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Mikrozatezna vezna čvrstoća smole za pečaćenje i predtretiranje različitim antibakterijskim sredstvima

Micro-Tensile Bond Strength of Fissure Sealants Treated with Different Antibacterial Agents

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Sažetak

Svrha - Ovim istraživanjem *in vitro* željela se na pojednostavljenom modelu caklinske fisure procijeniti mikrozatezna snaga smole za pečaćenje tretirana antibakterijskim samojetkajućim adhezivnim sustavom, antibakterijskim predtretmanom prije nanošenja samojetkajućeg adhezivnog sustava i samojetkajućim sustavom. **Materijali i metode:** Tek izvađeni kravlji mandibularni sjekutići nasumce su podijeljeni u četiri skupine: A. 35-postotna fosforna kiselina + Clinpro sealant®; B. 35-postotna fosforna kiselina + Clearfil protect bond + Clinpro sealant®; C. 35-postotna fosforna kiselina + Consepsis Scrub® + Clearfil SE bond® + Clinpro sealant®; D. 35-postotna fosforna kiselina + Clearfil SE bond® + Clinpro sealant®. Dva komadića cakline učvršćena su voskom na sterilna stakalca s razmakom od 0,6 ± 0,1 milimetar. Zatim su okomito razrezani štapići (ff1mm x 1mm) podvrgnuti testu čvrstoće u univerzalnom stroju s konstantnom poprečnom brzinom glave (1mm/min). Nakon toga je popucalim površinama određena vrsta frakture. Za statističku analizu korištena je jednosmjerna analiza varijance te Tukeyev i hi-kvadrat test. **Rezultati:** Mikrozatezna snaga veze u skupini C (34,63 ± 15,59 MPa) bila je mnogo veća nego u skupini A (19,86 ± 7,08 MPa) (p: 0,0001), B (24,49 ± 9,38 MPa) (*p*: 0,002) i D (19,84 ± 9,92 MPa) (*p*: 0,0001). Frakturne pukotine uglavnom su se nalazile u adhezivnom sloju, a među skupinama nije bilo statistički značajne razlike u obliku fraktura (*p*: 0,343). **Zaključak:** Mikrozatezna snaga veze samojetkajućeg adhezivnog sustava bila je na površinama tretiranima klorheksidinskim kavitetnim dezinficijensom značajno veća nego ona kod primijenjenog antibakterijskog samojetkajućeg sustava zasebno, samojetkajućeg adhezivnog sustava ili konvencionalnog jetkajućeg sustava kiselinom.

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Uvod

U jamicama i na fisurama (PiF) koje se ne mogu lako čistiti nakupljaju se organski ostatci te su pogodna mjesta za razvoj karijesa. Nakon njihova čišćenja primjena PiF-smola stvara fizičku zapreku između površine zuba i oralnog okoliša i tako smanjuje rizik od nastanka karijesa (1,2). PiF se obično postavlja nakon čišćenja i jetkanja fisuralne cakline fosfornom kiselinom kako bi se postigla trajna veza. Danas je prihvaćeno da se najveća snaga vezivanja na humanu caklinu postiže jetkanjem fosfornom kiselinom i adhezivnim sustavom ispod pečatnih smola (3,4). Prema podatcima iz literature duboke, uske i ampularne fisure mogu ograničiti prodiranje kiselina ili smola (5,6). Ta anatomska ograničenja mogu pratiti i mikroorganizmi duž napuknuća (7,8). Važno je postići stabilnu vezu PiF-a na intaktnu aprizmatsku caklinu te stvoriti fizičku i antibakterijsku barijeru od pečatnog materijala koji će izolirati okluzalne površine i zaštiti ih od oralnih opasnosti i spriječiti karijes (9,10).

Introduction

Pits and fissures (P&F), which cannot be cleansed easily, accumulate organic debris and provide suitable sites for the development of dental caries. Following the cleaning of pits and fissures, the application of a P&F sealant would provide a physical barrier between tooth surface and oral environment, therefore reducing the risk of dental caries (1,2). P&F sealants are usually placed after cleansing and phosphoric acid etching of the fissure enamel to achieve a sustainable bond. Today it is widely accepted that the highest bond strengths to human enamel were attained using phosphoric acid etching and adhesives underneath the sealants (3,4). According to literature, deep, narrow and bottleneck fissures may limit the penetration of acid etchants or sealants (5,6). These anatomical restrictions may be accompanied with presence of microorgansims along the fissure walls (7,8). At this point, it is also important to apply a stable bonding to the intact aprismatic enamel of P&F where there is utilization of a physical and

