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Bond Strength Comparison Among 10-MDP-Containing and Non-10-MDP-Containing Adhesives in Different Degrees of Dental Fluorosis

Comparación de fuerza de unión entre adhesivos que contienen y no contienen 10-MDP en diferentes grados de fluorosis dental

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ABSTRACT: Dental fluorosis can cause changes in the enamel surface, conditioning its functionality and esthetics. The application of dental adhesives is a treatment option; however, their use on fluorotic teeth can result in limitations. The aim of this study was to compare the shear bond strength of two different adhesives, one with 10-MDP and one without 10-MDP, in different degrees of dental fluorosis.This is an *in vitro* study on dental enamel samples, a total of 180 samples with the inclusion criteria were treated, randomly divided into two groups of 90, according to the type of dental adhesive, where each group was again divided into three groups of 30 samples, representing different degrees of dental fluorosis according to the Thylstrup-Fejerskov index (Group I: TF1 and TF2; Group II: TF3; Group III: TF4). Normality tests, two-factor ANOVA, and post-hoc tests were used to determine differences between the groups, with a significance level of 95%. As results, a statistically significant difference was shown between the use of dental adhesive with 10-MDP and the three groups of

dental fluorosis (p=0.011), in addition, a Tukey post-hoc test on the groups treated with 10-MDP adhesive revealed a statistically significant difference between group I versus group II, and group I versus group III, (p=<0.05). It is concluded that the use of adhesive systems with 10-MDP presents a better shear bond strength on enamel with dental fluorosis grades I and II in the Thylstrup-Fejerskov index.

KEYWORDS: Dental fluorosis; Dental Adhesive; 10-MDP; Shear strength; Dental bonding; Dental resin.

RESUMEN: La fluorosis dental puede ocasionar cambios en la superficie del esmalte, condicionando su funcionalidad y estética, la aplicación de adhesivos dentales es una opción de tratamiento, sin embargo, su uso en dientes fluoroticos puede resultar en limitaciones. El objetivo de este estudio consistió en comparar la fuerza de adhesión a la cizalladura de dos diferentes adhesivos, uno con 10-MDP y otro sin 10-MDP, en diferentes grados de fluorosis dental. Se trata de un estudio *in vitro* en muestras de esmalte dental, un total de 180 muestras con los criterios de inclusión fueron tratadas, aleatoriamente divididas en dos grupos de 90, de acuerdo al tipo de adhesivo dental, donde cada grupo fue dividido nuevamente en tres grupos de 30 muestras, representando diferentes grados de fluorosis dental según el índice de Thylstrup-Fejerskov (Grupo I: TF1 y TF2; Grupo II: TF3; Grupo III: TF4). Para determinar diferencias entre los grupos se utilizaron pruebas de normalidad, ANOVA de dos factores, y pruebas post-hoc, con un nivel de significancia de 95%. Como resultados, se mostró una diferencia estadísticamente significativa entre el uso de adhesivo dental con 10-MDP y los tres grupos de fluorosis dental (p=0.011), además, una prueba post hoc de Tukey sobre los grupos tratados con adhesivo 10-MDP revelaron una diferencia estadísticamente significativa entre el grupo I frente al grupo II, y el grupo I frente al grupo III, (p=<0.05) Se concluye que el uso de sistemas adhesivos con 10-MDP presentan una mejor resistencia de adhesión al cizallamiento en esmalte con grados de fluorosis dental I y II en el índice de Thylstrup-Fejerskov.

PALABRAS CLAVE: Fluorosis dental; Adhesivo dental; 10-MDP; Fuerza de unión; Adhesión dental; Resina dental.

INTRODUCTION

Currently, the use of fluorides is accepted and indicated for the prevention of dental caries (1), either through the fluoridation of water for human consumption (2), by the consumption of fluoridated table salt, the use of fluoride toothpaste and mouthwashes, and the topical oral application of fluorides (3). The main route of exposure to fluorides is artificial, intentional, and controlled with the fluoridation of drinking water, where the limit set by the World Health Organization (WHO) for this action is 0.5-1.0 mg/l (4). However, when the permissible limits are exceeded, the risk of developing dental and skeletal fluorosis increases (4). Dental fluorosis is an alteration of the enamel surface as a consequence of excessive exposure to fluorides, solely and exclusively, in the develo-

