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Effect of bonding technique on microleakage around ceramic laminate veneers (*In-vitro* comparative study)

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Abstract
This *In-vitro* study was conducted to assess the marginal sealing provided by Self-Adhesive resin cement compared to the traditional Total-Etch cement on Porcelain laminate veneers, by studying Microleakage. 24 intact human upper premolars extracted without caries and restorations. They were stored in physiologic saline solution. An Incisal Overlapp preparation was made on enamel surface to receive 24 Porcelain laminate veneers (PLV). The lithium disilicate PLV were fabricated by burnout/ heat pressing of wax pattern with a thickness of 0.5 mm. Sample was divided into two groups of twelve teeth each according to the bonding techniques.
Group1: Total-Etch, light cure resin cement (Variolink Esthetic LC, Ivoclar Vivadent) was used. Group2: Self-Adhesive resin cement (SA cement plus, Panavia, Kuraray Noritake) was used without any pre-conditioning of the tooth surfaces of the teeth. The sample was immersed in 0.5% methylene blue dye, temperature of 37C for 12 hours. Each tooth was sectioned Longitudinally and horizontally and evaluated using a stereomicroscope (1500 stereo microscope Nikon Stereo Microscopes-SMZ). The values of dye penetration were recorded. values were documented and statistically analyzed by Mann-Whitney U-test. There were significant differences in the values of microleakage between the two groups. Study concluded that the use of Total Etch technique reduced the microleakage around PLV.

Keywords: Porcelain laminate veneer, microleakage, self-adhesive cements, bonding techniques

Introduction
The increase in aesthetic demands in the modern era and the development of metal-free ceramic prosthetics have led to a wider application of ceramic veneers. These cosmetic veneers have recently gained a prominent position thanks to their aesthetic appearance mimicking the natural teeth, and they are considered one of the least destructive alternatives to dental tissues.
ceramic veneers are indirect permanent restorations that represent an esthetic treatment option for anterior teeth, and are a conservative alternative to full crowns [1].
Ceramic veneers are characterized by high breaking resistance after they were bonded to the dental structures, which clearly reduced their breaking ratios in most studies [2]. Many clinical studies showed the ability of these veneers to maintain a good marginal fit for a long time [3], while a few researchers indicated the formation of a small marginal void in a number of veneers after 5 years of follow-up [4]. Resin cement is the first choice for bonding this type of dental prosthesis, due to its high physical properties and its resistance to decomposition [5]. The clinical success of ceramic veneers depends on the strength and durability of the bonding of the resin cement [6]. Bonding ceramic veneers requires sensitive techniques that are time-consuming to achieve long restoration durability. Therefore, bonding systems have been developed with the aim of simplifying the procedures of the bonding process [7]. Resin cements are classified according to the bonding technique into total-etch and self-etch cements. Total-etch bonding techniques have multiple application steps and are sensitive to contaminants surrounding the adhesive medium, as they require the application of multiple isolation procedures [8]. While self-etch cement has simpler clinical application procedure and lower sensitivity to moisture contamination, self-adhesive cement is classified within this technique, it is considered one of the modern generations of resin cements; It does not require the use of any bonding agents and does not require any pre-treatment of the tooth structure. Therefore, it becomes widely used by practitioners and is known as one-step cement [9, 10].

Marginal sealing and resistance to microleakage were considered one of the most important criteria affecting the permanence of the prosthetics in the oral cavity [11].

The micro leakage can be defined as the penetration of small amounts of liquids, particles, electrolytes, and microorganisms and their toxins into the microscopic gaps between the surfaces of the restorations or its cements on one side, and the surface of the prepared tooth on the other side, which can pass into the dental pulp through the dentin, causing bad biological effects on the dental structures and the periodontium [12].

Micro leakage is considered the main cause of secondary caries, pulpitis, hypersensitivity and tooth pigmentation, and thus subsequent clinical failure of ceramic veneers [13]. Marginal sealing can be measured *in vitro* by following multiple procedures, including the study of micro leakage using dyes, electron microscopy, radioisotopes, and 3D methods [14]. Pigment penetration technology has been widely used in research in this field due to its ease of handling, cheapness, and leakage in a manner that simulates bacterial toxins.

