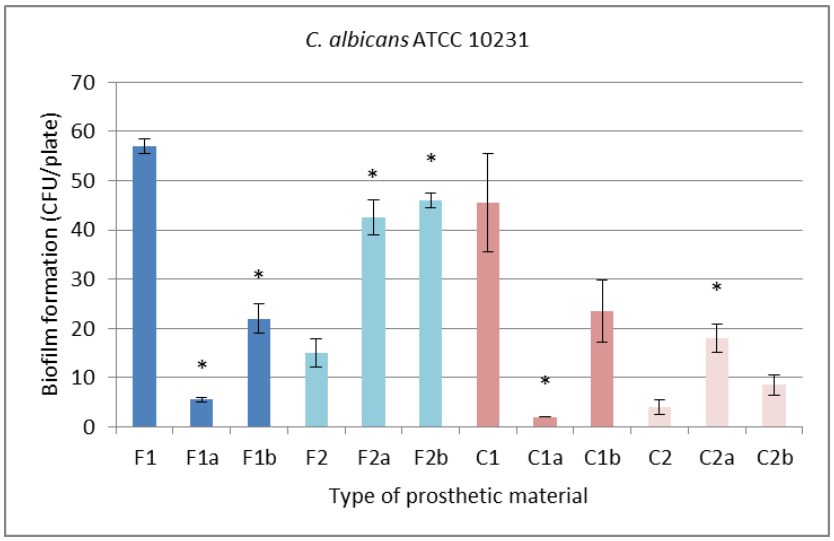

Figure 6. Biofilm formation of *Candida albicans* ATCC 10556 on different zirconia samples. The error bars indicate standard deviations. The data were presented as the mean ± SD of two replicates. * = $p \leq 0.05$. F1—zirconia veneered and polished samples; F1a—zirconia veneered and polished samples treated with ultrasonic scaling; F1b—zirconia veneered, polished and brushed samples; F2—zirconia veneered and glazed samples; F2a—zirconia veneered and glazed samples treated with ultrasonic scaling; F2b—zirconia veneered, glazed and brushed samples; C1—zirconia milled and polished samples; C1a—zirconia milled and polished samples treated with ultrasonic scaling; C1b—zirconia milled, polished and brushed samples; C2—zirconia milled and glazed samples; C2a—zirconia milled and glazed samples treated with ultrasonic scaling and C2b—zirconia milled, glazed and brushed samples.

In most of the veneered zirconia samples, *S. sanguinis* was detected in the lowest number (Figure 5). The exceptions among the veneered zirconia samples were the polished samples treated with ultrasonic scaling (F1a) and glazed and brushed samples (F2b), while, among the zirconia milled samples, the polished ones and those treated with a brush (C1b) had the lowest CFUs.

Regarding *C. albicans* biofilm, veneered samples showed higher CFUs then milled ones (Figure 6). Among the veneered zirconia samples, the lowest number of *C. albicans* colonies was detected on the polished zirconia samples treated with ultrasonic scaling (F1a), while, among the zirconia milled samples, the polished ones treated with ultrasonic scaling (C1a) had the lowest CFUs.

Overall, biofilm formation depends on the type of samples, surface properties and different sample treatments (ultrasonic scaling/brushing). Also, based on the data obtained by counting colonies, it can be concluded that the density of biofilms on different samples also depends on the biofilm-forming species of bacteria/fungi.

5. Discussion

This in vitro study investigated the effects of professional oral hygiene treatments on surface microtopography and microbial biofilm adhesion on zirconia obtained by different techniques (CAD/CAM milled and veenered). Both forms of zirconia had surfaces treated differently: glazed and polished.

The results of this study showed that the null hypothesis was rejected, because the microtopography of the sample surfaces and biofilm formations were different, depending on the oral hygiene maintenance treatment.

