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## Influence of Different Enamel Pretreatment on Bond Strength of Fissure Sealant

### *Utjecaj različitih predtretmana cakline na čvrstoću adhezivnog vezivanja pri pečaćenju fisura*

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#### Abstract

**Objective:** The aim of this *in vitro* study was to evaluate the bond strength of a resin composite fissure sealant to enamel which was pre-treated with different laser pulse modes and additional acid etching. **Materials and Methods:** Forty-two healthy molars and premolars were collected for this study and randomly divided into 6 groups ( $n=7$ ). Group 1: Quantum Square Pulse (QSP); Group 2: Medium-Short Pulse (MSP) mode; Group 3: Super Short Pulse (SSP) mode; Group 4: QSP + acid etching; Group 5: MSP + acid etching; Group 6: SSP + acid etching. The occlusal surfaces of the teeth were pre-treated according to the defined group. Laser conditioning of the enamel was performed using an Er:YAG laser Fotona Light Walker AT-S (Fotona, Ljubljana, Slovenia) with a wavelength of 2940 nm + acid etching (EN etch Ivoclar Vivadent AG, Schaan, Liechtenstein). Occlusal surfaces were sealed with a resin-based composite fissure sealant (Helioseal F, Ivoclar Vivadent AG, Schaan, Liechtenstein). Micro-tensile bond strength ( $\mu$ TBS) test and stereomicroscope evaluations of the failure mode were performed. The  $\mu$ TBS was tested using the Games-Howell method. The failure mode between groups was tested using the chi-square test. The significance level for all tests was set at  $p < 0.05$ . **Results:** The highest bond strength was measured using laser etching in MSP mode combined with acid etching (36.09 MPa). This combination showed a significantly higher bond strength than the other combinations (SSP + ETCH,  $p < 0.001$ ; QSP + ETCH,  $p < 0.001$ ). **Conclusion:** The SP laser followed by acid etching of enamel yielded the highest bond strength. Thus, the MSP with a 140  $\mu$ s pulse mode might be the preferred choice as a pre-treatment procedure for fissure sealing.

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## Introduction

Dental caries is “a biofilm-mediated, diet-modulated, multifactorial, non-communicable, dynamic disease that results in net mineral loss of dental hard tissues” (1). Although smooth surfaces have benefited from caries-prevention protocols, the high prevalence of occlusal caries remains a challenge (2). This can be attributed to the complex pit and fissure morphologies, which are highly susceptible to caries development. Dental biofilms easily accumulate in these areas and cannot be effectively removed.

Dental fissure sealants prevent the pit and fissure caries. They provide a mechanical barrier to prevent dental plaque accumulation (3). Therefore, fissure sealant application is one of the most reliable and effective methods to prevent occlusal caries. The advantages of sealant application are significant caries risk reduction compared to non-sealed controls and lower biological and economical cost compared to restoration placement (4). The success rate of fissure sealants depends

## Uvod

Zubni karijes je *biofilmom posredovana, prehranom modulirana, multifaktorijalna, nezarazna, dinamička bolest koja rezultira miligramskim gubitkom minerala iz tvrdih zubnih tkiva*. (1). Iako glatke površine imaju koristi od protokola za prevenciju karijesa, izazov ostaje visoka prevalencija okluzalnoga karijesa (2). To se može pripisati složenoj morfologiji jama i fisura koje su vrlo osjetljive na pojavu karijesa. U tim područjima lako se nakuplja dentalni biofilm koji se ne može učinkovito ukloniti.

Sredstva za pečaćenje fisura sprječavaju karijes jama i fisura i mehanička su barijera za sprječavanje nakupljanja zubnoga plaka (3). Zato je primjena toga sredstva jedna od najpouzdanijih i najučinkovitijih metoda za sprječavanje okluzalnog karijesa. Prednosti primjene materijala za pečaćenje jesu značajno smanjenje rizika od karijesa u usporedbi s nezapećaćenim Zubima te niži biološki i ekonomski trošak u usporedbi s postavljanjem ispuna (4). Uspješnost pečaćenja

on the quality of adhesion between the sealant material and the enamel (5). To enhance the bond strength between the composite fissure sealant and enamel, different pre-treatment techniques have been investigated. Each method aims to modify the enamel surface, creating micropores, increased surface roughness, or chemical interactions to facilitate mechanical or chemical bonding (6, 7).

Improving the bond and retention of the sealant material would increase the primary and secondary preventive benefits of sealants, as it has been reported that the loss of sealant is directly related to subsequent caries development.

Acid etching of the enamel with phosphoric acid at 30–40% concentration (8) is one of the most common procedures in resin-based pit and fissure sealant applications in clinical practice. Acid etching is a widely accepted and effective pre-treatment method that enhances sealant adhesion. Several studies have shown that acid etching significantly improves the bond strength. However, careful application and rinsing are required to avoid over-etching or enamel damage (9, 10). Over-etching or leaving acid on the enamel surface for an extended period can lead to excessive demineralization, enamel prismatic dissolution, or enamel surface roughness (11).

In addition to the conventional acid-etching method, laser etching has emerged as an alternative technique for enamel preparation before sealant placement. Pre-treatment of enamel with Er: YAG laser involves the use of laser energy to create micro texturing on the enamel surface, which can enhance the bond strength between the sealant material and the tooth. Some studies have demonstrated that the micro-roughened surface morphology after laser irradiation of permanent enamel is similar to that obtained with conventional acid etching (12, 13). Other studies reported that pre-treatment with Er: YAG laser alone prior to sealant application led to increased resin microléakage and lower bond strength of the sealant to enamel (14, 15).