That is why the detection of micro leakage around ceramic veneers upon bonding using Self-Etch technique compared to conventional cements (Total-Etch) is an important issue that cannot be overlooked; hence the research idea came.

Haralur in 2018 evaluated the effects of bonding techniques on the micro leakage around ceramic veneers, the sample consisted of 40 premolars that were randomly distributed according to the bonding technique into 4 groups as follows: The first group used (etching-washing) light-cured cement technique, the second group used (etching - washing) with a dual-curing cement technique, the third group: used a self-etching cement, the fourth group: used a self-adhesive cement. He concluded that the use of (etching and washing) technique in bonding ceramic veneers formed the strongest bonding surface between the tooth and the cement to resist the micro leakage with the lowest recorded value [13].

In 2004, Behr and his colleagues evaluated the marginal fit of a self-adhesive cement (Rely X Unicem) compared to conventional dual-curing resin cements on the surface of dentin. The sample consisted of 32 molars prepared to receive lithium di-silicate crowns (Empress II). They were distributed into 4 groups according to the type of adhesive cement used; then the marginal integrity was evaluated using the dye penetration technique in addition to evaluating the formation of voids with a scanning electron microscope. They concluded that the self-adhesive cement without any pre-treatment reinforces the marginal fit at the level of dentin compared to other bonding techniques [8].

In 2007, Ibarra and his colleagues evaluated the efficiency of a self-adhesive resin cement (Rely-X UniCem) compared to conventional self-etch cement, by assessing the micro leakage around ceramic veneers using an Ammoniacal Silver Nitrate dye solution. The sample consisted of 36 premolars prepared to receive ceramic veneers, made of glass porcelain reinforced with lucite crystals (Empress I) by thermal injection technique, and distributed into 4 groups according to the applied bonding technique. The micro leakage was evaluated using both optical and scanning electron microscopy on the entire surface of the dentin and enamel. The study concluded that at the dentine level, the self-adhesive cement without any bonding agents and the traditional total-etch cement (Variolink-Excite) showed less micro leakage than other cements; while the self-adhesive cement at the enamel level without any bonding agents showed higher micro leakage than the rest of the groups and more formation of gaps between the cement and the surface of the enamel was observed [7].

Materials and Methods

The study sample consisted of 24 maxillary premolars [8] freshly extracted for orthodontic or periodontal diseases reasons, free of caries and restorations and preserved in sterile physiological serum 0.9% (NaCl) [13]. The premolars were cleaned with a scaling device (UDS-J, Woodpecker), then with rotary cleaning brushes under running water, then were preserved again in neutral physiological solution (saline). Each tooth of the sample was dipped in an acrylic mold 2 mm before the cemento-enamel junction, in a vertical position in order to facilitate the process during the research stages, unified standards were followed to prepare all the specimen teeth, where the buccal surface was prepared with a depth-cutting bur (LVS-1, Komet Dental, Gebr, Brasseler, Lemog, Germany,) by making deep horizontal scales (0.5 mm) and then colored, then a round-headed diamond bur (Diatech, coltane, AG Switzerland) was used to remove the remaining enamel between the fissures; a chamfer finishing line was prepared with a depth of (0.5 mm) at the anatomical neck of the tooth [15]. The preparation was extended to the mesial and distal buccal linear angles; thus a prepared tooth of 0.5 mm thickness was obtained over the entire buccal surface. The design of the occlusal surface preparation was Incisal Overlap, in which the buccal cusp of the premolar was reduced (1.5 mm). All corners were rounded and the preparation was finished with fine diamond burs and discs to obtain a smooth finish line.