In this research, ultrasonic scaling and brushing were performed to simulate professional plaque control. These methods belong to the mandatory and recommended oral hygiene maintance procedure after prosthetic treatment. There is the recommended control and the performing of routine oral hygiene procedures to be carried out at least tvice a year after prosthetic and periodontal surgical treatment [24]. Previously reported data [21] showed that the the time required for the treatment of one tooth with ultrasonic scaler during periodontal therapy ranges from 0.35 to 3.90 min. Based on this study, ultrasonic scaling was performed for 1 min and repeated 10 times, imitating a procedure carried out over 5 years.

The microtopography properties of the tested zirconia samples were qualitatively evaluated by SEM. Observation with a SEM microscope was performed at 150× magnification. This magnification allowed us to see comprehensive changes on the surfaces of the tested samples. Other research performed the analysis at a higher magnification [25–27]. In this case, higher magnification would not show the changes on the surfaces caused by the applied ultrasonic scaling and brushing techniques described in this experiment in such an obvious and striking way.

In this study, the biggest surface defect was caused by the ultrasonic scaling treatment, especially on the glazed surfaces of the zirconia samples, which could be explained by the glassy structure of the glaze, which was literally broken up by ultrasound vibrations.

Some studies have showed a significant increase in surface roughness after scaling [24,28], while others have not [27]. This diversity could be caused by different timings of ultrasonic scaling in previous studies and the differently treated surfaces and types of ceramics.

Disruption of the surface topography by brushing was reported in other studies [26,29,30], but in this study, it was particularly pronounced in the glazed zirconia samples. Damage caused by brushing was less but not insignificant, especially when we knew that the microtopography of the ceramic restorations surface was related to the formation of the biofilm [31,32].

Biofilm has several stages in its formation. It represents dense microcommunities, which have the ability to adapt to changes in the environment by changing their gene expression patterns. The formation and composition of the biofilm is influenced by the method of adhesion to the surface. Each microorganism has its own unique method (binding using flagella, saws, proteins or polysaccharides). The initial phase of attachment

of microorganisms is the key phase for the formation of a biofilm, and this process can further go in two directions: the microorganisms can continue to stick to the surface and to each other, or they can return to their free form (planktonic). It could be said that microorganisms have created a unique way to survive by forming a biofilm [33].

It has been proven that a biofilm is formed on all surfaces in the oral cavity, both natural tissues and artificial materials. It is the microorganisms from the biofilm with their products that affect the inflammatory processes in the mouth and the durability of dental restorations. Scanning electron microscopy (SEM) showed a significant difference in biofilm formation on different materials and tooth enamel, and this was related to the roughness of the surfaces of the examined materials [34].

Biofilm adhesion is influenced by several factors. Most often, these are the surface characteristics of the materials, such as microtopography, surface roughness, surface free energy and chemical characteristics [35].

The adhesion of microorganisms on the surface of 3Y-TZP, after the routine procedure of ultrasonic scaling and brushing was evaluated using *S. aureus, S. sanguinis* and *C. albicans*, which are known to be initial colonizers among the microorganisms who compose dental plaque [36]. Therefore, these three representative microorganisms were chosen for this study.

The results of some studies have shown that glazed surfaces of monolithic zirconia samples have a greater surface roughness, and that was why they tend to accumulate more biofilm [37].

S. aureus showed the highest adhesion on the veneered and polished zirconia samples after ultraconic scaling, while *S. sanguis* mostly adhered to the zirconia surfaces after ultrasonic scaling. It was obvious that the defects created after the action of ultrasonic scaling favored the retention of microorganisms.

In a mutual comparison of microorganisms, *Streptococcus* showed a greather growth, independent of the type of the surface. Also, in support of the previous study, a greater colonization of microorganisms was detected on the glazed surfaces in comparison to the polished surfaces [37].

C. albicans adhered more to the vennered zirconia samples, but there was no difference in fungal adhesion between the polished and glazed sample groups, which was confirmed by the results [25,38].