pmental stages of enamel (5). With respect to the incidence of fluoride worldwide, to date there is a vast list of countries around the world, about 25, with high levels of fluoride that affect their water resources for human consumption, highlighting that approximately 200 million people depend on the supply of these water sources with high levels of fluoride, which results in consequences for their health (6). Regarding Mexico, the prevalence of dental fluorosis ranges from 15.5%-87.1% in areas with low or optimal fluoride levels in drinking water (<1.5 parts per million), and 92%-100% in areas where fluoride levels exceed 1.5 parts per million (7); for the city of Durango, the prevalence of dental fluorosis in children aged 12 and 15 years reaches 87.5% (7); at the same time, fluoride levels between 2.22 and 7.23 parts per million have been recorded in wells that supply the city's drinking water distribution network (8). Although dental fluorosis is an irreversible condition, there are a series of treatments aimed at minimizing and eliminating the ravages that this condition creates in the dental enamel, these treatments consist of the implementation of minimally invasive aesthetic management with micro-abrasion, whitening, and infiltrated resins (9), some other interventions involve a more invasive management, such as the use of dental crowns (10), the above is conditioned by the degree of dental fluorosis that is present in each case. It has been reported that the implementation of sandblasting (11), deproteinization with 5% sodium hypochlorite (NaOCl) (12), and the use of high concentrations of phosphoric acid (13), in the presence of dental fluorosis, can greatly improve the enamel surface, conditioning an improvement for the reception of adhesive systems. With regard to dental adhesive systems, their beginnings date back to the work of Oskar Hagger and the development of glycerolphosphoric acid dimethacrylate implementing chemical adhesion in dental structures (14), later in 1955 Buonocore proposed an improvement in adhesion to dental surfaces with the use of orthophosphoric acid at 85% concentration on dental enamel thus obtaining a micromechanical retention (15), since then, the progress that dental adhesive systems have had until today is vast and undoubted. Among the advances in dental adhesion, the implementation of functional adhesive monomers (FAM) stands out, which allow the formation of a hybrid layer that allows mechanical interlocking and is responsible for the adhesion of the restorations to the dental surfaces and dentin sealant coating formation (14,15). An example of these FAM is 10-MDP (10-Methacryloyloxyalkyl Dihydrogen Phosphate), where its use was characteristic in third generation dental bonding systems, also known as dentin conditioner generation (16) and which is still in use today. Although the use of 10-MPD adhesives has shown an improvement not only in bond strength, but also in bond durability, it is important to note that this is only present in enamel not affected by fluorosis (17). Even with the above, in our population of interest, there is no research aimed at studying the behavior of adhesive materials for use and restoration on fluorotic enamel, so the objective of this work was focused on comparing the bond strength between adhesives containing and not containing MDP in different degrees of dental fluorosis. We hypothesize that none of the factors, both the type of adhesive and the degree of fluorosis (nor their interaction) influence the shear bond strength results.

MATERIALS AND METHODS

The present *in vitro* study was performed within the facilities of the Faculty of Dentistry of the Universidad Juarez del Estado de Durango, Mexico, and under the approval of the research ethics committee obtaining a unique identification folio (Folio No. 153), and following the guidelines according to the General Health Law on Health Research in its article 17.

COLLECTION OF EXPERIMENTAL SAMPLES AND CLASSIFICATION

The samples were obtained from subjects who attended the clinics of the School of Dentistry and who had a diagnostic indication for dental extraction (orthodontic, periodontal, prosthodontic or prosthetic), and who signed a written informed consent to use the extracted teeth for research purposes. The inclusion criteria were: permanent teeth with a presumptive diagnosis of dental fluorosis, extracted from any arch, without caries, without coronal fracture, without forceps cracks, without history of any type of endodontic, orthodontic, prosthetic, esthetic and/or restorative treatment. The classification and establishment of the degree of dental fluorosis was done by applying the Thylstrup-Fejerskov Index (10) and through two examiners trained and calibrated for this purpose, offering a Kappa concordance of 0.001. The sample size corresponded to 180 extracted teeth, which were divided into 3 groups by degree of dental fluorosis (n=60) being Group 1-TF1 and TF2, Group 2-TF3, and Group 3-TF4; subsequently the teeth of each group were randomly subdivided into 2 groups (n=30) to be subjected to dental adhesive contact, one group with 10-MDP (Universal adhesive) and another group without 10-MDP (2-Step Etch&Rinse Adhesive).

