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Abstract

Purpose: This study aimed to evaluate the effect of two types of computer-aided design/computer-aided manufacturing ceramic occlusal veneers cemented to the teeth with or without immediate dentin sealing (IDS) on the shear bond strength of posterior occlusal veneers after thermodynamic loading. **Patients and methods:** In all, 24 anonymous extracted sound permanent mandibular second molars were prepared to replicate flat occlusal surfaces. Samples were distributed into two groups according to the received occlusal veneer material: lithium disilicate ceramic (IPS-e.max press, Ivoclar Vivadent) and ultra-translucent zirconia (Katana UTML Kuraray Noritake Dental Inc., Japan). Samples were divided into two equal groups ($n = 12$). Each group was subdivided into two subgroups ($n = 6$) regarding the dentin conditioning method [IDS vs. delayed dentin sealing, (DDS)]. The samples were cemented with adhesive resin cement and subjected to thermomechanical fatigue, and then the shear bond strength was measured using the universal testing machine. **Results:** Statistical analysis showed that the highest statistically significant shear load mean value was recorded for the e.max group with the IDS subgroup (519.7 N) followed by the e.max group with the DDS subgroup (510.88 N) and then the zirconia group with the IDS subgroup (332.625 N). The lowest statistically significant shear load mean value was recorded for the zirconia group with DDS subgroup (260.238 N) as proved by the one-way ANOVA ($P \leq 0.0001 < 0.05$). Pairwise Tukey's post hoc test showed nonsignificant difference between (treated and nontreated) dentin subgroups with the e-max group. **Conclusion:** IDS improves the bond strength of occlusal veneers. Furthermore, the bonding technique may have critical effects on the dentin bond strength of occlusal veneer materials.

Keywords: Immediate dentin sealing, Occlusal veneers, Shear bond strength, Thermomechanical aging

1. Introduction

One of the main aims of modern restorative dentistry is to provide good aesthetic results without renouncing the preservation of biological structures. Restorative methods have grown simpler, more predictable, and capable of giving satisfying aesthetic and functional results in both clinical and laboratory settings [1].

The wear of the occlusal surfaces of teeth over time is a natural part of existence. Excessive wear of the occlusal surface, however, can cause pulpal pathology, occlusal disharmony, reduced function, and aesthetic deformity. Tooth wear has a multifaceted etiology that includes dietary habits, medical problems, and oral habits that cause enamel and dentine attrition and abrasion [2].

One type of design for less invasive preparation of severely worn teeth is occlusal veneer, the non-retaining restoration that entirely covers the occlusal surface [3].

Recently; computer-aided design/computer-aided manufacturing (CAD/CAM) technology has been progressively used to manufacture dental prostheses. Industrially manufactured CAD/CAM ceramic blocks have been introduced to dentistry to improve the mechanical qualities of restorations [4]. The use of recent generations of all-ceramic restorations and

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adhesive techniques can increase the conservation of remaining tooth structures, particularly in single-tooth therapy [1].

The availability of ceramic materials that could be fabricated in thin disks and still have good mechanical characteristics accompanied by enhanced bonding properties make occlusal veneers a credible effective prosthetic treatment option [5]. Zirconia and lithium disilicate are suitable materials for this kind of restoration. They can be fabricated by a variety of processes, including milling, heat-pressing, and the most recent three dimensional printing [6].

The introduction of a more advanced lithium disilicate, IPS e-max, has significantly enhanced this ceramic material. The bonding capability of lithium disilicate glass-ceramic has been well documented, giving it a combination of being strong ceramic and yet still etchable to achieve durable bonded strong restorations [5].

Heat-pressed lithium disilicate restorations exhibit better mechanical qualities with improved flexural strength and fracture toughness compared with machinable lithium disilicate restorations. Wax patterns can be fabricated through a recently standardized process with the use of CAD/CAM machines and millable higher synthetic wax and polymers. Therefore, it is believed that this advanced process leads to a decrease in errors caused by manual processing and prolonged laboratory steps [5].

Ultra-translucent multilayer yttria-stabilized katana zirconia is a recent generation material with great aesthetics and translucency, especially for the anterior teeth. It combines high-profile mechanical and excellent optical properties [7]. The functionalization process or surface roughening process is essential when using zirconium material because it increases the shear bonding strength between the tooth structure and the restoration's surface. One of the most effective techniques for surface abrasion is sandblasting with 50 μm Al_2O_3 particles [8].

