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Considerations on indirect adhesive restorations at the material and tooth interfaces

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Abstract

Objective: Lithium-silicate (LiSi) ceramic is nowadays widely used as a restorative material in dentistry due to its excellent mechanical and esthetic properties. However, for the longevity of LiSi indirect restorations, it is important to pretreat the material and the dental substrate adequately. The dental market is shifting toward simplification and therefore new products that are aimed to simultaneously condition and decontaminate the LiSi ceramics are being developed. Furthermore, the CAD/CAM technique for manufacturing LiSi crowns requires less processing time compared to the traditional pressing technique. However, is not certain how the simplification of the manufacturing and conditioning procedures influences the bonding performances of LiSi ceramic restorations. Accordingly, the aims of this thesis were to investigate the effect of: 1) different LiSi ceramic surface decontamination procedures on the shear bond strength (SBS) to resin composite; 2) different types of lithium-disilicate (LiDi) (pressed vs CAD-CAM) on SBS to resin composite; 3) an experimental metal salt-based zirconium oxynitrate etchant [$ZrO(NO_3)_2$] on bonding performances to dentin.

Materials and Methods: A series of 3 studies was performed to investigate these research questions. In the first two studies a SBS test was used to investigate the influence of different cleaning protocols applied for 20s (water rinsing, 37% H_3PO_4 etching, 70% ethanol, Ivoclean (Ivoclar), 5% hydrofluoric acid (HF), Monobond Etch&Prime (MEP) with or without prior HF etching), or different processing techniques (CAD or PRESS) on the bond strength to composite resin. The third study tackled the interface between restorative materials and dentin, and investigated the

microtensile bond strength test (μTBS), nanoleakage expression analysis (NL), gelatin zymography and *in situ* zymography of dentin conditioned with an experimental metal salt-based zirconium oxynitrate etchant [$ZrO(NO_3)_2$].

Results: MEP (with or without HP etching) showed comparable bond strength to the double HP etching and higher compared to other groups. BS of press LiSi to composite was higher than that of CAD/CAM LiSi. ZON pretreatment increased bond strength to dentin when used with a universal adhesive, and inhibited dentinal endogenous enzymes.

Conclusions: While simplification of the LiSi conditioning and cleaning procedures seems to yield bond strength comparable to the traditional procedures, it could be recommended in the clinical practice. However, pressed LiSi still seems to perform better in terms of bond strength compared to the CAD/CAM LiSi. Further, the novel ZON etchant seems to perform better compared to the traditional phosphoric dentin etching and should be considered in the adhesive procedures for the direct and indirect restorations.

Introduction

Teeth that underwent a significant loss of substance due to carious processes, fracture or failure of previous restorations, are indicated to be treated with indirect restorations. In the past, the standard treatment in this clinical situation was the fabrication of crowns with complete dental preparations in order to obtain mechanical retention from the abutment design, therefore the cement only served as a sealer of the tooth-restoration interface. The advent of modern adhesive techniques applied to indirect restorations, has allowed the realization of non-retentive partial restorations (inlays, veneers, Maryland bridges and partial crowns), guaranteeing good long-term clinical results avoiding healthy dental tissue removal during the preparation of the abutment. Despite the great progress in the field of adhesive dentistry and improved characteristics of resin cements, clinical failure can occur, on the interface ceramics/composite or ceramics/tooth tissue, depending on the structure of the abutment (i.e. whether a composite build-up was made after the removal of carious tissue/prior restorations). The failure of the indirect restoration can be caused by the inadequate procedure of conditioning and decontaminating the restoration, but also in the interface with the tooth tissues due to the hydrolytic degradation of the Hybrid Layer caused by endogenous dentinal enzymes, such as matrix metalloproteinases (MMPs). When performing adhesive bonding or cementation to dentin, it was shown that the phosphoric acid etching is a particularly sensitive procedure, that can lead to MMPs activation and hydrolysis of the denuded collagen fibrils. In addition, in order to obtain good results during adhesive procedures, strict protocols must be followed, particularly

when glass-ceramic materials such as lithium disilicate are used. However, complex technique-sensitive cementation procedures can often lead to poor results in the hands of an inexperienced practitioner. Hence, it can be noted in the recent scientific and commercial literature that there is a tendency towards simplification of the adhesive cementation procedures, including the conditioning of ceramic surfaces and their decontamination after the try-in procedures. Also, alternative dentin conditioning protocols are being investigated, with no consensus in the literature on their efficiency.

Lithium disilicate clinical overview

Mechanical Properties

According to the new classification published by Gracis et al.(38), lithium disilicate (LiSi) is a glass matrix ceramic. IPS Empress 2 was the first formulation available on the market, in the nineties, composed of 65% lithium disilicate, needle shaped crystals, immersed in a glassy matrix, showing remarkable mechanical properties (flexural strength: 350 MPa; fracture toughness (KIC): 3.3 MPa√m; heat extrusion temperature: 920 ° C; thermal expansion co-efficient (CTE): 10.6 + 0.25 ppm / ° C). Initially, this material was marketed in ingots, usable with the die-casting technique, similar to the lost wax process for metal alloys, the material was injected at high temperatures and high pressures into a mold. In order to obtain better optical and aesthetic characteristics, the disilicate core was layered with a highly translucent fluorapatite ceramic(114). In 2005 a new formulation of the product was marketed as "IPS e.max Press (Ivoclar Vivadent), in particular the presence of smaller and more uniformly distributed crystals allowed a significant improvement of the mechanical and optical properties (flexural strength: 370-460 MPa; fracture toughness (KIC): 2.8-3.5 MPa√m). The improvement of the mechanical properties is due to a better propagation of stresses around the crystals, due to the different coefficient of thermal expansion of the disilicate crystals and the resinous matrix(115). On the other hand, the improved optical properties of IPS e.max press extended the clinical indications of lithium disilicate to monolithic restorations, i.e. anatomically shaped restorations that are only stained and characterized on the surface, with better fatigue resistance than bilayered restorations.

In addition to the heat-pressed technique, the continuous development of cad-cam technologies in the dental market has allowed the introduction of ceramic blocks, which allow the production of lithium disilicate restorations by milling devices also suitable for chairside production. These blocks are marketed in pre-crystallized form of purple color, in fact they contain 40% of metasilicate (Li2SiO3) as well as disilicate crystal nuclei. In this state the flexural strength is lower, around 130 MPa, this implies a better cutting capacity of the block, resulting in easier and faster workability and in less wear of the milling devices(105,114,115). Once the milling process has been completed, the heat treatment of the restoration (840 ° -850 ° C for 10 min) transforms the metasilicate into lithium disilicate, consequently improving the mechanical properties (flexural strength 262 ± 88 MPa; fracture toughness 2.5MPa · m1 /2). The dispersion of staining ions in the glass matrix allows the realization of the disilicate in different colors, while the different size and distribution of the crystals in the matrix allows the creation of blocks with different translucencies. However, it a question was raised in the research literature whether this aspect could have an influence on flexural strength. In particular, the flexural strength of IPS e.max PRESS and CAD is comparable and the different manufacturing processes do not seem to influence the mechanical properties of the lithium disilicate(11,100,114,115). Nevertheless, it has been shown that the different levels of translucency determine significantly influences the flexural strength of the disilicate in blocks.

As far as mechanical strength is concerned, it has been clearly demonstrated that monolithic crowns have much higher load fracture values (2665.4 ± 759.2 N) compared

to bilayered crowns (1431.1 ± 404.3 N)(34,39). Furthermore, to date there is strong evidence that unlike bilayered crowns, monolithic crowns have sufficient mechanical properties to be indicated for posterior restorations, both on natural teeth and implants, up to small 3-unit bridges(114,115). Monolithic LS2, as well as Zirconia reinforced-Lithium Silicate ceramics (ZLS), offers higher fracture resistance than bilayered, hand-veneered zirconia(42), while a recent in vitro research has shown that load-to-fracture values of monolithic zirconia are higher than those of Lithium disilicate; the latter, in turn, are higher than those of Zirconia reinforced lithium disilicate(81).

It has to be pointed out, however, that, particularly as regards Lithium disilicate, fatigue resistance is strongly influenced by many experimental variables, like amount of cyclic loading, abutment and antagonist design and material, thermocycling parameters and test environment; for this reason, the heterogeneity and lack of standardization in research designs, tested materials and experimental conditions make a comparison of data not easily feasible(72).

Abrasiveness and wear

Nowadays wear and abrasion are important properties for a restorative material and lithium disilicate appears to have favorable characteristics regarding this aspect. When properly polished, lithium disilicate has been shown to have restorative friction and wear values similar to natural enamel in vitro, however it is more aggressive than type III gold and polished monolithic zirconia in in vitro simulations(7,53,117). Such favorable wear behavior and durability have been also confirmed by some in vivo evidence(90).

On the other hand, it is reported in literature that glazing or ceramic layering significantly increases the wear of both the restoration and the antagonist element. Furthermore, the surface roughness of the ceramic can be increased by the presence of a basic pH and by brushing with particularly abrasive toothpastes. For these reasons it is not recommended to layer or glaze the monolithic disilicate on purely functional occlusal surfaces, but only on mainly aesthetic surfaces. Moreover, it is advisable to carefully polish the surfaces that undergo retouching, since scanning electron microscopy (SEM) studies have shown that lithium disilicate is one of the most critical materials to be corrected intraorally, due to the fact that requires more strength and energy compared than other materials, whereby retouching can cause micro cracks that can propagate into the restoration(92).

Biocompatibility

Biocompatibility is one of the best characteristics of lithium disilicate, in fact this material has shown excellent results in vitro both in terms of plaque accumulation and proliferation of epithelial cells and fibroblasts, especially when the surface is carefully polished(32). In in vivo studies, no signs of inflammation were found in crevicular fluid, checking the levels of inflammation markers. these data are confirmed by tissue culture data. Clinically it is also possible to state that the periodontal and peri-implant soft tissues surrounding the lithium disilicate restorations maintain a healthy and natural appearance(8,32,99).

Clinical indications and performances

As far as clinical applications are concerned, it is possible to state that lithium disilicate is one of the most versatile metal free materials, due to its esthetic, mechanical, and adhesive properties thanks to its silica content. Lithium disilicate can be used for tooth and implant supported restorations, ranging from partial adhesive restorations such as inlays, onlays, overlays or veneers, to full monolithic crowns, up to small 2 or 3-unit bridges. However, due to their recent entry into the dental market, there is a lack of data in the literature regarding the long-term outcomes of lithium disilicate dedicated to cad-cam production. Although, medium-term prospective studies report promising results for both dental and implant-supported prostheses (94.8% at 8 years and 100% at 5 years) made from conventional impressions. A recent prospective study reports 100% success rate without any technical or biological complications at 2 years for single implant-supported crowns made through a full digital workflow. Similarly, retrospective studies have shown that LiSi can yield satisfactory clinical performance with favorable survival rates and low incidence of mechanical failures, like debonding, fractures and chipping (28,90). For chairside restorations, complete lithium disilicate crowns reported a 10-year survival rate of 83.5%. the main causes of failure were secondary caries, loss of retention and hypersensitivity. The high reliability of glass ceramic with resin cements and the mechanical properties of these materials mean that they are also widely used for the realization of non-retentive partial restorations such as tabletops, maryland bridges and veneers. In the anterior sectors, the use of hand layered lithium disilicate veneers, allows very high aesthetic results, while maintaining mechanical performances. Therefore, clinical and laboratory studies have shown that

the presence of long teeth, with a margin beyond the CEJ with abundant areas of exposed dentin, represents a risk factor for the veneers' integrity, because of the high flexural strength stress and high functional loads. the mechanical properties of lithium disilicate can be very useful in the production of ceramic veneers in less than optimal biomechanical conditions. In fact, it has been demonstrated that the more rigidity of the material has a shield effect on the underlying tissues, strengthening the tooth-restoration complex. Thanks to its good optical properties and the possible treatment with Hydrofluoric acid and silane, lithium disilicate has also been proposed for the realization of cantilevered prostheses in anterior sites such as single wing maryland bridges. In a systematic review, this type of prosthesis showed a lower failure rate compared to traditional two-wing marylands, according to the author this is due to the greater biomechanical stresses during protrusive and lateral movements(93). In another recent review, a survival rate of 91.2% was recorded at 5 years of follow-up for all-ceramic marylands, exhibiting a higher debonding rate for zirconia restorations compared to glass ceramics, which however reported a higher fracture rate, although a greater degree of evidence is needed to correctly delineate the long-term performance of this type of restorations(36)(22). This type of restorations is indicated in the presence of anatomical anomalies that would require expensive and invasive surgery for implant insertion, or other surgical contraindications such as the young age of the patient. However, it is necessary to make an accurate aesthetic and functional evaluation of the treatment plan before proceeding with this type of solution in order to avoid fracture and debonding of the single wing Maryland. In the posterior region, lithium disilicate

is indicated for the fabrication of single resin bonded restorations such as inlays, onlays, non-retentive partial crowns and full coverage tabletops in monolithic form. The material offers undisputable advantages, like high fracture resistance, showed by high load-at-fracture values in tabletops/occlusal veneers, allowing reduced thickness of the restorations (1–1.5 mm), low wear and abrasive potential, adhesive bonding strength and high biocompatibility. This type of prosthetic solution in the most recent literature has shown favorable results but the reduced follow-up making it impossible to draw conclusions on the outcomes of long-term disilicate when used for resin bonded restorations. A recent 3-years randomized, controlled prospective trial has shown that LiSi partial crowns can be used as successful restorative solutions for endodontically treated posterior teeth, with no significant differences between premolar or molars and with or without the use of fiber posts(31).

Marginal accuracy and internal fit

Many studies in the literature have decided to compare the fit of lithium disilicate crowns obtained with the heat pressing technique with those obtained with CAD-CAM technology. (25)According to the most recent publications, no statistically significant differences were found in marginal precision during the fabrication of traditional lithium disilicate crowns compared with those obtained a with full digital protocol. However, some authors have noted a better fit of the crowns obtained by heat pressing and a polyvinylsiloxane impression compared to the crowns obtained with CAD-CAM disilicate(12). Another aspect that must be taken into consideration is the machinery

used for the production of CAD-CAM lithium disilicate crowns. In fact, the crowns produced in milling centers appeared to have a better fit compared to those made with chairside machines present in dental offices(41). Conversely, other studies reported that marginal and internal fit of lithium disilicate crowns were more accurate when using digital impression technique; in any case, whatever the workflow used, the adaptation was shown to be within clinical acceptability range(67).

To date, drawing univocal conclusions about adaptation accuracy of lithium disilicate restorations is not easy, due to the high number of variables involved in the final prosthetic fit, like digital impression system and technique, used material and fabrication procedure, so there is still a noticeable amount of controversial debate(79,114,115).

Bonding Protocol

Posterior indirect restorations are widely used in modern restorative dentistry to overcome the problems resulting from direct techniques. The adhesive concepts that have been used for direct restorative procedures are now being applied to indirect restorations and have been incorporated into daily practice(17,74). This allowed to restore teeth in a reliable way, avoiding invasive techniques of the traditional fixed prosthesis, in which the preparation of the tooth must provide mechanical retention of the restoration, at the expense of healthy dental substance that is arbitrarily removed. The success of adhesive restorations depends primarily on the compliance with the adhesive protocol both on the tooth and on the restoration surface. In the next

paragraphs an overview of the literature regarding the treatment of the two interfaces will be described:

Lithium disilicate surface conditioning

The presence of a silica-based vitreous matrix makes lithium disilicate an acid-sensitive ceramic, for this reason, high adhesion values can be achieved due to both micromechanical and chemical bonding mechanisms. Micromechanical interlocking between ceramics and resin cement at the intaglio surface can be obtained through the creation of microporosity and roughness through acid etching or mechanical procedures such as surface sandblasting(10,115). Regarding acid etching, hydrofluoric acid certainly represents the gold standard for the treatment of lithium disilicate, however both concentration and exposure time of the restoration to the action of the acid must be taken into consideration in the treatment protocol. Lithium disilicate requires a shorter and less concentrated etching compared to felspathic ceramics, in fact the ideal treatment is represented by the application of 5% HF for 20 sec, while 9-10% HF for 90 -120 seconds is usually applied for felspathic ceramics(10). Treatments with a higher concentration or prolonged etching time have proven to be harmful for lithium disilicate, as they can modify the internal microstructure of the material by acting in depth, and consequently affect the mechanical performance such as flexural strength, especially if reduced material thicknesses are taken into consideration(82). As mentioned previously, another system for obtaining microporosity on the lithium disilicate surface is sandblasting with aluminum oxide particles. However, it has been shown that this procedure, like overetching, involves a modification of the flexural

strength, combined with an excessive removal of the material and an inhomogeneous surface conditioning. In addition to improving the mechanical interlocking, the presence of silica in the disilicate structure allows us to improve the chemical adhesion between the resin agents of the cements and the ceramic, through the application of silane, which involves forming strong siloxane linkages formation. Recently, it has been shown that the use of silane combined to a phosphate functional monomer, the 10- Methacryloyloxydecyl-Dihydrogen-Phosphate (10-MDP), creating an acidic environment further improves the bond strength of resin-based luting cement to lithium disilicate ceramics(95).

Lithium Disilicate surface decontamination

Before bonding a definitive ceramic restoration, it is mandatory to control some parameters by trying the restoration directly in the mouth such as, marginal precision, fit on the abutment, contact and occlusal point. However, the try-in procedure of all-ceramic restorations causes the contamination of the intaglio bonding surface of the restoration with saliva, blood, or try-in silicone remnants, making the adhesive cementation of the restorations even more difficult. Failure to remove fluids or try-in materials results in reduction of bond strength. Thus, any inorganic or organic contaminants should be eliminated before adhesive cementation(3,4).

Saliva contains organic materials such as salivary proteins, enzymatic molecules, bacteria, and food debris, and inorganic compounds, such as mineral ions, in water solution.13 Adhesion of salivary proteins to dental materials and tooth surfaces (saliva contamination) results in formation of acquired enamel pellicle of 10 to 20 nm

thickness within a few minutes, which is free of bacteria(3,30,116). When the protein transmission from saliva increases, the thickness of the proteinaceous layer reaches 100 to 1000 nm between 30 and 90 min.17 It is almost impossible to avoid saliva contamination of all-ceramic restorations during the try-in procedure. Therefore, during adhesive cementation procedures, contaminant removal plays an important role in the durable adhesion and clinical performance of the restoration(45).

The sole water rinsing was not efficient in removing the biofilm of saliva from restorations(58). In literature several cleaning methods have been proposed in order to find the best way to provide surface decontamination, such as Phosforic acid, Alcohol, Sodium hypoclorite, Ivoclean, preliminary silanization and re-etching. (Literature overview in Table 1). Even if some results may be contradictory, it is possible to sum up that all the methods mentioned seemed to be more effective compared to the sole water rinsing, on the other hand, the gold standard procedure is to perform the surface conditioning of HF and silanization after try-in procedure(20,58,59,110). However, in most cases, etching is performed by the dental technician prior to delivery to the dental office, since due to the high toxic potential it could be a dangerous procedure to perform in a dental office. In fact, in some countries its use is even banned.

Recently, new possibilities are emerging with the introduction of new products on the market with alternative compositions able to provide etching and priming of the surface in just one application, it is possible to conclude that Monobond Etch & Prime (MEP; Ivoclar Vivadent) is the most investigated product in literature. MEP is a single bottle ceramic primer which allows etching and silanization of the glass ceramic surface in

one step. It contains a trimethoxypropyl methacrylate for silanization and a new polyfluoride for the etching step. Polifluoride provides a milder etching of the surface compared to HF, for this reason the product is safer to use both for user safety and for the risk of over etching lithium disilicate. On the other hand, MEP creates a roughness pattern which is less pronounced than with HF gel but as efficient for bonding(70,101). In last years some authors evaluated the efficiency on MEP in the surface treatment of Lithium disilicate (58,84)reporting equivalent results in terms of bond strength. On the other hand, Wille et al. reported opposite results concluding that Bonding systems utilizing a self- etching primer showed a significantly lower TBS than group using a conventional ceramic bonding system. However only Lyann et al. (59) decided to test MEP after surface contamination with human saliva, in order evaluate if MEP is efficient also for surface decontamination, and no research group has simulated a complete try-in procedure by contaminating samples with both saliva and try-in silicone before applying MEP, compared to the gold standard technique.