Pulse Duration and Laser energy influence enamel surface modification. Different pulse modes, such as long-pulse, short-pulse, and ultrashort-pulse lasers, can affect the interaction with enamel. Longer pulse durations allow for deeper penetration and more thermal effects, whereas shorter pulse durations provide more precise ablation with minimal thermal side effects (16). Regarding pulse shaping, three pulse-forming technologies are currently used in erbium dental lasers: Pulse-Forming Network (PFN), Variable Square Pulse (VSP), and the latest Adaptive Structured Pulse (ASP) technology (17). Adaptive Structured Pulsing (ASP) is a powerful technology enabling precise manipulation of the temporal envelope of laser pulses, transitioning from a simple square pulse shape to more complex forms. This includes breaking individual pulses in a train into multipulse burst sequences, with each pulse uniquely shaped. A notable example of this adaptive approach is the Quantum Square Pulse (QSP) modality, which optimizes Er: YAG laser performance by meeting the three fundamental requirements for dental tissue ablation: rapid ablation, minimal heat deposition, and reduced vibration (18, 19).

However, it should be noted that the optimal laser parameters for enamel etching may vary depending on the laser

fisura ovisi o kvaliteti adhezije između materijala za pečaće- nje i cakline (5). Kako bi se povećala čvrstoća veze između kompozitnog sredstva za pečaćenje fisura i cakline ispitane su različite tehnike predtretmana. Svaka metoda ima za cilj modificirati površinu cakline stvarajući mikropore, poveća- nu hrapavost površine ili kemijske interakcije da bi se olakša- lo mehaničko ili kemijsko spajanje (6, 7).

Poboljšanje veze i zadržavanje materijala za pečaćenje po- većalo bi primarnu i sekundarnu preventivnu korist materijala za pečaćenje, jer je prijavljeno da je gubitak materijala za pečaćenje izravno povezan s kasnjim razvojem karijesa.

Jetkanje cakline fosfornom kiselinom u koncentraciji od 30 do 40 % (8) jedan je od najčešćih postupaka pri primjeni pečatnih smola u kliničkoj praksi. Jetkanje kiselinom široko je prihvaćena i učinkovita metoda koja poboljšava adhezivno vezivanje. Autori više studija pokazali su da jetkanje kiselinom značajno poboljšava čvrstoću veze. No potrebno je pažljivo nanošenje i ispiranje kako bi se izbjeglo prekomjerno jetkanje ili oštećenje cakline (9, 10). Prekomjerno jetkanje, ili ako je kiselina dulje ostavljana na površini cakline, može rezultirati pretjeranom demineralizacijom, otapanjem prizmi cakline ili njezinom površinskom hrapavošću (11).

Uz konvencionalnu metodu jetkanja kiselinom, lasersko jetkanje predloženo je kao alternativna tehnika za pripremu cakline prije postavljanja materijala za pečaćenje. Predtretman cakline Er:YAG laserom uključuje korištenje laserske energije za stvaranje mikroteksture na površini cakline, što može poboljšati snagu veze između materijala za pečaćenje i zuba. U nekim je studijama istaknuto da je morfologija mikrohrapave površine nakon laserskoga zračenja trajne cakline slična onoj dobivenoj konvencionalnim jetkanjem kiselinom (12, 13). Autori drugih studija izvjestili su pak da je pret- hodna obrada samo Er:YAG laserom prije nanošenja materijala za pečaćenje povećala mikropropuštanje smole i smanjila čvrstoću materijala za pečaćenje s caklinom (14, 15).

Trajanje pulsa i energija lasera utječu na modifikaciju površine cakline. Različiti načini aplikacije pulsa, kao što su laseri dugoga pulsa, kratkoga pulsa i ultrakratkoga pulsa, mogu utjecati na interakciju s caklinom. Dulje trajanje pulsa omogućuje dublje prodiranje i veći termalni učinak, a kraće precizniju ablaciju s minimalnim termalnim spo- rednim učinkom (16). Pri upotrebi erbijevih dentalnih la- sera trenutačno se koriste tri tehnologije za oblikovanje pulsa: mrežno formiranje pulsa (engl. *Pulse-Forming Network – PFN*), varijabilni kvadratni puls (engl. *Variable Square Pulse – VSP*) i najnovija tehnologija, adaptivno strukturirano pulsiranje (engl. *Adaptive Structured Pulse – ASP*) (17). Adaptivno strukturirano pulsiranje moćna je tehnologija koja omogućuje preciznu vremensku manipulaciju laser- skih impulsa prelazeći iz jednostavnoga četvrtastog oblika pulsa u složenije oblike, što uključuje razbijanje individualnih impulsa u nizu sekvencije višestrukih impulsa, pri čemu je svaki impuls jedinstveno oblikovan. Primjer takva prilagodljivoga pristupa jest način rada varijabilnoga kvadratnog pulsa koji optimizira učinak Er:YAG lasera ispunjavanjem triju temeljnih zahtjeva u slučaju ablacije zubnoga tkiva: brzu ablaciju, minimalno taloženje topline i smanjenu vibra- ciju (18, 19).

type, wavelength, energy density, and pulse duration. Further research is needed to determine the most effective laser parameters for sealant placement and evaluate the long-term clinical performance of laser-etched sealants. The aim of this study was to evaluate the bond strength of a composite fissure sealant to the enamel surface after pre-treatment with different laser pulse modes of an Er:YAG laser alone or using laser modes associated with acid etching, and to compare the results among all pre-treatment procedures. The null hypotheses were: H<sub>1</sub><sub>0</sub> there is no difference in the bond strength whether the materials were applied to the enamel surface pretreated with Quantum Square pulse mode (QSP), Super Short Pulse (SSP mode), and Medium-Short Pulse mode (MSD); H<sub>2</sub><sub>0</sub> there is no difference in bond strength of composite fissure sealant to enamel surface pre-treated with QSP, SSP, MSP laser mode with additional acid etching; H<sub>3</sub><sub>0</sub> there is no difference in the failure mode among the groups.

## Materials and Methods

The study was approved by the Ethics Committee of the University of Zagreb School of Dentistry and the University of Pristina "Hasan Pristina" Dental Faculty. A total of 42 noncarious human premolars and molars were collected for this study. Periodontal tissue remnants were cleanly removed. After disinfection with 0.5% chloramine solution for a week, the teeth were placed in distilled water and stored until sample preparation at 4 °C until use. All teeth were examined at 10X magnification using a stereomicroscope, and teeth with caries, cracks, and other enamel structure anomalies were excluded from the study.