Ispod PiF-ova pečata mogu biti korisni kavitetni dezinficijensi s antibakterijskim svojstvima. Danas zubni adhezivi postoje u dvama oblicima – tradicionalni *adhezivi za jetkanje i ispiranje* (3 ili 2 stupnja) i suvremeni s hidrofilnim funkcijskim monomerom, sustavom nazvanim *samojetkajući adhezivi* [2 ili 1 stupanj (jedna bočica)] (11,12). Prvi sustav sadržava fosfornu kiselinu za jetkanje i primer/adheziv u bočici, a drugi sustavi kombiniraju jetkanje i adheziv u jednoj bočici, a adhezivnu smolu u drugoj, ili u postupku postavljanja sadržavaju sve – jetkanje, primanje i adheziv. Antibakterijski učinak vrlo je važan jer inaktivacija bakterija znači neposrednu strategiju za uklanjanje uzroka karijesa. Antibakterijsko djelovanje dentinskih adhezivnih sustava zaustavlja stvaranje zubnog karijesa, posebice na caklinskim rubovima (13,14).

Nedavno je razvijen novi monomer – *12-methacryloyloxydodecyl pyridinium bromid* (MDPB) s antibakterijskim djelovanjem na oralne streptokoke koji omogućuje adhezivnu vezu s antibakterijskim svojstvom. Ovaj monomer je kvartarni amonijev spoj s metakrilatnom skupinom (15) i kao samojetkajući adhezivni sustav ima zadovoljavajuća fizikalna svojstva za uporabu ispod PiF-smola za pečaćenje na nezakošenoj/ravnoj caklini, uz zadržavanje antibakterijskih karakteristika (16). Posljednjih dvadeset godina za antibakterijska djelovanja korišteni su različiti spojevi s etanolom, etilnim acetatom, acetonom, ili klorheksidinovim diglukonatom (17). Nedavno je ustanovljeno da je aprizmatska caklina fisura manje pogodna za mikromehaničko vezanje s pomoću kiselog jetkanja (5,18). Kako bi procijenili adhezivno vezivanje, Sano i suradnici (19) obavili su test mikronapetosti veze (μTBS). To je precizna metoda za mjerenje adhezivne veze između zubne strukture i kompozitne smole. Do tada su se PiF-smole za pečaćenje procjenjivale testovima čvrstoće (18,20-23). U jednom istraživanju procjenjivala se mikrosnaga vezivanja tih smola unutar caklinske fisure te su autori primijenili model pojednostavljene caklinske fisure (5). Naš model sastoji se od tankog sloja smole istodobno vezane za dvije, blizu postavljene, intaktne aprizmatske kravlje caklinske površine. Svrha ovog istraživanja bila je procijeniti mikrozateznu čvrstoću vezivanja smola za pečaćenje tretiranih samojetkajućim antibakterijskim sredstvom, antibakterijskim sredstvom prije postavljanja samojetkajućeg adhezivnog sustava i zasebno samojetkajućeg adhezivnog sustava, a koristili smo se pojednostavljenim modelom caklinske fisure.

Materijali i metode

U ovom istraživanju bili su korišteni uzorci 40 ekstrahiranih kravljih mandibularnih inciziva. Odmah su uronjeni u fiziološku otopinu temperature 4 °C, gdje su ostali najdulje četiri tjedna. Zubi su pregledani bez pomagala, samo okom, te su isključene površinske pukotine, dekalcifikacije i znakovi trošenja. Nisu obavljeni nikakvi pripremni postupci. Nakon

an antibacterial barrier in the form of a P&F sealant, which isolates the occlusal surfaces from the oral threats in order to prevent the dental caries (9-10).

An adhesive bond or a cavity disinfectant possessing antibacterial properties may be useful underneath P&F sealants. Today dental adhesives constitute both traditional 'etch-and-rinse adhesives' (3-step or 2-step) and contemporary systems containing hydrophilic functional monomers called 'self-etch adhesives' (2-step or 1-step (all-in-one)) (11-12); the first system uses phosphoric acid etching and primer/adhesive resin in one bottle, and the latter combines etching and priming in one bottle and an adhesive resin in another, or uses etch-prime adhesive all in one procedure. The antibacterial effect is an important property because inactivation of bacteria means a direct strategy to eradicate the cause of dental caries. The antibacterial effects of dentin bonding systems indicate the inhibition of dental caries formation, especially along the enamel margins (13,14). Recently a new monomer, 12-methacryloyloxydodecyl pyridinium bromide (MDPB) with antibacterial activity against oral *Streptococci*, has been developed to provide adhesive systems with antibacterial activity. This monomer is a quaternary ammonium compound with a methacryloyl group (15) where its usage with a self-etching adhesive has sufficient physical qualities to be used under P&F sealant and composite resin on uncut enamel, its antibacterial efficiency notwithstanding (16). In addition to antibacterial adhesive systems, different cleaning agents containing ethanol, ethyl acetate, acetone or chlorhexidine digluconate have been used for the last two decades (17).