Table 1: *Overview of the clinical studies that investigated the effect of different surface decontamination and MEP on lithium disilicate*

Author, Year	**Study Design**	**Decontamination groups**	**Contamination**	**Conclusions**
Marfenko et al., 2020	Shear bond strenght test	4 Groups: Water - Ethanol 80% - Phosphoric acid	Saliva, dental stone	Preliminary silanization of hydrofluoric prior to saliva or dental stone

		- Cleaning gel		contamination re-established resin luting cement adhesion, irrespective of the cleaning regimen used
Lyann et al. , 2019	Tensile Bond Strenght and X-ray Microscopy	5 Groups, with or without previous saliva contamination: - Control - Monobond Plus - Phosporic acid + Monobond plus - HF + Monobond plus - Monobond etch & Prime	Saliva	Surface treatments with PA or HF followed by silane or by MEP alone were effective in removing saliva contamination and enhancing the resin bond strength
Klosa et al. 2009	Tensile bond strenght, thermocycling	4 Groups: - PA 37% - HF 5% - Isopropanol 96% - Sodium Bicarbonate	Saliva, silicone	Ceramic cleaning methods after try-in procedures have a significant influence on the resin bond strength and are dependent on the type of contamination.

				Re-etching lithium disilicate ceramic with 5% hydrofluoric acid is most effective in removing contamination with saliva and/or a silicone disclosing medium.
Borges et al. 2017	Shear Bond Strenght, Thermocycling	4 groups: - Water - PA 37% - Ivoclean - Isopropanol	Saliva	In case of saliva contamination of acid-etched glass-ceramics, mechanical cleaning can restore adhesion to the baseline situation.
Yoshida et al. 2020	Tensile Bond Strenght, Thermocycling	5 groups: -Water sray - 40% PA - Ivoclean - AD gel - Silane before saliva	Saliva	Water spray did not restore the bond strength, while PA, IC, ADG, and SCA all benefited.
Aladag et al. 2015	Shear Bond Strenght test	4 Groups: - Control - Water	Saliva	The leucite-reinforced glass-ceramic group bene-fited from 0.5%

		- Sodium hypochlorite - Ivoclean		sodium hypochlorite solution cleaning with increased bond strengths.
Yoshida et al. 2015	Shear Bond Strenght test	4 Groups: - Water - PA 37% - HF 5% - Non contaminated control group	Saliva	Hydrofluoric acid and phosphoric acid etching may be effective methods of removing the contaminants a thin layer of contaminants remained on the lithum disilicate and leucite glass ceramic surface after exposure to saliva.
Lapinska et al. 2019	Shear bond strenght	5 groups: - Control - Ultrasonic bath - PA - Ivoclean - Re-etching	Saliva	Re-etching was the most effective cleaning methods on lithium disilicate
Vichi et al. 2021	Shear Bond strenght test	3 groups: - HF - HF + Silane - Monobond E&P	No contamination	The self-etching primer coyld be a good alternative to hydrofluoric acid and silane

				conditioning protocols.
Fagan et al. 2022	Shear Bond strenght test	- Water spray - Alcohol 70% - 35% phosphoric acid - - Ivoclean	Saliva, Human blood	All the assessed cleaning methods were effective in removing saliva; however, only Ivoclean was able to restore the adhesion quality when the silanized EMX surface was contaminated with human blood
Yu et al. 2021	Shear Bond strength	- HF - Silane - HF + Silane - Monobond E&P	No contamination	μSBS of resin cement to lithium disilicate glass ceramic etched with MEP is as efficient as that treated with HF and silane.
Wille et al. 2021	Tensile Bond strenght	- HF + Silane - HF + Primer - MEP - Primer	No contamination	Bonding systems utilizing a self-etching primer showed a significantly lower TBS than group using a conventional ceramic

				bonding system.
Rodriguez et al 2017	Shear Bond test strength	- HF + Silane - MEP	No contamination	Monobond Etch&Prime appears to obtain equivalent results in terms of bond strength

CAD CAM vs PRESSED lithium disilicate

As described above, a lithium disilicate restoration can be produced by vacuum injection method or by milling a cad cam block. The two production processes are completely different, and researchers investigated whther the two materials have different mechanical properties that can consequently lead to different clinical performances. Using Pressed lithium disilicate, ingots are already crystallized. By heating, the ingots become viscous and pressable. Conversely, CAD lithium disilicate the blocks exhibit an intermediate status (Li2 SiO3), necessary for the milling procedures. After milling, the restoration undergoes a heat-mediated chemical reaction, resulting in the lithium disilicate crystallization (Li2 Si2 O5). This crystallization process consists of two major events, nucleation and crystals growth. Due to the nucleating agents, the reaction is controlled, and the final crystals shape, size, and content are determined. Particularly, in order to achieve the desired shade and translucency, some oxides are used, acting as co-nucleating agents. These oxides

interact with the described nucleation and crystallization processes, thus affecting the size of the crystals and, consequently the mechanical and physical properties. In fact, if for Pressed lithium disilicate translucency does not affect the flexural strength, on the other hand for CAD lithium disilicate it is an influential factor(54). In the literature various in vitro aspects have been investigated regarding the differences between cad and pressed disilicate, such as marginal fit, cyclic fatigue, flexural strength, with controversial results between the two materials(1–3,8). According to Schestatsky *et al.* Pressed lithium-disilicate monolithic crowns showed better fatigue performance in comparison to CAD/CAM milled crowns, El rashid *et al.* concluded LD all ceramic crowns fabricated by using CAD-CAM techniques showed lesser marginal gap and better marginal fit compared to the conventional technique. Controversely Azar found out that lithium disilicate crowns fabricated with the press technique have measurably smaller marginal gaps compared with those fabricated with CAD/CAM technique within in vitro environments. However, the only observable clinical difference between these two materials arises when we etch the inner surface of the restoration with hydrofluoric acid. The surface of pressed disilicate appears much more opaque and chalky compared to the cad-cam lithium disilicate after the etching process. For this reason, since the two surfaces on which adhesion are made appear very different, it is desirable to ask if there were any differences in terms of bond strength between these materials. In the literature this aspect does not seem to have been investigated yet.

Bonding to dentin

Resin-based dental composites are the most commonly used restorative materials in

everyday dental practice due to their good mechanical and esthetic characteristics and handling properties(17). In order to achieve long term bonding to enamel and dentin, composite materials require the use of adhesive systems(74). Based on their interaction with the smear layer and number of steps used during bonding procedures, dental adhesives can be classified into etch-and- rinse (EAR) systems (3- and 2-step) and self-etch (SE) systems (2- and 1-step)(66,74).

The application of the adhesive system (either EAR or SE) on dentin surface results in the formation of hybrid layer, a structure that is composed of demineralized collagen fibrils reinforced by resin matrix.(69) Etch-and-rinse systems are the oldest adhesives in the evolution of dentin bonding agents. When supplied in the 3-step version, they involve acid-etching with phosphoric acid, priming and application of a separate adhesive. In the 2-step version, after acid-etching, dentin is simultaneously primed and bonded since the hydrophilic primer and the hydrophobic resin are blended in one solution (74) On the other hand, simplified self-etch adhesives do not require separate etching step with phosphoric acid. They either come as two- or one-step adhesives, depending whether the self-etching primer and the adhesive resin are provided separately or combined into one single solution. Simplified adhesives are composed from acidic monomers that simultaneously condition and prime dentin, through a partially dissolved smear layer. Since they do not include a separate etching step, the initial substrate for one-step self-etch adhesive systems is mineralized dentin (66).Generally speaking, thicker hybrid layers are observed when using EAR adhesive systems when compared to SE system(5). However, thicker hybrid layers do not

necessarily mean higher bond strengths, since both adequate immediate bond strength and good clinical behavior was observed when using SE systems(51). Interestingly, neither EAR or SE adhesive systems are able to prevent the phenomenon of nanoleakage - the diffusion of small ions or molecules within the hybrid layer in the absence of gap formation. (59, 60) Unlike self-adhesive resin cements which do not form a typical hybrid layer, multi-step or conventional resin cements rely on the application of adhesive system, and therefore, a true hybrid layer is formed when using this group of cements for luting procedures. Adhesive cementation of FRC posts can be achieved using also multi-step resin cements, which are modified composite resins with a higher fluidity to improve flow during cementation(94). Multi-step resin cements require more chair-side time and clinical steps compared to self-adhesive ones, due to the dentin pretreatment which is necessary when using these cements. Also, they are considered to be more technique-sensitive than self-adhesive cements. Although conventional resin cements are more technique sensitive, because they require adhesive cements, these cements are more capable of interpenetrating the demineralized dentin substrate(103). A recent systematic review and meta-analysis investigated the data from laboratory studies that assessed the adhesion performance of indirect restorations to dentin of two different resin cement types: conventional and self-adhesive. The overall results of this article reported that the conventional adhesive approach (resin cement applied in combination with an adhesive system or primer agent) tends to promote higher immediate- and long term bond strength of indirect coronal restorations to dentin(68)

. On the other hand, another systematic review revealed that self-adhesive cements seem to improve the retention of FRC posts to radicular dentin when compared to multi-step resin cements(86).

The application of adhesive systems, whether EAR or SE, results in incomplete hybridization of dentin substrate, leaving unprotected collagen fibrils surrounded with water on the bottom of the hybrid layer(63). Two important aspects, described in the following sections, should be taken into consideration for better understanding of processes that lead to degradation of resin-based restorations. Two main mechanisms are considered to be responsible for HL degradation: the disintegration and solubilization of collagen fibers and the hydrolysis and leaching of the adhesive resin material from the interfibrillar spaces. The most important reason for resin degradation between the hybrid layer is hydrolysis(33). In an attempt to overcome this problem, contemporary adhesive systems contain a mixture of hydrophilic resin monomers, such as two-hydroxyethyl methacrylate (HEMA),in diluents and organic solvents, usually water, ethanol or acetone. These hydrophilic resin monomers are important for infiltration of the adhesive systems through the wet and demineralized dentin causing the hybridization of the adhesive with the substrate(65). Still, the mentioned hydrophilic resin monomers in adhesives formulations cause high water sorption by the resin systems and generate a HL that behaves as a pours membrane after polymerization, which permits moving of water throughout the bonded interface(97). The penetration of water into the hydrophilic domains of the adhesive enables the leaching of the solubilized resin material. Consequently, resin-infiltrated collagen

matrix is solubilized and is slowly leached-out, the underlying insoluble collagen fibrils become exposed and become prone to attack by enzymes, such as matrix metalloproteinases (MMPs)(16). Furthermore, the presence of residual water in the pretreated (etched) dentin can decrease the polymerization of the adhesive monomers which further leads to the increased permeability of the adhesive layer(48). Even though great advances have been made in the field of adhesive dentistry, all adhesives show variable degrees of incomplete polymerization that correspond to the extent of fluid movement throughout the adhesive layer(17). Finally, long-term exposure of resin-based restorations to masticatory forces and repeated changes in temperature and pH which are present in oral cavity may cause deformation of restorative materials (contraction and expansion), affecting resin-dentin interface and allowing penetration of oral fluids(35). The infiltration of water molecules into the hydrophilic domains of resin-infiltrated matrix collagen matrix can become trapped during the process of photopolymerization. This “trapped” water can further enhances the hydrolysis of collagen and resin polymers, accelerating degradation by abrading the surface, and allowing entrance of both water and salivary enzymes, that can accelerate ester bond hydrolysis, leading to the failure of the adhesive interface(16). One of the pioneers in explaining collagen degradation over time even in aseptic conditions was Pashley et al., who suggested that this phenomenon occurs due to the endogenous enzymes(75). The most widely studied group of enzymes which are considered responsible for resin-dentin degradation are MMPs and cysteine cathepsins(56). In order to understand MMPs mechanism on degradation of HLs as well as hybridization process that occur

during adhesive procedures, a short overview of dentin's structure should be given. Dentin is a collagen-based mineralized tissue consisting of inorganic apatite crystallites embedded in an extracellular matrix. Type I collagen is the main component of the ECM compartment of dentin, representing up to 90% of the organic material(56). In addition, several proteins, collectively referred to as noncollagenous proteins, constitute approximately 10% of the matrix. The noncollagenous dentin proteins include proteoglycans, phospholipids, and enzymes(16). The composition of dentin can vary in different areas of the tooth, depending on its proximity to the pulp tissue, as well as whether the matrix is demineralized or caries affected/infected. These differences can have an effect on the mechanical properties of dentin, as well as the success of bonding to dentin(37). A collagen molecule is composed of three α-chains, two α-1and one α-2 chain intertwined into a left-handed triple helix. Collagen chains have 3 main domains: a central triple helical region (>95%), a non-helical amino terminal (N-telo peptide) region and a car-boxyterminal (C-telopeptide) region.These peptide chains organize unsoluble collagen fibers by aggregating and stacking in parallel. These collagen fibers contain a 67 nm gap between the adjacent collagen molecules, and are further organized in bundles(16). During dentin maturation, apatitic mineral crystallites precipitate and inactivate enzymes that are present in the extracellular matrix and were active during the dentinogenesis(106). Interestingly, dentinal collagen can withstand adhesive procedures that would otherwise destroy the structure of the dermal collagen(16). However, it is important to underline that dentin over-etching with phosphoric acid (etching longer than 15s) may lead to structural

changes in collagen molecules and therefore it is important to limit etching time to not more than 15s. MMPs are endogenous Zn2+- and Ca2+-dependent enzymes, capable of degrading almost all extracellular matrix components. In human species, the MMPs family consists of 23 members, classified into 6 groups based on substrate specificity and homology. MMPs are typically present as inactive enyzmes in dentin, and the pro-domain requires to be dissociated from the catalytic one in order to be activated(44). In their non-active form MMPs, the unpaired cysteine in the pro-domain forms a bridge with the catalytic zinc (known as "cysteine switch" mechanism), preventing enzymatic activity and acting as a ligand for the catalytic zinc atom in the active site, excluding water molecules and rendering the enzyme inactive.Moreover, tissue inhibitors of MMPs have an important role in the local control of MMP activities in tissues, and represent the main inhibitors of MMPs. The MMPs inhibitor family consists of 4 members that all together inhibit MMP activities and prevent breakdown of extracellular matrix(76). The most abundant MMP in human dentin is MMP-2, followed by MMP-9. The gelatinases MMP-2 and -9 are not considered to be true collagenases. Yet, they are crucial for the process of collagen degradation. The presence of other enzymes such as collagenase MMP-8, stromelysin-1, MMP-3 and MMP-20 that have been discovered in dentin using different methods(16).

True collagenases such as MMP-1, -8, -13, -18 are not capable of cleaving intact collagen molecule at the cleavage site, because of the collagen molecule orientation and the position of the C-terminal end, which blocks access to the peptide bonds(77). Gelatinases, that belong to the large group of telopeptidases, can remove blocking C-

terminal telopeptides, allowing access to the true collagenases. Consequently, collagenases can come in contact with the collagen at the cleavage site, turning it into fragments: a 3/4 N-terminal and a 1/4 C-terminal fragment. Removal of the telopeptides also eliminates the C-terminal cross-links, most likely making the collagen more prone to non-specific degradation(16).

As previously explained, when the dentin is mineralized, its proteases remain structurally stable and inactive(63). One of the first studies that investigated the influence of application of EAR and SE adhesive systems on MMP-2 and MMP-9 activity by means of gelatin zymography was carried out by Mazzoni et al. (2013)(64). Briefly, the authors mixed dentin powder of sound human teeth with different brands of EAR or SE adhesives, after which the adhesives were rinsed off with acetone. The treated dentin powder was then subjected to zymographic analysis in accordance with the previously established protocol(15). Interestingly, the activity of MMP-2 and - 9 after treatment with either EAR or SE adhesives were adhesive-dependent. Likewise, with SE systems, the exposure of matrix-bound MMPs was followed by increased activity, but sometime showed reduced level of activation. To sum up, the authors concluded that there was direct evidence of increased MMP-2 and -9 activities following adhesive application, regardless of the adhesive system used (EAR or SE). (88) Another interesting approach using in situ instead of only using gelatin zymography was suggested by the same groups of authors. This study was one of the firsts to evaluate the activity of endogenous proteases of the HL by means of in situ

zymography, showing obvious gelatinolytic activity within HLs created with a two-step EAR adhesive(62).

Strategies for preservation of the HL

As introduced before, degradation of collagen fibers and hydrophilic resin components lead to degradation of the hybrid layer and can cause the loss of dentin bond strength over time. Currently,

the literature suggests two distinct methods of preserving HLs (16)

1. inhibition of enzymatic activity (mostly referring to MMPs activity)
2. increasing the collagen resistance to degradation

Ultimately, alternative phosphoric acid etchants containing MMPs inhibitors have been developed and investigated (108). These are classified according to their own individual organic components and functional monomers and designed to be used not only on dental tissues but also on restoration materials such as ceramic or zirconia (87). A novel experimental zirconium oxynitrate conditioner [$ZrO(NO_3)_2$] has been recently proposed in adhesive dentistry, while previously has only been used in applied chemistry or as radiopacifying material for endodontic cements (18). Promising results have been obtained when the $ZrO(NO_3)_2$ etchant was used in combination with different adhesive systems to enamel (108). The use of an etching product that can selectively solubilize the hydroxyapatite of the dentin, reduce nanoleakage and counteract the MMPs-mediated proteolytic activity at the adhesive interface is desirable to maintain the stability of the bond over time and could be attractive in term of enhanced adhesive technology.

Research Question

As previously reported, the application of lithium disilicate in dentistry is nowadays very widespread and the literature recognizes good mechanical and optical properties and reports good long-term clinical results in the realization of indirect restorations, especially if cemented using the latest adhesive materials and following carefully the adhesive protocols. However, adhesion of lithium disilicate is still a hotly debated topic in scientific journals, in order to find a solution for the "weak points" previously reported, both at the level of the tooth-cement interface and at the cement-restoration interface. In addition, from the initial overview it emerged that some aspects of the topic have not been treated at all or at least require further analysis; the aim of this thesis was to investigate these aspects, through 3 distinct research protocols, in particular:

1. to evaluate the influence of different decontamination approaches on shear bond strength of a multi-step resin cement to LiSi restorations
2. to evaluate the effect of different types of LiSi (pressed vs CAD-CAM) on the shear bond strength (SBS) to resin cement.
3. to evaluate the immediate (T_0) *vs* 1 year (T_{12}) microtensile bond strength and interfacial nanoleakage expression and the effects on endogenous enzymatic activity within the HL of two representative simplified adhesive systems (one 2-step adhesive and one universal adhesive) when applied after a traditional H_3PO_4 acid etchant or an experimental metal-based $ZrO(NO_3)_2$ conditioner to dentin.

RESIN BONDING TO LITHIUM-DISILICATE CERAMIC AFTER DIFFERENT SURFACE CLEANING APPROACHES

Introduction

Computer-aided design and computer-aided manufacturing (CAD/CAM) represent the last frontiers of dental technology for the fabrication of ceramic restorations. CAD/CAM lithium-disilicate (LiSi) ceramics have revealed more uniform surface characteristics and less susceptibility to discoloration compared to traditionally fabricated ceramics (54), making them materials of excellence for the manufacturing of reliable restorations with high aesthetics (61).

The quality of the bond between ceramic restoration and tooth substrates relies on the luting procedures. At this stage, the selection of the adequate cement materials along with the suitable ceramic surface treatments are pre-requisites for the achievement of enhanced bonding performances and clinical success (50,58,113). The universal adhesives in combination with resin cements have demonstrated good *in vitro* effectiveness and clinical performances when used for bonding to LiSi ceramic restorations (19,55). In general, adhesion to ceramic restorations occurs after treatment of the restoration with hydrofluoric acid followed by silanization. The microporosities and surface modifications due to acid etching and the chemical coupling provided by silane solutions contribute to high retentive patterns (20). In most cases, etching is performed by the dental technician prior to delivery to the dental office, reducing chairside time. Alternatively, this procedure can also be performed by the dentist.

Despite several studies that support the beneficial effects of hydrofluoric acid etching as all-ceramic surface treatment, this substance has a high toxic potential and in some countries its use is even banned (80,102). Moreover, the high reactivity of hydrofluoric acid makes silica-based glass ceramics easily contaminable (60). Contamination is impossible to avoid during the try-in of the prosthetic manufact in

the patient's mouth prior to cementation, as saliva, blood or fitting silicone paste may be deposited on the ceramic surface and hinder subsequent proper interaction with the luting material.