Teeth were randomly divided into six groups, with seven specimens in each group (n=7) depending on the pre-treatment variety. These pre-treatments were performed on the occlusal surfaces of each tooth prior to fissure sealant application.

The groups were as follows:

1. Group QSP (Quantum Square pulse mode) Adaptive Structured Pulse (ASP) – QSP
2. Group MSP (Medium-Short Pulse mode) 140 µs pulse mode – MSP
3. Group SSP (Super Short Pulse mode) 50 µs pulse mode – SSP
4. QSP + Acid etching – QSP+ETCH
5. MSP + Acid etching – MSP+ETCH
6. SSP + Acid Etching – SSP+ETCH

After cleaning, rinsing, and drying, the specimens were conditioned with different laser modes, and acid etching was used for Groups 4, 5, and 6.

Laser conditioning of the enamel surface in all groups was carried out using the Adaptive Structured Pulse (ASP) mode with an Er: YAG laser device, the Fotona Light Walker AT-S (Fotona, Ljubljana, Slovenia) with a wavelength of

No treba napomenuti da se optimalni laserski parametri za jetkanje cakline mogu razlikovati ovisno o vrsti lasera, valnoj duljini, gustoći energije i trajanju impulsa. Potrebna su daljnja istraživanja da bi se odredili najučinkovitiji laserski parametri za postavljanje materijala za pečaćenje i procjenjila dugoročna klinička učinkovitost materijala za pečaćenje pri upotrebi laserskoga jetkanja. Svrha ove studije bila je procijeniti čvrstoću veze kompozita za pečaćenje fisura s površinom cakline poslije predtretmana različitim načinima laserskoga pulsa samog Er:YAG lasera ili korištenjem laserskih metoda povezanih s jetkanjem kiselinom, te usporediti rezultate između svih postupaka predtretmana. Nulte hipoteze bile su: H<sub>1</sub><sub>0</sub> nema razlike u čvrstoći veze bilo da su materijali naneseni na površinu cakline koja je prije toga tretirana metodom kvantnoga kvadratnoga pulsa (engl. *Quantum Square pulse* – QSP), metodom super kratkoga pulsa (engl. *Super Short Pulse* – SSP) ili metodom srednje kratkoga pulsa (engl. *Medium-Short Pulse* – MSD); H<sub>2</sub><sub>0</sub> nema razlike u čvrstoći kompozitnog sredstva za pečaćenje fisura na površini cakline prethodno tretiranoj QSP, SSP ili MSP laserskim načinom rada u kombinaciji s dodatnim jetkanjem kiselinom; H<sub>3</sub><sub>0</sub> nema razlike između skupina kod vrsta neuspjeha.

## Materijali i metode

Istraživanje je odobrilo Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu i Etički odbor Stomatološkog fakulteta Sveučilišta u Prištini *Hasan Prishtina*. Za ovu studiju prikupljena su ukupno 42 nekariozna ljudska pretkutnjaka i kutnjaka. Ostatci parodontnog tkiva uredno su uklonjeni. Nakon jednotjedne dezinfekcije 0,5-postotnom otopinom kloramina zubi su stavljeni u destiliranu vodu i čuvani do pripreme uzorka na temperaturi od 4 °C. Svi su zubi pregledani stereomikroskopom pod povećanjem od 10 puta, a zubi s karijesom, pukotinama ili drugim anomalijama strukture cakline isključeni su iz istraživanja.

Zubi su postupkom randomizacije podijeljeni u šest skupina sa sedam uzoraka u svakoj (n = 7), ovisno o načinu predtretmana. Predtretmani su provedeni na okluzalnim površinama svih zuba prije nanošenja sredstva za pečaćenje fisura.

Skupine su bile:

1. QSP adaptivnoga strukturiranoga pulsa (QSP)
2. MSP 140 µs pulsnii način rada (MSP)
3. SSP 50 µs pulsnii način rada (SSP)
4. QSP + jetkanje kiselinom (QSP+ETCH)
5. MSP + jetkanje kiselinom (MSP+ETCH)
6. SSP + jetkanje kiselinom (SSP+ETCH)

Nakon čišćenja, ispiranja i sušenja, uzorci su kondicionirani različitim laserskim metodama, a jetkanje kiselinom korišteno je u skupinama 4, 5 i 6.

Lasersko kondicioniranje površine cakline u svim skupinama provedeno je s pomoću adaptivnoga strukturiranoga načina rada pulsa Er:YAG laserskim uređajem (Fotona LightWalker AT-S, Ljubljana, Slovenija) s valnom duljinom od 2940 nm. Pritom je korišten nasadnik H14 s vrhom od konusnih safirnih vlakana (13 do 0,8/8 mm). Izlazna snaga bila je 120 mJ, odnosno 10 Hz. Dodatno jetkanje kiselinom

2940 nm, using a handpiece H14 with a conical sapphire fiber (13 up to 0.8/8 mm) tip. The power outputs were 120 mJ and 10 Hz, respectively. Additional acid etching was performed using orthophosphoric acid 37% (EN etch Ivoclar Vivadent AG, Schaan, Liechtenstein) for 20 s. After pretreatment of the occlusal surface, the composite fissure sealant (Helioseal F, Ivoclar Vivadent AG, Schaan, Liechtenstein) was placed on the occlusal surfaces according to the manufacturer's instructions and polymerized in high-power mode at 1200 mW/cm<sup>2</sup> (Bluephase Ivoclar Vivadent AG, Schaan, Liechtenstein) for 20 seconds. All teeth were placed in a self-curing acrylate to obtain a square surface so that cutting could be performed in a universal cutting machine (Isomet). All teeth were cut into 1-1.5 mm thick and at least 6 mm long beams. From each tooth we were able to obtain one or two beams making the total number of samples ten for each group.