The sample was divided into two groups equally, each group consisted of 12 premolars according to the bonding technique used to bond the ceramic veneers: The first group (Total-Etch): ceramic veneers in this group were bonding with a total-etch resin cement (Variolink Esthetic LC, Ivoclar Vivadent). The second group (Self-Adhesive): The ceramic veneers were bonded with a self-adhesive resin cement (SA Cement Plus, Panavia, Kuraray), Table (1). The samples were numbered in each group, red labels were placed on the samples of the first group (Total Etch) and numbered from 1 to 12, and green labels were placed on the samples of the second group (Self-Adhesive) and numbered from 1 to 12. Figure (1).

Fig 1: Sample distribution and numbering

Table 1: shows the chemical composition and the bonding system used for the cements that is used in this study [16]

Material	Type	Composition	Polymerization Type	Manufacturer
Variolink-Esthetics (LC)	Resin cement	Ivocerin and thiocarbamide, hydroperoxide UDMA, Urethane dimethacrylate monomers, filler, initiator and stabilizers, pigments	Light-cured	Ivoclar-Vivadent
Panavia SA Cement Plus	Self-adhesive resin cement	Bis-GMA, TEGDMA, HEMA, 10-MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, sodium fluoride, silanated barium glass filler, silanated colloidal silica (70% wt %/40 vol) Peroxide, Catalysts, Pigments	Dual-cured	Kuraray (Osaka Japan)
Tetric N-Bond	bonding agent	UDMA, Bis-GMA, dimethacrylate,HEMA,Phosphonic acid acrylate,nanofillers (SiO2), Ethanol, initiator and stabilizers	Dual cured	Ivoclar Vivadent
Monobond Plus	Coupling agent (silane)	Ethanol,3-trimethoxysilypropyl methacrylate,10-MDP, disulfide acrylate		Ivoclar Vivadent

The ceramic veneers were manufactured directly on the teeth using the burnout of wax pattern technique, The prepared surfaces of the teeth were coated with a layer of die-spacer to perform a manual waxing of the veneer directly on the prepared sample using modeling wax (GEO Crowax, gray-opaque, Renfert) the margins were sealed with Cervical wax (GEO Crowax Cervical and undercut wax, red-transparent, Renfert), to give the final anatomical shape of the veneer.
The thickness of the wax was confirmed by using a thickness gauge. The ceramic veneer was made using the thermal pressing technique of the medium-density ceramic ingot (MO-1, GC Initial® LiSi Press Ingots), then the ceramic veneer were cleaned from the investment powder by sandblasting, the reaction layer was removed using a diluted liquid of hydrofluoric acid of less than 1% concentration for a period of 10-30 minutes, then the injection rods were cut and the veneers were trimmed with diamond burs and carborundum stones. Figure (5), the thickness of the veneers was confirmed using a thickness scale at three points.

Fig 2: medium-density ceramic ingot

Fig 3: Ceramic veneers waxing and placing the wax rod

Fig 4: ceramic pressing oven (Programat EP 3010, Ivoclar Vivadent) and crucible (100g)