Saliva is a water-based fluid mixed with blood, enzymes, nitrogenous compounds (urea and ammonia), glycoproteins, epithelial cells, bacteria, food debris, several electrolytes (sodium, potassium, calcium, magnesium, bicarbonate) and other components. When saliva comes into contact with the ceramic surface, an acquired film is formed that causes surface changes (lower surface free energy and decreased wettability) and counteracts the adhesive effectiveness of luting materials (4,30,116).

The sole water rinsing was not efficient in removing the biofilm of saliva from restorations (1). To recompose the material's characteristics and simultaneously provide surface decontamination, several surface cleaning methods have been approached (58,109). Re-etching with hydrofluoric acid is a viable method (52), although some authors have hypothesized detrimental over-etching effects on the physical characteristics of ceramics (71,109). Recently, new possibilities are emerging with the introduction of new products on the market with alternative compositions able to provide etching and priming of the surface in just one application (101).

Determining the most appropriate decontamination strategy for a LiSi restoration is critical for the clinician to enable the stability of the luting system and enhance the micromechanical and chemical adhesion to dental substrates (83). Accordingly, the objective of this laboratory study was to evaluate the influence of different decontamination approaches on shear bond strength of a multi-step resin cement to LiSi restorations. The null hypothesis tested was that no differences exist between the different cleaning protocols on the bond strength to LiSi restorations.

Material & Methods

Specimens' preparations

Seventy ceramic specimens were obtained by sectioning lithium disilicate CAD/CAM blocks (IPS e.max CAD – Ivoclar Vivadent, Schaan, Liechtenstein) with a slow-speed

diamond saw (Micromet - Remet, Bologna, Italy) under water cooling. All specimens were sintered in a laboratory furnace (Programat P500/G2 - Ivoclar Vivadent). Afterwards, each LiSi ceramic specimen was embedded in self-curing polymethylmethacrylate (PMMA) resin (Technovit 4071, Kulzer, Hanau, Germany) leaving one side of the specimen free. After complete setting of the resin, the ceramic surface was flattened by wet polishing with #600 grit silicon carbide paper for 120 s. The specimens were ultrasonically cleaned (Transsonic T460/H – Elma) in 50% ethanol for 2 min. Fifty out of seventy ceramic blocks were pre-etched with 5% hydrofluoric acid gel (Ips ceramic gel, Ivoclar Vivadent) for 20 s (Group 1,2,3,4,6), water rinsed for 1 min and air-dried, to simulate surface etching as performed by the dental laboratory. These specimens were further ultrasonicated in 50% ethanol for 2 min. The remaining 20 ceramic specimens (Groups 5 and 7) did not receive any prior hydrofluoric acid etching. The preparation, conditioning, cleaning and luting procedures were carried out by the same operator.

The LiSi specimens were contaminated with fresh human saliva obtained from a healthy male donor who refrained from consuming food and drink the 2 hours prior to saliva collection. The saliva was gathered with a cotton pellet and applied on the specimens' surface for 10 min. Each specimen was treated with fresh saliva, collected on the spot from the same donor. Afterwards, specimens were squeezed with finger pressure into the freshly mixed silicone disclosing medium (Fit Checker, GC; Tokyo, Japan) for 2 min. The contaminated specimens were randomly allocated to one of the following groups, according to the surface cleaning method (n=10):

Group 1: specimens were only water rinsed for 20 s followed by air drying for 20s (Control, C);

Group 2: 37% H_3PO_4 was applied for 20 s, then the specimens were water rinsed and air dried (PA);

Group 3: 70% ethanol was brushed for 20 s and air drying was performed for 20 s (E);

Group 4: Ivoclean (Ivoclar Vivadent) was applied for 20 s, then the specimens were thoroughly water rinsed and air dried for 20 s (IVO);

Group 5: Hydrofluoric acid gel (Ips ceramic gel, Ivoclar Vivadent) was applied for 20 s, water rinsed and air dried for 20 s (HF);

Group 6: After application of HF, the single solution Monobond Etch&Prime (Ivoclar Vivadent) was rubbed for 20 s, water rinsed and air dried for 20 s (HFMEP);

Group 7: MEP applied as in group 6, without previous HF acid etching (MEP).

A silane coupling agent (Monobond Plus, Ivoclar Vivadent) was applied for 60s on the ceramic surfaces and gently air-dried for 10s (Groups 1-5), with the exception of Groups 6 and 7 (HFMEP and MEP) as the MEP single product already contain priming solutions as prompted by manufacturers.

Two 2-mm layers of a nano-hybrid resin composite material (Empress direct; Ivoclar Vivadent) were compacted into cylindrical silicone molds (inner dimensions: 4 mm diameter and 4 mm height). Each layer was light-cured for 40s (Bluephase G2; Ivoclar Vivadent) and, after removal of the cylinder from the mold, additional polymerizations were performed from all sides for 40 s. The composite cylinders were wet-polished by #600 grit silicon carbide paper for 120 s and then ultrasonically cleaned in 50% ethanol for 2 min.

A universal bonding in conjunction with a resin cement (Adhese Universal and Variolink Esthetic DC, respectively; Ivoclar Vivadent) were used to lute composite cylinders to ceramic surfaces. The resin cement excesses were removed with a microbrush, and the surfaces were light-cured for 40 s. After polymerization, the specimens were stored in deionized water at 37 °C for 24 h before being submitted to the micro shear bond strength test (μSBS). The ceramic blocks were inserted into a customized specimens support and the shear bond strength test was performed using a universal testing machine (Instron 4301, IL, USA) at a crosshead speed of 0.5 mm/min. Shear forces were applied at the ceramic/composite interface until debonding occurred.

After testing, the debonded specimens were observed under a stereomicroscope at 50x to assess the failure pattern, as follows: adhesive between ceramic and resin cement (A), cohesive within the resin cement or composite (C) or mixed when A and C occurred simultaneously (M).

Two specimens per group were randomly selected, sputter-coated and observed with a scanning electron microscope (SEM, Jeol, Tokyo, Japan) at different magnifications to evaluate ceramic surface morphologies after SBS test.

After failing the normality validation (Shapiro-Wilk test), the data were statistically analyzed (SigmaPlot, Systat Software Inc., Chicago, IL, USA) with the Kruskal-Wallis test followed by pairwise multiple comparisons (Dunn's test) ($p<0.05$).

Results

Table 2 shows the mean shear bond strength values and standard deviations with statistically significant differences and mode of failure of the tested groups.

Statistical analysis revealed that the type of ceramic surface cleaning approach influenced the shear bond strength ($P<0.001$). The highest bond values were obtained when Monobond Etch&Prime was used as cleaning solution (Groups 6 and 7), regardless of the previous etching of the ceramic surface with hydrofluoric acid. The MEP groups did not differ significantly from Group 5, where re-etching with HF acid was performed. The lowest bonding values were registered when water rinsing, ethanol and phosphoric acid etching were used as cleaning procedures. In particular, the following comparisons were observed: HFMEP=MEP=HF≥IVO≥HP=E=C.

All groups homogeneously recorded a majority of adhesive fractures at the resin cement/ceramic surface level. However, mixed failures were observed for the HFMEP, MEP and HF groups. No cohesive failures were detected.

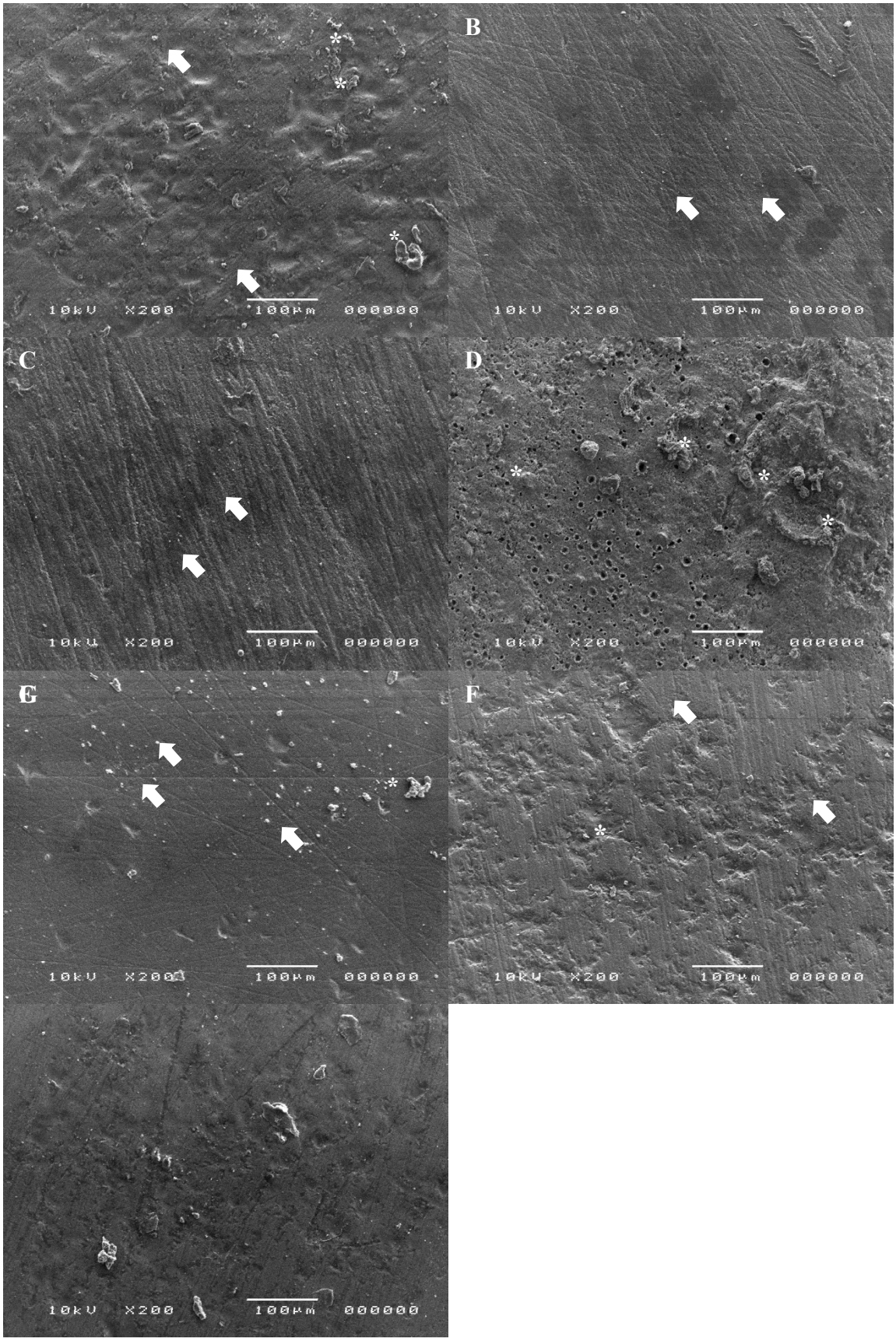
Figure 1 shows a representative panel with SEM images of LiSi ceramic surfaces after decontamination procedures performed in the tested groups. None of the cleaning methods tested resulted in a surface completely free of salivary contamination. In general, no signs of defects or cracks were observed in the examined LiSi ceramic

surfaces. The specimens of the water rinsed group (C) were smooth and covered by organic and inorganic components (Fig. 1A), while HF was able to procure the roughest surface among groups (Fig. 1E). The presence of resin cement remnants still attached to the ceramic surface were observed in groups previously cleaned with HFMEP, MEP and HF (Fig. 1E-G). Among the MEP groups, a higher presence of organic contaminants was observed when the primer was applied on unetched ceramic surface. A preponderance of residues of the silicon material encompassing the entire adhesive surface were observed after Ivoclean cleaning (Fig. 1D).

***Table 2**. Mean shear bond strength (standard deviations) among the different ceramic surface cleaning approaches. C: water rinsing; PA: 37% phosphoric acid etching; E: 70% ethanol; IVO: 20s Ivoclean; HF: 5% hydrofluoric acid re-etching; MEP: Monobond etch&Prime after 5% HF; MEP2: Monobond Etch&Prime without prior HF etching.*

Cleaning methods	**Mean (SD)**
C	145 (43) **c**
PA	130.4 (22) **c**
E	128.6 (15.1) **c**
IVO	179.9 (44.7) **bc**
HF	211 (39.2)**ab**
HFMEP	195.9 (27) **a**
MEP	212.3 (20.7) **a**

***Figure 1.** Representative panel of LiSi ceramic surfaces after cleaning with the tested methods. A) water rinsing; B) 37% phosphoric acid etching; C) 70% ethanol; D) Ivoclean; E) 5% hydrofluoric acid etching; F) Monobond Etch&Prime after HF; G) Monobond Etch&Prime. White arrows indicate the presence of organic contaminants. Traces of silicone residuals were also observed (asterisks).*


B
C
D
F
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PRESSED VS CAD/CAM LITHIUM DISILICATE BOND STRENGTH EVALUATION

Introduction

Metal-free restorations have become increasingly popular into the daily clinical practice in order to satisfy the high esthetic demand. Among these materials, ceramics have undergo to a series of improvement processes becoming very perfomant from both mechanical and esthetic point of view (Dolev E, Bitterman Y, Meirowitz A. Comparison of marginal fit between CAD-CAM and hot-press lithium disilicate crowns. J Prosthet Dent 2019;121: 124-8; Biscaro L, Bonfiglioli R, Soattin M, Vigolo P. An in vivo evaluation of fit of zirconium-oxide based ceramic single crowns, generated with two CAD- CAM systems, in comparison to metal ceramic single crowns. J Prosthodont 2013;22:36-41). The long-term success of restorative materials largely depends on the retention to the dental substrates, that, in turn, is obtained through the interconnection with the resin cement (Tan PL, Gratton DG, Diaz-Arnold AM, Holmes DC. An in vitro comparison of vertical marginal gaps of CAD-CAM titanium and conventional cast res- torations. J Prosthodont 2008;17:378-83; Demir N, Ozturk AN, Malkoc MA. Evaluation of the marginal fit of full ceramic crowns by the microcomputed tomography (micro-CT) technique. Eur J Dent 2014;8:437-44). Debondings at the cement/dentin or cement/restoration interfaces are adverse events that lead to premature failure of the restoration, resulting in discomfort for the patient and operator and increased expense. Different chemo/mechanical treatment of the

intaglio surface of the restorations have been proposed over time, mainly differentiated according to the type of material the restoration is made of.

Advances in material technology led to the development of lithium disilicate (LiSi) that is a particle-filled glass-ceramic increasingly used in dental office thanks to the excellent biocompatibility properties, optical and mechanical characteristics. Nowadays, LiSi has become one of the most used materials for indirect monolithic and veneered restorations.(52,110,113,115).

LiSi is commercially available in two forms, namely heat-pressed crystalized ingots (i.e. IPS e.max Press) and blocks to be used with the CAD/CAM technology (i.e. IPS e.max CAD). The use of CAD/CAM technology has increased exponentially both in the laboratory and in the dental office possibly simplifying the prosthetic workflow by reducing clinical steps and operator's mismanipulation (Papadiochou S, Pissiotis LA. Marginal adaptation and CAD-CAM technol- ogy: a systematic review of restorative material and fabrication techniques. J Prosthet 2017;7:1-7). Traditionally, after impression and cast pouring, the models are sent to the laboratory for restoration manufacturing. However, nowadays technologies has led to the introduction of ceramic blocks aimed at the production of restorations by milling devices (IPS e.max CAD), also suitable for chairside production of restorations(115).

CAD/CAM LiSi ceramics have revealed more uniform surface characteristics and less susceptibility to discoloration compared to traditionally fabricated ceramics (54), making them materials of excellence for the manufacturing of reliable restorations with high aesthetics (61). In the literature various in vitro aspects have been investigated

regarding the differences between CAD and pressed disilicate, such as marginal fit, cyclic fatigue, flexural strength, with comparable results between the two materials(9,26,29,89).

The data in the literature do not clearly indicate which of the two techniques may be preferred over the other, leaving the dentist to choose during the treatment plan. One of the difference between the two techniques can be identified during the surface treatment with hydrofluoric acid (HF) before bonding procedures. Indeed, the surface of pressed disilicate appears much opaquer and chalkier compared to the CAD/CAM LiSi after the etching process.

Regardless of the surface pre-treatment of both materials, contamination with saliva and silicone paste may occur during the try-in phases of the restoration before cementation. Residuals of these materials may deposit on the surface and hamper the proper interaction between luting materials and the restoration (REF). To the author's best knowledge, there are no data in literature regarding the effect of saliva and silicone paste on the bond strength of pressed and CAD/CAM LiSi to resin cement.

Accordingly, the aim of this study was to evaluate the shear bond strength of a multi-step resin cement to pressed vs CAD LiSi after contamination with saliva and silicone paste. In particular, the null hypotheses tested were that: 1) there is no differences between pressed and CAD LiSi on the bond strength both to resin cements; 2) decontamination of the ceramic surface do not influence the bond strength; and that 3) aging do not affect the bond strengths of the materials tested.

Material & Methods

Specimens' preparations

Sixty CAD-CAM specimens were obtained by sectioning lithium disilicate CAD/CAM blocks (IPS e.max CAD – Ivoclar Vivadent, Schaan, Liechtenstein) with a slow-speed diamond saw (Micromet - Remet, Bologna, Italy) under water cooling. All specimens were sintered in a laboratory furnace (Programat P500/G2 - Ivoclar Vivadent). On the other hand pressed samples were obtained by pressing lithium disilicate ingots in sixty wax samples. Afterwards, each LiSi ceramic specimen was embedded in self-curing polymethylmethacrylate (PMMA) resin (Technovit 4071, Kulzer, Hanau, Germany) leaving one side of the specimen free. After complete setting of the resin, the ceramic surface was flattened by wet polishing with #600 grit silicon carbide paper for 120 s. The specimens were ultrasonically cleaned (Transsonic T460/H – Elma) in 50% ethanol for 2 min.

The preparation, conditioning, cleaning and luting procedures were carried out by the same operator.

Half of the LiSi specimens were contaminated with fresh human saliva obtained from a healthy male donor who refrained from consuming food and drink the 2 hours prior to saliva collection. The saliva was gathered with a cotton pellet and applied on the specimens' surface for 10 min. Each specimen was treated with fresh saliva, collected on the spot from the same donor. Afterwards, the contaminated specimens were squeezed with finger pressure into the freshly mixed silicone disclosing medium (Fit Checker, GC; Tokyo, Japan) for 2 min.

Summing up, the specimens formed the four following groups:

Group 1: CAD/CAM lithium disilicate contaminated samples (Cad C);

Group 2: CAD/CAM lithium disilicate non-contaminated samples (Cad NC);

Group 3: Pressed lithium disilicate contaminated samples (Press C);

Group 4: Pressed lithium disilicate non-contaminated samples (Press NC).

A single bottle ceramic self-etching silane primer (Monobond Etch & Prime, Ivoclar Vivadent) was applied for 60s on the ceramic surfaces and gently air-dried for 10s

Two 2-mm layers of a nano-hybrid resin composite material (Empress direct; Ivoclar Vivadent) were compacted into cylindrical silicone molds (inner dimensions: 4 mm diameter and 4 mm height). Each layer was light-cured for 40s (Bluephase G2; Ivoclar Vivadent) and, after removal of the cylinder from the mold, additional polymerizations were performed from all sides for 40 s. The composite cylinders were wet-polished by #600 grit silicon carbide paper for 120 s and then ultrasonically cleaned in 50% ethanol for 2 min.

A universal bonding in conjunction with a resin cement (Adhese Universal and Variolink Esthetic DC, respectively; Ivoclar Vivadent) were used to lute composite cylinders to ceramic surfaces. The resin cement excesses were removed with a microbrush, and the surfaces were light-cured for 40 s. After polymerization, half of the specimens were stored in deionized water at 37 °C for 24 h, the other half underwent to thermocycling process before being submitted to the micro shear bond strength test

(μSBS) The thermocycling protocols was as follows: 10.000 cycles, 5-55°C (dwell time 30 s).

Shear Bond Strenght test

The ceramic blocks were inserted into a customized specimens support and the shear bond strength test was performed using a universal testing machine (Instron 4301, IL, USA) at a crosshead speed of 0.5 mm/min. Shear forces were applied at the ceramic/composite interface until debonding occurred.

After testing, the debonded specimens were observed under a stereomicroscope at 50x to assess the failure pattern, as follows: adhesive between ceramic and resin cement (A), cohesive within the resin cement or composite (C) or mixed when A and C occurred simultaneously (M).

Two specimens per group were randomly selected, sputter-coated and observed with a scanning electron microscope (SEM, Jeol, Tokyo, Japan) at different magnifications to evaluate ceramic surface morphologies after SBS test.