Surface microtopography plays an important role in the mechanism of the adhesion of microorganisms. Microtopographic changes of the surface properties (at the micro or nano level) can occur as a consequence of the applied technique of processing of the material surface and can affect the inhibition or development of a biofilm in relation to the same material processed differently [39]. In this study, however, the highest bacterial adhesion was on the zirconia sample surfaces, with most drastic defects after ultrasonic scaling, which could be explained by the depth and rough edges of the defects that retained microorganisms.

One of the most important factors in the formation of an oral biofilm is surface roughness, which is correlated with bacterial adhesion. The surface finishing protocols (grinding directions, pressure and water coolants), as well as treatment during the routine maintenance of oral hygiene procedures, can affect the roughness of the surfaces of zirconia prosthetic restorations [40].

In the treatment of periodontal disease, patients are offered modern surgical procedures; after which, it is recommended to make prosthetic ceramic restorations in order to maintain the achieved therapeutic results. Furthermore, patients are scheduled for regular hygiene procedures that include the use of ultrasound, brushes and abrasive paste. This experiment has shown that these routine hygiene procedures should be carried out very carefully, as they can damage the surface of the prosthetic restoration.

The importance of this study is that SEM technology provided extensive data of the surface properties of zirconia samples obtained by CAD/CAM and veenering with different finishing procedures while simulating routine professional procedures for maintaining oral hyhiene in daily dental practice.

Finally, it should be pointed out that the results of this in vitro study were based on data of viable microorganism counts (microbial growth), which might show uneven results due to the general problem of achieving the reproduction of microorganisms in microbial tests and the reliable determination of cell viability. The estimation of the cell count by CFU, as used in this experiment, usually underestimates the number of viable cells present in a sample, as clumps of microbial cells can be miscounted as single colonies. This can pose a problem for statistical analyses. This study did not simulate all the relevant conditions, such as the influence of saliva, rinsing effects or changes in the pH. Therefore, it would be necessary to prove the results under in vivo conditions as well.

6. Conclusions

This work has shown that, after ultrasonic scaling and brushing, the resulting mechanical damage favors the greater adhesion of microorganisms, and this is in contrast to maintaining the achieved therapeutic results in the surgical treatment of periodontal disease. That is why it is recommended to apply ultrasonic scaling and brushing with the greatest care, especially on surfaces that are glazed, because this experiment proved that these routine hygiene procedures damage the glazed surfaces of prosthetic zirconia restorations more than polished zirconia surfaces.

Author Contributions: Conceptualization, M.P.; Methodology, A.Č. and V.B.P.; Software, A.Š.G.; Validation, M.K.; Formal analysis, M.P., A.Š.G., M.I. and J.G.; Investigation, M.P., A.Č., M.K. and V.B.P.; Resources, D.S., A.Ć. and J.G.; Data curation, D.S.; Writing—original draft, M.P. and V.B.P.; Writing—review & editing, A.Č. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No. 451-03-47/2023-01/200007).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors are grateful to DDS_Dental Lab and Jelačić Dental Lab for the laboratory support. The authors respectfully thank Vladimir Pavlović for his dedicated help with the SEM.