The standard approach of bonding indirect restorations to dentin surface by resin cement was previously known as 'delayed dentin sealing.' This method relies on delayed dentin cementation following a provisional restorative time. Although, this approach does not offer ideal bonding circumstances because dentin contamination weakens the bond between the dentin substrate and restorations [9]. To get optimum cementation efficiency, different techniques were developed by researchers. The concept of bonding the dentin surface immediately after the dentin is prepared and before the impression is taken known as 'immediate dentin sealing' offers significant benefits [9].

The immediate dentin sealing technique is based on four basic principles. First, only freshly cut, uncontaminated dentin gives the most suitable substrate for bonding; otherwise, the bond strength will be low. Second, the hybrid layer can collapse due to pressure from composite placement or restoration, if the dentin bonding agent and overlaying composite are polymerized together. Thus, procuring the dentin bonding agent leads to enhanced bond strength. Third, immediate dentin sealing and delayed restoration installation allow dentin bonds to mature in an environment without occlusal pressure and superimposed composite shrinkage. Fourth, immediate dentin sealing minimizes fluid and bacterial penetration [10].

In-depth research and considerable advancements in immediate dentin sealing have produced favorable outcomes in terms of adhesion strength, gap formation, bacterial leakage, and hypersensitivity after cementation [11].

Therefore, the aim of the study was to evaluate the effect of immediate dentine sealing on the bond strength of e-max press and ultra-translucent multilayered zirconia occlusal veneers after thermodynamic loading.

2. Patients and methods

To evaluate the effect of immediate dentine sealing on the bond strength of two types of occlusal ceramic veneers (lithium disilicate and ultra-translucent zirconia) after thermodynamic loading cemented with/without immediate dentine sealing, *t*-test, or an equivalent non-parametric test will be used for comparison between groups. According to a previous study, the mean bond strength ranged from 15.2 ± 3.3 to 21.2 ± 6.1 without sealing, while using immediate dentine sealing the mean ranged from 15.5 ± 2.2 to 26.6 ± 6.5 [12].

A total sample size of 24 (12 in each group; 6 in each subgroup) will be sufficient to detect a large effect size (d) ranging from 1.22 to 2.28, with an actual power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for the two-sided hypothesis test.

In all, 24 anonymous extracted sound mandibular permanent second molars were selected and used in this study. Selected molars were free from caries, old restorations, cracks, or any defects. Ethical approval for the use of extracted human teeth was obtained in accordance with the guidelines of Research Ethic Committee (REC) of the Faculty of Dental Medicine for Girls, Al Azhar University. Ethical code: (REC-CR-22-06).

Selected molars had similar dimensions measured using a digital caliper (Mastercraft Digital Caliper;

Canada) in mesiodistal and buccolingual directions at the cementoenamel junction and at the highest convexity of the height of contour. The molars were of average dimensions of (7.5 mm ± 0.5) crown length (from the cementoenamel junction to the mesiobuccal cusp), (10.5 mm ± 0.5) in the buccolingual width, and (11 mm ± 0.5) in mesiodistal width. Tissues and calculus deposits were removed with an ultrasonic scaler; the teeth were kept in distilled water at room temperature.

2.1. Sample grouping

The samples were randomly divided into two equal main groups according to ceramic material ($n = 12$).

Group I Lithium-disilicate glass-ceramic occlusal veneers (IPS E-max press HT). Group II: Ultra-translucent multilayared zirconia ceramic occlusal veneers (UTML Katana Zirconia).

All samples of each group were subdivided into two subgroups, according to the bonding technique of occlusal veneers ($n = 6$).

Subgroup A: Occlusal veneers cemented by adhesive resin cement with immediate dentin sealing (IDS).

Subgroup B: Occlusal veneers cemented by adhesive resin cement with delayed dentin sealing (DDS).

2.1.1. Teeth preparation

Molars were inserted in epoxy resin (Chemapoxy resin, CMB Chemicals, Egypt) blocks using round plastic rings as molds. A dental surveyor was used for centralizing the molars vertically up to 2 mm under cementoenamel junction (CEJ) in plastic rings during epoxy resin block construction similar to the bone level. All teeth received standardized preparation similar to a flat surface using a diamond saw (ISOMET 4000, Buehler, and Lake Bluff, IL, USA). The preparation was made 4 mm occlusal to the cementoenamel junction measured at the maximum convexity of the cervical line at the mid-mesial surface using a digital caliper exposing peripheral enamel and central bare dentin [13]. Two round shallow indentations (1 mm in diameter) were prepared on the occlusal surface of every prepared tooth using a high-speed 1 mm sized round-ended diamond rotary bur (#ZR 850 FG.01, Komet USA), 2 mm from the mid-mesial and mid-distal external surfaces of each tooth to aid in seating of occlusal veneers and prevent rotation of the restoration during cementation (Fig. 1).