For the shear bond strength test the data were homogeneous (modified Levin test) and normally distributed (Shapiro-wilk test). Consequently 3 way analysis of variance (ANOVA) and multiple comparison procedure (Tukey method) were performed to investigate the effect of three variables “material”, “contamination”, “aging”, and their interactions on shear bond strength. The level of significance was set to p=.05

Results

The statistical analysis revealed that the type of material (press vs CAD), the contamination (contaminated vs decontaminated) and aging (baseline vs

thermocycling) influenced the bond strengths ($p<0.05$). No differences were found between the interaction between factors ($p>0.05$).

In particular, IPS e.max Press resulted in higher shear bond strength values compared to IPS e.max CAD, independent of the aging period ($P<0.001$). At baseline, contamination significantly influenced the results for both materials ($P<0.001$). However, no differences were found between contaminated and decontaminated surfaces after thermocycling for IPS e.max Press ($p=0.05$). Instead, contamination of the intaglio surface with saliva and try-in paste significantly influenced the results for IPS e.max CAD that obtained inferior results than when bonded to the decontaminated surface ($P<0.001$) and, in general, the lowest values among the groups ($p<0.05$), and this was present both before and after thermocycling ($p<0.05$).

Morphological anlysis with SEM showed different morphologies between the two materials tested. The presence of residues were observed on the contaminated surfaces, independent of the ceramic material.

BONDING TO DENTIN USING AN EXPERIMENTAL ZIRCONIUM OXYNITRATE ETCHANT

Introduction

Since the advent of dental bonding techniques, adhesion to dentin has represented a challenge for researchers and clinicians. Structural complexities of the dentinal substrate, such as the hydration state that is wet in nature (66), the high heterogeneity of the intrinsic constituents (74) and the sensitivity of the organic matrix component to the operator experience (66), make its treatment inquisitive.

Dentin bonding mechanism is a form of tissue engineering (74), based on consecutive clinical steps aimed at "conditioning" and "priming" the tooth substrate as to create the surface conditions necessary to receive the resin-based adhesive and form together the hybrid layer (HL). Clinical errors occurring during one of these stages, along with chemical (i.e. hydrolysis of the resin functional monomers, plasticization of the uncured resin mesh) (13,57) and physical problems (i.e. incomplete infiltration of the resin blend into the collagen matrix) (16,74) at the resin-dentin level, contribute to premature deterioration of the dentin/adhesive seal (16,17).

The adhesion process begins with superficial dentin demineralization, either with a specific acid etching or with an acidic self-etch approach. After the treatment of the dentin surface, dentin collagen fibrils are denuded, and ideally, the adhesive resin should fully infiltrate the fibrils and envelope and interact with them to form a stable HL. However, a thick but poorly impregnated hybrid layer, characterized by denuded

collagen fibrils surrounded by water molecules of dental origin at the bottom of the HL after the adhesive procedures have been observed (2,46). Different studies have shown it might be susceptible to enzymatic degradation by host-derived collagen-bound matrix metalloproteinases (MMPs) (63,73) and by cysteine cathepsins (75) that in presence of water can hydrolyze collagen fibrils, degrade the hybrid layer and undermine the longevity of the resin-dentin bonds (16),(15).

Unfortunately, despite the continuous attempts to improve the bonding performances of the adhesive materials and to promote more stable adhesive interfaces through the introduction of therapeutic compounds that could act as preservative intermediate agent, degradation phenomena of the adhesive interface are present in all types of dentin bonding system approaches (16,17,74).

Therapeutic systems, blended to the adhesive or used as primer in a separate step, have been proposed to prevent the degradation of the bonded layer mediated by MMPs (6,16,40). Ultimately, alternative phosphoric acid etchants containing MMPs inhibitors have been developed and investigated (108). These are classified according to their own individual organic components and functional monomers and designed to be used not only on dental tissues but also on restoration materials such as ceramic or zirconia (87). A novel experimental zirconium oxynitrate conditioner [$ZrO(NO_3)_2$] has been recently proposed in adhesive dentistry, while previously has only been used in applied chemistry or as radiopacifying material for endodontic cements (18). Promising results have been obtained when the $ZrO(NO_3)_2$ etchant was used in combination with different adhesive systems to enamel (108). The use of an etching product that can

selectively solubilize the hydroxyapatite of the dentin, reduce nanoleakage and counteract the MMPs-mediated proteolytic activity at the adhesive interface is desirable to maintain the stability of the bond over time and could be attractive in term of enhanced adhesive technology [9].

Single-component universal adhesives are the latest category of simplified bonding materials used to reduce the possibility of iatrogenically induced clinical mis-manipulation. Despite their acclaimed versatility allowing them to adapt to different clinical situations alongside the feasibility to choose the bonding strategy to be combined (self-etch, etch-and-rinse or selective enamel etching), concerns have been expressed regarding their ability to simultaneously demineralize and interact with dentin when used in the self-etch mode, as to create effective and durable bonds (21). However, when applied in the etch-and-rinse modality, resin-poor but water-rich dentin nanopores were observed on the adhesive interfaces [2], whereabouts the dentin MMPs, once activated, have decreed for a premature degradation of the resin-dentin bond and have reduced the lifetime of the restoration (76). The association of universal adhesives with therapeutic primers has demonstrated improvements in the adhesion strength to dentin and in maintaining the adhesive bond stability over time (15,16,74).

Therefore, according to the abovementioned considerations, the aim of the present *in vitro* study was to evaluate the immediate (T_0) *vs* 1 year (T_{12}) microtensile bond strength and interfacial nanoleakage expression and the effects on endogenous enzymatic activity within the HL of two representative simplified adhesive systems (one 2-step adhesive and one universal adhesive) when applied after a traditional

H_3PO_4 acid etchant or an experimental metal-based $ZrO(NO_3)_2$ conditioner to dentin. In particular, the null hypothesis tested were that the experimental $ZrO(NO_3)_2$ etchant material: 1) does not influence the immediate bonding performances of the tested adhesive systems to dentin; 2) does not decrease dentin bond durability over time; 3) has no effect on the activity of endogenous dentin MMPs immediately or over time**.**

Material & Methods

Microtensile bond strength test (μTBS)

Sixty-four freshly extracted non-carious sound human third molars were obtained from anonymous individuals following their informed consent under a protocol approved by the Ethical Committee of the University of Bologna, Bologna, Italy (protocol N°: 71/2019/OSS/AUSLBO).

Tooth crowns were removed with a low-speed diamond saw under water cooling (Microremet, Remet, Bologna, Italy) to expose enamel-free deep coronal dentin. The absence of enamel remnants was assessed under an optical microscope. A standardized smear layer was created on dentin surface with 600-grit wet silicon carbide (SiC) paper. Two adhesive approaches were used for bonding procedures in this study: a universal adhesive,Adhese Universal (AU), Ivoclar Vivadent, Schaan, Liechtenstein) used in the etch-and-rinse mode, and a 2-step self-etch adhesive,Excite F (EF), Ivoclar Vivadent). Prior to adhesive application, dentin conditioning was performed with one of the following products: a conventional 37% H_3PO_4 etchant, Total Etch (TE, Ivoclar Vivadent) or an experimental zirconium oxynitrate gel (ZON, Ivoclar Vivadent).

So, at the end, four groups could be formed (n=16): 1) ZON + AU; 2) TE + AU; 3) ZON + EF; 4) TE + EF. Chemical composition and instruction for use are presented in Table 1.

After adhesive polymerization, a single 4 mm-thick layer resin composite build-up was placed with a nanohybrid composite (Tetric Evo Ceram Bulk Fill, Ivoclar Vivadent) and light polymerization was performed for 20 s with a LED curing lamp (Demi™Plus, Kerr Dental; light output > 500 mW/cm² and wavelength 440-480 nm).

Specimens were serially sectioned to obtain approximately 1-mm-thick beams in accordance with the non-trimming technique of the microtensile test. The dimension of each stick (ca. 0.9 × 0.9 × 8 mm) was recorded using a digital caliper (± 0.01mm) and the bonded area was calculated for subsequent conversion of microtensile strength values into units of stress (MPa). Beams were stressed to failure after 24 h (T_0) or 1 year (T_{12}) of storage in artificial saliva at 37 °C using a simplified universal testing machine (Bisco Inc., Schaumburg, IL, USA) at a crosshead speed of 1 mm/min. The number of prematurely debonded sticks in each test group was registered, but these values were not included in the statistical analysis because they did not exceed 3% of the total number of tested specimens and were similarly distributed within the groups. A single observer evaluated each side of the fractured sticks with optical microscopy at 50× magnification to determine the mode of failure, and classified them as adhesive (A), cohesive in dentin (CD), cohesive in composite (CC), or mixed failures (M; adhesive and cohesive fractures occurred simultaneously).

The study involved three independent variables: the adhesive systems, the dentin conditioner and the aging. After checking the normal and equal distribution (Kolmogorov-Smirnoff and Levene's tests respectively), microtensile bond strength data were submitted to the three-way analysis of variance (ANOVA), while the Tukey test was used for pairwise multiple comparisons. The nanoleakage results were analyzed using the Chi-squared test. The significance threshold was set at $p=0.05$. All the statistical analyses were performed using Stata v. 12.0. software (StataCorp LLC, College Station, TX, USA).

Nanoleakage expression assessment

Four additional teeth per group were sectioned into 1 mm-thick slices of mid-coronal dentin and treated with the same bonding procedures as previously described for μTBS test. The 1-mm thick composite build-up was made using TetricEvo Flow composite (Ivoclar Vivadent). The specimens were cut vertically into 1-mm-thick sticks to expose the bonding surfaces. Half of the sticks were stored in the artificial saliva at 37 °C for 24h (T_0), while the remaining half was stored for 12 months (T_{12}). After aging, specimens were immersed in 50 wt% ammoniacal $AgNO_3$ solution for 24 h following the protocol described by Tay *et al.* (98), thoroughly rinsed in distilled water, and immersed in a photo-developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interfaces.

The specimens were subsequently fixed on glass, flattened on a grinding device (LS2; Remet) under water irrigation using a series of abrasives (180-, 600-, 1200-,

2400-, and 4000-grit SiC) and observed using a light microscope (E800; Nikon, Tokyo, Japan). Images of the adhesive interfaces were obtained (original magnification: 20x) and the degree of interfacial nanoleakage expression was quantified using a four-point scale by one experienced investigator. Scoring was performed using the methodology described by Saboia *et al.* (85).

Gelatin zymographic analysis

Zymographic analysis was performed in accordance to Mazzoni *et al.* (64). In brief, mineralized dentin powder was obtained from eight human third molars. Teeth were ground free of enamel, pulpal soft tissue, and cementum; dentin powder was obtained by freezing the dentin in liquid nitrogen and triturating it by means of a Retsch mill (Reimiller, Reggio Emilia, Italy). The fine mineralized dentin powder was pooled, dried, and kept frozen until use. Aliquots of mineralized dentin powder were divided into 3 groups as follows:

- Mineralized dentin, that served as control (C);
- Demineralized dentin powder with ZON for 10 min (ZON);
- Demineralized dentin powder with TE for 10 min (TE).

In the groups treated with liquid form of etchants, the acid was neutralized after 10 min and centrifuged. The supernatant was removed, and the powder was washed 2 more times with distilled water and re-centrifuged for 20 min at 4 °C (20.800 G). For protein extraction, dentin powder aliquots were re-suspended in extraction buffer (50 mM Tris–HCl pH: 6, containing 5 mM $CaCl_2$, 100 mM NaCl, 0.1% Triton X-100, 0.1%

nonionic detergent P-40, 0.1 mM $ZnCl_2$, 0.02% NaN_3) for 24 h at 4 °C, intermittently sonicated for 10 min (ca. 30 pulses), centrifuged for 20 min at 4 °C (20.800 G), then the supernatant was removed and re-centrifuged. The protein content was further concentrated using Vivaspin centrifugal concentrator (10.000kDa cut off; Vivaspin Sartorius Stedim Biotech, Goettingen, Germany) for 30 min at 25 °C (15.000 G × 3 times). Total protein concentration in the dentin extracts was determined by the Bradford assay (Bio-Rad, Hercules, CA, USA). Dentin proteins aliquots (60 µg) were diluted in Laemmli sample buffer in a 4:1 ratio and subjected to electrophoresis under non-reducing conditions in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) containing 1mg/mL fluorescein-labeled gelatin. Pre-stained low-range molecular weight SDS-PAGE standards (Bio-Rad, Hercules, CA) were used as molecular-weight markers. After electrophoresis, the gels were washed for 1 h in 2% Triton X-100, and then were incubated in zymography activation buffer (50 mmol/L Tris–HCl, 5 mmol/L $CaCl_2$, pH: 7.4) for 48 h. Proteolytic activity was evaluated and registered under long-wave UV light scanner (ChemiDoc Universal Hood, Bio-Rad). Gelatinase activities in the samples were analyzed in duplicate by gelatin zymography. The images were qualitatively assessed, and gelatinases activity quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

In situ zymography of resin-dentin interfaces

One millimeter-thick slabs of middle/deep dentin were obtained from three extracted human third molars using a low-speed saw (Micromet) with water-cooling.

Two slabs were obtained from each tooth and then further divided into 4 pieces, so that testing of all groups was performed using the same dentinal substrate. A standardized smear layer was created on each dentin surface using 600- grit SiC paper. The samples were then randomly assigned to the same 4 groups (n= 3) described for μTBS and identical bonding procedures were performed. Each bonded specimen was light-cured for 20 s using a LED light- curing unit (Demi™Plus). The adhesive-dentin interfaces were then exposed by cutting the bonded specimens vertically into 1 mm-thick sticks using a slow-speed saw under water cooling. The sticks were glued to glass slides, ground down and polished to obtain specimens of approximately 50 μm-thick, using a series of wet SiC papers. Self-quenched fluorescein-conjugated gelatin was used as the MMP substrate (E-12055, Molecular Probes, Eugene, OR, USA) for *in situ* zymography at the baseline (T_0) and after 12 months (T_{12}) of storage in artificial saliva at 37 °C as previously described (24). Briefly, the fluorescent gelatin mixture was placed on top of each slab and covered with a coverslip. The slides were incubated in a humidified chamber at 37 °C overnight. During incubation, the assemblies were prevented from direct contact with water and were protected from exposure to light. After incubation, the microscopic slides were examined using a confocal laser scanning microscope (excitation wavelength 488 nm; emission wavelength 530 nm; Model A1-R; Nikon, Tokyo, Japan). For each specimen, a series of images were made to visualize hydrolysis of the quenched fluorescein-conjugated gelatin substrate as an indicator of endogenous gelatinolytic activity. Enzymatic activity was quantified as the integrated density of the fluorescence signals using ImageJ software. Since data were normally

distributed (Kolmogorov-Smirnoff test), data were analyzed with one-way ANOVA and Tukey's *post hoc* test ($p < 0.05$).

Results

Microtensile bond strength test (μTBS)

Bond strength values at T_0 and T_{12} are reported in Table 3. The three-way ANOVA test showed that all the investigated factors ("adhesive systems", "dentin conditioners" and "aging"), significantly influenced the results ($p<0.05$). In addition, statistically significant interactions were evidenced between the adhesives/dentin conditioners and the adhesive/aging variables ($p<0.05$). At T_0, ZON/AU registered the higher mean bond strength values among the tested groups ($p<0.05$). No differences were observed between TE/AU, ZON/EF and TE/EF. Laboratory aging statistically influenced the bond strengths of ZON/AU, but these values were comparable to the mean values of TE/AU, that from its side registered the higher bonding values among the aging group ($p<0.05$). ZON/EF and TE/EF showed decreased bond strengths at T_{12}, and no differences could be observed among these groups.

Table 4 graphically summarize the failure mode distribution and percentage after μTBS test. Pattern failures were not homogeneously distributed between groups. At T_0, a set of adhesive, cohesive in composite, cohesive in dentin and mixed failures were recorded among the tested groups. Assessment of mixed fractures was inferior at T_{12} and an increase in cohesive in composite debondings was observed in the AU groups, irrespective of the dentin conditioner combination.

Nanoleakage expression assessment

Descriptive statistics of interfacial nanoleakage expression scores are reported in Figure 1. Representative light microscopy images of nanoleakage expression for tested groups either at T_0 and T_{12} are reported in Figure 3 and 4, respectively. The Chi-squared test showed differences in the amount of silver particles deposition at the resin-dentin interfaces among all the tested groups. At T_0 quantification of nanoleakage expression was similar among the experimental groups. Aging resulted in higher silver grain deposits at the adhesive interfaces compared to immediate evaluation, independent of the tested group ($p<0.05$). However, differences were assessed between the different etchant groups, with TE collecting higher silver grain accumulation into the HL compared to the ZON groups ($p<0.05$).

Gelatin zymographic analysis

Zymography results are shown in Figs. 5 and 6. The qualitative and quantitative results of the zymographic assay revealed enzymatic activity of pro- and active form of MMP-9 (92 kDa and 86 kDa respectively) in the mineralized dentin. In dentin demineralized with TE, protease expression of MMP-9 was more pronounced, and also a band in the molecular weight of the active MMP-2 was observed. ZON showed enzymatic activity in the band that corresponded to molecular weight of pro-MMP-9 slightly lower to that presented in the TE-demineralized dentin, and complete inhibition of MMP-2 and 9 activities was evidenced.

In situ zymography of resin-dentin interfaces

Qualitative and quantitative *in situ* zymography results are shown in Figs. 6-8. The green fluorescence signal observed in AU groups either at T_0 and T_{12} was lower compared to EF groups. All the groups demonstrated a general trend in enzymatic activity increase after aging. Overall, the experimental groups treated with ZON showed a lower level of fluorescence immediately as well as over time, compared to TE, regardless of the bonding system employed ($\alpha < 0.05$).

Table 3. Chemical composition and mode of employ of the materials used in the study.

Material	**Composition**	**Ph**	**Mode of use**
Adhese Universal Ivoclar Vivadent, Schaan, Liechtenstein	MDP, MCAP, HEMA, Bis-GMA, D3MA, Water, Ethanol Highly dispersed silicon dioxide Initiators and Stabilizers	2.5- 3	1. Upon etching, the adhesive is scrub on dentin for at least 20 s; 2. Air-spray with oil- and moisture-free compressed air until a glossy, immobile film layer results; 3. Light-cure using a LED light-curing unit for 20 s.
Excite F Ivoclar Vivadent, Schaan, Liechtenstein	Phosphonic acid acrylate, HEMA, D3MA Highly dispersed silica Ethanol Catalysts, stabilizers, fluoride	2.5	1. Upon etching, apply the adhesive to dentin by scrubbing for at least 10 s; 2. Disperse the adhesive with an oil- and moisture-free compressed air until a glossy, immobile film layer results; 3. Light-cure using a LED light-curing unit for 20 s.
Experimental etchant (ZON) Ivoclar Vivadent, Schaan, Liechtenstein	$ZrO(NO_3)_2$, water, glycerol, fumed silica, polyethylene oxide	0.56	1. Apply ZON and allow it to interact with the tooth surface without agitation for 30 s; 2. Thoroughly rinse off the etchant with water spray and dry with oil-free air;

			3. Continue with the bonding procedures.
Total Etch Ivoclar Vivadent, Schaan, Liechtenstein	Phosphoric acid (37 wt% in water), thickening agent and colour pigments.	0,1-0,4	Apply the etchant and allow it to interact with the tooth surface without agitation for 15 s; Thoroughly rinse off the etchant with water spray and dry with oil-free air. Continue with the bonding procedures.

Table 4. Mean values ± SD (MPa) of the tested groups after microtensile bond strength test, immediately (T_0) and after 12 months of laboratory aging (T_{12}). ZON, experimental zirconia oxynitrate [$ZrO(NO_3)_2$] etchant; TE, Total Etch conventional 37% phosphoric acid (H_3PO_4) etchant; AU, universal adhesive Adhese Universal; EF, 2-step self-etch adhesive Excite F.