The microtensile bond strength ( $\mu$ TBS) of the samples was tested using a Bisco Microtensile tester (Bisco, Schaumburg, Illinois, USA). Each sample was fixed at both ends to microtensile device test jaws using cyanoacrylate glue (Zapit, Dental Ventures of America, and Corona, CA, USA). The force was applied at a crosshead speed of 1 mm/min until the failure of the sample. The force obtained at the moment of sample failure was recorded. The microtensile bond strength values (MPa) were calculated as the ratio of the applied force (N) at the moment of sample failure to the cross-sectional bonding area (mm<sup>2</sup>) of the sample. The cross-sectional bonding area was determined by measuring the width of the sample. The type of failure of the sample was classified as adhesive, cohesive, or mixed using a stereomicroscope at 40 $\times$  magnification.

Statistical analyses were performed using the statistical software SPSS version 20 (IBM, New York, USA). Individual entities obtained by  $\mu$ TBS analysis were described with two independent nominal variables and one dependent continuous variable: three different pulse modes, with and without additional acid etching treatment, and  $\mu$ TBS. The answer to the hypotheses was determined using two-way analysis of variance (ANOVA). The significance of the effects was determined by hypothesis testing using F-tests.

The normality of the distribution of the dependent variables was tested using the Kolmogorov-Smirnov test. Because the equality of error variances condition was not met for multiple comparisons, the  $\mu$ TBS was tested using the Games-Howell method.

The failure mode between groups (three without etching and three with etching) was tested using the chi-square test. The significance level for all tests was set at  $p < 0.05$ .

## Results

Group SSP (24.79 MPa  $\pm$ 3.92) had the lowest bond strength, followed by QSP + ECTH (27.92 MPa  $\pm$ 2.24), and SSP + ECTH (28.16 MPa  $\pm$ 3.69). The highest bond strength was achieved in the MSP + ECTH group (36.09 MPa  $\pm$ 4.42), while almost the same bond strength was achieved in the MSP and QSP (32.42  $\pm$ 5.91 and 32.03 MPa  $\pm$ 6.13 respec-

to obavljeno je korištenjem 37-postotne ortofosforne kiseline (Ivoclar Vivadent AG, Schaan, Lihtenštajn (ETCH)) tijekom 20 sekunda. Nakon predtretmana okluzalne površine, pečatna smola (Helioseal F, Ivoclar Vivadent AG, Schaan, Lihtenštajn) postavljena je na okluzalne površine u skladu s uputama proizvođača i 20 sekunda polimerizirana snagom od 1200 mW/cm<sup>2</sup> (Bluephase Ivoclar Vivadent AG, Schaan, Lihtenštajn). Svi su zubi stavljeni u samopolimerizirajući akrilat da bi se dobila četvrtasta površina za rezanje u univerzalnom stroju (Isomet). Svi su zubi izrezani na presjek debljine od 1 do 1,5 mm i duljine najmanje 6 mm. Iz svakoga zuba dobiven je jedan do dva presjeka, što čini ukupno 10 uzoraka za svaku skupinu.

Mikrovlačna čvrstoća veze ( $\mu$ TBS) uzoraka ispitana je uređajem Bisco Microtensile (Bisco, Schaumburg, Illinois, SAD). Svaki uzorak fiksiran je cijanoakrilatnim ljepilom (Zapit, Dental Ventures of America, Corona, CA, SAD) na oba kraja uređaja. Sila je primjenjivana brzinom od 1 mm/min. do puknuća uzorka. Zabilježena je sila pri lomu uzorka. Vrijednosti mikrovlačne čvrstoće veze (MPa) izračunate su kao omjer primjenjene sile (N) u trenutku loma uzorka u području adhezivne površine presjeka uzorka (mm<sup>2</sup>). Adhezivna površina određena je mjeranjem širine uzorka. Vrsta neuspjeha uzorka klasificirana je kao adhezivna, kohezivna ili mješovita s pomoću stereomikroskopa pri povećanju od 40 puta.

Statistička analiza obavljena je u softveru SPSS verzija 20 (IBM, New York, SAD). Pojedinačni entiteti dobiveni  $\mu$ TBS analizom opisani su dvjema neovisnim nominalnim varijablama i jednom ovisnom kontinuiranom varijablu: tri različita pulsna načina s dodatnim tretmanom jetkanjem kiselinom i bez toga tretmana i  $\mu$ TBS-om. Odgovor na postavljene hipoteze određen je dvosmjernom analizom varijance (ANOVA). Značajnost učinaka određena je testiranjem hipoteza s pomoću F-testova.

Normalnost distribucije zavisnih varijabli testirana je Kolmogorov-Smirnovljevim testom. Budući da uvjet jednakosti varijanci pogreške nije ispunjen za višestruke usporedbe,  $\mu$ TBS je testiran uporabom Games-Howellove metode.

Način neuspjeha između skupina (tri bez jetkanja i tri s jetkanjem) testiran je s pomoću hi-kvadrat testa. Razina značajnosti za sve testove bila je postavljena na  $p < 0,05$ .

## Rezultati

Skupina SSP (24,79 MPa  $\pm$ 3,92) imala je najmanju vrijednost čvrstoće veze, zatim QSP + ECTH (27,92 MPa  $\pm$ 2,24) i SSP + ECTH (28,16 MPa  $\pm$ 3,69). Najveća vrijednost čvrstoće adhezivne veze postignuta je u skupini MSP + ECTH (36,09 MPa  $\pm$ 4,42), a gotovo jednaka zabilježena je u MSP-u i QSP-u (32,42  $\pm$ 5,91, odnosno 32,03 MPa  $\pm$ 6,13).