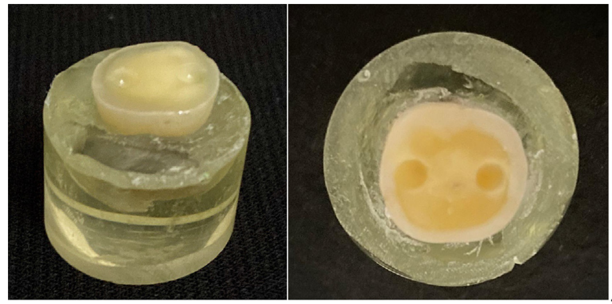

Fig. 1. Exposed dentin surface.

2.2. Immediate dentin sealing

In IDS subgroups, freshly cut and uncontaminated surfaces were etched with phosphoric acid 35% (Select HV Etch, Bisco, IL, USA) as it was applied onto the prepared enamel margins first and then to the exposed dentin. The etchant was left to react on the enamel for 30 s and on the dentin for 15 s. Then, the etchant was rinsed thoroughly from all surfaces with vigorous water spray for 30 s and then dried with oil-free air spray.

Subsequently, the bonding agent (All-Bond Universal, Bisco, IL, USA) was applied to the surfaces of teeth and rubbed with microbrush for 10–15 s, air-dried for 10 s, then cured with light cure (Elipar LED curing unit, 3 M ESPE, Seefeld, Germany) of 1200 mW/cm^2 for 20 s followed by the application of a thick layer of glycerin gel to the sealed surface.

An air-blocking barrier was applied to polymerize the oxygen-inhibited layer, and then another 10 s of light curing was applied. Following rinsing with water was applied.

2.3. Fabrication of occlusal veneers

All the prepared teeth were digitally scanned using the smart optics scanner. Each veneer restoration was designed using the CAD-CAM software. The thickness of all veneer restorations was 1.5 mm at the cusp tip and 1 mm at the central fossa. Then, the restorations were milled (CAD/CAM Roland machine, Japan) from zirconia blank (Katana UTML Kuraray Noritake Dental Inc., Japan), and CAD wax disk (Yamahachi wax disc, japan). After wax milling, e.max samples were fabricated using the heat press technique where the wax was eliminated, and the IPS e-max ceramic ingots (IPS e-max press LT, Ivoclar Vivadent AG, Schaan Liechtenstein) were pressed in the pressing furnace (Programat EP3010; Ivoclar Vivadent AG) to produce IPS e-max occlusal veneers for group I. Zirconia samples were sintered and glazed according to the manufacturer's recommendations to produce occlusal veneers for group II.

2.4. Cementation of the occlusal veneers

2.4.1. Surface treatment of the E-max occlusal veneers

Etching of the bonding surface of the veneers was made using a Porcelain Etch with 9.5% hydrofluoric acid gel (BISCO, Illinois, USA) for 20 s. Occlusal veneers were then rinsed with water spray thoroughly for another 60 s and then dried with oil-free air. The surfaces were salinated with the silane coupling agent (Porcelain primer, Bisco, Illinois, U.S.A.) and left to react for 60 s, then gently dried for 5 s with water/oil-free compressed air according to manufacturer's instructions.

2.5. Treatment of the fitting surfaces of zirconia occlusal veneers

Intaglio surfaces of all zirconia occlusal veneers were air-borne particles abraded with 50 μm alumina at a pressure of 2 bars from 10 mm distance, and then cleaned with a steam cleaner and dried with compressed air, and then treated with ceramic primer (Z-Primer Plus, Bisco, IL, USA) for 10 s and then dried with air for 5 s.

2.6. Surface treatment of teeth

For the IDS subgroup, air abrasion for sealed dentin was made using 50 μm AL_2O_3 at a distance of 10 mm under pressure of 2 bars, until it became dull and then enamel margins were etched by 35% phosphoric acid (Select HV Etch, Bisco, IL, USA) for 30 s, and then rinsed and dried followed by applying a bonding agent (All-Bond Universal, Bisco, IL, USA) to the whole surface for 20 s. Then, thinning of the adhesive was done with a gentle air stream for 5 s with no polymerization.