Recently the surface aprismatic enamel layer covering the fissure walls has been reported to be less conductive to micromechanical bonding via acid conditioning (5, 18). To evaluate strenghts of adhesive bondings, Sano et al. (19) had introduced the microtensile bond test (μTBS) which is an accurate method to measure the adhesive strenght between tooth structure and composite resin. So far P&F sealants have been evaluated regarding their bond strenght to enamel using tensile tests (18, 20-23). However, only one study has evaluated the microtensile strenght of a P&F sealant within an enamel fissure where authors introduced the model as the simplified enamel fissure model (5). The present model consists of a thin layer of P&F sealant simultaneouly to two layers of closed approximated, intact, aprismatic bovine enamel.

Thus, the objective of this *in vitro* study was to evaluate the micro-tensile enamel bond strength of fissure sealants treated by an antibacterial self-etching agent, antibacterial pretreatment prior to application of a self-etching adhesive system and self-etching adhesive system alone using the simplified ename l fissure model.

Materials and Methods

Samples from 40 freshly extracted bovine mandibular incisors were used in the present study. They were stored in saline at 4 °C for no longer than 4 weeks. Teeth were examined with the naked eye, and determined to be free of surface cracks, decalcification or any sign of previous grinding. No other preparation was performed. After dissecting the roots

što su odrezani korijeni, središnje trećine kruna nasumce su uvrštene u jednu od četiriju skupina. Svaki uzorak zuba učvršćen je ljepljivim voskom na akrilatni cilindar postavljen na niskobrzinsku dijamantnu pilu (Isomet*, Buehler, Lake Bluff, Il, SAD). Njome su odrezana, uz vodeno hlađenje, četiri komadića (4 x 6 mm) bukalne cakline. Zatim su po dva komadića pričvršćena ljepljivim voskom na sterilna predmetna stakalca sa srednjom udaljenošću od 0,6 ± 0,1 mm mjerenom mikrometrom (Mitutoyo*, Hampshire, Velika Britanija). U prostor između uzoraka cakline stavljeno je sredstvo za jetkanje i kondicioniranje, te je zapečaćeno prema uputama proizvođača (slika 1.), ovisno o skupini u koju je uvršteno (tablica 1.). Skupine su bile sljedeće:

A. 35-postotna fosforna kiselina (Scotchbond Etchant*, 3M, ESPE, St Paul, MN, SAD) + Clinpro sealant* (3M, ESPE, St Paul, MN, SAD)
B. 35-postotna fosforna kiselina (Scotchbond Etchant*) + Clearfil protect bond* (Kuraray America Inc, New York, NY, SAD) + Clinpro sealant*
C. 35-postotna fosforna kiselina (Scotchbond Etchant*) + Consepsis Scrub* (Ultradent, South Jordan, Utah, SAD) + Clearfil SE bond* + Clinpro sealant*
D. 35-postotna fosforna kiselina (Scotchbond Etchant*) + Clearfil SE bond* (Kuraray America Inc, New York, NY, SAD) + Clinpro sealant*

at the middle third, crowns were randomly assigned to one of the four groups. Each tooth was secured with sticky wax on an accrylic cylinder which was mounted on a slow-speed diamond saw (Isomet*, Buehler, Lake Bluff, Il, USA). Each tooth was sectioned under water cooling into four 4 x 6 mm pieces of buccal enamel. Two pieces of enamel were secured with sticky wax on a sterile glass slide to a mean distance of 0.6 ± 0.1 mm measured with a micrometer (Mitutoyo*, Hampshire, England). The space between enamel samples were then etched and conditioned and sealed according to manufacturer's instructions (Figure 1) regarding the test group (Table 1). The groups were:

A. 35% phosphoric acid (Scotchbond Etchant*, 3M, ESPE, St Paul, MN, USA) + Clinpro sealant* (3M, ESPE, St Paul, MN, USA)
B. 35% phosphoric acid (Scotchbond Etchant*) + Clearfil protect bond* (Kuraray America Inc, New York, NY, USA) + Clinpro sealant*
C. 35% phosphoric acid (Scotchbond Etchant*) + Consepsis Scrub* (Ultradent, South Jordan, Utah, USA) + Clearfil SE bond* + Clinpro sealant*
D. 35% phosphoric acid (Scotchbond Etchant*) + Clearfil SE bond* (Kuraray America Inc, New York, NY, USA) + Clinpro sealant*

Slika 1. Priprema prostora između uzoraka cakline
Figure 1 Conditioning of the space between enamel samples

Višak materijala uklonjen je špatulom, a pečatni materijal polimeriziran svjetiljkom (Optilux 501®, Kerr Corporation, West Collins Orange, CA, SAD), uz snagu svjetla prema uputama proizvođača, no ne manju od 550 mW/cm^2 za sve proučavane adhezivne sustave.