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

References

1. Gomes, A.L.; Montero, J. Zirconia implant abutments: A review. *Med. Oral Patol. Oral Cir. Bucal.* **2011**, *16*, e50–e55. [CrossRef] [PubMed]
2. Piconi, C.; Maccauro, G. Zirconia as a ceramic biomaterial. *Biomaterials* **1999**, *20*, 1–25. [CrossRef] [PubMed]
3. Hisbergues, M.; Vendeville, S.; Vendeville, P. Zirconia: Established facts and perspectives for a biomaterial in dental implantology. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2009**, *88*, 519–529. [CrossRef] [PubMed]
4. Lee, B.C.; Jung, G.Y.; Kim, D.J.; Han, J.S. Initial bacterial adhesion on resin, titanium and zirconia in vitro. *J. Adv. Prosthodont.* **2011**, *3*, 81–84. [CrossRef]
5. Saravi, B.; Vollmer, A.; Hartmann, M.; Lang, G.; Kohal, R.-J.; Boeker, M.; Patzelt, S.B.M. Clinical Performance of CAD/CAM All-Ceramic Tooth-Supported Fixed Dental Prostheses: A Systematic Review and Meta-Analysis. *Materials* **2021**, *14*, 2672. [CrossRef]
6. Carvalho, I.F.A.; Santos Marques, T.M.S.; Araújo, F.M.; Azevedo, L.F.; Donato, H.; Correia, A. Clinical Performance of CAD/CAM Tooth-Supported Ceramic Restorations: A Systematic Review. *Int. J. Periodontics Restor. Dent.* **2018**, *38*, e68–e78. [CrossRef]
7. Vitti, R.P.; Catelan, A.; Amaral, M.; Pacheco, R.R. Zirconium in dentistry. In *Advanced Dental Biomaterials*; Woodhead Publishing: Sawston, UK, 2019; pp. 317–345.
8. Cantner, F.; Cacaci, C.; Mücke, T.; Randelzhofer, P.; Hajtó, J.; Beuer, F. Clinical performance of tooth- or implant-supported veneered zirconia single crowns: 42-month results. *Clin. Oral Investig.* **2019**, *23*, 4301–4309. [CrossRef]
9. de Matos, J.D.M.; Lopes, G.R.S.; Queiroz, D.A.; Nakano, L.J.N.; Ribeiro, N.C.R.; Barbosa, A.B.; Anami, L.C.; Bottino, M.A. Dental Ceramics: Fabrication Methods and Aesthetic Characterization. *Coatings* **2022**, *12*, 1228. [CrossRef]