For occlusal veneers cemented by adhesive resin cement without immediate dentin sealing in the DDS subgroup, the enamel margins were etched with 35% phosphoric acid for 30 s and dentin for 15 s. Then, they were rinsed and dried, followed by applying a bonding agent, to the tooth structure for 20 s and then thinning the adhesive with a gentle air stream for 5 s, with no polymerization.

All occlusal veneers were cemented to their respective teeth using resin luting cement (Duo-Link Universal, Bisco, IL, USA), which was applied to the fitting surface of all veneers. The veneers were placed gently to their respective teeth under 3 Kg weight, after 3 s of light curing, excess cement was removed with cotton pellets and complete light curing of each side of the restoration was done for 40 s.

2.7. Thermomechanical aging

Mechanical aging was performed using a four-station multimodal Robota chewing simulator integrated with thermo-cyclic protocol operated on servomotors with parameters (Model ACH-9075DC-T, AD-Tech Technology CO, LTD., Germany). Vertical and horizontal movements were applied simultaneously in the thermodynamic condition. Each of the chambers consists of an upper Jackob's chuck as a hardened steel antagonist holder that can be tightened with a screw and a lower plastic sample holder in which the sample can be embedded. A weight of 5 kg, comparable to 49 N of chewing force was exerted with thermal cycles of between 5 °C and 55 °C. The test was repeated 37 500 cycles to clinically simulate 3 months of chewing [14].

2.8. Shear bond strength

The baseline shear bond strength (SBS) tests were performed for all the samples 24 h after cementation while the samples were immersed in distilled water at 37 °C. Tests were performed using a universal testing machine (Fig. 2); (0.5 mm/min) the shear load was applied by a steel rod at the cement line between the tooth and the occlusal veneer. SBS values were recorded in newton's (N) and converted into megapascals (Mpa).

2.9. Failure mode analysis

After shear bond test, samples in the test groups were viewed using a USB digital microscope (U500x

Fig. 2. Universal testing machine.

Digital Microscope, Guangdong, China), and the images were captured and transferred to a personal computer equipped with the image-tool software (Image J 1.43U, National Institute of Health, USA) to determine failure mode pattern according to the following categorization: adhesive failure (at the ceramic–cement interface or at the dentin–cement interface), cohesive failure (the fracture occurring either within the ceramic or within dentin), and mixed failure (combination of adhesive and cohesive failures were present simultaneously) [15].

3. Results

The results were analyzed using GraphPad InStat (Graph Pad, Inc.) software for Windows. A value of $P \leq 0.05$ was considered statistically significant. Continuous variables were expressed as the mean and standard deviation. After homogeneity of variance and normal distribution of errors had been confirmed, a one-way analysis of variance was performed followed by Tukey's post hoc test if showed significance. Student's t-test was done for compared pairs. Two-way ANOVA compared the effect of each factor (material and dentin treatment). Chi-square test was used to compare failure mode patterns. Sample size ($n = 6$) was large enough to detect large effect sizes for main effects and pairwise comparisons, with the satisfactory level of power set at 80% and a 95% confidence level.

3.1. Results of shear bond test

3.1.1. Effect of dentin sealing

Descriptive statistics showing mean values and standard deviation of shear bond strength test results measured in Newton (N) for both material groups as a function of prior dentin surface treatment are summarized in Table 1.

Table 1. Results of shear bond strength test (Mean values ± SDs) comparing between both groups as a function of dentin treatment.

Variables	Mean ± SD	95% CI	
		Low	High
E.max			
Subgroup A (IDS)	519.7^{A} ± 81.08	454.82	584.58
Subgroup B (DDS)	510.88^{A} ± 40.784	478.25	543.51
Zirconia			
Subgroup A (IDS)	332.625^{B} ± 18.1	318.14	347.11
Subgroup B (DDS)	260.238^{C} ± 16.25	247.24	273.24
ANOVA			
P value	<0.0001*		

Different superscripted capital letters in the same column indicate statistically significant difference (Tukey's $P < 0.05$) ns: nonsignificant ($P > 0.05$)*, significant ($P < 0.05$).