Svaki uzorak bio je pohranjen 24 sata u vodi temperature 37 °C. Dobiveni blokovi vertikalno su rezani na kvadratičaste štapiće (≈ 1mm x 1mm) na mikrotomu (Isomet*, Buehler, Lake Bluff, Il, SAD). Štapići koji su sadržavali pečatnu smolu u sredini i oko dvaju slojeva cakline i dentina zalijepljeni su cijanoakrilatnim adhezivnim gelom (Pattex*, Henkel, İstanbul, Turska) na testnu platformu i podvrgnuti sve do pucanja testu mikrozatezne čvrstoće (μTBS) uz konstantnu brzinu okretanja glave (1mm/min). To je učinjeno na univerzalnom stroju za testiranje (Instron 3345*, Norwood, Mass,

Any excess material was removed with a spatula and the sandwiched sealants were polymerized using a light-curing unit (Optilux 501®, Kerr Corporation, West Collins Orange, CA, USA) with a light output not less than 550 mW/cm^2 for all tested adhesives according to the instructions of the manufacturers. Each specimen was stored in water at 37°C for 24 h. The build-ups were vertically sectioned into quadrangular (≈ 1mm x 1mm) compound bars with a microtome (Isomet*, Buehler, Lake Bluff, Il, USA). The bars, consisting of sealant layer in the middle and two layers of unground enamel and underlying dentin on the sides, were glued with a cyanoacrylate adhesive gel (Pattex*, Henkel, İstanbul, Turkey) to the probe and submitted to a micro-tensile bond strength tensile tests at constant crosshead speed (1mm/min) using a universal testing machine (Instron 3345*, Norwood, Mass,

Tablica 1. Korišteni materijali (Bis-GMA: Bis-phenol A diglycidylmethacrylate, CQ: Camphorquinone, HEMA: 2-hydroxy ethyl methacrylate, BHT: butylhydroxytoluene, EDMAB: Ethyl P-Dimethylamino Benzoate, MDP: 10- Methacryloyloxydecyl dihydrogen phosphate, MDPB: 1 2-Methacryloyloxydodecylpyridinium bromide, TBATFB: tetrabutyl dimethacrylate, UDMA: urethane dimethacrylate)
Table 1 Materials used (Bis-GMA: Bis-phenol A diglycidylmethacrylate, CQ: Camphorquinone, HEMA: 2-hydroxy ethyl methacrylate, BHT: butylhydroxytoluene, EDMAB: Ethyl P-Dimethylamino Benzoate, MDP: 10- Methacryloyloxydecyl dihydrogen phosphate, MDPB: 12-Me thacryloyloxydodecylpyridinium bromide, TBATFB: tetrabutyl dimethacrylate, UDMA: urethane dimethacrylate)

Materijal • Materials	Sastav •Components	Br. serije • Batch no	Proizvođač • Manufacturer	Primjena •Application
Scotchbond Etchant*	35% H_3PO_4		3M, ESPE, St Paul, MN, SAD • USA	Nanijeti i ostaviti 30 s, isprati i posušiti do kredaste boje cakline • Apply for 30 s, dry until enamel is chalky
Clinpro sealant*	Bis-GMA, TEG-DMA, EDMAB, CQ, BHT, silikat, TBATFB, titanijev dioksid, rose Bengal soda • Bis-GMA, TEG-DMA, EDMAB, CQ, BHT, silica, TBATFB, titanium dioxide, rose Bengal sodium	20090501	3M, ESPE, St Paul, MN, SAD • USA	Nanijeti i polimerizirati 20 s • Apply and polymerize for 20 s
Clearfil protect bond*	Adheziv (samojetkajući adheziv) • PRIMER (self-etching primer): MDP, MDPB, HEMA, hidrofilni dimetakrilat • Hydrophilic dimethacrylate, voda • Water BOND (fluoride-bonding agent): MDP, Bis-GMA, HEMA, hidrofobni dimetakrilat • Hydrophobic dimethacrylate, dl-CQ, N, N-Diethanol-p-toluidine, silanizirani koloidalni silikat • silanated colloidal silica, površina obrađena natrijevim fluoridom • surface treated sodium fluoride	41190	Kuraray America Inc, New York, NY, SAD • USA	Nanijeti adheziv i ostaviti 20 s, lagano posušiti, nanijeti BOND, lagano posušiti i polimerizirati 10 s • Apply PRIMER for 20 s, gently dry apply BOND, gently dry and for polymerize for 10 s
Clearfil SE bond*	Adheziv • PRIMER: MDP, HEMA, dl-CQ, N, N-Dietanol-p-toluidin, hidrofilni dimetakrilat, voda • MDP, HEMA, dl-CQ, N, N-Diethanol-p-toluidine, Hydrophilic dimethacrylate, Water BOND: MDP, Bis-GMA, HEMA, hidrofobni dimetakrilat, dl-CQ, N, N-Dietanol-p-toluidin, silanizirani koloidalni silikatni prah • MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, dl-CQ, N, N-Diethanol-p-toluidine, silanated colloidal silica	041742	Kuraray America Inc, New York, NY, SAD • USA	Nanijeti PRIMER i ostaviti 20 s, lagano posušiti nanijeti BOND, lagano posušiti i polimerizirati 10 s • Apply PRIMER for 20 s, gently dry apply BOND, gently dry and polymerize for 10 s
Consepsis Scrub*	2-postotni klorheksidinov glukonat • 2% chlorhexidin gluconate etilni alkohol • Ethyl alcohol	S732-CE B3F7N	Ultradent, South Jordan, Utah, SAD • USA	Učetkati, čekati 60 s, sušiti 15 s • Brush, wait 60 s, dry 15 s