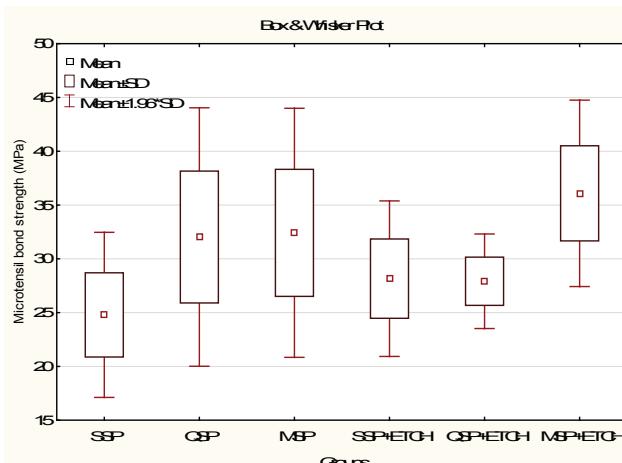
tively). The bond strength dispersion, expressed by the standard deviation, was the highest in the QSP group (6.13 MPa) and almost the same in the MSP group (5.91 MPa). The other groups showed less bond strength dispersion, especially in the QSP + ETCH group (2.24 MPa), Figure 1.

The above results of the  $\mu$ TBS analysis using two-way analysis of variance contradict hypotheses  $H_0_1$  and  $H_0_2$ ; namely, there are statistically significant differences among the SSP, MSP, and QSP groups regarding  $\mu$ TBS, despite the fact that etching alone was not a statistically significant factor. A statistically significant difference was found in the interaction between the studied groups, Figure 2.

Multiple comparisons of the  $\mu$ TBS test with the Games-Howell method are shown in Table 1. The method enables mutual comparison of all six groups (SSP, MSP, QSP, SSP + ETCH, MSP + ETCH, and QSP + ECTH), pair by pair.

For example, SSP showed significantly lower bond strength compared to QSP and MSP, but it did not increase significantly by etching (SSP + ETCH); it was also lower than those in the other two groups with ECTH. Neither the QSP, nor MSP changed significantly with etching, Table 1.

Failure mode analyses showed 100% success of sealing (adhesive) outcomes in the SSP, MSP, and MPS + ETCH groups. In the QSP group, the success rate was 87% (three failed sealing outcomes), whereas in the same group with etching, the success rate was 90.5% (two failed sealing outcomes). In the SSP group, one failed experiment was registered in the case of etching, which reduced the success rate by 5%, Table 2.



**Figure 1** Box and whisker plot microtensile bond strength (MPa) by the group

**Slika 1.** Kutijasti dijagram (box and whisker plot) mikrovlačne čvrstoće veze (MPa) po skupinama

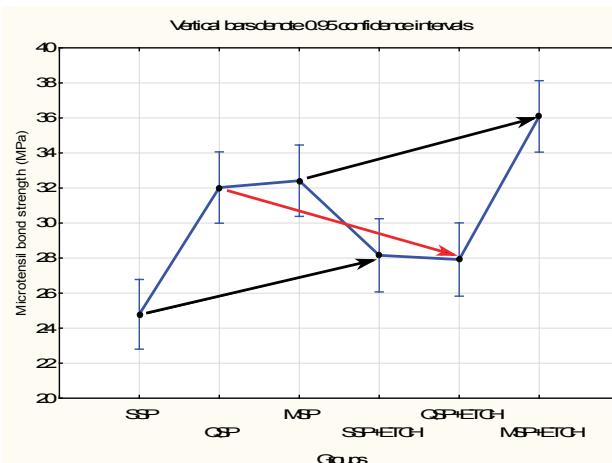
Disperzija čvrstoće veze, izražena standardnom devijacijom, bila je najveća u QSP skupini (6,13 MPa) i gotovo jednaka u MSP skupini (5,91 MPa). Ostale skupine pokazale su manju disperziju čvrstoće veze, posebno u skupini QSP + ETCH (2,24 MPa) (slika 1.).

Gore navedeni rezultati  $\mu$ TBS analize, korištenjem dvo-smjerne analize varijance, proturječe hipotezama  $H_0_1$  i  $H_0_2$ . Naime, postoje statistički značajne razlike između skupina SSP, MSP i QSP kod  $\mu$ TBS analize, unatoč činjenici da samo jetkanje nije bilo statistički značajan čimbenik. Utvrđena je statistički značajna razlika u interakciji između ispitivanih skupina (slika 2.).

Višestruke usporedbe  $\mu$ TBS analize s Games-Howellovom metodom nalaze se u tablici 1. Metoda omogućuje ujamnu usporedbu svih šest skupina (SSP, MSP, QSP, SSP + ETCH, MSP + ETCH i QSP + ECTH) u parovima.

Na primjer, SSP je pokazao znatno manju čvrstoću veze u usporedbi s QSP-om i MSP-om koja se nije značajno povećala jetkanjem (SSP + ETCH). Čvrstoća veze također je bila manja u usporedbi s drugim dvjema ECTH skupinama. Ni QSP ni MSP nisu se značajno promjenili postupkom jetkanja (tablica 1.).

Analiza vrste neuspjeha pokazala je 100-postotnu uspješnost ishoda adhezivnog vezivanja u skupinama SSP, MSP i MPS + ETCH. U QSP skupini uspješnost je bila 87 % (tri neuspješna ishoda pečaćenja), a u istoj skupini s jetkanjem stopa uspješnosti bila je 90,5 % (dva neuspjela ishoda pečaćenja). U SSP skupini zabilježen je jedan neuspjeh u slučaju jetkanja što je smanjilo uspješnost za 5 % (tablica 2.).