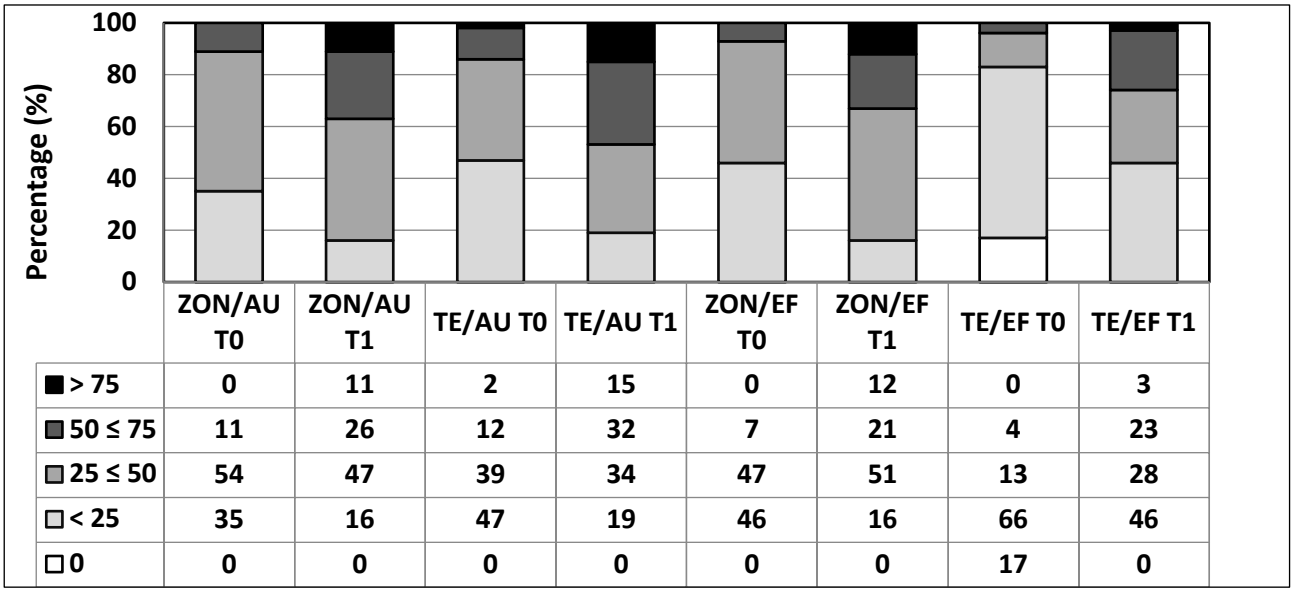
Adhesive	**T_0 (MPa)**	**T_{12} (MPa)**
ZON/AU	54.3 ± 15.04 ^{a,A}	43.91 ± 14.23 ^{a,B}
TE/AU	39.12 ± 14.20 ^{b,A}	35.97 ± 14.14 ^{a,b,A}
ZON/EF	42.11 ± 17.85 ^{b,A}	26.86 ± 14.27 ^{b,c,B}
TE/EF	37.85 ± 16.15 ^{b,A}	20.83 ± 14.83 ^{c,B}

Different lower-case letters indicate significant differences within the same column ($p < 0.05$),different upper-case letters indicate significant differences within the same row ($p < 0.05$).

Table 5. Failure mode distributions and percentages observed in the experimental groups after testing. A, adhesive; CC, cohesive in composite; CD, cohesive in dentin; M, mixed. ZON, experimental $ZrO(NO_3)_2$ etchant; TE, Total Etch 37% H_3PO_3; AU, Adhese Universal; EF, Excite F,

	Failure mode (%)							
	T_0				**T_{12}**			
	A	**CC**	**CD**	**M**	**A**	**CC**	**CD**	**M**
ZON/AU	43%	34%	0%	23%	19%	73%	6%	2%
TE/AU	28%	43%	6%	24%	24%	61%	11%	4%
ZON/EF	57%	22%	2%	20%	81%	11%	3%	5%
TE/EF	47%	21%	8%	25%	52%	32%	0%	16%

Figure 2. Percentage of interfacial nanoleakage expression in resin-dentin interfaces created among the different groups, at T_0 and T_{12}.



	ZON/AU T0	ZON/AU T1	TE/AU T0	TE/AU T1	ZON/EF T0	ZON/EF T1	TE/EF T0	TE/EF T1
> 75	0	11	2	15	0	12	0	3
50 ≤ 75	11	26	12	32	7	21	4	23
25 ≤ 50	54	47	39	34	47	51	13	28
< 25	35	16	47	19	46	16	66	46
0	0	0	0	0	0	0	17	0

Figure 3. Representative light microscopy images (20x magnification) of the tested materials in the experimental conditions and submitted to NL with silver nitrate after 24 h (T_0). D = Dentin; HL = Hybrid Layer; C = Composite. Arrows indicate areas of silver nitrate particles deposition.

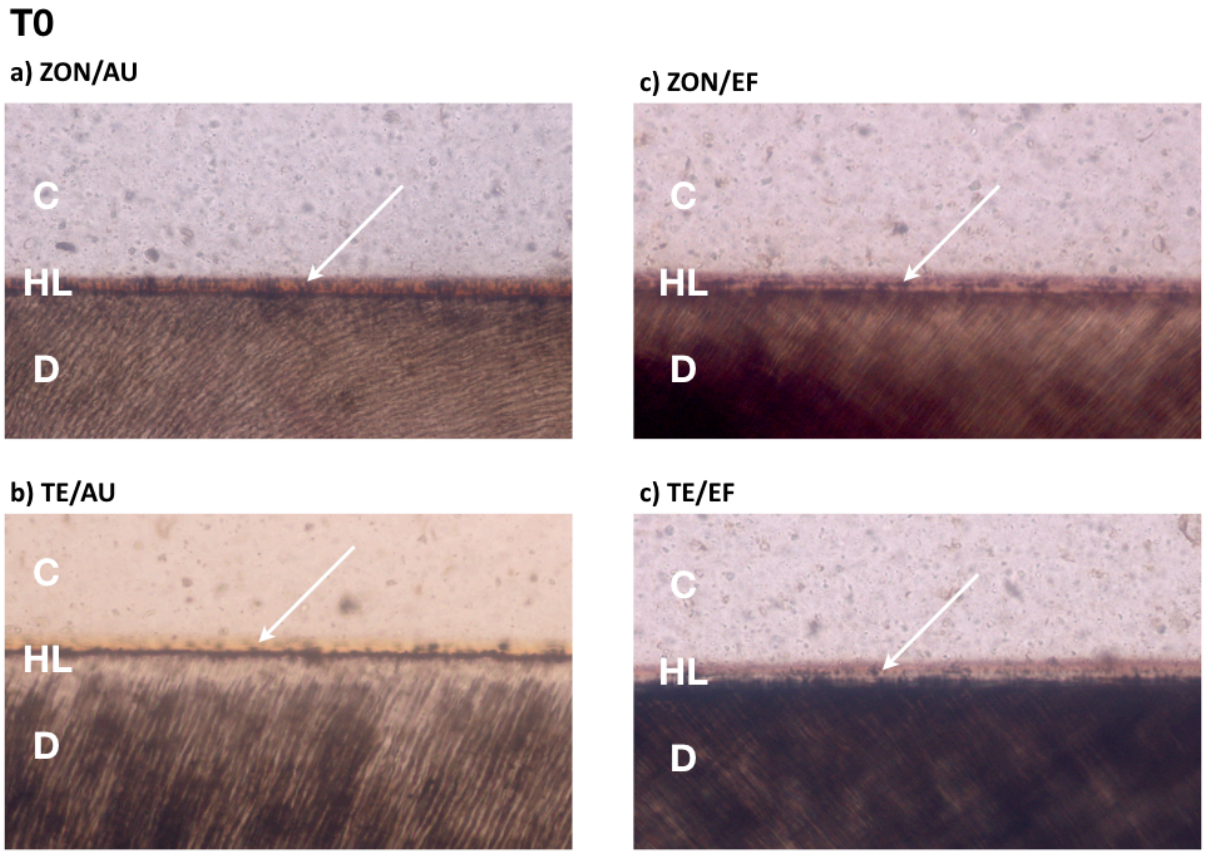


Figure 4. Representative light microscopy images (20x magnification) of the tested materials in the experimental conditions and submitted to NL with silver nitrate after 12 months of storage in artificial saliva (T_{12}). D = Dentin; HL = Hybrid Layer; C = Composite. Arrows indicate areas of silver nitrate particles deposition.

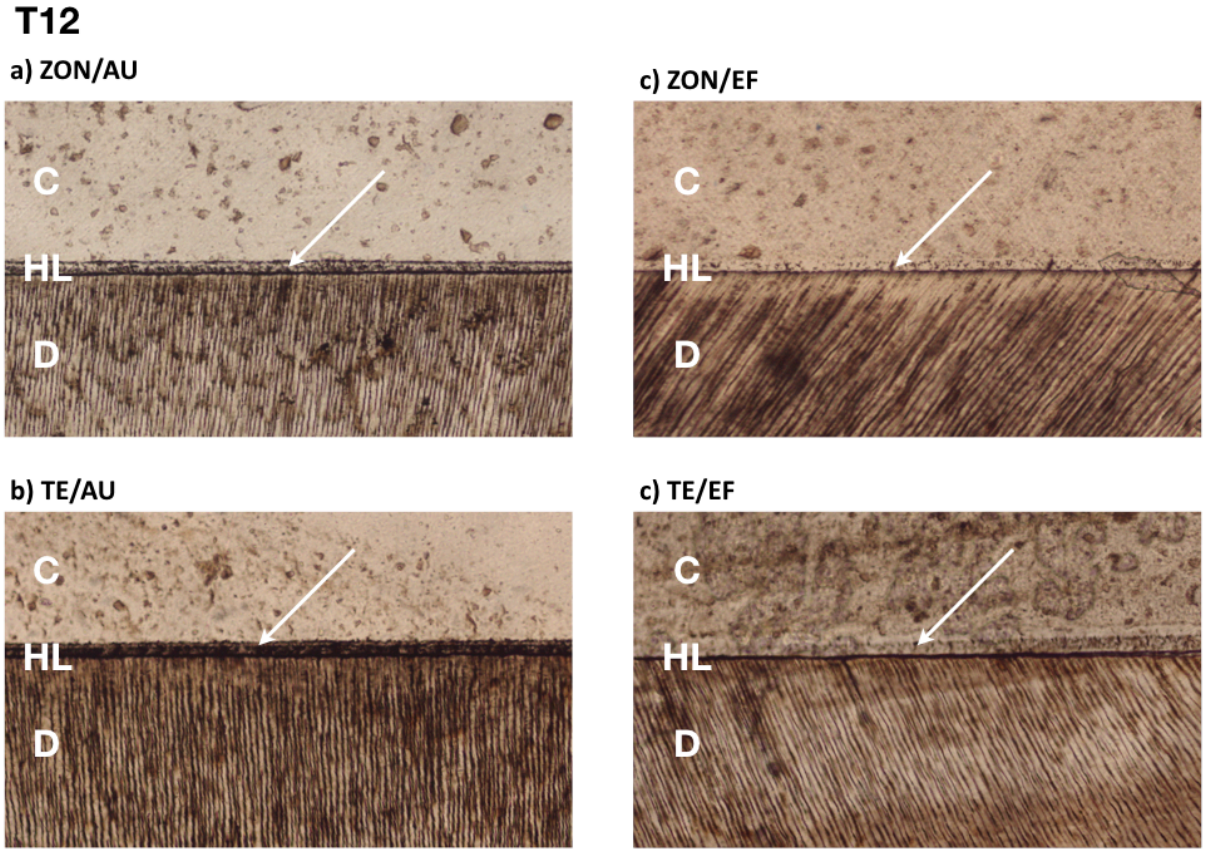
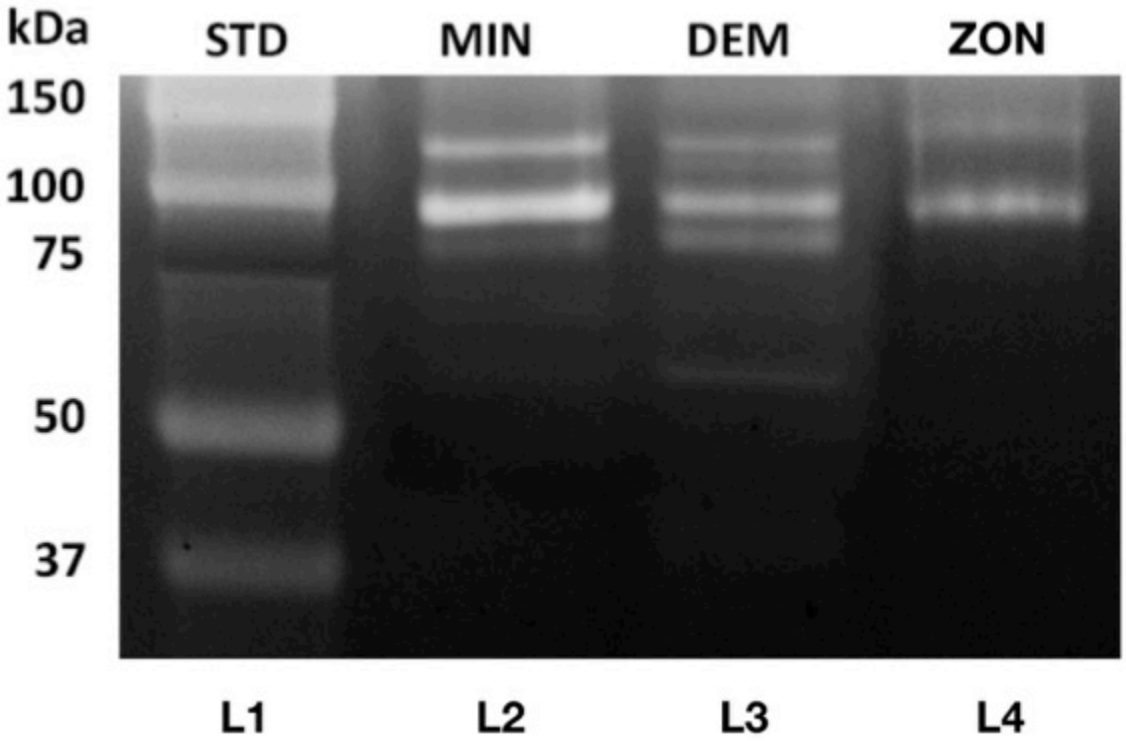


Figure 5. Zymographic analysis of proteins extracted from dentin powder. L1: Standards (STD); L2: mineralized dentin (MIN) showing the presence of activity of pro-form of MMP-9 (92 kDa) and active form of MMP- 2 (66 kDa); L3: demineralized dentin powder (DDP) showing an increase of MMP-9 (86 kDa) and the presence of the active form of MMP-2 (66 kDa); L4: Dentin powder demineralized with ZON showing inhibition of MMP-9 and MMP-2 activity.


kDa
STD
MIN
DEM
ZON
150
100
75
50
37
L1
L2
L3
L4

Discussion

According to the results of the first study, the null hypothesis must be rejected since the type of surface cleaning approach influenced the shear bond strength of the resin cement to CAD/CAM LiSi ceramics.

Digital technology has simplified the manufacturing of prosthetic restorations, greatly reducing the number of the required clinical steps. Among all these steps, the one that cannot be underestimated, however, is the fitting of the restoration in the patient's mouth before definitive cementation. This testing phase is important to confirm the correctness of the fitting, marginal adaptation, esthetic, and occlusal parameters of the prosthesis, but it encompasses the risk of exposing the restoration to contamination. Once the restoration comes into contact with saliva during the try-in phase, a glycoproteic layer which, besides creating an obstacle to the direct interaction with the adhesive system, is also capable of altering the composition of the material, is deposited in the intaglio surface of the restoration (4,45,107). Because of these critical adhesion issues, cleaning of the surface must be performed immediately after contamination.

Usually, after the fabrication of the prosthetic framework, the technician in is charged to pre-etch the intaglio surface of the restoration with hydrofluoric acid. From a practical point of view, this helps the clinician to save valuable time at the chair. On the other hand, the quality of the acid surface treatment may be compromised during the try-in of the restoration in the mouth due to contamination with saliva, blood or

other materials. Consequently, the work done by the technician is nullified and a new type of surface treatment is required. In this situation, after the try-in procedures, the dentist can choice to re-etch the inner surface of the restoration with hydrofluoric acid. Required time considerations aside, this procedure raised doubts as to how much the double exposure to hydrofluoric acid could affect the mechanical surface characteristics of the ceramic.

Several decontamination methods have been proposed over time. Common cleaning approaches consist in scrubbing the intaglio LiSi ceramic surface with water, ethanol, phosphoric acid or hydrofluoric acid(3,58,59). Alternative extra-oral solutions for decontamination of ceramic, zirconia or metal restorations have recently been introduced.

The results of the present investigation recorded the greatest adhesion values after decontamination with MEP, both without prior etching and after etching, as well as by re-etching with hydrofluoric acid (Table 1). To the best of the authors' knowledge, this is the first study present in the literature in which MEP has been used as a cleaning approach of LiSi surfaces after both saliva and silicone paste. Previous studies investigating the efficacy of MEP as ceramic surface treatment reported good bond strength results (70,101), but the eventual contamination with saliva or silicone pastes has not been taken into consideration. The possibility of using a single product that can have both decontaminating and surface conditioning capabilities has obvious clinical advantages. Manufacturers boast self-limiting etching capability as a possibility to avoid damage to structural material compositions. There were no

statistically significant differences when MEP was used, with or without previous acid etching (Table 1), likely confirming the self-limiting effect. However, higher percentage of organic residuals were observed when the simplified primer was used without previous HF acid etching, suggesting that the combined action of the two materials may have greater decontamination effectiveness than the single product. Future studies will focus on evaluating the long-term effects MEP has on material bonding and chemistry.

Ivoclean is a suspension of zirconium dioxide-based particles in an alkaline solution, intended to absorb salivary phosphate contaminants, leaving behind a clean surface. This alkaline solution has previously obtained good bond strength results when used as zirconia or glass-ceramic surface treatment (4,23,111). However, in the present study, no differences in bonding values were observed between Ivoclean and water rinsing, ethanol or phosphoric acid etching (Table 1). The presence of silicone residues from the fitting paste supposedly affected the adhesive capacity to the ceramics, and the solution seemed to not be able to adequately remove it from the surface (Fig. 1). The manufacturers of Ivoclean recommend not using this solution as a cleaning material in the presence of silicone pastes in order to avoid undesirable disturbances in the adhesion mechanisms.

Previous studies found that water rinsing and ethanol were ineffective in removing fluid residues from glass ceramic surfaces (4,14), and incapable of restoring bond strength to LiSi ceramic after saliva contamination (111). Although phosphoric acid etching was previously described as an effective method to decontaminate ceramic

surface though acidic dissolution of organic debris (109,111), the present study found no differences between phosphoric acid, water rinsing and ethanol. Contrary to previous studies, in the present protocol ceramic surfaces were contaminated with both saliva and fitting paste to simulate more realistically the clinical try-in procedures. It is highly likely that the acidic dissolution of the organic contaminants followed by the removal of the silicone debris from the subsequent water wash left no room for the acidic gel to create the micro-roughness necessary to ensure adequate retention with the adhesive system. In fact, microscopy images showed a rough ceramic surface with grinding lines present, and sparsely distributed bacterial debris.

These findings have an important clinical implication since contamination of the intaglio surface of an indirect restoration is an absolutely unavoidable event during the try-in procedure of the restoration prior to cementation. The formation of a salivary biofilm layer, or the presence of silicone residues, result in a reduced ability of resin cements to interact with the ceramic surface, decreasing their durability. Complete surface cleaning cannot be expected (23). Therefore, efficient procedures must be implemented to decontaminate the surface of the restoration and ensure adequate retention of the resin cement, preserving the restoration from premature failure, fracture, or secondary caries.

On the other hand, the first and third null hypothesis of the second research protocol must be rejected since significant differences in bond strength between pressed and CAD/CAM LiSi were found both immediately and after artificial aging. However,

decontamination of the ceramic surface statistically influenced the bonding values of CAD ceramic, independent of the storage time, and that of IPS e.max press at baseline, thus requiring a partial rejection of the second null hypothesis.

The continuous improvement of digital technologies by dental companies and the demands of the market, has led to a significant increase in blocks or pods materials production and development in order to perform increasingly restorations with CAD/CAM workflow. In addition, this technique entails several advantages, such as the possibility of working model-free, reducing production costs, and limiting the number of dependent operator steps in the production process that can be influenced by human error. (aggiungere 1-7).

LiSi is available on the market both in ingots and in CAD/CAM blocks, so it can be used both with analogue or digital techniques. Since LiSi is an etchable glass ceramic, one of the most important characteristics that determines the wide use of this material compared to others, in addition to the mechanical and aesthetic properties, are the favorable bond values, which broaden the indications for the use of this type of material even in partial non-retentive restorations such as veneers, table tops or onlays.