SAD). Za skupinu A testirano je 26 štapića, za skupinu B njih 27, za skupinu C odabrano ih je 30 i 21 uzorak za skupinu D. Nakon testa mikrozatezne čvrstoće (μTBS-testiranja) površine su se proučavale pod velikim povećanjem (25x) (Opmi* Pico Carl Zeiss Meditec Inc, Dublin, CA, SAD) kako bi se odredio frakturni tip. Frakture su klasificirane kao adhezivne, miješane, kohezivne unutar smole ili kohezivne unutar cakline. Statističkom analizom procjenjivala se srednja vrijednost dentalnih adheziva jednosmjerne analize varijance, te rezultati Tukeyeva i hi-kvadrat testa u sklopu programskog paketa NCSS 2007* (Kaysville, UT, SAD). Za razinu statističke značajnosti p-vrijednost je postavljena na <0,5.

Rezultati

Mikrozatezna čvrstoća caklinske veze antibakterijskog samojetkajućeg adhezivnog sustava, kavitetnog dezinficijensa nanesenog prije samojetkajućeg sustava i zasebnog samojetkajućeg sustava na intaktnu caklinu ispod smole za pečaćenje kao funkcija broja nanošenja, prikazani su u tablici 2. Re-

USA) until fracture. For group A, B, C and D; 26, 27, 30 and 21 specimens were tested respectively. Specimen samples were not equally distributed as there were natural losses. After the μTBS tests, fractured surfaces were inspected at magnification (25x) (Opmi* Pico Carl Zeiss Meditec Inc, Dublin, CA, USA) to determine the mode of fracture. Fractures were classified as adhesive, mixed, cohesive in resin or cohesive in enamel. Regarding statistical analysis, the mean dentin bond strengths were evaluated by One-way variance, Tukey and Chi-square tests using NCSS 2007* (Kaysville, UT, USA) package programme. A *p*-value <0.05 was considered as statistically significant.

Results

The mean micro-tensile enamel bond strength of an antibacterial self-etching adhesive system, a cavity disinfectant applied antibacterial self-etching adhesive system and a self-etching adhesive system on intact enamel under a resin-based fissure sealant as a function of the number of applications

zultati jednosmjerne analize varijance, mikrozatezne čvrstoće veze kavitetnog dezinficijensa i nanesenog antibakterijskog samojetkajućeg adhezivnog sustava (skupina C) bili su statistički značajno različiti od skupina A, B, i C (*p*: 0,0001). Tipovi fraktura za svaku skupinu nalaze se u tablici 3. Tijekom testiranja μTBS-a tipovi fraktura bili su uglavnom adhezivni, pri čemu je skupina B imala najmanji adhezivni obrazac (59 % adheziveni, 22,2 % kohezivni u smoli, 7,4 % kohezivni u caklini i 11,1 % miješani). Statistička analiza hi-kvadrat testa pokazala je da između skupina nema značajne razlike u tipu frakture (*p*: 0,343).

are shown in Table 2. The results for the One-way variance test, the micro-tensile bond strengths of a cavity disinfectant applied antibacterial self-etching adhesive system (Group C) were significantly different Groups A, B and C (*p*:0.0001). The modes of failure for each group are shown in Table 3. With the μTBS tests, failures were predominantly adhesive in nature, where group B showed the least adhesive failure pattern (59% adhesive, 22.2% cohesive in resin, 7.4% cohesive in enamel and 11.1% mixed). Statistical analysis of chi-square test showed that there were no statistically significant differences in fracture pattern between groups (*p*:0.343).