The highest statistically significant shear bond strength mean value was recorded for the e.max group with IDS subgroup (519.7 N) followed by e.max group with the DDS subgroup (510.88 N) and then the Zirconia group with IDS subgroup (332.625 N).

The lowest statistically significant shear load mean value was recorded for Zirconia group with the DDS subgroup (260.238 N) as proved by one-way ANOVA ($P \leq 0.0001 < 0.05$).

Pairwise Tukey's post hoc test showed nonsignificant difference between IDS and DDS subgroups with the e.max group as shown in Table 1.

For the e.max group, it was found that e.max samples recorded statistically nonsignificant higher shear load mean value (519.7 N) with the immediate dentin sealing subgroup than the DDS subgroup (510.88 N) as demonstrated by Student's paired *t*-test ($P = 0.8167 > 0.05$) as shown in Table 1.

For the zirconia group, it was found that zirconia samples group recorded statistically significantly higher shear load mean value (332.625 N) with the treated dentin subgroup than the nontreated dentin subgroup (260.238 N) as indicated by Student's paired *t*-test ($P \leq 0.0001 < 0.05$) as shown in Table 1.

3.2. Effect of material on e.max vs. zirconia

For subgroups IDS: it was found that the e.max group recorded statistically significant higher shear bond strength mean value (519.7 N) than the zirconia group (332.625 N) as shown by the Student's t-test ($P = 0.0003 < 0.05$).

For subgroups DDS: it was found that the e.max group recorded statistically significant higher shear bond strength mean value (510.88 N) than the zirconia group (260.238 N) as exhibited by Studen's *t*-test ($P \leq 0.0001 < 0.05$).

3.3. Total effect of material type

Regardless of dentin treatment, totally it was found that the e.max group recorded statistically significant higher shear bond strength mean value than te zirconia group as indicated by two-way ANOVA test ($P = 0.0193 < 0.05$) Table 2.

Table 2. Comparison of total shear load results (Mean values SDs) as a function of material type.

Variables	Mean ± SD	Statistics
		P value
Material type		
E.max group	515.29 ± 60.93	<0.0001*
Zirconia group	296.431 ± 17.18	

3.4. Failure mode pattern

For e.max group with immediate dentin sealing, majority of samples showed adhesive and cohesive failure mode patterns (40%) while minority exhibited a mixed pattern (20%). For the e.max group with DDS, a mixed pattern was predominant (60%), while a minority exhibited adhesive and cohesive failure mode patterns (20%).

For the Zirconia group with immediate dentin sealing, majority of samples showed adhesive pattern (80%) while the minority exhibited a mixed pattern (20%) with no record for cohesive failure mode (0%). For the Zirconia group with DDS, all samples showed an adhesive pattern predominantly (60%) with no record for mixed or cohesive failure mode (0%).

The variance in failure modes between both groups was statistically significant as revealed by the χ^2 test ($P \leq 0.0001 < 0.05$).

4. Discussion

The null hypothesis of the present study was partially rejected according to the statistical analysis of the obtained data. Tabletop occlusal veneers are viewed as an appealing prosthetic alternative to restore function and anatomy for teeth with severe occlusal wear as adopting these minimally invasive techniques could preserve 40% of the prepared tooth structure [5]. The tolerance of these innovative restorations is affected by a number of items, including the type of restoration and its thickness, mechanical qualities of restorative materials, position of the tooth being restored, patient's occlusal forces, bonding substrate, and the bonding technique [16].

In this study, human natural teeth were used because their elastic properties, bonding ability, and strength better simulate the clinical conditions [4]. To prevent them from drying out and becoming brittle, all teeth were kept in saline until use [1]. The irregular occlusal anatomy of human natural teeth crowns makes it difficult to use occlusal veneers having a uniform thickness. In this study, CAD/CAM technology was selected because it allows for precise control over the thickness and geometry of the restorations during fabrication. It also offers a standardized fabrication technique that prevents laboratory variations [4].

In addition, the preparation of the occlusal veneer was restricted to the occlusal surface alone, with no finishing line extending to the axial walls. In comparison to conventional onlays and complete-coverage crowns, occlusal veneers, thin overlay restorations with a non-retentive design, represent a conservative treatment option. Occlusal veneers covering only the occlusal surface created minimal stresses within the restoration [17]. A previous study discovered that occlusal veneers that covered the occlusal surface only exhibited lower maximum stresses in the restoration and higher fracture strength compared with conventional full-coverage crowns and occlusal veneers that only partially covered the axial surfaces [3].