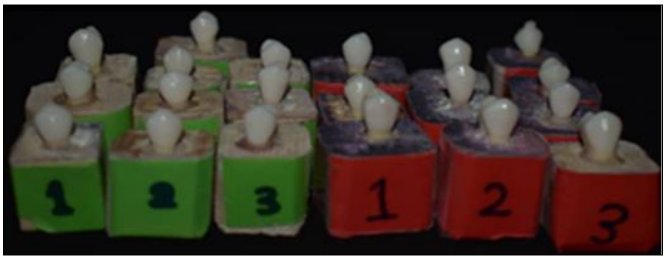

Fig 5: Ceramic veneers after finishing and trimming

The inner surfaces of all the ceramic veneers of the two groups were disinfected with 99% alcohol, then washed with a stream of water and air, and dried with air, the inner surfaces of the veneers were etched with hydrofluoric acid 10 % (FGM) for 60 seconds [17], then washed with a stream of water and air and dried With air until the appearance of a chalky surface of the etched ceramic, then a coupling agent (Silane) is applied (Monobond Plus, Ivoclar Vivadent) and is kept for 60 seconds to complete the reaction.
The ceramic veneers of the first group were fixed using a light-cured resin cement (Variolink Esthetic LC) according to the manufacturer's instructions, following the following steps: The prepared surfaces of the teeth were cleaned with the fluorine-free pumice powder using a brush, and rinsed with a stream of water and air, then the tooth surfaces were etched with phosphoric acid (N-Etch, Ivoclar Vivadent) at a concentration of 37% for 30 seconds and then rinsed with a stream of water and air and dried with air. The bonding agent (Tetric N-bond, Ivoclar Vivadent) was applied to the prepared surfaces and spread with a stream of air. The bonding agent was light-cured for 30 seconds. light-cured resin cement was applied to the inner surfaces of the veneers, then placed on the teeth with finger pressure [7]. The excess cement was removed using a microbrush, Then the cement was light-cured for 4 seconds per surface. The excess was removed again using a sharp instrument after which the resin cement was light-cured again (Led curing light, hemao, china) for 40 seconds on each surface (mesial, distal, buccal, occlusal). The veneers were finished with composite finishing burs and rubber tips. The sample was kept in distilled water at room temperature until the tests were carried out.
The ceramic veneers were bonded in the second group using dual-cured self-adhesive resin cement (SA Cement Plus, Panavia, Kuraray Noritake) according to the manufacturer's instructions following the following steps: The prepared tooth surfaces were cleaned with a rotating brush and water, then dried with a gentle, water-free and oil-free air stream. There isn't any pre-treatment of tooth surfaces in this technique. The mixing tip supplied by the manufacturer was installed and the cement was injected directly onto the inner surfaces of the ceramic veneers and distributed evenly over the entire surface. The veneers were placed and finger pressure was

applied, their position was confirmed by checking the marginal fit with direct vision. The resin cement was light-cure for a period of 2 to 5 seconds, then the cement excess was removed using a sharp instrument, then the resin cement was light-cured again for 10 seconds per surface (mesial, distal, buccal, occlusal). The sample was preserved with distilled water at room temperature until the tests were carried out. The preparation for the leakage tests was done after all the ceramic veneers were bonded, as follows: The teeth were separated from their acrylic bases, the root apexes of the teeth were sealed with glass ionomer cement in order to prevent the return of the pigment through the apex, which may affect negatively on the results. All the surfaces of the teeth were covered with two layers of nail polish 1mm far of the line separating the margin of the veneer and the tooth from all directions (Figure 6), then the samples were immersed with methylene blue dye at a concentration of 0.5% for 12 hours, at a temperature of 37°C. Samples were removed from the pigment and washed thoroughly with water then placed in pure water for 12 hours to remove any remaining traces of the pigment, then left to dry, (Figure 7).

Fig 6: All surfaces of the teeth are covered with two layers of nail polish, 1 mm far from the veneer-tooth junction.

Fig 7: The samples after removing them from the water, drying them and preparing them for the cutting stage

The cutting process was performed in two stages by cutting each tooth from the sample from the center in a buccal-lingual direction (sagittal section) and in a mesial-distal direction (cross section) [18], using a diamond separation disc of 0.5 mm thickness at a low speed using a straight handpiece with generous irrigation, thus obtaining four parts for each sample. The cut parts were examined with a magnifying glass to determine the points that will be evaluated later under the microscope. The points for measuring microleakage are designated as follows: For the longitudinal section: Two incisal points and two cervical points are obtained for each sample. for the cross-section: two mesial points and two distal points are obtained for each sample. Consequently, the total number of studied points becomes 8 points to assess the microleakage for each studied sample. The following criteria were defined to evaluate the marginal leakage process:

Value (0) no dye penetration. Value (1) dye penetration distance is less than 0.5 mm.

Value (2) dye penetration distance is from 0.5 to 1 mm.

Value (3) dye penetration distance is from 1 to 2 mm.

Value (4) Dye penetration distance is from 2 to 3 mm.