TREATMENT OF THE EXPERIMENTAL SAMPLES

After extraction the teeth were subjected to ultrasonic cleaning to remove any remaining dental calculus, periodontal tissue, organic debris or any remaining soft tissue debris, and prior to the in vitro study the teeth were preserved in deionized water grade 3 at 4±4 °C until use. In addition, ISO 29022:2013 was followed which specifies the substrate selection, storage and handling of the tooth structure, as well as the testing procedure.

PREPARATION OF THE EXPERIMENTAL SAMPLES

A crown-root odontosection of the extracted dental organ was performed with a diamond disc, followed by a second disto-mesial odontosection to obtain a vestibular side and a palatal/lingual side, and 2 pieces (2 mm^2) were obtained from each side, which were embedded in class IV gypsum with the enamel side free. The class IV gypsum was subsequently coded by letters according to the level of fluorosis and the type of adhesive to be used in each test (following recommendations of ISO 29022: 2013).

BONDING PROTOCOL FOR THE EXPERIMENTAL SAMPLES

The characteristics, indications and procedure for each adhesive in the different degrees of dental fluorosis are shown in Table 1.

SHEAR BOND STRENGTH TEST

A Teflon bar former and press by UltradentTM (South Jordan, UT, USA) was used to create resin cylinder with specifications of 2.38±0.03 millimeters (according to ISO 29022), the resin used was Filtek™ Z250 by 3M-ESPE (Saint Paul, MN, USA), where each cylinder was bonded to the tooth surface following the manufacturer's instructions, and using a Translux Power Blue LED light curing light from Heraeus-KulzerTM. The gypsum block with the samples was placed between the lower and upper bar, the conformers were placed in the presses in its upper bar, these conformers have a hole that was made to coincide with the enamel sample where it was going to be adhered and in that hole the resin was placed which took the shape of the hole with the standardized measures for resin adhesion tests.

For the measurement of shear forces, a universal testing machine Model-LS1 by Lloyd Instruments/Ametek (FL,USA) was used in conjunction with a software NexygenTM Plus to obtain test results for each group and then convert them to megapascals (MPa) using the following formula:

Load in N/ Surface area in mm^2= MPa

STATISTICAL ANALYSIS

Normality and homoscedasticity tests were applied. To compare multiple means, the two-factor ANOVA test and Tukey's post-hoc tests were used at a statistical significance level of 5%. The freely distributable statistical package R Studio, 2020 (18) was used.

Table 1. Adhesive type, composition, degree of dental fluorosis, shaping sequence and application of resin bars.

Adhesive type	Composition	Degrees of dental fluorosis, mode of application of adhesive* and resin rods.
		Group 1: TF1 y TF2 Group 2: TF3 Group 3: TF4
Adhesive without 10-MDP (2-Step Etch&Rinse Adhesive)		
OptiBondTM S (Kerr Italia. Scafati,Italy)	Ethanol base, 15% filled with 0.4 micron barium glass	Samples in gypsum 1. Apply 35% phosphoric acid (Ultra-Etch™) for 30 seconds. 2. Rinse for 60 s and dry with cotton swab. 3. Adhesive application, gentle air for 10 seconds. 4. Photopolymerize for 20 seconds at 1200 mW/s. 5. The gypsum block is placed in the press and aligned with the resin former. 6. Placement of resin in the resin conformer making sure that the resin passes through the hole until it is in contact with the tooth enamel. 7. Photopolymerize for 20 seconds at 1200 mW/s by placing the lamp on the top of the former. 8. Remove the block from the press.
Adhesive with 10-MDP (Universal Adhesive)		
ScotchbondTM Universal Adhesive (3M ESPE, St Paul MN, USA).	1.Scotchbond universal etchant: 34% phosphoric acid. 2.Adhesive: Methacrylo-yloxyalkyl dihydrogen phosphate monomer, dimethacrylate resins, hydroxyethyl methacrylate, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane.	Samples in gypsum 1. Apply 35% phosphoric acid (Ultra-Etch™) for 30 seconds. 2. Rinse for 60 s and dry with cotton swab. 3. Adhesive application, gentle air for 10 seconds. 4. Photopolymerize for 20 seconds at 1200 mW/s. 5. The gypsum block is placed in the press and aligned with the resin former. 6. Placement of resin in the resin conformer making sure that the resin passes through the hole until it is in contact with the tooth enamel. 7. Photopolymerize for 20 seconds at 1200 mW/s by placing the lamp on the top of the former. 8. Remove the block from the press.