Tablica 2. Mikrozatezna snaga vezanja (μTBS) testiranih uzoraka na caklinu
Table 2 The micro-tensile bond strength (μTBS) to enamel of the tested samples.

Skupina • Groups	Ar. sredina μTBS (Mpa) ± SD • mean μTBS (Mpa) ± SD
A. 35-postotna fosforna kiselina + Clinpro sealant® (n: 26) • 35% phosphoric acid + Clinpro sealant® (n: 26)	19.86 ± 7.08 *
B. 35-postotna fosforna kiselina + Clearfil protect bond® + Clinpro sealan® (n: 27) • 35% phosphoric acid + Clearfil protect bond® + Clinpro sealant® (n: 27)	24.49 ± 9.38 **
C. 35-postotna fosforna kiselina + Consepsis Scrub® + Clearfil SE bond® + Clinpro sealant® (n: 30) • 35% phosphoric acid + Consepsis Scrub® + Clearfil SE bond® + Clinpro sealant® (n: 30)	34.63 ± 15.59 Φ,*,**
D. 35-postotna fosforna kiselina + Clearfil SE bond® + Clinpro sealant® (n:21) • 35% phosphoric acid + Clearfil SE bond® + Clinpro sealant® (n:21)	19.84 ± 9.92 *

Jednosmjerni test varijance • One-way varians test, Φ *p*:0.0001
Tukeyev test • Tukey test, * *p*:0.0001,* *p*:0.002 **

Tablica 3. Broj i postotak uzoraka kategoriziran u frakturne mostove
Table 3 The number and percentage of specimens categorized into fracture modes.

Skupina • Groups	Adheziv • Adhesive n (%)	Miješano • Mixed n (%)	Kohezivno • Cohesive	
			U smoli • in resin n (%)	U caklini • in enamel n (%)
A	23 (88.5%)	1 (3.8%)	2 (7.7%)	0 (0%)
B	16 (59.3%)	3 (11.1%)	6 (22.2%)	2 (7.4%)
C	24 (80.0%)	3 (10.0%)	2 (6.7%)	1 (3.3%)
D	18 (85.7%)	1 (4.8%)	2 (9.5%)	0 (0.0%)

Hi-kvadrat test • Chi-square test, *p*:0.343 NS

Rasprava

PiF-smole za pečaćenje uspješno se primjenjuju u prevenciji karijesa. Ta učinkovitost je u neposrednoj vezi s retencijom tih smola, a retencija ovisi o načinu primjene. Predloženo je da se kod dubokih fisura poboljša retencija pečatnih smola s pomoću dentinskih adhezivnih sustava, osobito ako napuknuća nisu potpuno suha prije postavljanja pečatne smole (24). To osobito vrijedi kod mladih ljudi kad je riječ o kutnjacima koji nisu potpuno niknuli, a pokušava ih se pečatiti. U tim slučajevima okluzalna površina može se kontaminirati vlagom i slinom te ugroziti učinkovitost smola (25). U takvim je slučajevima uporaba adhezivnih sustava pokazala slabije mikrocurenje (26, 27). No ako nema takve kontaminacije, prednost adhezivnog sustava je nejasna. Dok se u nekim istraživanjima ističe pozitivni učinak nakon primjene tih sustava (3,24), u drugima se govori o suprotnom učinku (28,29). U ovom istraživanju svi su uzorci najprije tretirani 35-postotnom fosfornom kiselinom kako bi se dobili uvjeti što sličniji kao kod navedene djece. Nedavna istraživanja

Discussion

P&F sealants have been used successfully to prevent dental caries. This effectiveness is directly related to P&F sealant retention while retention is dependent upon method of application. It is proposed that the dentine adhesive systems may improve the retention rate of sealants in deep fissures particularly if the fissure is not completely dry prior to resin placement (24). Especially in young children, it is attempted to seal the molar teeth which are not fully erupted. At this moment, the occlusal surface may get contaminated by moisture and saliva, compromising the effectiveness of the sealant (25). In such a situation, the use of an adhesive system was shown to result in less microleakage (26-27). However, in the absence of such a contamination, the advantage of applying an adhesive system is unclear. Although in some studies its use has been reported to be beneficial (3, 24), other studies reported the opposite effect (28,29). In the present study, all samples were pretreated with 35% phosphoric acid to mimic natural conditions in children mentioned above.

snage vezivanja potvrdila su na kontaminiranoj slini prednosti, tj. povećanu snagu vezivanja adhezivnog sredstva ispod PiF-smola (30, 31). U ovom istraživanju najetkani caklinski uzorci neposredno zapečaćeni smolom i samojetkajućim smolama nisu pokazali značajne razlike u snazi veze. Naši se rezultati slažu s hipotezom Pinara i njegovih suradnika (32) prema kojoj uspješnost smole za pečaćenje ovisi o optimalnim uvjetima u kojima je postavljena.