10. Vieira Colombo, A.P.; Magalhães, C.B.; Hartenbach, F.A.; Martins do Souto, R.; Maciel da Silva-Boghossian, C. Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance. *Microb. Pathog.* **2016**, *94*, 27–34. [CrossRef]
11. Offenbacher, S.; Barros, S.P.; Singer, R.E.; Moss, K.; Williams, R.C.; Beck, J.D. Periodontal disease at the biofilm–gingival interface. *J. Periodontol.* **2007**, *78*, 1911–1925. [CrossRef]
12. Heitz-Mayfield, L.J.; Lang, N.P. Comparative biology of chronic and aggressive periodontitis vs.peri-implantitis. *Periodontology* **2010**, *53*, 167–181. [CrossRef]
13. Pita, P.P.C.; Rodrigues, J.A.; Ota-Tsuzuki, C.; Miato, T.F.; Zenobio, E.G.; Giro, G.; Figueiredo, L.C.; Gonçalves, C.; Gehrke, S.A.; Cassoni, A. Oral streptococci biofilm formation on different implant surface topographies. *BioMed Res. Int.* **2015**, *2015*, 159625. [CrossRef]
14. Li, J.; Helmerhorst, E.J.; Leone, C.W.; Troxler, R.F.; Yaskell, T.; Haffajee, A.D.; Socransky, S.S.; Oppenheim, F.G. Identification of early microbial colonizers in human dental biofilm. *J. Appl. Microbiol.* **2004**, *97*, 1311–1318. [CrossRef]
15. Øilo, M.; Bakken, V. Biofilm and dental biomaterials. *Materials* **2015**, *8*, 2887–2900. [CrossRef]
16. Cinquini, C.; Alfonsi, F.; Marchio, V.; Gallo, F.; Zingari, F.; Bolzoni, A.R.; Romeggio, S.; Barone, A. The Use of Zirconia for Implant-Supported Fixed Complete Dental Prostheses: A Narrative Review. *Dent. J.* **2023**, *11*, 144. [CrossRef]
17. Hjerppe, J.; Rodas, S.; Korvala, J.; Pesonen, P.; Kaisanlahti, A.; Özcan, M.; Suojanen, J.; Reunanen, J. Surface Roughness and *Streptococcus mutans* Adhesion on Metallic and Ceramic Fixed Prosthodontic Materials after Scaling. *Materials* **2021**, *14*, 1027. [CrossRef]
18. Jaeggi, M.; Gyr, S.; Astasov-Frauenhoffer, M.; Zitzmann, N.U.; Fischer, J.; Rohr, N. Influence of different zirconia surface treatments on biofilm formation in vitro and in situ. *Clin. Oral Implants Res.* **2022**, *33*, 424–432. [CrossRef]
19. Chen, H.L.; Yang, S.; Yu, P.; Wu, J.; Guan, H.; Wu, Z. Comparison of bacterial adhesion and biofilm formation on zirconia fabricated by two different approaches: An in vitro and in vivo study. *Adv. Ceram. Dent. Struct. Funct. Bioceram.* **2020**, *119*, 323–331. [CrossRef]
20. Alves, L.M.M.; Contreras, L.P.C.; Bueno, M.G.; Campos, T.M.B.; Bresciani, E.; Valera, M.C. The wear performance of glazed and polished full contour zirconia. *Braz. Dent. J.* **2019**, *30*, 511–518. [CrossRef]
21. Tunkel, J.; Heinecke, A.; Flemmig, T.F. A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. *J. Clin. Periodontol.* **2002**, *29* (Suppl. S3), 72–81. [CrossRef]
22. Ivanovic, V.; Popovic, D.; Petrovic, S.; Rudolf, R.; Majerič, P.; Lazarevic, M.; Djordjevic, I.; Lazic, V.; Radunovic, M. Unraveling the Antibiofilm Activity of a New Nanogold Resin for Dentures and Epithesis. *Pharmaceutics* **2022**, *14*, 1513. [CrossRef] [PubMed]
23. Brugger, S.D.; Baumberger, C.; Jost, M.; Jenni, W.; Brugger, U.; Mühlemann, K. Automated Counting of Bacterial Colony Forming Units on Agar Plates. *PLoS ONE* **2012**, *7*, e33695. [CrossRef] [PubMed]
24. Nakazawa, K.; Nakamura, K.; Harada, A.; Shirato, M.; Inagaki, R.; Örtengren, U.; Kanno, T.; Niwano, Y.; Egusa, H. Surface properties of dental zirconia ceramics affected by ultrasonic scaling and low-temperature degradation. *PLoS ONE* **2018**, *13*, e0203849. [CrossRef]
25. Zupancic Cepic, L.; Dvorak, G.; Piehslinger, E.; Georgopoulos, A. In vitro adherence of Candida albicans to zirconia surfaces. *Oral Dis.* **2020**, *26*, 1072–1080. [CrossRef]
26. Lee, W.F.; Iwasaki, N.; Peng, P.W.; Takahashi, H. Effect of toothbrushing on the optical properties and surface roughness of extrinsically stained high-translucency zirconia. *Clin. Oral Investig.* **2022**, *26*, 3041–3048. [CrossRef] [PubMed]
27. Lee, J.H.; Kim, S.H.; Han, J.S.; Luke Yeo, I.S.; Yoon, H.I.; Lee, J. Effects of ultrasonic scaling on the optical properties and surface characteristics of highly translucent CAD/CAM ceramic restorative materials: An in vitro study. *Ceram. Int.* **2019**, *45*, 14594–14601. [CrossRef]
28. Yoon, H.I.; Noh, H.M.; Park, E.J. Surface changes of metal alloys and high-strength ceramics after ultrasonic scaling and intraoral polishing. *J. Adv. Prosthodont.* **2017**, *9*, 188–194. [CrossRef]
29. Sulaiman, T.A.; Camino, R.N.; Cook, R.; Delgado, A.J.; Roulet, J.F.; Clark, W.A. Time-lasting ceramic stains and glaze: A toothbrush simulation study. *J. Esthet. Restor. Dent.* **2020**, *32*, 581–585. [CrossRef]
30. Fiorin, L.; Oliveira, P.E.B.S.; Silva AOd Faria, A.C.L.; Macedo, A.P.; Ribeiro, R.F.; Rodrigues, R.C.S. Wear Behavior of Monolithic Zirconia after Staining, Glazing, and Polishing Opposing Dental Restorative Materials: An In Vitro Study. *Coatings* **2023**, *13*, 466. [CrossRef]
31. Yuan JC, C.; Barão VA, R.; Wee, A.G.; Alfaro, M.F.; Afshari, F.S.; Sukotjo, C. Effect of brushing and thermocycling on the shade and surface roughness of CAD-CAM ceramic restorations. *J. Prosthet. Dent.* **2018**, *119*, 1000–1006. [CrossRef]
32. Kreve, S.; Dos Reis, A.C. Effect of surface properties of ceramic materials on bacterial adhesion: A systematic review. *J. Esthet. Restor. Dent.* **2022**, *34*, 461–472. [CrossRef] [PubMed]
33. O'Toole, G.; Kaplan, H.B.; Kolter, R. Biofilm Formation as Microbial Development. *Annu. Rev. Microbiol.* **2000**, *54*, 49–79. [CrossRef]
34. Engel, A.S.; Kranz, H.T.; Schneider, M.; Tietze, J.P.; Piwowarcyk, A.; Kuzius, T.; Arnold, W.; Naumova, E.A. Biofilm formation on different dental restorative materials in the oral cavity. *BMC Oral Health* **2020**, *20*, 162. [CrossRef] [PubMed]
35. Song, F.; Koo, H.; Ren, D. Effects of material properties on bacterial adhesion and biofilm formation. *J. Dent. Res.* **2015**, *94*, 1027–1034. [CrossRef] [PubMed]