The comparison between the pressed and CAD/CAM LiSi is not new to the literature, as the differences on the composition and processing of the two formulations would influence the mechanical properties of the restoration and led to different translucent parameters (Flexural resistance of heat-pressed and CAD-CAM lithium disilicate with different translucencies.(100). Regarding the processing step, it is first related to the crystallization phase, that is industrially-mediated for the IPS e.max press while it is

performed in the clinic for IPS e.max CAD. Moreover, the two materials show different crystal distribution and shape, resulting in different compositions that characterize their bonding behavior.

However, no information exists on the bond strength of the two ceramic materials after decontamination.

Decontamination is an important issue to consider during laboratory simulation of clinical procedures, i.e. the cementation of restorations. It is a good clinical practice to try-in the restoration before the cementation procedure, as soon as the manufact is received from the laboratory. After the try-in in the oral cavity, saliva, blood and, in case of use, silicone paste, can adhere to the ceramic surface and, if not properly removed, hamper the intimate contact of the cement influencing the durability of the restoration. It was noteworthy that the solely water or air were not able to clean the surface. Afterwards, different cleaning procedures were investigated into literature, Nowadays, manufacturer presented their own solutions with decontamination purposes while enhancing bonding capabilities to resin cements. MEP is a single bottle solution able to etch and prime at the same time, offering the possibility of decontaminating, clean and condition the intaglio surface of the restoration. According to its manufacturer, MEP possess a self-limiting etching capacity, that would be highly desirable during the pre-treatment of ceramic as to avoid any deterioration of the surfaces that would inevitably result in crack formation and decreased mechanical properties. According to the results of the present study, decontamination was essential at baseline for both materials investigated, while it was fundamental after

thermocycling solely for IPS e.max CAD. In general, the latter showed inferior bonding values when compared to the pressed format. This agrees with the information present in the literature, that showed decreased physio/mechanical properties and different marginal fit between the two materials, with the pressed ceramic obtaining higher performances than the CAD counterpart (REF). Accordingly, since the production process and the microstructure of pressed and CAD/CAM ceramic is quite different, this could influence their responsiveness to the contamination protocols, thereby leading to differences in the bonding behaviour.

The results of this research have an impact on the clinical activity since the type of disilicate has a statistically significant influence on the adhesion values. Clinicians should be aware of these differences when choosing the workflow for lithium disilicate restorations, in particular in presence of other clinical variables that can affect bond values, such as low amount of available enamel or impossibility in isolate with rubber dam. In these situations, it is possible to obtain pressed lithium disilicate through a digital workflow, by producing the restoration in castable material such as wax, through milling or 3D printing, which can subsequently be subjected into die casting of the disilicate in ingots. The comparison between the latter procedure and the two materials tested in the present study should be furtherly addressed.

Finally the first null hypothesis of the third research protocolfixed in the present study has to be rejected since the experimental ZON etchant not only did not impair the immediate bond strengths, but it even increased those of the universal adhesive AU.

However, since there was a general reduction in the adhesion values after 1 year of laboratory storage, regardless of the adhesive system used, the second null hypothesis is, on the contrary, confirmed. The dentin etching with ZON decreased the activity of MMPs in the HL, immediately and over time, independently of the adhesive system used; hence, the third null hypothesis has to be rejected.

Clinicians dispose of easily manageable adhesive systems that exhibit adhesion performances to dentin unimaginable up to a couple of decades ago. While in the past, clinically acceptable results were achieved in terms of "quantity", nowadays researchers redefined the concept by focusing on the "quality" of the adhesive bonds that could guarantee a longer duration of the clinical restorations over time. Thanks to the identification of the proteolytic mechanisms that work at the origin of degradative mechanism (63,64), efforts have been made to identify a potential therapeutic agent that could counteract the enzymatic action of dentin MMPs and stabilize the adhesive bond over time (16). Chlorhexidine is the first therapeutic agent to demonstrate efficacy at 10 years (15), but several molecules have been currently proposed either as additional primers or directly blended in the adhesive composition (74,76).

An experimental therapeutic etching agent $ZrO(NO_3)_2$ was used in this study prior resin adhesive application to dentin. The same etching agent has shown promising bond strengths and stable adhesive bonds after thermocycling when used in combination with different adhesive systems on enamel (108). However, to the knowledge of the authors, there is currently no information on the effects that the

experimental etchant has on the bonding performances of simplified adhesive systems to dentin.

According to manufacturer's disclaim, the experimental etchant $ZrO(NO_3)_2$ has a self-limiting etching ability, which should allow it to limit the etching potential on the dentin surface and therefore facilitate the interaction with the adhesive resins. ZON is a Lewis acidic metal salt zirconium oxynitrate [$ZrO(NO_3)_2$] highly soluble in water. The presence of water is necessary to dissolve the zirconium salt and to instigate hydrolysis phenomena so to form an acidic environment (pH= 0.56) imperative for dentin conditioning. During the demineralization process, Ca^+ and P^+ ions are released from dentin and they can bind to the Zr ions as to form a solid complex (91). Due to the limited information available, we can only speculate that the advocated self-limiting capacity of the experimental etchant is related to the sedimentation of the Ca^{2+}-Zr and P^+- Zr complexes at the bottom of the dentin surface. This hypothesis needs to be confirmed in future studies focused on the evaluation of the etching potential on dentin and the effects on the tooth substrate.

The ZON etchant increased the immediate bond strength of AU to dentin, resulting in higher adhesion values among all the tested groups (Table 2). It was supposed that the chemical interaction between the dentin substrate and MDP does not have any influence on the immediate bond strength to dentin, but it may help to improve the bond stability (27). Indeed, this was confirmed in the present study, where AU obtained increased bond strength when used with the conventional etching and after 1 year of aging (Table 2). However, a significant decrease was observed for

ZON/AU at T_{12}, even though these results were comparable to those of TE/AU. Accordingly, the *in situ* zymography analysis showed a stronger ZON enzymatic activity inhibition when compared with TE, immediately as well as overtime (Figure 8). This was particularly evident in the ZON/AU group that showed the lower enzymatic activity among all groups at T_{12}. The 10-MDP and the methacrylated carboxylic acid polymer incorporated within AU, can bond effectively and durably to dentin (47,78). The creation of nano-layering at the adhesive interface and the deposition of a more secure MDP-Ca salt influence the creation of reliable and stabilized MDP-based bonding (112). The results of the zymographic analysis revealed an important inhibition of enzymatic activities when ZON etchant was used to treat the dentin surface and compared to TE. These results could be explained by the ability of ZON to bind to Ca ions, conferring it a chelating ability. Through this bond, the etching agent can subtract calcium ions from the MMPs's surface, as they require calcium to maintain their typical tertiary structure (104), in a modality that recalls that of EDTA (88), but it also ensures a greater dentin etching depth typical of conventional etchants (108).

The 2-step self-etch adhesive EF obtained the lowest bond strengths among the groups, either at T_0 and T_{12}, irrespective of being used in combination with TE or ZON. A decrease in bonding values was observed after 1 year of artificial aging (Table 2). Moreover, even if ZON/EF showed the lowest enzymatic activity among all T_0 groups, after 1 year of artificial aging the enzymatic activity was comparable to the TE/EF aged group (Figure 8). The presence of HEMA, a hydrophilic resin functional monomer, in

its formulation confirms previous suggestions according to which these monomers are liable agglomerates of water proceeding through dentinal tubules from the underlying perfused dentin (96). As the resin was incapable of penetrating into the water-rich network of fibril collagen, a series of hydrolytic degradations occurred at the bottom of the HL, welling in incomplete resin infiltration of the entire depth of demineralized dentin (43). Plasticization phenomena of the resinous matrix bulk were also observed, preparing the adhesive interface for inevitable premature degradation (96). Although the possible reduced demineralization effect of ZON, it should be plausible that the HEMA functional monomer would have de-bound the etchant with consequently lower amount of Zr-Ca complexes bound to demineralized dentin after bonding (49). Higher enzymatic activity at the bottom of the HL was observed in the EF groups, irrespective of the dentin conditionings, and levels of fluorescence were indeed increased after laboratory aging (Figs 7-8).

Nanoleakage expression resulted in silver uptake increase at the adhesive interfaces when the adhesives were used in combination with the conventional etchant material (Figure 1). Considering the high acidity of the etchant, deeper demineralization effects could be expected increasing the possibility that the resin blends cannot envelope the exposed collagen fibers in the entire depth. In the light of these considerations, it could be asserted that the self-limiting etching capacity of the experimental etchant, as manufacturers claim, could prevent nanoleakage phenomena.

Conclusions

- The single product Monobond Etch & Prime solution, with or without pre-treatment with hydrofluoric acid, as well as re-etching with hydrofluoric acid demonstrated a greater ability than other ceramic surface cleaning methods to enhance bond strength after saliva and try-in paste contamination.

  Monobond Etch & Prime could simplify lithium disilicate surface procedures preceding bonding without bond strength decreese.

- Pressed ceramic showed higher bond strength than CAD ceramic counterpart, independent of the aging period. In addition the decontamination of ceramic surface was fundamental, in particular for CAD ceramic; Finally Thermocycling decreased the bond values, independent of the material and decontamination approach.

- It could be speculated that the experimental zirconium oxynitrate etchant, showed an inhibitory potential on dentinal endogenous enzymes, paving the way for possible uses as a preservative agent of the hybrid layer in the long-term period, when universal adhesives are used in the etch-and-rinse mode. However, further studies are needed to confirm the results obtained before clinically validating the use of the zirconium oxynitrate etchant as an alternative material to conventional H_3PO_4 etching.

Acknowledgements

References

1. Aboush YE. Removing saliva contamination from porcelain veneers before bonding. J Prosthet Dent 1998;80:649–653.
2. Abu-Hanna A, Gordan V V., Mjor I. The effect of variation in etching times on dentin bonding. Gen Dent 2004;52:28–33.
3. Aladağ A, Elter B, Çömlekoğlu E, Kanat B, Sonugelen M, Kesercioğlu A, Özcan M. Effect of Different Cleaning Regimens on the Adhesion of Resin to Saliva-Contaminated Ceramics. J Prosthodont 2015;24:136–145.
4. Aladağ A, Elter B, Çömlekoğlu E, Kanat B, Sonugelen M, Kesercioğlu A, Özcan M. Effect of Different Cleaning Regimens on the Adhesion of Resin to Saliva-Contaminated Ceramics. J Prosthodont 2015;24:136–145.
5. Albaladejo A, Osorio R, Toledano M, Ferrari M. Hybrid layers of etch-and-rinse versus self-etching adhesive systems. Med Oral Patol Oral Cir Bucal 2010;15:e112-8.
6. Almahdy A, Koller G, Sauro S, Barts JW, Sherriff M, Watson TF, Banerjee A. Effects of MMP inhibitors incorporated within dental adhesives. J Dent Res 2012;91:605–611.
7. Amer R, Kürklü D, Kateeb E, Seghi RR. Three-body wear potential of dental yttrium-stabilized zirconia ceramic after grinding, polishing, and glazing treatments. J Prosthet Dent 2014;112:1151–1155.
8. Ariaans K, Heussen N, Schiffer H, Wienert A-L, Plümäkers B, Rink L, Wolfart

S. Use of molecular indicators of inflammation to assess the biocompatibility of all-ceramic restorations. J Clin Periodontol 2016;43:173–179.

9. Azar B, Eckert S, Kunkela J, Ingr T, Mounajjed R. The marginal fit of lithium disilicate crowns: Press vs. CAD/CAM. Braz Oral Res 2018;32:1–7.
10. Bajraktarova-Valjakova E, Grozdanov A, Guguvcevski L, Korunoska-Stevkovska V, Kapusevska B, Gigovski N, Mijoska A, Bajraktarova-Misevska C. Acid Etching as Surface Treatment Method for Luting of Glass-Ceramic Restorations, part 1: Acids, Application Protocol and Etching Effectiveness. Open access Maced J Med Sci 2018;6:568–573.
11. Belli R, Geinzer E, Muschweck A, Petschelt A, Lohbauer U. Mechanical fatigue degradation of ceramics versus resin composites for dental restorations. Dent Mater 2014;30:424–432.
12. Benic GI, Mühlemann S, Fehmer V, Hämmerle CHF, Sailer I. Randomized controlled within-subject evaluation of digital and conventional workflows for the fabrication of lithium disilicate single crowns. Part I: digital versus conventional unilateral impressions. J Prosthet Dent 2016;116:777–782.
13. Betancourt DE, Baldion PA, Castellanos JE. Resin-Dentin Bonding Interface: Mechanisms of Degradation and Strategies for Stabilization of the Hybrid Layer. Int J Biomater 2019;2019:5268342.
14. Borges ALS, Posritong S, Özcan M, Campos F, Melo R, Bottino MC. Can Cleansing Regimens Effectively Eliminate Saliva Contamination from Lithium Disilicate Ceramic Surface? Eur J Prosthodont Restor Dent 2017;25:9–14.

15. Breschi L, Maravic T, Comba A, Cunha SR, Loguercio AD, Reis A, Hass V, Cadenaro M, Mancuso E, Mayer-Santos E, Niu L, Pashley DH, Tay FR, Mazzoni A. Chlorhexidine preserves the hybrid layer in vitro after 10-years aging. Dent Mater 2020;
16. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, Pashley DH, Tay FR, Mazzoni A. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34:78–96.
17. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: Aging and stability of the bonded interface. Dent. Mater. 2008;24:90–101.
18. Camilleri J, Cutajar A, Mallia B. Hydration characteristics of zirconium oxide replaced Portland cement for use as a root-end filling material. Dent Mater 2011;27:845–854.
19. Canatan S, Oz FD, Bolay S. A randomized, controlled clinical evaluation of two resin cement systems in the adhesion of CAD/CAM-fabricated resin nanoceramic restorations: 18-month preliminary results. J Esthet Restor Dent 2022;
20. Chen, liang and In Suh B. Bonding of Resin Materials to All-Ceramics : A Review Liang Chen and Byoung In Suh Research and Development , Bisco Inc ,. Sci Publ 2012;3:7–17.
21. Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH,

Tay FR. Bonding of universal adhesives to dentine-Old wine in new bottles? J Dent 2015;43:525–536.

22. Chen J, Cai H, Ren X, Suo L, Pei X, Wan Q. A Systematic Review of the Survival and Complication Rates of All-Ceramic Resin-Bonded Fixed Dental Prostheses. J Prosthodont 2018;27:535–543.
23. Cinel Sahin S, Celik E. The effect of different cleaning agents and resin cement materials on the bond strength of contaminated zirconia. Microsc Res Tech 2022;85:840–847.
24. Comba A, Scotti N, Mazzoni A, Maravic T, Ribeiro Cunha S, Michelotto Tempesta R, Carossa M, Pashley DH, Tay FR, Breschi L. Carbodiimide inactivation of matrix metalloproteinases in radicular dentine. J Dent 2019;82:56–62.
25. Dolev E, Bitterman Y, Meirowitz A. Comparison of marginal fit between CAD-CAM and hot-press lithium disilicate crowns. J Prosthet Dent 2019;121:124–128.
26. Elrashid A, AlKahtani A, Alqahtani S, Alajmi N, Alsultan F. Stereomicroscopic evaluation of marginal fit of E.Max press and E.Max computer-aided design and computer-assisted manufacturing lithium disilicate ceramic crowns: An in vitro study. J Int Soc Prev Community Dent 2019;9:178.
27. Erhardt MCG, Pisani-Proença J, Osorio E, Aguilera FS, Toledano M, Osorio R. Influence of laboratory degradation methods and bonding application parameters on microTBS of self-etch adhesives to dentin. Am J Dent

2011;24:103–108.

28. Fabbri G, Zarone F, Dellificorelli G, Cannistraro G, De Lorenzi M, Mosca A, Sorrentino R. Clinical evaluation of 860 anterior and posterior lithium disilicate restorations: retrospective study with a mean follow-up of 3 years and a maximum observational period of 6 years. Int J Periodontics Restorative Dent 34:165–177.
29. Fabian Fonzar R, Carrabba M, Sedda M, Ferrari M, Goracci C, Vichi A. Flexural resistance of heat-pressed and CAD-CAM lithium disilicate with different translucencies. Dent Mater 2017;33:63–70.
30. Fagan J, Vesselovcz J, Puppin-Rontani J, Correr-Sobrinho L, Freitas K, Robertson T, Pacheco R, Pini N, Sundfeld D. Evaluation of Cleaning Methods on Lithium Disilicate Glass Ceramic Surfaces After Organic Contamination. Oper Dent 2022;
31. Ferrari M, Ferrari Cagidiaco E, Goracci C, Sorrentino R, Zarone F, Grandini S, Joda T. Posterior partial crowns out of lithium disilicate (LS2) with or without posts: A randomized controlled prospective clinical trial with a 3-year follow up. J Dent 2019;83:12–17.
32. Forster A, Ungvári K, Györgyey Á, Kukovecz Á, Turzó K, Nagy K. Human epithelial tissue culture study on restorative materials. J Dent 2014;42:7–14.
33. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, Pashley DH, Cadenaro M. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability--A literature

review. Dent Mater 2016;32:e41-53.

34. Furtado de Mendonca A, Shahmoradi M, Gouvêa CVD de, De Souza GM, Ellakwa A. Microstructural and Mechanical Characterization of CAD/CAM Materials for Monolithic Dental Restorations. J Prosthodont 2019;28:e587–e594.

35. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent 1999;27:89–99.

36. Ge C, Green CC, Sederstrom DA, McLaren EA, Chalfant JA, White SN. Effect of tooth substrate and porcelain thickness on porcelain veneer failure loads in vitro. J Prosthet Dent 2018;120:85–91.

37. Goldberg M, Kulkarni AB, Young M, Boskey A. Dentin: structure, composition and mineralization. Front Biosci (Elite Ed) 2011;3:711–735.

38. Gracis S, Thompson V, Ferencz J, Silva N, Bonfante E. A New Classification System for All-Ceramic and Ceramic-like Restorative Materials. Int J Prosthodont 2016;28:227–235.

39. Guess PC, Zavanelli RA, Silva NRFA, Bonfante EA, Coelho PG, Thompson VP. Monolithic CAD/CAM lithium disilicate versus veneered Y-TZP crowns: comparison of failure modes and reliability after fatigue. Int J Prosthodont 23:434–442.

40. Guo J mei, Makvandi P, Wei C chuan, Chen J hua, Xu H kun, Breschi L, Pashley DH, Huang C, Niu L na, Tay FR. Polymer conjugation optimizes EDTA as a calcium-chelating agent that exclusively removes extrafibrillar

minerals from mineralized collagen. Acta Biomater 2019;90:424–440.

41. Haddadi Y, Bahrami G, Isidor F. Accuracy of crowns based on digital intraoral scanning compared to conventional impression-a split-mouth randomised clinical study. Clin Oral Investig 2019;23:4043–4050.

42. Hamza TA, Sherif RM. Fracture Resistance of Monolithic Glass-Ceramics Versus Bilayered Zirconia-Based Restorations. J Prosthodont 2019;28:e259–e264.

43. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, De Munck J. Bonding effectiveness of a new “multi-mode” adhesive to enamel and dentine. J Dent 2012;40:475–484.

44. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1–13.

45. Hannig C, Hannig M, Attin T. Enzymes in the acquired enamel pellicle. Eur J Oral Sci 2005;113:2–13.

46. Hashimoto M, Ohno H, Kaga M, Sano H, Tay FR, Oguchi H, Araki Y, Kubota M. Over-etching effects on micro-tensile bond strength and failure patterns for two dentin bonding systems. J Dent 2002;30:99–105.

47. Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, Sano H, Van Meerbeek B. Hydrolytic stability of self-etch adhesives bonded to dentin. J Dent Res 2005;84:1160–1164.

48. Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. Dent Mater 1995;11:132–136.

49. Kim J, Gu L, Breschi L, Tjäderhane L, Choi KK, Pashley DH, Tay FR. Implication of ethanol wet-bonding in hybrid layer remineralization. J Dent Res 2010;89:575–580.

50. Klosa K, Wolfart S, Lehmann F, Wenz H-J, Kern M. The effect of storage conditions, contamination modes and cleaning procedures on the resin bond strength to lithium disilicate ceramic. J Adhes Dent 2009;11:127–135.

51. Kurokawa H, Takamizawa T, Rikuta A, Tsubota K, Miyazaki M. Three-year clinical evaluation of posterior composite restorations placed with a single-step self-etch adhesive. J Oral Sci 2015;57:101–108.