The application protocol was followed depending on the type of adhesive and following the manufacturer's instructions.

RESULTS

The total sample used in this study corresponded to 180 tooth surfaces: of which 90 were treated with adhesive with 10-MDP, and 90 were treated with adhesive without 10-MDP.

Overall, no statistically significant differences were found in the comparison of shear bond strength between these two groups (p=0.75); however, a higher mean bond strength was observed in the group treated with 10-MDP adhesive (Table 2).

Further examination of the variances of the mean shear bond strength between the different fluorosis groups and types of dental adhesive showed a non-statistically significant difference (p=0.188), while between the use of dental adhesive with 10-MDP and the three dental fluorosis groups a statistically significant difference was observed (p=0.011), however, this significance is lost in the group treated with dental adhesive without 10-MDP (p=0.752), still even without statistical significance, group I shows the highest bond strength (Table 3). In addition, a post hoc test was performed on the groups treated with 10-MDP adhesive to determine which groups were different from each other, finding a statistically significant difference between group I versus group II, and group I versus group III (Table 3).

Table 2. Comparative shear bond strength data between the two types of dental adhesives and the total samples tested.

	Shear bond strength (MPa)		
Group	**Normality test***	**Media ± SD**	**P-value****
Samples treated with 10-MDP (n=90)	0.005	11.86 ± 4.81	0.75
Samples treated without 10-MDP (n=90)	0.092	11.64 ± 4.75	

* Kolmogorov-Smirnov normality test; ** Welch´s t-test; SD, standard deviation; MPa, Megapascals.

Table 3. Comparative data of the mean shear bond strength between each type of dental adhesive and the different dental fluorosis groups.

	Shear bond strength (MPa)		
Group	**I (TF1 y TF2) (n=30) Mean ± SD**	**II (TF3) (n=30) Mean ± SD**	**III (TF4) (n=30) Mean ± SD**
Samples treated with 10-MDP (n=90)	14.13 ± 4.85	10.44 ± 4.11a*	11.02 ± 4.74b*
Samples treated without 10-MDP (n=90)	12.37 ± 4.77	11.80 ± 5.36	10.75 ± 4.04

*Two-way ANOVA test, with Tukey post-hoc test ($p < 0.05$), shown with superscript letter: a Group I vs Group II, b Group I vs Group III; SD, standard deviation; MPa, Megapascals.
p-value [Interaction model] 0.188; p-value [Adhesive] 0.752; p-value [Degree of fluorosis] 0.011.

DISCUSSION

Some studies establish that dental fluorosis and enamel hypomineralization are conditioning factors that affect the shear bond strength on composite resins bonded to human enamel as a consequence of an affected etching surface (19-21), however, there is scientific literature that mentions a similar pattern of enamel etching regardless of whether the surface is fluorotic or not (22). This becomes relevant if we remember that the success of dental bonding relies on several factors (23), and mainly on enamel etching with phosphoric acid, considered the gold standard for bonding resin-based materials to the tooth structure (24). In this context, the present study was performed following a similar tooth surface etching protocol for all groups of samples.

Currently, the 10-MDP monomer presents a proven potential for interaction with hydroxyapatite; in addition, it offers a very stable bond, with low calcium salts dissolution rate, and a broad, hydrophobic chain that results in an adhesive MDP-Ca salt interface, which enhances bond strength and remains stable (25). However, in the results of this investigation, no statistically significant difference was found between the use of adhesives with w10-MDP and adhesives without 10-MDP and the shear bond strength in the total treated samples, but a higher shear bond strength is observed in the samples treated with adhesives with 10-MDP, which is in agreement with that reported by Sachdeva *et al.*, (26) who indicate that eighth generation adhesives offer higher average shear strength in dentin from primary teeth without fluorosis. Regarding the mean shear strength in the different degrees of dental fluorosis, the results of this study have a certain similarity with those reported by Torres-Gallegos *et al.* (27), who determine that the bond strength to shear is minimized the higher the degree of dental fluorosis. Another study (28), compares dental adhesion between healthy enamel and fluorotic enamel, using self-etching adhesives and the etching and rinsing technique, determining that the latter favors the increase of adhesion in teeth with fluorosis, which is relevant to our results, since in all the samples the technique of etching and rinsing of enamel was carried out, with which a greater mean shear bond strength was observed in the samples treated with 10-MDP adhesive.