The samples were examined using an optical microscope (Nikon Stereo Microscopes-SMZ1500) located at the Faculty of Veterinary Medicine - University of Hama, under (40X) magnification. Images of the samples were taken under that magnification to be examined by computer using (MicroDicom software), where a measurement reference is taken to evaluate the results, the reference taken on this study is a parallel ruler, as the vertical distance between each two parallel lines is 1 mm. The ruler was placed under the studied sample tooth under the microscope to become at the same degree of magnification of the sample. a vertical line was drawn between the parallel lines with (MicroDicom) software, and was set as a reference measure to determine the values of the selected points. The degree of leakage was evaluated according to the distance traveled by the methylene blue dye within the gap between the tooth surface and the ceramic veneer (in millimeters), then the value it represents was determined based on the previously determined criteria. The same process was applied on all the specified points on the samples.

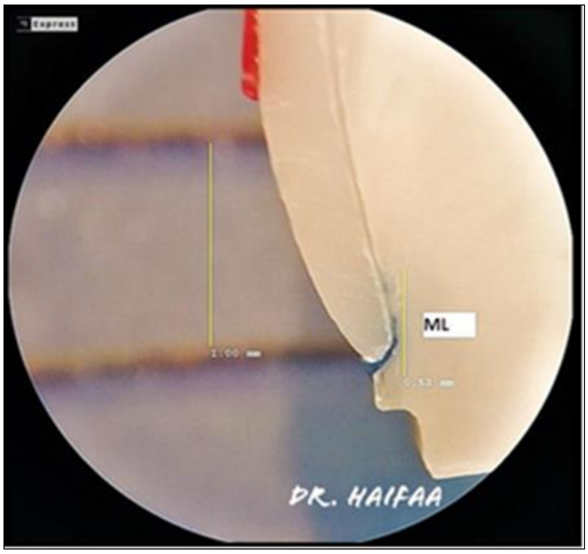

Fig 8: a sample under the microscope shows a 2 degrees value of microleakage (ML)

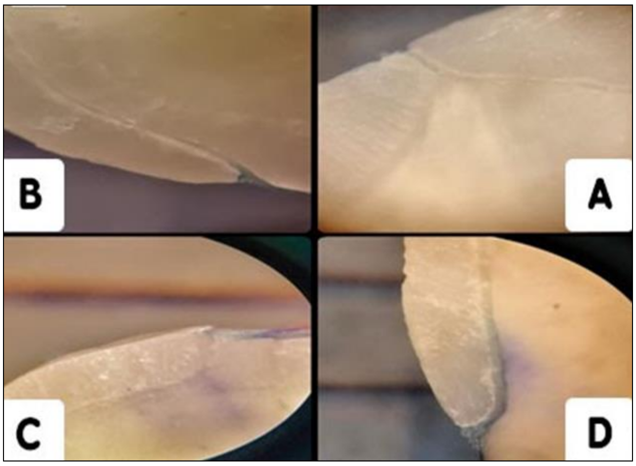

Fig 9: A micrograph of one of the samples of the total-etch group where (A) the occlusal part, (B) the cervical part, (C) the distal part, (D) the mesial part

Results and statistical analysis

The degree of micro leakage was examined in four different sites (occlusal, cervical, mesial, and distal) for each tooth in the sample, where the degree of micro leakage was calculated in two points at each site and then the arithmetic mean of the total of eight points for each tooth was taken, the degree of micro leakage and the number of repetitions were determined according to the above mentioned micro leakage criteria and then its percentage was calculated, then the significance of differences in the micro leakage degrees between the self-adhesive group and the total-etch group was studied using the Mann-Whitney U test to study any significant difference between the two groups. In the self-etch group, the percentage of teeth in which the leakage was less than 0.5 mm was 33.3% (4 teeth) and in which the leakage was between 0.5-1 mm 50% (6 teeth) and in which the leakage was between 1 - 2 mm 16.7% (2 Teeth), while in the total-etch group the percentage was 83.3% (10 teeth), 16.7% (2 teeth) and 0%, respectively, Table (2).