**Figure 2** Average values of microtensile bond strength without and with the etching procedure in the groups (with associated 0.95 confidence intervals)

**Slika 2.** Prosječne vrijednosti mikrovlačne čvrstoće veze s postupkom jetkanja po skupinama i bez toga postupka (s pridruženim intervalima pouzdanosti od 0,95)

Table 1	Multiple comparisons microtensile bond strength (MPa) with Games-Howell method	First group	Second group *	Mean Difference	Std. Error	p
<b>Tablica 1.</b> Multiple usporedbe mikrovlačne čvrstoće veze (MPa) – Games-Howellova metoda	<u>SSP</u>	<u>QSP</u>	QSP	-7.24*	1.615	<u>0.001</u>
			MSP	-7.63*	1.573	<u>&lt;0.001</u>
			SSP+ETCH	-3.37	1.202	<u>0.079</u>
			QSP+ETCH	-3.13*	0.997	<u>0.039</u>
			MSP+ETCH	-11.30*	1.306	<0.001
	<u>MSP</u>	<u>QSP</u>	SSP	7.24*	1.615	<u>0.001</u>
			MSP	-0.39	1.903	<u>1.000</u>
			SSP+ETCH	3.87	1.611	<u>0.187</u>
			QSP+ETCH	4.11	1.464	<u>0.090</u>
			MSP+ETCH	-4.06	1.690	0.183
	<u>QSP+ETCH</u>	<u>SSP+ETCH</u>	SSP	7.63*	1.573	<u>&lt;0.001</u>
			QSP	0.39	1.903	<u>1.000</u>
			SSP+ETCH	4.26	1.568	<u>0.100</u>
			QSP+ETCH	4.50*	1.417	<u>0.041</u>
			MSP+ETCH	-3.67	1.649	0.252
	<u>MSP+ETCH</u>	<u>QSP+ETCH</u>	SSP	3.37	1.202	<u>0.079</u>
			QSP	-3.87	1.611	<u>0.187</u>
			MSP	-4.26	1.568	<u>0.100</u>
			QSP+ETCH	0.24	0.990	<u>1.000</u>
			MSP+ETCH	-7.93*	1.301	<0.001
	<u>QSP</u>	<u>MSP</u>	SSP	3.13*	0.997	<u>0.039</u>
			QSP	-4.11	1.464	<u>0.090</u>
			MSP	-4.50*	1.417	<u>0.041</u>
			SSP+ETCH	-0.24	0.990	<u>1.000</u>
			MSP+ETCH	-8.17*	1.114	<0.001
	<u>MSP</u>	<u>SSP</u>	SSP	11.30*	1.306	<u>&lt;0.001</u>
			QSP	4.06	1.690	<u>0.183</u>
			MSP	3.67	1.649	0.252
			SSP+ETCH	7.93*	1.301	<0.001
			QSP+ETCH	8.17*	1.114	<0.001

\*The mean difference is significant at the 0.05 level.

**Table 2.** Stereomicroscope analysis results  
**Tablica 2** Rezultati stereomikroskopske analize

Pulse mode		Stereomicroscope analysis				
		Adhesive	Cohesive-material	Cohesive-teeth	Pretest Failure-Adhesive	Total
<u>SSP</u>	n	21	0	0	0	<u>21</u>
	rp	100.0%	0.0%	0.0%	0.0%	100.0%
<u>QSP</u>	n	20	0	1	2	<u>23</u>
	rp	87.0%	0.0%	4.3%	8.7%	100.0%
<u>MSP</u>	n	20	0	0	0	<u>20</u>
	rp	100.0%	0.0%	0.0%	0.0%	100.0%
<u>SSP+ETCH</u>	n	19	1	0	0	<u>20</u>
	rp	95.0%	5.0%	0.0%	0.0%	100.0%
<u>QSP+ETCH</u>	n	19	2	0	0	<u>21</u>
	rp	90.5%	9.5%	0.0%	0.0%	100.0%
<u>MSP+ETCH</u>	n	20	0	0	0	<u>20</u>
	rp	100.0%	0.0%	0.0%	0.0%	100.0%
Total	n	119	3	1	2	<u>125</u>
	rp	95.2%	2.4%	0.8%	1.6%	100.0%

\*Legend: n – number of cases, rp – row percentages

## Discussion

The aim of this study was to test and compare the bond strength of composite fissure sealant to enamel after pre-treatment with QSP, SSP, and MSP laser pulse modes and additional acid etching. The results have shown that SSP had the lowest bond strength (24.79 MPa  $\pm 3.92$ ), followed by QSP + ETCH (27.92 MPa  $\pm 2.24$ ), SSP + ETCH (28.16 MPa  $\pm 3.69$ ), and MSP + ETCH (36.09 MPa  $\pm 4.42$ ).

Comparisons between the groups revealed significant differences. The SSP exhibited lower bond strength than both the QSP and MSP modes ( $p=0.001$ ), with MSP showing the highest overall strength ( $p<0.001$ ). However, no significant difference was observed between the QSP and MSP modes ( $p = 1.000$ ). This confirms that SSP results in lower bond strength, while the QSP and MSP modes provide higher strength, refusing the first hypothesis that there is no difference between the SSP, MSP and QSP bond strength.

The impact of acid etching in combination with laser modes was further examined. Although SSP + ETCH showed higher bond strength than SSP alone, the difference was not statistically significant ( $p=0.079$ ). The combination of MSP + ETCH resulted in the highest bond strength (36.09 MPa  $\pm 4.42$ ), thus showing that etching with MSP improves adhesion, while QSP + ETCH decreases the bond strength compared to QSP alone.

The analysis rejected the null hypothesis and confirmed the significant effect of the laser pulse mode on the bond strength, particularly when combined with etching. A post-hoc analysis using the Games-Howell method showed that MSP + ETCH had a significantly higher bond strength than the other groups, suggesting that the MSP was the preferred pre-treatment mode.

Stereomicroscope analysis found that the MSP and SSP groups showed 100% success in sealing outcomes, whereas the QSP group had a higher failure rate, further supporting MSP as the best pre-treatment option. This study aligns with and expands upon the findings from previous research on bond strength using laser pre-treatment techniques. Previous studies have reported varying results depending on the laser type and parameters used. For example, AlHumaid et al. (20) demonstrated similar bond strengths for Er, Cr: YSGG laser pre-treatment compared to traditional acid etching. In contrast, Drummond et al. (21) and Shahabi et al. (22) observed that acid etching generally provided superior bond strength compared with laser treatment. However, Wanderley et al. (23) found that Er: YAG lasers can achieve bond strengths comparable to or even exceeding those of acid etching.