52. Lapinska B, Rogowski J, Nowak J, Nissan J, Sokolowski J, Lukomska-Szymanska M. Effect of surface cleaning regimen on glass ceramic bond strength. Molecules 2019;24:1–17.

53. Lee A, Swain M, He L, Lyons K. Wear behavior of human enamel against lithium disilicate glass ceramic and type III gold. J Prosthet Dent 2014;112:1399–1405.

54. Li RWK, Chow TW, Matinlinna JP. Ceramic dental biomaterials and CAD/CAM technology: State of the art. J Prosthodont Res 2014;58:208–216.

55. Lima RBW, Muniz I de AF, Campos D e S, Murillo-Gómez F, Andrade AKM de, Duarte RM, de Souza GM. Effect of universal adhesives and self-etch ceramic primers on bond strength to glass ceramics: A systematic review and meta-analysis of in vitro studies. J Prosthet Dent 2022;

56. Linde A, Goldberg M. Dentinogenesis. Crit Rev Oral Biol Med 1993;4:679–

728.

57. Liu Y, Tjäderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, Tay FR. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J. Dent. Res. 2011;90:953–968.

58. Lyann SK, Takagaki T, Nikaido T, Uo M, Ikeda M, Sadr A, Tagami J. Effect of different surface treatments on the tensile bond strength to lithium disilicate glass ceramics. J Adhes Dent 2018;20:261–268.

59. Lyann SK, Takagaki T, Nikaido T, Wada T, Uo M, Ikeda M, Sadr A, Tagami J. Efficacy of Various Surface Treatments on the Bonding Performance of Saliva-contaminated Lithium-Disilicate Ceramics. J Adhes Dent 2019;21:51–58.

60. Magne P, Cascione D. Influence of post-etching cleaning and connecting porcelain on the microtensile bond strength of composite resin to feldspathic porcelain. J Prosthet Dent 2006;96:354–361.

61. Mazza LC, Lemos CAA, Pesqueira AA, Pellizzer EP. Survival and complications of monolithic ceramic for tooth-supported fixed dental prostheses: A systematic review and meta-analysis. J Prosthet Dent 2021;1–9.

62. Mazzoni A, Nascimento FD, Carrilho M, Tersariol I, Papa V, Tjäderhane L, Di Lenarda R, Tay FR, Pashley DH, Breschi L. MMP activity in the hybrid layer detected with in situ zymography. J Dent Res 2012;91:467–472.

63. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M, Pashley EL, Tay FR. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse

adhesives. Biomaterials 2006;27:4470–4476.

64. Mazzoni A, Scaffa P, Carrilho M, Tjäderhane L, Di Lenarda R, Polimeni A, Tezvergil-Mutluay A, Tay FR, Pashley DH, Breschi L. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res 2013;92:82–86.

65. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, Yoshida Y, Inoue S, Suzuki K. Technique-sensitivity of contemporary adhesives. Dent Mater J 2005;24:1–13.

66. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.

67. Memari Y, Mohajerfar M, Armin A, Kamalian F, Rezayani V, Beyabanaki E. Marginal Adaptation of CAD/CAM All-Ceramic Crowns Made by Different Impression Methods: A Literature Review. J Prosthodont 2019;28:e536–e544.

68. Miotti LL, Follak AC, Montagner AF, Pozzobon RT, da Silveira BL, Susin AH. Is Conventional Resin Cement Adhesive Performance to Dentin Better Than Self-adhesive? A Systematic Review and Meta-Analysis of Laboratory Studies. Oper Dent 2020;45:484–495.

69. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. J Esthet Dent 3:133–138.

70. Nascimento A, Mantovani M, Mendonça Lc, Vesselovcz J, Pacheco R, Pini N, Sundfeld D. Two-Year Follow-Up of Self-Etching Ceramic Primer as Surface Treatment for Feldspathic Veneers: A Clinical Case Review. Oper Dent

2021;46:126–135.

71. Naves LZ, Soares CJ, Moraes RR, Gonçalves LS, Sinhoreti MAC, Correr-Sobrinho L. Surface/Interface Morphology and Bond Strength to Glass Ceramic Etched for Different Periods. Oper Dent 2010;35:420–427.

72. Nawafleh N, Hatamleh M, Elshiyab S, Mack F. Lithium Disilicate Restorations Fatigue Testing Parameters: A Systematic Review. J Prosthodont 2016;25:116–126.

73. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, Carvalho RM, Tjaderhane L, Tay FR, Pashley DH. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci 2006;114:160–166.

74. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, Tezvergil-Mutluay A. State of the art etch-and-rinse adhesives. Dent Mater 2011;27:1–16.

75. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, Ito S. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83:216–221.

76. Perdigão J, Reis A, Loguercio AD. Dentin adhesion and MMPs: A comprehensive review. J. Esthet. Restor. Dent. 2013;25:219–241.

77. Perumal S, Antipova O, Orgel JPRO. Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. Proc Natl Acad Sci U S A 2008;105:2824–2829.

78. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B. Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. Dent Mater 2010;26:1176–1184.

79. Politano G, Van Meerbeek B, Peumans M. Nonretentive Bonded Ceramic Partial Crowns: Concept and Simplified Protocol for Long-lasting Dental Restorations. J Adhes Dent 2018;20:495–510.

80. Prasad A, Ibrahim H, Mortimore K, Vandabona R. Critical care management of hydrofluoric acid burns with a negative outcome. BMJ Case Rep 2021;14:e242187.

81. Preis V, Weiser F, Handel G, Rosentritt M. Wear performance of monolithic dental ceramics with different surface treatments. Quintessence Int 2013;44:393–405.

82. Prochnow C, Venturini AB, Guilardi LF, Pereira GKR, Burgo TAL, Bottino MC, Kleverlaan CJ, Valandro LF. Hydrofluoric acid concentrations: Effect on the cyclic load-to-failure of machined lithium disilicate restorations. Dent Mater 2018;34:e255–e263.

83. Ramakrishnaiah R, Alkheraif A, Divakar D, Matinlinna J, Vallittu P. The Effect of Hydrofluoric Acid Etching Duration on the Surface Micromorphology, Roughness, and Wettability of Dental Ceramics. Int J Mol Sci 2016;17:822.

84. Román-Rodríguez JL, Perez-Barquero JA, Gonzalez-Angulo E, Fons-Font A, Bustos-Salvador JL. Bonding to silicate ceramics: Conventional technique compared with a simplified technique. J Clin Exp Dent 2017;9:e384–e386.

85. Saboia VPA, Nato F, Mazzoni A, Orsini G, Putignano A, Giannini M, Breschi L. Adhesion of a two-step etch-and-rinse adhesive on collagen-depleted dentin. J Adhes Dent 2008;10:419–422.

86. Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: a systematic review and meta-analysis of in vitro studies. Oper Dent 39:E31-44.

87. Sato T, Takagaki T, Baba Y, Vicheva M, Matsui N, Hiraishi N, Ikeda M, Nikaido T, Tagami J. Effects of Different Tooth Conditioners on the Bonding of Universal Self-etching Adhesive to Dentin. J Adhes Dent 2019;21:77–85.

88. Sauro S, Mannocci F, Toledano M, Osorio R, Pashley DH, Watson TF. EDTA or H3PO4/NaOCl dentine treatments may increase hybrid layers' resistance to degradation: A microtensile bond strength and confocal-micropermeability study. J Dent 2009;37:279–288.

89. Schestatsky R, Zucuni CP, Venturini AB, de Lima Burgo TA, Bacchi A, Valandro LF, Rocha Pereira GK. CAD-CAM milled versus pressed lithium-disilicate monolithic crowns adhesively cemented after distinct surface treatments: Fatigue performance and ceramic surface characteristics. J Mech Behav Biomed Mater 2019;94:144–154.

90. Silva NRFA, Thompson VP, Valverde GB, Coelho PG, Powers JM, Farah JW, Esquivel-Upshaw J. Comparative reliability analyses of zirconium oxide and lithium disilicate restorations in vitro and in vivo. J Am Dent Assoc 2011;142 Suppl:4S-9S.

91. Solovkin AS, Tsvetkova ZN. THE CHEMISTRY OF AQUEOUS SOLUTIONS OF ZIRCONIUM SALTS (DOES THE ZIRCONYL ION EXIST?). Russ Chem Rev 1962;31:655–669.

92. Song X-F, Ren H-T, Yin L. Machinability of lithium disilicate glass ceramic in in vitro dental diamond bur adjusting process. J Mech Behav Biomed Mater 2016;53:78–92.

93. Sorrentino R, Apicella D, Riccio C, Gherlone E, Zarone F, Aversa R, Garcia-Godoy F, Ferrari M, Apicella A. Nonlinear visco-elastic finite element analysis of different porcelain veneers configuration. J Biomed Mater Res B Appl Biomater 2009;91:727–736.

94. Sousa SJL, Poubel DL do N, Rezende LVM de L, Almeida FT, de Toledo IP, Garcia FCP. Early clinical performance of resin cements in glass-ceramic posterior restorations in adult vital teeth: A systematic review and meta-analysis. J Prosthet Dent 2020;123:61–70.

95. Taguchi S, Komine F, Kubochi K, Fushiki R, Kimura F, Matsumura H. Effect of a silane and phosphate functional monomer on shear bond strength of a resin-based luting agent to lithium disilicate ceramic and quartz materials. J Oral Sci 2018;60:360–366.

96. Tay FR, King NM, Chan K PD. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? | Request PDF. J Adhes Dent. 2002;4: 255-269.

97. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step

adhesives are permeable membranes. J Dent 30:371–382.

98. Tay FR, Pashley DH, Yoshiyama M. Two Modes of Nanoleakage Expression in Single-step Adhesives. J Dent Res 2002;81:472–476.

99. Tetè S, Zizzari VL, Borelli B, De Colli M, Zara S, Sorrentino R, Scarano A, Gherlone E, Cataldi A, Zarone F. Proliferation and adhesion capability of human gingival fibroblasts onto zirconia, lithium disilicate and feldspathic veneering ceramic in vitro. Dent Mater J 2014;33:7–15.

100. Vichi A, Fabian Fonzar R, Carrabba M, Louca C, Scotti N, Mazzitelli C, Breschi L, Goracci C. Comparison between hydrofluoric acid and single-component primer as conditioners on resin cement adhesion to lithium silicate and lithium disilicate glass ceramics. Materials (Basel) 2021;14.

101. Vichi A, Fabian Fonzar R, Carrabba M, Louca C, Scotti N, Mazzitelli C, Breschi L, Goracci C. Comparison between Hydrofluoric Acid and Single-Component Primer as Conditioners on Resin Cement Adhesion to Lithium Silicate and Lithium Disilicate Glass Ceramics. Materials (Basel) 2021;14:6776.

102. Vijayan SM, Göen T, Dennerlein K, Horch RE, Ludolph I, Drexler H, Kilo S. Calcium, magnesium and aluminium ions as decontaminating agents against dermal fluoride absorption following hydrofluoric acid exposure. Toxicol Vitr 2021;71:105055.

103. Viotti RG, Kasaz A, Pena CE, Alexandre RS, Arrais CA, Reis AF. Microtensile bond strength of new self-adhesive luting agents and conventional multistep

systems. J Prosthet Dent 2009;102:306–312.

104. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ. Res. 2003;92:827–839.

105. Willard A, Gabriel Chu T-M. The science and application of IPS e.Max dental ceramic. Kaohsiung J Med Sci 2018;34:238–242.

106. Yamauchi M, Shiiba M. Lysine hydroxylation and cross-linking of collagen. Methods Mol Biol 2008;446:95–108.

107. Yang B, Scharnberg M, Wolfart S, Quaas AC, Ludwig K, Adelung R, Kern M. Influence of contamination on bonding to zirconia ceramic. J Biomed Mater Res Part B Appl Biomater 2007;81B:283–290.

108. Yao C, Ahmed MH, Yoshihara K, Mercelis B, Parise Gré C, Van Landuyt KL, Huang C, Van Meerbeek B. Bonding to enamel using alternative Enamel Conditioner/etchants. Dent Mater 2019;35:1415–1429.

109. Yoshida F, Tsujimoto A, Ishii R, Nojiri K, Takamizawa T, Miyazaki M, Latta MA. Influence of surface treatment of contaminated lithium disilicate and leucite glass ceramics on surface free energy and bond strength of universal adhesives. Dent Mater J 2015;34:855–862.

110. Yoshida K. Influence of cleaning methods on the bond strength of resin cement to saliva-contaminated lithium disilicate ceramic. Clin Oral Investig 2020;24:2091–2097.

111. Yoshida K. Influence of cleaning methods on the bond strength of resin cement

to saliva-contaminated lithium disilicate ceramic. Clin Oral Investig 2020;24:2091–2097.

112. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, Meerbeek B Van. Self-assembled nano-layering at the adhesive interface. J Dent Res 2012;91:376–381.
113. Yu P, Wang XY. Effects of Surface Treatment Procedures on Bond Strength of Lithium Disilicate Glass Ceramic. Chin J Dent Res 2021;24:119–124.
114. Zarone F, Ferrari M, Mangano FG, Leone R, Sorrentino R. "Digitally Oriented Materials": Focus on Lithium Disilicate Ceramics. Int J Dent 2016;2016:9840594.
115. Zarone F, Di Mauro MI, Ausiello P, Ruggiero G, Sorrentino R. Current status on lithium disilicate and zirconia: A narrative review. BMC Oral Health 2019;19:1–14.
116. Zhang S, Kocjan A, Lehmann F, Kosmač T, Kern M. Influence of contamination on resin bond strength to nano-structured alumina-coated zirconia ceramic. Eur J Oral Sci 2010;118:396–403.
117. Zurek AD, Alfaro MF, Wee AG, Yuan JC-C, Barao VA, Mathew MT, Sukotjo C. Wear Characteristics and Volume Loss of CAD/CAM Ceramic Materials. J Prosthodont 2019;28:e510–e518.

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REVIEW ARTICLE

WILEY

The effect of chlorhexidine primer application on the clinical performance of composite restorations: a literature review

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Abstract

To discuss the effectiveness of chlorhexidine (CHX) used as therapeutic dentin primer in adhesively bonded composite restorations.

Overview: An electronic search in MEDLINE database, accessed through PubMed was conducted. No restrictions of languages and date of publication were made. The following key words were used: "chlorhexidine", "composite" and "composite resins." Clinical studies in which CHX was used during bonding procedures were included in this review. Six studies met the inclusion criteria. Of these, five studies were carried out on noncarious cervical lesions (NCCL). Only one study was carried out on class II preparation of permanent molars. In all studies, either etch-and-rinse and self-etch adhesive systems were used during bonding procedures. On the basis of the reviewed clinical trials, it can be concluded that CHX primer application does not seem to influence clinical outcome of composite restorations.

Clinical significance: Current scientific evidence cannot neither strongly recommend nor discourage the application of CHX as therapeutic primer in composite restorations. Studies with longer follow-up periods with adhesive restorations placed on dentin after caries removal, rather than only on NCCL, are desirable to further investigate the therapeutic effect of CHX during bonding procedures.

KEYWORDS

adhesive systems, chlorhexidine, composite restoration, hybrid layer, matrix metalloproteinases

1 | INTRODUCTION

Recent advances in dental materials made resin composites the materials of choice for the restoration of caries-affected teeth, exhibiting enhanced mechanical properties and improved esthetic behavior.[1] Resin composites rely on the application of adhesive systems to establish a reliable interaction with dentin, through the formation of the hybrid layer (HL) – a structure that is composed of demineralized collagen fibrils reinforced by resin matrix.[2] Different dentin adhesive systems have been proposed over time with the intent to simplify clinical procedures, limit operator mismanagement and improve bond durability of the restorations.[3] However, regardless of the adhesive strategy employed, HL remains the weakest portion within the adhesive-dentin interface, impacting the prognosis of the restoration. Secondary caries, in fact, is more likely to occur because of degradation of HL components, being responsible for failures of resin composite restorations, in particular in the posterior region of the mouth.

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Is clinical behavior of composite restorations placed in non-carious cervical lesions influenced by the application mode of universal adhesives? A systematic review and meta-analysis

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ARTICLE INFO

Keywords:
Universal adhesives
Non-carious cervical lesions
Systematic review

ABSTRACT

Objective. To answer the following PICOS question: "Is the risk of retention loss, marginal discoloration, marginal adaptation and postoperative sensitivity (POS) equal for etch-and-rinse (EAR) compared to self-etch (SE) or selective-enamel etch (SEE) mode when restoring non carious cervical lesions (NCCLs) with universal adhesives?".

Methods. PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, Scientific Electronic Library Online, LILACS, OpenGrey and Google Scholar™ were searched. Randomized controlled clinical trials in which resin composites and universal adhesives were used for restoration of NCCLs were considered. Cochrane Risk of Bias Tool was used to assess the risk of bias. Meta-analyses were performed using Revman; random-effects models were applied, and heterogeneity was tested using the I^2 index. The significance level was set at $p < 0.05$. Certainty of evidence was assessed by GRADE tool.

Results. After screening, 20 articles were included in qualitative, while 14 articles were used for quantitative synthesis. Twelve studies ranked as "low", while 8 studies scored as "unclear" for risk of bias. At 12- and 18/24-months the risk for retention loss was higher for SE than for EAR groups ($p = 0.005$; RR = 0.22, 95% CI [0.08, 0.63],[moderate certainty of evidence and $p = 0.0002$; RR = 0.32, 95% CI [0.17, 0.58], moderate certainty of evidence, respectively). No significant differences were observed for marginal discoloration and adaptation ($p > 0.05$). The probability of POS occurrence was less in SE than in EAR groups (RR = 2.12, 95% CI [1.23, 3.64], moderate certainty of evidence). The certainty of evidence for other outcomes was scored as "low" or "moderate", depending on the follow-up period.

Significance. Using universal adhesives in EAR or SEE mode provides more predictable retention, while SE strategy reduces the risk of POS occurrence.

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Bonding to dentin using an experimental zirconium oxynitrate etchant

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ARTICLE INFO

Keywords:
Adhesive
Dentin etchant
Longevity
MMP inhibition
Zirconium oxynitrate
Zymography

ABSTRACT

Objective: To investigate, by means of microtensile bond strength test (μTBS), nanoleakage expression analysis (NL), gelatin zymography and *in situ* zymography, the effects of an experimental metal salt-based zirconium oxynitrate etchant [$ZrO(NO_3)_2$,] – ZON with two simplified adhesives on long-term bond strength and endogenous enzymatic activities.

Methods: Middle/deep coronal dentin surfaces (N = 32) were conditioned either with a traditional 37 % H_3PO_4 etchant (TE) or with ZON. Further, a single-component etch-and-rinse adhesive (EF) or a universal adhesive (AU) were applied and μTBS and NL tests were performed. Additional freshly extracted teeth were processed for gelatin zymography and *in situ* zymography evaluation. The tests were performed at baseline and (T0) and after 1-year-aging (T12). Bond strength and *in situ* zymography results were analyzed using analysis of variance (ANOVA) (three-way and one-way, respectively), while Chi-squared test was used for the NL results. Statistical significance was preset at $\alpha = 0.05$.

Results: All the investigated factors (adhesive system, dentin conditioner and aging) significantly influenced μTBS, with the AU and ZON performing better compared to EF and TE, respectively, and with lower bond strength values after aging ($p < 0.05$). Incremented silver nitrate deposits were observed at the adhesive interfaces after aging, especially for the TE groups ($p < 0.05$). Further, the experimental groups treated with ZON had significantly lower levels of enzymatic activity compared to TE, as shown by gelatin and *in situ* zymography ($p < 0.05$).

Conclusions: The experimental etchant demonstrated promising results in hybrid-layer preservation over time when used with simplified bonding systems, and could therefore be recommended in the clinical practice.

1. Introduction

Adhesion to dentin has been a challenge for researchers and clinicians ever since dentin bonding became a clinical procedure. This is attributed to the structural complexity of the dentin substrate. Attributes such as the intrinsic wetness of deep vital dentin [1], heterogeneity of its intrinsic constituents [2] and the sensitivity of the organic matrix to operator manipulation [1] renders bonding to dentin extremely taxing to be performed well.