The two materials used (e.max and UTML zirconia) have different qualities and benefits that could raise the survival rates of occlusal veneers. The high fracture toughness and good bonding ability due to effective surface roughness by applying hydrofluoric acid etchant provides lithium disilicate glass-ceramic its unique nature [18,19].

Regardless of the microstructure of zirconia, the adhesive luting technique requires surface treatment of the restoration's intaglio surface; it strengthens the bond between resin cement and the restorative material [20]. So, in the current study, the bond strength of the two materials is compared.

Dentinal adhesion is more complicated due to the porous structure, wettability, and hydroxyapatite composition of the collagen−protein matrix. The fundamental concept for bonding adhesive systems to dentin is micromechanical adhesion. Therefore, the bonding efficiency of traditional resin cement was related to the quality of hybridization produced by the dentin bonding agents applied to the dentin surface [9]. In the present study, immediate dentin sealing approach was used, which represents the gold standard in this situation as the freshly cut dentin gives the most suitable substrate for bonding [4].

Different techniques were used to test the bond strength of cement to substrates, and shear tests were used in this study. It should be noted that the shear bond strength depends on two interfaces (resin cement−restorative material and dentin−resin cement). According to data of present study, the shear bond strength values of the groups showed statistically significant differences according to the type of ceramic and dentin treatment.

In addition, higher bond strengths to dentin can be achieved by pre-curing dentin adhesives. In the DDS technique, the delayed unpolymerized adhesive layer that remained until the cement was applied could result in collagen network or uncured dentin−resin hybrid layer collapse during restoration seating because of the pressure exerted at this stage [21]. However, in the IDS process, the resin has cured and has penetrated the hybrid layer. Besides, the application of IDS allows the release of all the stresses and strains developed at the dentin bonding area before the insertion of the restoration and the real functional forces that will be applied on this tooth [14].

The findings of this study are congruent with research result which concluded that the use of IDS has a significant positive impact. According to their findings, the freshly cut dentin gives the most promising bonding substrate at preparation time and before being contaminated by any remnants of the impression material or temporary cement [21]. A previous study concluded the same finding that immediate dentin sealing improves the bond strength of indirect restorations [9].

The exception in the present study was with group A. One hypothesis would be the nonsignificant variance between IDS and DDS. The observed variations in failure loads were probably primarily due to the slight anatomical changes in tooth size and characteristics. There was an attempt to use teeth of similar size. In addition, all occlusal veneers are designed with identical occlusal anatomy, thickness, and subjected to the same pre-cementation treatment applied to their fitting surfaces. However, natural changes in the properties of the tooth structure and morphology could contribute in bonding, and variations in failure load can be argued to represent clinical reality [22]. The present study results were in accordance with another research [23], which concluded that immediate dentin sealing with tested dentine bonding agents has no statistical effect on the shear bond strength of etched press ceramics bonded to dentin with Rely X Uni sem (RXU) compared with controls [23]. However, another research found no significant difference between IDS and DDS in the survival rate of posterior lithium disilicate partial restorations [24].

As the result shows, groups with the e.max ceramic showed higher bonding strength than the groups of zirconia ceramic because tooth bonding capacity of e.max are better than zirconia.

Laboratory research attempt to replicate the clinical conditions but are unable to accurately represent actual clinical conditions. For use of water during thermodynamic loading as opposed to artificial saliva, and the absence of dentin fluids and their potential impact on both bonding and the slow acceleration of crack propagation should be seen as clear limitations of this laboratory research. Therefore, the effective outcomes of this laboratory research provide suggestions about the possibilities of these thin occlusal ceramic restoration when properly cemented [21].

4.1. Conclusions

Within the limitations of this *in vitro* experiment, the following conclusions were made:

1. E.max has better bond strength than UTML zirconia when used as occlusal veneer. 2. Immediate dentin sealing improves bond strength of occlusal veneer made of UTML zirconia. Furthermore, the bonding technique can have a significant effect on the dentin bond strength of occlusal veneer materials.

4.2. Recommendations

In the light of the present study:

(1) It is recommended to use IPS e.max press as occlusal veneers with and without immediate dentin sealing.
(2) It is recommended to use immediate dentin sealing before cementation of UTML zirconia occlusal veneers as it increases the SBS significantly.

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Conflicts of interest

The authors declare that there was no conflict of interest.

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