In this study, MSP + ETCH provided the highest bond strength, supporting the notion that combining laser etching (particularly the MSP mode) with acid etching improves bond strength. This finding aligns with the research by Borsatto et al. (24, 25), who showed that combining etching methods, that is laser and acid method, can enhance the tensile strength and reduce microleakage. However, unlike AlHumaid's results, which indicated that higher laser power settings (3.5 W) are required to match the acid etching

## Rasprava

Svrha ove studije bila je ispitati i usporediti čvrstoću ahezivne veze kompozitnog materijala za pečaćenje fisura nakon predtretmana cakline QSP, SSP i MSP laserskim pulsnim načinima rada i dodatnim jetkanjem kiselinom. Rezultati pokazuju da je SSP imao najnižu čvrstoću veze (24,79 MPa  $\pm 3,92$ ), zatim slijede QSP + ETCH (27,92 MPa  $\pm 2,24$ ), SSP + ETCH (28,16 MPa  $\pm 3,69$ ) i MSP + ETCH (36,09 MPa  $\pm 4,42$ ).

Usporedbe između skupina otkrile su značajne razlike. SSP je imao manje vrijednosti čvrstoće veze od QSP i MSP načina ( $p = 0,001$ ), pri čemu je MSP pokazao najveću ukupnu čvrstoću ( $p < 0,001$ ). No nije primijećena značajna razlika između QSP i MSP načina rada ( $p = 1,000$ ). To potvrđuje da SSP pokazuje manje vrijednosti čvrstoće veze, a QSP i MSP načini rada pokazuju veće vrijednosti čvrstoće veze. Time je odbačena prva hipoteza.

Dodatno je ispitana utjecaj jetkanja kiselinom u kombinaciji s laserskim načinima rada. Iako je SSP + ETCH pokazao veće vrijednosti čvrstoće veze nego sam SSP, razlika nije bila statistički značajna ( $p = 0,079$ ). Kombinacija MSP + ETCH rezultirala je najvećim vrijednostima čvrstoće veze (36,09 MPa  $\pm 4,42$ ) pokazujući da jetkanje MSP-a poboljšava vezivanje, a QSP + ETCH smanjuje čvrstoću veze u usporedbi sa samim QSP-om.

Na temelju rezultata analize odbačena je nulta hipoteza i potvrđen značajan učinak načina rada laserskoga pulsa na jačinu adhezijske veze, osobito u kombinaciji s jetkanjem. Post-hoc analiza korištenjem Games-Howellove metode pokazala je da MSP + ETCH ima značajno veće vrijednosti čvrstoće veze od ostalih skupina, što bi moglo dati prednost MSP načinu predtretmana.

Stereomikroskopskom analizom utvrđeno je da su skupine MSP i SSP pokazale 100-postotnu uspješnost u ishodima pečaćenja, a skupina QSP imala je višu stopu neuspjeha, što dodatno podupire MSP kao bolju opciju predtretmana. Ova se studija uskladjuje i proširuje na rezultate ranijih istraživanja o čvrstoći adhezivnog vezivanja korištenjem tehnika laserskoga predtretmana. Autori dosadašnjih studija izvjestili su o različitim rezultatima, ovisno o vrsti lasera i korištenim parametrima. Na primjer, AlHumaid i suradnici (20) dobili su slične vrijednosti čvrstoće veze pri primjeni predtretmana Er, Cr:YSGG laserom u usporedbi s tradicionalnim jetkanjem kiselinom. Suprotno tomu, Drummond i suradnici (21) te Shahabi i suradnici (22) primjetili su da jetkanje općenito osigurava bolju čvrstoću veze u usporedbi s laserskim tretmanom. Međutim, Wanderley i suradnici (23) pokazali su da Er:YAG laser mogu postići snagu veze koja se može usporediti s onom pri jetkanju ili može biti čak i veća.

U ovoj studiji je MSP + ETCH pružio najveće vrijednosti čvrstoće veze, podupirući ideju da kombinacija laserskoga jetkanja (osobito MSP način) s jetkanjem kiselinom poboljšava čvrstoću veze. Ti rezultati u skladu su s istraživanjem Borsatta i suradnika (24, 25) koji su pokazali da kombiniranje obiju metoda jetkanja može povećati vlačnu čvrstoću i smanjiti mikropropuštanje, za razliku od AlHumaidovih rezultata koji su pokazali da su potrebne veće postavke sna-

bond strengths, our study found that the MSP mode provided superior bond strength even without a particularly high laser power setting. This suggests that the laser pulse mode (e.g., MSP) may play a critical role in achieving high bond strength, rather than just the laser power output.

While some studies indicate that lasers can negatively affect bond strength owing to enamel surface irregularities, our findings contrast with this by showing that the MSP, in particular, significantly enhances bond strength. Additionally, the stereomicroscope analysis used in this study indicated high success rates for the MSP and SSP groups in sealing, further reinforcing MSP's reliability as a pre-treatment method, which contrasts with findings in studies using lasers such as Er, Cr:YSGG, where surface damage reduced bond strength (26, 27).

Although this study offers valuable insights, several limitations must be acknowledged. First, the relatively small sample size may have reduced the statistical power and generalizability of our results. Future research with a larger sample size would strengthen the reliability of these findings. Second, this study focused exclusively on the  $\mu$ TBS without evaluating other important factors, such as retention rates or clinical performance. Including these additional parameters in future studies would provide a more complete picture of the effectiveness of laser etching as a pre-treatment for fissure sealants. Third, the study did not directly compare laser etching with conventional acid etching; instead, it compared different laser modes and the combination of laser and acid etching, which means that the conventional method was not used as a reference.

Thus, this study adds to the existing body of literature by demonstrating that the MSP mode combined with acid etching provides the highest bond strength, reinforcing the findings of previous studies while offering a new perspective on the effectiveness of specific laser modes in improving composite bond strength.

## Conclusion

These findings contribute to the growing body of literature on pre-treatment methods for fissure sealants and emphasize the potential benefits of alternative approaches, such as the QSP and MSP laser enamel pre-treatment. Future studies with larger sample sizes and comprehensive evaluations are warranted to elucidate the role of laser etching in enhancing the effectiveness of fissure sealant placement.