Different clinical steps are used to "condition" and "prime" the tooth substrate. These steps enable a clinician to create dentin surface conditions that are conducive to receiving a methacrylate resin-based adhesive. Errors can occur during these clinical steps [2–4]. The adhesion process begins with conditioning of the dentin surface with an acid etchant or a solution of acidic resin monomers to create a layer of partially-demineralized or fully-demineralized collagen-rich organic matrix for infiltration of adhesive resin. During this process a water-rich, resin-sparse zone might be produced at the base of the hybrid layer. This zone contains denuded collagen fibrils that are surrounded by water molecules of dental origin [5,6]. Many studies have reported that this zone is susceptible to degradation by host-derived matrix metalloproteinases (MMPs) [7,8] and cysteine cathepsins [9]. Following degradation, the longevity of resin-dentin bonds is undermined [5,10]. Despite attempts to improve adhesive performance and to promote more stable resin-dentin interfaces through the use of protease inhibitors, degradation of these interfaces have been reported with all types of bonding approaches [2,5,6]. Therapeutic systems, blended to an adhesive or used as primer in a separate step, have been used to prevent the degradation of the resin-dentin interface mediated by MMPs [5,11,12]. Phosphoric acid etchants containing MMP inhibitors have also been

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29° CONGRESSO NAZIONALE
Collegio dei Docenti Universitari di discipline Odontostomatologiche

Alma Mater Studiorum
Università di bologna

Riabilitazione di elementi gravemente compromessi attraverso l'utilizzo di restauri indiretti in disilicato di litio

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CO08

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OBIETTIVO

Restauro funzionale ed estetico di elementi gravemente compromessi sfruttando procedure adesive in modo da evitare preparazioni coronali complete

METODI

Un paziente maschio di 55 anni, si presenta alla nostra attenzione presso la clinica universitaria lamentando dolore e discomfort masticatorio nel secondo quadrante. Esami clinici e radiografici mostrano restauri incongrui sugli elementi 2.7, 2.6, 2.5, 2.4 e 2.3, insieme ad una carie secondaria destruente sul 2.7 ed una frattura cuspidale sul 2.4. Il piano di trattamento prevedeva la realizzazione di restauri indiretti da 2.7 a 2.4 e il restauro diretto del 2.3. Gli elementi vengono isolati sotto diga di gomma da 2.7 a 2.2 previa infiltrazione locale di anestetico. Durante le procedure di rimozione della carie sul 2.7 si riscontra esposizione pulpare, per questo motivo è stato eseguito il trattamento endodontico dell'elemento stesso. Le restanti cavità sono state deterse simultaneamente alternando frese diamantate a grana media e fine a frese multiama al carburo di tungsteno. 2.3.

Dopo aver fatto adesione (Optibond FL, Kerr) sono stati eseguiti i build-ups in composito (Filtek Supreme, 3M) sugli elementi 2.7, 2.6, 2.5, 2.4 e il restauro diretto del Una volta ultimate le preparazioni coronali per gli intarsi è stata rilevata un impronta di precisione in silicone. Dopo 7 giorni gli intarsi sono stati cementati sotto diga con composito riscaldato (Filtek Supreme, 3M)

RISULTATI

Esami clinici e radiografici dopo 1 mese mostrano una buona integrazione, funzione ed estetica. IL pz non ha riportato sintomi o discomfort.

CONCLUSIONI

Restauri parziali adesivi possono essere considerati una buona soluzione anche in casi digrave perdita di sostanza dentale e ridotte porzioni di smalto disponibile, comportando un trattamento più conservativo e con un minor impatto economico.

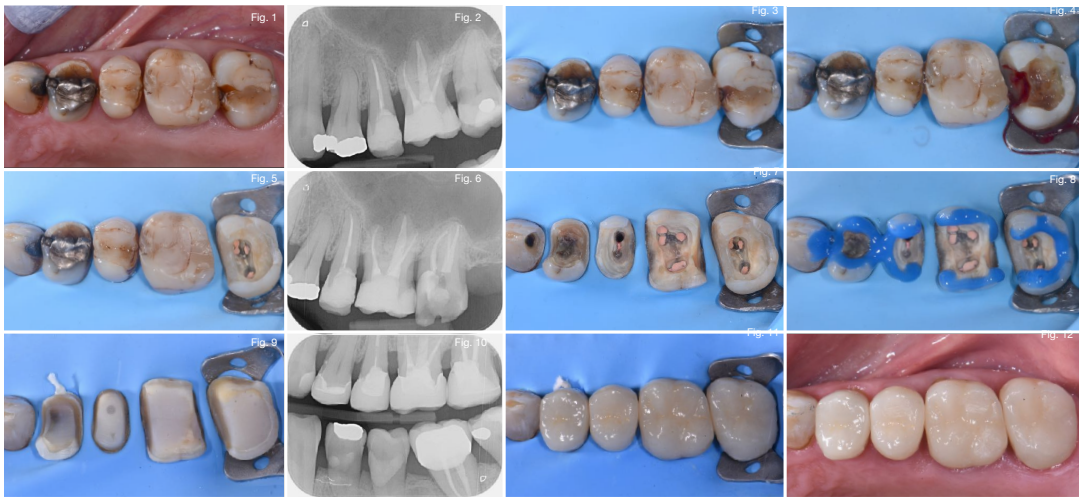

Fig.1 Visione occlusale pre-operatoria; **Fig.2** Radiografia pre-operatoria; **Fig.3** Isolamento con diga; **Fig.4** Sanguinamento pulpare su 2.7; **Fig.5** Rilocazione del margine e terapia canalare su 2.7; **Fig.6** Controllo radiografico post-endodontico; **Fig.7** Cavità preparate; **Fig.8** Procedure adesive; **Fig.9** Restauro diretto su 2.3 e preparazioni finali da 2.4 a 2.7 **Fig.10** Controllo radiografico finale; **Fig.11** Intarsi in disilicato di litio cementati sotto diga; **Fig.12** Controllo ad un mese

References

Chen, Y., Chen, D., Ding, H., Chen, Q., & Meng, X. (2022). Fatigue behavior of endodontically treated maxillary premolars with MOD defects under different minimally invasive restorations. Clinical Oral Investigations, 26(1), 197–206. https://doi.org/10.1007/s00784-021-03991-9

Saravi, B., Vollmer, A., Hartmann, M., Lang, G., Kohal, R. J., Boeker, M., & Patzelt, S. B. M. (2021). Clinical performance of cad/cam all-ceramic tooth-supported fixed dental prostheses: A systematic review and meta-analysis. Materials, 14(10). https://doi.org/10.3390/ma14102672

SIPRO
SOCIETÀ ITALIANA DI PROTESI DENTARIA E RIABILITAZIONE ORALE

Alma Mater Studiorum
Università di Bologna

No-prep ultra-thin CAD/CAM temporary esthetic veneers rehabilitation in a young patient

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Federico Del Bianco

PURPOSE

To contribute to the esthetic and occlusal results after ortho treatment of a young patient with minimally invasive CAD/CAM hybrid ceramic veneers.

METHODS

A 16 year old male patient came to our attention after ortho treatment, demanding for diastema closure with very high esthetic expectations of him and his parents. After anamnestic data recording and professional hygiene, intraoral scans and pictures of the dental arches were taken. The esthetic analysis was performed with a digital wax-up. As no areas of undercuts were detected, the treatment plan consisted in the realization of 6 no-prep veneers (from 1.3 to 2.3). A mockup was fabricated and used to facilitate aesthetic and treatment goals with the patient and his family. A second digital impression of the upper arch was taken with retraction cords on site. The two digital impressions were matched to obtain both subgingival teeth profile and the position of gingiva. Six ultra-thin monolithic hybrid ceramic veneers with thickness from 0.4 to 0.6 mm (Vita Enamic® multiColor – shade 2 M2-HT) were projected following the digital wax-up, milling, finishing, and staining. Veneers were bonded one by one under rubber dam isolations following a strict bonding protocol with a dual curing resin cement (Variolink DC–Ivoclar).

RESULTS

The patient reported aesthetic satisfaction with no pain or discomfort, immediately and after 6 months follow-up. During the recalls, the periodontal tissues were monitored, and no signs of gingival inflammation were observed. Marginal accuracy, esthetic stability and emergence profiles were preserved over time.

CONCLUSIONS

No-prep ultra-thin CAD/CAM veneers were used as alternative to direct composite veneering in young patients demanding aesthetic improvement of the smile, especially as post-ortho treatments in case of tooth-alveolar discrepancy or additive rehabilitations. Digital technologies are crucial in order to make the technique sustainable. Once occlusal stability has been attained and proper periodontal tissue maturation has been reached at the end of the patient's growth, there will then be the option of improving the esthetics by replacing these restorations with ceramic veneers.

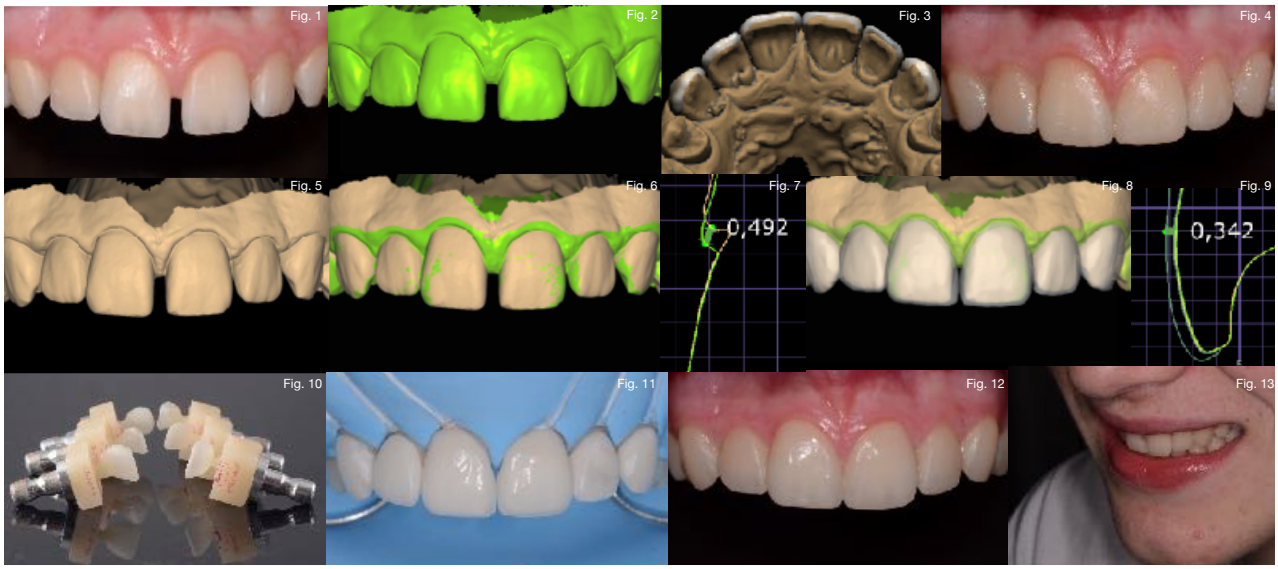

Fig.1 Pre-op buccal view; **Fig.2** Pre-op Intraoral scan; **Fig.3** Digital wax-up; **Fig.4** Mock-up; **Fig.5** Intraoral scan with retraction cords **Fig.6** Match of the two scans; **Fig.7** Sagittal view of the two scans matched; **Fig.8** Restorations design; **Fig.9** Sagittal view of restorations design **Fig.10** Restorations milled; **Fig.11** Rubber dam isolation and try-in procedures; **Fig.12-13** Intraoral and extraoral view at 6 months follow-up

Reference

Patroni, S., & Cocconi, R. (2017). From orthodontic treatment plan to ultrathin no-prep CAD/CAM temporary veneers. *The International Journal of Esthetic Dentistry*, *12*(4), 504–522.

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AESTHETIC RESTORATION OF SEVERELY WORN DENTITION

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AIM

The treatment of severe worn dentition can be challenging because of the alteration in the vertical dimensions. This clinical case presents the management of severe toothwear dentition in a functionally and aesthetically compromised patient.

MATERIAL & METHODS

A 55-yr old male patient referred to the dental clinic complaining difficulties during mastication and the desire to improve the aesthetic of his smile. After medical and dental anamnesis, photographs were taken of the face and the mouth and a radiographical status was performed. The dental examination confirmed the diagnosis of severe tooth wear due to attrition (clenching and bruxism) with considerable reduction in the VDO. The treatment plan foresaw a combination of adhesively-bonded indirect restorations and implant-supported crown rehabilitations. An interim mock-up was used to achieve the comfortable VDO before bite registration. Definitive restorations were performed with zirconia screw retained crowns on implants (1.6, 2.6, 3.3 4.6)(Katana - Kuraray) and lithium disilicate restoration on teeth (1.1-1.5, 2.1-2.5, 3.1-3.2, 3.4-3.6 4.1-4.5)(E.Max press; Ivoclar) bonded under rubber dam isolation with a 3-steps Etch&Rinse adhesive (Optibond FL, Kerr; 30s enamel etching, 15s dentin etching) and a dual-cure resin cement (Variolink DC - Ivoclar Vivadent)

RESULTS

The case showed the analytical diagnostic steps taken to formulate the treatment plan. After 1 month of mock-up, a comfortable VDO was encountered. The plan was finalized in 4 months from first consultation and after 1yr of follow-up no debondings, chippings or dental/periodontal problems were observed.

Fig 1-5: Pre-op **Fig 6-7:** Mock-up **Fig 9-10:** VDO registration **Fig. 11-16:** Bonding procedures **Fig 17:** Radiographic status after 1 year follow-up **Fig.18-20; 23:** Photographic status after 1 year follow-up **Fig. 21:** Pre-op, extaoral view **Fig. 22:** Mock-up, extra oral view

CONCLUSIONS

Minimally-invasive rehabilitation with indirect approaches was presented for the restoration of generalized tooth wear in general practice. After 1yr, the aesthetic and the function completely fulfilled patient's requirements.

Bibliografia:
Fradeani M, Barducci G, Bacherini L. Esthetic rehabilitation of a worn dentition with a minimally invasive prosthetic procedure (MIPP). Int J Esthet Dent. 2016 Spring;11(1):16-35. PMID: 26835522.
Saeidi Pour R, Engler MLPD, Edelhoff D, Prandtner O, Frei S, Liebermann A. A patient-calibrated individual wax-up as an essential tool for planning and creating a patient-oriented treatment concept for pathological tooth wear. Int J Esthet Dent. 2018;13(4):476-492. PMID: 30302438.

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28° CONGRESSO NAZIONALE
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DI DISCIPLINE ODONTOSTOMATOLOGICHE

RESTAURI INDIRETTI IN COMPOSITO CON TECNICA CHAIRSIDE: CASE REPORT

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OBIETTIVO

Presentare con documentazione step by step di un caso di restauri indiretti con tecnica chairside

MATERIALI E METODI

Paziente di 47 anni di sesso Femminile, si presenta alla nostra attenzione lamentando forte sensibilità agli stimoli termici nel 4° sestante, con dolore alla percussione dell'elemento 3.6. Anamnesi medica negativa. L'esame clinico e radiografico hanno evidenziato la presenza di carie secondaria sugli elementi 3.7 e 3.5 e di pulpite sul 3.6. Durante la detersione della cavità del 3.6 parte della camera pulpare è stata esposta. Si è deciso così di procedere al trattamento endodontico prima della realizzazione dei restauri indiretti. Dopo aver eseguito build-up in composito, le preparazioni dentali sono state ultimate con frese a grana rispettivamente media e fine. E' stata rilevata impronta con scanner intraorale. I restauri sono stati progettati con software dedicato e mandati in produzione. Una volta rifiniti, la superficie interna degli intarsi è stata sabbiata con ossido di alluminio a 50µm (distanza 5 cm per 10 secondi), dopodiché sono stati detersi in bagno ad ultrasuoni in soluzione alcolica per 1 minuto, silanizzati (5 minuti) e l'adesivo etch 6 rinse a 3 passaggi(Optibond Fl-Kerr) è stato applicato senza essere polimerizzato. Dopo aver isolato gli elementi con diga di gomma, questi sono stati detersi con spazzolino e miscela acqua-pomice, sabbiati, e le procedure adesive sono state applicate polimerizzando l'adesivo per 20 secondi. La cementazione è stata ultimata con composito in pasta pre-riscaldato (Filtek supreme, 3M) polimerizzato per 60 sec per lato (vestibolare, linguale occlusale. Dopo aver rifinito e lucidato i restauri, è stato eseguito un controllo occlusale e radiografico.

RISULTATI

Ad un follow-up di 16 mesi non sono state rilevate tracce di frattura, discoloration, infiltrazione, carie secondaria, sensibilità e/o dolore.
Le radiografie non mostrano processi di infiammazione apicale.

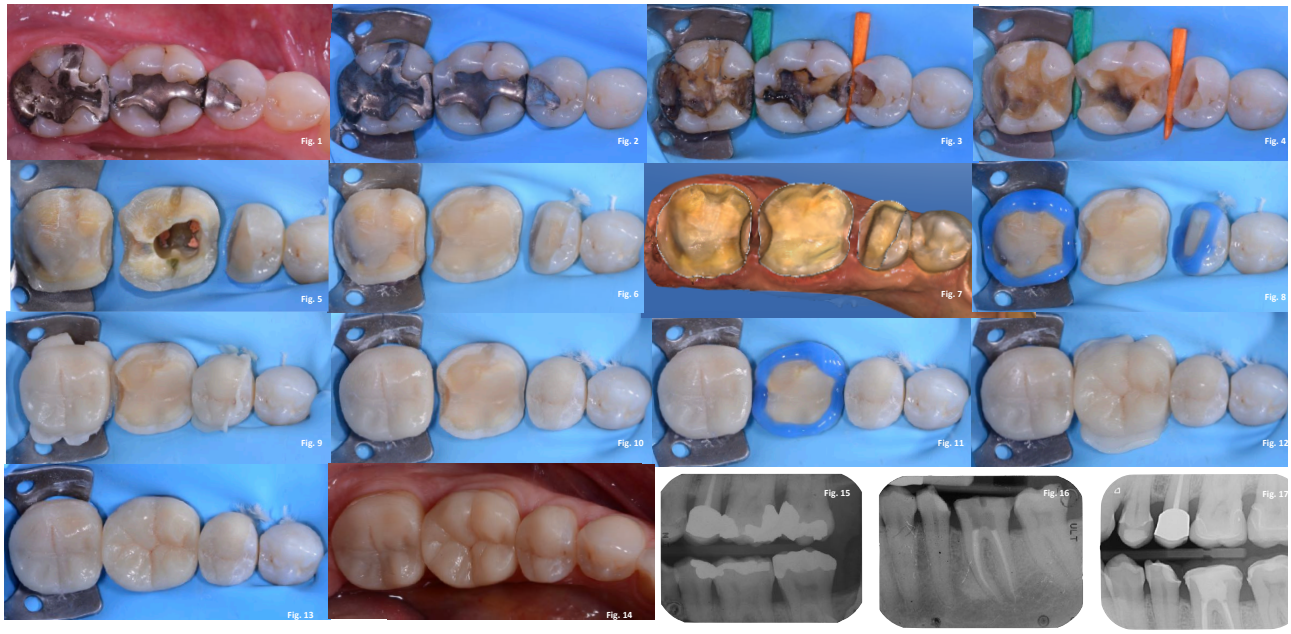

Fig 1: Situazione iniziale **Fig 2:** Isolamento con diga **Fig 3:** Rimozione vecchi restauri **Fig 4:** Detersione cavità (esposizione pulpare) **Fig. 5:** Terapia canalare ultimata **Fig 6:** Build-up e preparazione **Fig.7:** Scansione intraorale **Fig 8-12:** Procedure adesive **Fig 13:** Restauri rifiniti e lucidati sotto diga **Fig 14:** Controllo a 16 mesi **Fig 15:** Bitewing pre-operatoria **Fig 16:** Rx endorale post trattamento endodontico **Fig 17:** Bitewing di controllo a 16 mesi

CONCLUSIONI

I restauri semi-diretti in composito sono una valida alternativa di trattamento, quando viene richiesto la copertura ruspale di uno o più elementi dentari. Il flusso di lavoro CAD-CAM Chairside rende le procedure più veloci, predicibilirispetto al protocollo tradizionale

Bibliografia:
IDel Curto F, Saratti CM, Krejci I. CAD/CAM-based chairside restorative technique with composite resin for full-mouth adhesive rehabilitation of excessively worn dentition. Int J Esthet Dent. 2018;13(1):50-64. PMID: 29379903.
Gold D, Hahnel SF, Schierz O, Rauch A. Practicability of a chairside approach for characterizing CAD/CAM resin-based composites. J Oral Sci. 2020 Sep 26;62(4):430-434. doi: 10.2334/josnusd.19-0507. Epub 2020 Aug 31. PMID: 32863318.

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