Table 2: frequencies and percentages of micro leakage degrees in the two study groups

Microleakage	Self-Adhesive		Total-Etch	
	Frequency	Percentages %	Frequency	Percentages %
Less than 0.5mm	4	33.3%	10	83.3%
0.5 – 1 mm	6	50%	2	16.7%
1 – 2 mm	2	16.7%	0	0%
Total	12	100%	12	100%

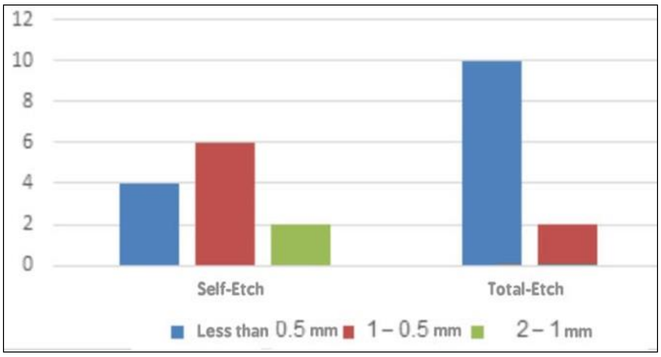

Chart 1: The frequencies of the microleakage degree in the two study groups

The Mann-Whitney U for independent samples test was used to study the significance of the differences in the degrees of microleakage between the group of self-etch and the group of total-etch (Table 3).

Table 3: The Mann-Whitney U to study the significance of the differences in the degrees of microleakage

Etching technique	Mean ranks	P value	Indication of differences
Self	15.67	0.028	There is a significant difference
Total	9.33		

The degrees of micro leakage in the self-etch group (average grade = 15.67) were greater than in the total=etch group (average grade = 9.33). The value of the significance level (P = 0.028) was less than the value (0.05) indicating that there are significant statistical differences between the two groups in the micro leakage values.

Discussion

The results of the statistical study showed a significant difference in the degree of micro leakage between the two study groups, namely: dual-cured self-adhesive resin cement (Kuraray's Panaiva SA) and total-etch resin cement (Variolink Esthetic for Ivoclar Vivadent), the self-adhesive cement showed greater values in micro leakage, this may be due to several factors, including: The insufficient ability of the self-adhesive cement (Panavia SA) to remove the smear layer surrounding the surface of the enamel as these cement modifies the smear layer, i.e. partially dissolves it and does not remove it [9] as the initial acidity of its monomers which are equivalent to (4 pH), was considered is insufficient to etch the enamel, as a study by Rohr and colleagues 2017 showed, in which self-adhesive cement (Panavia SA) was used without any pre-treatment of the surface of the enamel. The samples appeared on a scanning electron microscope with a smooth surface. It also showed lower values of shear forces resistance at the surface of the enamel compared to other cements that depend on pre-treatment of the enamel surface [19], this may be due to the fast polymerization of its monomers and the ionic reactions that lead to the modification of the acidic environment as a result, and thus does not allow sufficient time for the acid monomers to etch the enamel [8]. This may also be due to the high viscosity of the self-adhesive cement after mixing it [19], which may impede its flow and the spread of the acid monomers.

All of these factors may lead to an imperfect shape of the microscopic pores formed on the surface of the enamel, thus limiting the insertion of monomers within the hybrid layer and the formation of microscopic voids between the adhesive cement and the surface of the enamel where the importance of a microscopic gap, however small, that permits a continuous leakage of liquids and water within the hybrid layer over the entire bonding interface, this allows dissolve of non-polymerized or poorly cured molecules, and as a result, the bond is broken over time, leading to micro leakage [20]

In addition, the hydrophilic nature of the acid monomers that are included in the composition of self-adhesive cement makes it vulnerable to liquid absorption and thus causes volumetric expansion, which affects the polymerization of the resin cement and leads to formation of microscopic bubbles known as (nano-leakage) [21] within the cement layers that constitute a pathway for invasion of fluids thus negatively affect the durability of the resin bonding, allowing the occurrence of micro leakage at the end, as the self-adhesive cement (Panaiva SA) recorded high values of stress resulted from water absorption in a study conducted by Sokolowski and colleagues, he explained that its role in micro leakage is greater than shrinkage [16].