**Conflict of Interest:** The authors declare no conflict of interest.

**Author Contributions:** D.D., H.J. - contributed to the design and implementation of the research; D.D. - was responsible for the analysis of the results and the writing of the manuscript; H.J. - conceived the original idea and supervised the project.

ge lasera (3,5 W) kako bi se uskladile s jetkanjem kiselinom. U našem smo istraživanju otkrili da je MSP način rada pokazao superiornu čvrstoću veze čak i bez posebno visoke postavke snage lasera. To ističe da način rada laserskoga pulsa (npr., MSP) može biti veoma važan u postizanju visoke čvrstoće veze, a ne samo izlazna snaga lasera.

Dok autori nekih studija ističu da laseri mogu negativno utjecati na čvrstoću veze zbog nepravilnosti na površini cakline, naša otkrića u suprotnosti su s time i pokazuju da MSP značajno povećava čvrstoću veze. Uz to, prema rezultatima stereomikroskopske analize u ovoj studiji postignuta je visoka uspješnost MSP i SSP skupine kod pečaćenja, dodatno pojačavajući pouzdanost MSP-a kao metode predtretmana što je u suprotnosti s rezultatima u studijama u kojima su se koristili laseri kao što su Er, Cr:YSGG, gdje oštećenja površine smanjuju čvrstoću veze (26, 27).

Moramo reći da ova studija ima nekoliko ograničenja. Prvo: razmjerno mala veličina uzorka mogla je smanjiti statističku snagu i mogućnost simplifikacije naših rezultata. Buduća istraživanja s većim uzorkom povećala bi pouzdanost tih rezultata. Drugo: autori ove studije usredotočili su se isključivo na  $\mu$ TBS bez procjene drugih važnih čimbenika, kao što su retencija ili klinička učinkovitost materijala. Uključivanje tih dodatnih parametara u buduće studije dalo bi širu sliku učinkovitosti laserskoga jetkanja kao predtretmana za pečaćenje fisura. Treće: u studiji nije izravno uspoređivano lasersko jetkanje s konvencionalnim jetkanjem kiselinom. Umjesto toga uspoređivani su različiti načini rada laserom i kombinacija lasera i jetkanja kiselinom, što znači da konvencionalna metoda nije korištena kao referencija.

Dakle, ova studija pridružuje se postojećoj literaturi i pokazuje da MSP način rada u kombinaciji s jetkanjem kiselinom osigurava najveću čvrstoću adhezijske veze i potvrđuje rezultate dosadašnjih istraživanja te nudi novu perspektivu o učinkovitosti specifičnih načina rada laserom u poboljšanju čvrstoće adhezijskoga vezivanja kompozitnoga materijala za pečaćenje.

## Zaključak

Ovi rezultati pridonose sve većem broju tekstova o metodama predtretmana pri pečaćenju fisura i ističu potencijalne prednosti alternativnih pristupa, kao što su QSP i MSP predtretmani cakline laserom. Potrebna su daljnja istraživanja s većim brojem uzoraka i sveobuhvatnim procjenama kako bi se razjasnila uloga laserskoga jetkanja u povećanju učinkovitosti postavljanja sredstva za pečaćenje fisura.

**Sukob interesa:** Autori nisu bili u sukobu interesa.

**Doprinos autora:** D. D., H. J. – planiranje i provedba istraživanja; D. D. – analiza rezultata i pisanje teksta; H. J. – izvorna ideja i nadzor projekta.

**Sažetak**

**Cilj:** Cilj ove studije *in vitro* bio je procijeniti čvrstoču adhezivnog vezivanja kompozitne smole za pečaćenje fisura na već pripremljenu caklinu s različitim načinima rada laserskoga pulsa i dodatnim jetkanjem kiselinom. **Materijali i metode:** Četrdeset i dva zdrava kutnjaka i pretkutnjaka prikljujena su za ovo istraživanje i randomizacijom podijeljena u 6 sljedećih skupina ( $n = 7$ ): Skupina: Kvantni kvalitativni puls (QSP), Skupina 2: Srednje kratki puls (MSP), Skupina 3: Super kratki puls (SSP), Skupina 4: QSP + jetkanje kiselinom, Skupina 5: MSP + jetkanje kiselinom, Skupina 6: SSP + jetkanje kiselinom. Okluzalne plohe zuba predtretirane su prema definiranoj skupini. Laserska priprema cakline obavljena je Er:YAG laserom Fotona LightWalker AT-S (Fotona, Ljubljana, Slovenija) s valnom duljinom od 2940 nm i jetkanjem kiselinom (ETCH) (Ivoclar Vivadent AG, Schaan, Lihtenštajn). Okluzalne površine započaćene su kompozitnom smolom za pečaćenje fisura (Helioseal F, Ivoclar Vivadent AG, Schaan, Lihtenštajn). Provedeno je ispitivanje mikrovlačne čvrstoče veze ( $\mu$ TBS) i stereomikroskop-ska procjena metode neuspjeha.  $\mu$ TBS ispitivan je s pomoću Games-Howellove metode. Metoda neuспjeha između skupina testirana je koristeći se chi-square testom. Razina značajnosti za sve teste postavljena je na  $p < 0,05$ . **Rezultati:** Najveće vrijednosti čvrstoče adhezivne veze izmjerene su pri upotrebi laserskoga jetkanja MSP načinom rada u kombinaciji s jetkanjem kiselinom (36,09 MPa). Ta je kombinacija pokazala značajno veću čvrstoču veze od ostalih (SSP + ETCH,  $p < 0,001$ ; QSP + ETCH,  $p < 0,001$ ). **Zaključak:** MSP način rada lasera praćen jetkanjem cakline kiselinom pokazao je najveće vrijednosti čvrstoče adhezivne veze. MSP s pulsnim modom od 140  $\mu$ s mogao bi imati prednost pri izboru postupka za predtretman cakline kad je riječ o pečaćenju fisura.

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**Autorske ključne riječi:** predtretman cakline, čvrstoča veze, kompoziti za pečaćenje fisura, Er:YAG laser

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