36. Karygianni, L.; Jähnig, A.; Schienle, S.; Bernsmann, F.; Adolfsson, E.; Kohal, R.J.; Chevalier, J.; Hellwig, E.; Al-Ahmad, A. Initial Bacterial Adhesion on Different Yttria-Stabilized Tetragonal Zirconia Implant Surfaces in Vitro. *Materials* **2013**, *6*, 5659–5674. [CrossRef]
37. Dal Piva, A.M.O.; Contreras, L.P.C.; Ribeiro, F.C.; Anami, L.C.; Camargo, S.E.A.; Jorge, A.O.C.; Bottino, M.A. Monolithic Ceramics: Effect of Finishing Techniques on Surface Properties, Bacterial Adhesion and Cell Viability. *Oper. Dent.* **2018**, *43*, 315–325. [CrossRef]
38. de Carvalho, I.H.G.; da Silva, N.R.; Vila-Nova, T.E.L.; de Fatima, D.; de Almeida, L.; Veríssimo, A.H.; de Melo, R.M.; Zhang, Y.; de Assunção, E.; Souza, R.O. Effect of finishing/polishing techniques and aging on topography, C. albicans adherence, and flexural strength of ultra-translucent zirconia: An in situ study. *Clin. Oral Investig.* **2022**, *26*, 889–900. [CrossRef]
39. Han, A.; Tsoi, J.K.-H.; Matinlinna, J.P.; Chen, Z. Influence of Grit-Blasting and Hydrofluoric Acid Etching Treatment on Surface Characteristics and Biofilm Formation on Zirconia. *Coatings* **2017**, *7*, 130. [CrossRef]
40. Lee, D.H.; Mai, H.N.; Thant, P.P.; Hong, S.H.; Kim, J.; Jeong, S.M.; Lee, K.W. Effects of different surface finishing protocols for zirconia on surface roughness and bacterial biofilm formation. *J. Adv. Prosthodont.* **2019**, *11*, 41–47. [CrossRef]