Whereas, conventional cement based on total-etch technique (Variolink Esthetic) has achieved greater resistance to micro leakage, as the use of phosphorous acid before applying the bonding agent increases the surface energy of the enamel and makes it a reactive surface by creating a microscopic porous layer within the enamel prisms, resulting from the complete removal of smear layer and degradation of mineral salts [19], thus a more deep penetration of the monomers and forming resin tags of greater length, thereby improving the durability and stability of the bond and increasing the resistance to micro leakage [22].

The results of this study agree with Haralur study in 2018, which evaluated the effect of bonding techniques on the micro leakage around ceramic veneers, this study concluded that the use of (etch and rinse technique) in bonding ceramic veneers formed the strongest bonding surface between tooth and cement to resist the micro leakage at the lowest recorded value [13].

The results of this study are also in agreement with the Ibarra and colleagues study in 2007, in which it showed the ability of self-adhesive cement (Rely-X UniCem) to ensure marginal sealing and resistance to leakage on the dentinal surface without any bonding agent or any prior etching of the surface, while the cement has shown a less capability of sealing at the level of untreated enamel, as it showed clear gap formation and thus obtained a higher value of micro leakage [7].

The results of this study differed with a study conducted by Rosentritt and colleagues in 2004, which assessed the effect of the type of adhesive cement on the marginal fit of ceramic inlays made of (Empress I) porcelain to both the surface of the enamel and the dentin by conducting a micro leakage test and evaluating the results with a scanning electron microscope, by using conventional cement based on pre-conditioning of the tooth surface and self-adhesive single-stage cement, this study concluded that there is no fundamental difference between the marginal sealing of conventional cement and self-adhesive cement. The reason for the difference may be that the researchers use another brand of self-adhesive cement [23].

Out study also disagreed with the results of a study conducted in 2012 that evaluated the marginal fit and sealing of inlays made of ceramic (Empress 1) bonded using four types of self-adhesive resin cement, the sample included 32 third molars that were prepared with finish lines within both the enamel surface and dentine surface and were divided into four groups depending on the type of self-adhesive cement (Clearfil SA Cement, Kuraray, Osaka, Japan), (iCEM Self Adhesive, Germany) (Bifix SE, Voco, Cuxhaven, Germany) (seT, SDI, Victoria, Australia)). They assessed both the marginal fit using an electron microscope and the micro leakage with pigment penetration test. They concluded that the four cements showed good performance on both enamel and dentin. The reason for the difference may be due to the different composition of the organic matrix of the self-adhesive cement used [24].

Conclusions

After studying and discussing the results of this research, and within the data of this study, the following can be concluded:

1. There is a clear correlation between the bonding technique used and micro leakage values when the ceramic veneers are bonded.
2. Total-etch technique-based cements gave greater resistance to micro leakage compared to self-adhesive cements.

Recommendations

1. Total-etch technique must be followed when bonding ceramic veneers to the surface of the enamel despite the numerous clinical steps involved in order to obtain a good marginal sealing.
2. It is not recommended to use self-adhesive cement without any pre-treatment to the tooth surface when bonding to the enamel surface due to the high values recorded of microleakage.

Suggestions

1. Conducting more *in-vitro* studies to find out the effect of the adhesive technique used (self-etch - total-etch) on the bonding strength with the study of the shear forces test.
2. Conducting a similar *in-vitro* study to assess the microleakage when bonding using the two techniques (total-etch self-etch) on the surface of the dentin.
3. Conducting a laboratory study to evaluate the effectiveness of self-adhesive cement using selective-etch technique when applied to mixed dentin enamel surfaces.

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