Pečaćenje PiF-om, primjenom adherentnih biokompatibilnih polimera na okluzalnoj površini, sprječava ulazak bakterija i hrane. Ova tehnika vrlo je učinkovita ako su smole pravilno postavljene. Drugi pristup uključuje primjenu različitih antimikrobnih tvari, pa se pristup temelji na stajalištu da je sredstvo sposobno inaktivirati svu mikrofloru iz jamica i fisura ili samo dio, što može spriječiti razvoj karijesa (33). Ima i studija u kojima se ističe da samojetkajući sustavi suprotno djeluju na trajnost restauracija (34,35). Nedavno je pronađeno da *Clearfill protect bond* djeluje antibakterijski (15, 36). Razlog za produljeni antibakterijski učinak toga preparata povezan je s antibakterijskim učincima MDPB-molekule (37, 38). Pirimidinska skupina, kao komponenta MDPB-monomera, pozitivno je nabijena. Stanične strukture bakterija također su pozitivno nabijene i automatski se od njega odbijaju. Tada gube električnu ravnotežu, što razara staničnu membranu bakterija. Bakterije se uništavaju procesom bakteriolize koja smanjuje broj zaostalih bakterija u pripremljenom kavitetu. Inače bi uzrokovale rekurentni karijes i oštetile pulpu (15).

Na tržištu se mogu nabaviti različita sredstva za uklanjanje debrisa (ostataka hrane) s površine cakline, poput onih s etanolom, etilnim acetatom, acetonom ili klorheksidinovim diglukonatom. Neki istraživači ističu da je snaga vezivanja na površine veća ako su tretirane klorheksidinovim diglukonatom negoli samojetkajućim adhezivnim sustavima (39, 40). Postoje i istraživanja u kojima se napominje da predtretman klorheksidinom prije jetkanja kiselinom ne djeluje izravno na vezu kompozita i adheziva na caklinu (41,42). U tim je studijama korištena 2-postotna otopina klorheksidinova glukonata pri pH 6 (Consepsis Scrub) prije samojetkajućeg adheziva *Clearfil SE bond*, kako bi se postigla dvostruka dezinfekcija kaviteta i samojetkajućeg adheziva. Uočeno je također da otopina *Consepsis* nije utjecala na brtvljenje *Clearfii SE Bondom* (43).

U ovom je istraživanju *Clearfil SE bond*, samojetkajući adheziv bez antibakterijskih svojstava (15,44), bio kontrola samojetkajućim adhezivnim sustavima s antibakterijskim svojstvima (15, 44). *Clearfil SE bond* kiseli je adheziv (pH=2), a nedavno je istaknuto da se antibakterijski učinak stvara niskim pH (45, 46). No antibakterijski učinak kiselih adheziva (niski pH) nije pouzdan jer ne djeluju na aciduричne bakterije poput laktobacila (46). Totu (47) i suradnici ustanovili su da je pri restauraciji mliječnih zuba kompomerima za dezinfekciju kaviteta, učinkovitu adhezivnu vezu i manje mikropopuštanja, bolje primijeniti antimikrobne adhezivne sustave negoli kavitetne dezinficijense. U nedavnim istraživanjima analizirano je korištenje klorheksidina nakon jetkanja koje je pokazalo inicijalnu snagu vezivanja sličnu kao kod kontrole (48 – 50). U našem je istraživanju na intaktnoj ca-

Recent studies carried out on bond strenght confirmed the benefit of bonding agents under P&F sealants on contaminated enamel to increase bond strength (30-31). In the present study, preetched enamel samples directly sealed with a fissure sealant and self-etched fissure sealants did not have significant differences regarding their bond strenghts. Our results are in accordance with the hypothesis of Pinar et al. (32), where they stated that the clinical success of a fissure sealant is related to whether the sealant is applied under optimal conditions.

Sealing of the P&F's by application of adherent biocompatible polymers to the occlusal surfaces prevents the ingress of bacteria and nutrients. The present technique has been shown to be very effective when the sealants are properly applied. Another approach involves the application of various antimicrobial agents, where the rationale for this approach is based on the concept that agents capable of inactivating all or a part of the pit and fissure microflora might suppress caries development (33).