Turp *et al.* (29) indicate that the etching and rinsing technique benefits adhesion in systems containing 10-MDP, which can be observed in the group treated with 10-MDP adhesive in the present study. In turn, Weerasinghe *et al.* (30) mention that the use of 37% phosphoric acid improves dental adhesion, in the present study a concentration of 35% phosphoric acid was used, so that this small difference could be a conditioning factor for changes in the results shown.

In general, research on changes and alterations in dental adhesion focus on studies comparing healthy tooth surfaces against fluorosed tooth surfaces (27,31-34), surfaces such as zirconia (35), some more between fluorotic dentin surfaces (36,37). In the study conducted by Liu *et al.* (38), it was determined that the use of the total etch adhesive system in different degrees of dental fluorosis resulted in improving the bond strength of enamel with mild fluorosis. This is in agreement with the results of this study, since independently of the use of adhesives with 10-MDP and adhesives without 10-MDP, the use of total etching prior to the application of the adhesive systems resulted in a higher mean shear bond strength in group I represented by grade I and II fluorosis in the Thylstrup-Fejerskov index (10).

Regarding the interaction between the fluorotic enamel surface and the adhesion mechanism of the dental adhesives implemented in this study, we consider that porosity, hypomineralization, loss of structure, and a weakness on the enamel are the main characteristics in dental fluorosis (10),

that generate a negative effect on dental adhesion, and would be clearly represented by the evident decrease in shear bond strength observed in the study groups corresponding to dental fluorosis TF3 and TF4, when compared to lower grades of this dental affectation (TF1-TF2. On the other hand, the presence of FAM 10-MDP has the characteristic of interacting strongly with the hydroxyapatite crystals on the enamel surface (25), as mentioned above, and it is in this sense that these conditions are represented in group I corresponding to dental fluorosis grades TF1 and TF2, which would contain a greater quantity and better structure of these crystals in comparison to the other groups, which would allow obtaining a better dental adhesion, just as it is observed in the results.

Finally, we can mention that the success of dental bonding with the use of adhesive systems containing 10-MDP depends on the type of mechanical test, the type of substrate, the monomer concentration, the surface pretreatment and the working times (39-42). The limitations of the present study include the lack of a control group represented by healthy enamel samples. Also, the lack of surface conditioning such as the use of NaOCl in some of its concentrations, as well as the implementation of more adhesive systems according to other classifications. Due to the impossibility of accessing specialized equipment at the time of the study, it was not possible to carry out the failure pattern evaluation and morphological surface analyses. It is important to mention that in vitro studies will always offer more encouraging results compared to in vivo studies, so it is suggested to take the results of this study with clinical relevance.

CONCLUSION

With the limitations of the study, it is possible to determine that the degrees of dental fluorosis have some influence on the shear bond strength. Furthermore, the use of adhesive systems with 10-MDP shows better shear bond strength on enamel with dental fluorosis grades I and II in the Thylstrup-Fejerskov index. In addition, in conjunction with the use of 10-MDP adhesives, the use of the total enamel surface etching technique is suggested in teeth with dental fluorosis.

CLINICAL SIGNIFICANCE

In areas with endemic dental fluorosis, such as the region of Durango, Mexico, it is important to have studies that evaluate the effects that different degrees of dental fluorosis can have when using different dental bonding systems, and this study helps in an important, although limited, way in the knowledge of these effects.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest and declare that they have no commercial, proprietary or financial interest in the products or companies described in this article.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: L.J.S.M., E.G.T.
Literature review: L.J.S.M., E.G.T.
Methodology and validation: L.J.S.M., K.Y.V.F., V.H.B.P.
Formal analysis: L.J.S.M., K.Y.V.F., V.H.B.P., N.V.C., O.E.A.O., E.G.T.
Research and data collection: L.J.S.M., K.Y.V.F.
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Data analysis and interpretation: N.V.C., E.G.T.
Writing-preparation of original draft: L.J.S.M., E.G.T.
Writing: revising and editing: L.J.S.M., K.Y.V.F., N.V.C., O.E.A.O., V.H.B.P., E.G.T.
Supervision and approval of final version: L.J.S.M., K.Y.V.F., N.V.C., O.E.A.O., V.H.B.P., E.G.T.

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