There are some reports where self-etching adhesive systems played controversial roles in the longevity of the restoration (34-35). Recently Clearfil protect bond was evaluated to have antibacterial activity (15, 36). The reason for the prolonged antibacterial effect of Clearfil protect bond is related to the antibacterial properties of the MDPB molecule (37-38). The pyridinium group as a component of the MDPB monomer is positively charged. The cell structures of bacteria are generally negatively charged and are automatically attracted by the positive charge of the MDPB monomer. They subsequently lose their electrical balance, which destroys the cell membranes of the bacteria. The bacteria are killed by this process known as bacteriolysis, which reduces the presence of residual bacteria in the prepared cavity, which would otherwise induce recurrent caries and damage to the pulp (15).

For removal of debris and remnants from the enamel surface, different cleaning agents containing ethanol, ethyl acetate, acetone or chlorhexidine digluconate have been marketed. Some recent studies have obtained superior results of bond strenghts for chlorhexidine digluconate than those treated with the self-etching adhesive systems (39-40). There are also previous studies demonstrating that chlorhexidine application prior to acid-etching has no adverse effects on immediate composite-adhesive bonds in enamel (41-42). At this point, Consepsis Scrub, a 2.0% chlorhexidine gluconate solution with a pH of 6, was used prior to a self-etching primer, Clearfil SE bond, to produce a double effect of a cavity disinfectant and a self-etch primer together. It was also noted that Consepsis solution did not adversely affect the sealing ability of Clearfii SE Bond (43).

In the present study, Clearfil SE bond, a self-etching primer that does not show any antibacterial activity (15, 44) was used as a control to an antibacterial self-etching adhesive system. Actually Clearfil SE Bond is a low pH (pH: 2) bond. Recently it was reported that antibacterial effects were produced at low pH (45-46). However, the bactericidal activities of self-etching primers elicited at a low pH are not reliable since they are ineffective against acid-tolerant bacteria such as lactobacilli (46).

klini ispod smole za pečaćenje fisura, kod primjene klorheksidina prije samojetkajućeg adhezivnog sustava, postignuta veća čvrstoća vezivanja negoli kod antimikrobnog samojetkajućeg adhezivnog sustava, samojetkajućeg sustava te u slučaju konvencionalnog adhezivnog sustava s jetkanjem. Naši rezultati slažu se s Brännströmovima (51), a on je istaknuo da kavitetni dezinficijensi mogu poboljšati brtvljenje dentinskih adheziva ako se kavitet navlaži prije postavljanja dentinskih adheziva koji se vežu na vlažnu zubnu površinu.

Na kraju – mikrozatezna čvrstoća samojetkajućeg adhezivnog sustava tretirana najprije klorheksidinskim kavitetnim dezinficijensom ima značajno veću vrijednost negoli antimikrobni samojetkajući adhezivni sustavi zasebno, samojetkajući sustavi bez antibakterijskog učinka i konvencionalni sustavi tehnikom jetkanja.

Recently Totu (47) stated that in order to obtain cavity disinfection, effective bond strength and less microleakage, application of an antibacterial bonding system rather than using a cavity disinfectant is more appropriate in restoring primary teeth with compomers. However, recent studies have examined the use of chlorhexidine after acid-etching, demonstrating initial bond strengths comparable with those of the controls (48-50). In the present study, the chlorhexide cavity disinfectant applied prior to the self-etching adhesive system exhibited higher bond strenghts than an antibacterial self-etching adhesive system, a self-etching adhesive system and a conventional etching system on intact enamel under a resin-based fissure sealant. Our finding is in accordance with Brännström (51) who suggested that cavity disinfectants could improve the sealing ability of dentin-bonding agents by remoistening the cavity prior to placing a dentin bonding agent that bonds to moist tooth structure.

In conclusion, the micro-tensile bond strength of a self-etching adhesive system previously treated with chlorhexidine cavity disinfectant was significantly higher than an antibacterial self-etching adhesive system alone, a self-etching adhesive system and a conventional acid etching system.

Zahvale

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Abstract

Objective of this *in vitro* study was to evaluate the micro-tensile enamel bond strength of fissure sealants treated by an antibacterial self-etching agent, antibacterial pretreatment prior to application of a self-etching adhesive system and self-etching adhesive system alone using the simplified enamel fissure model. **Materials and Methods:** Fresh extracted bovine mandibular incisors were randomly divided into four groups: A. 35% phosphoric acid + Clinpro sealant®; B. 35% phosphoric acid + Clearfil protect bond + Clinpro sealant®; C. 35% phosphoric acid + Consepsis Scrub® + Clearfil SE bond® + Clinpro sealant®; D. 35% phosphoric acid + Clearfil SE bond® + Clinpro sealant®. Two pieces (4 x 6 mm) of enamel were secured with wax on a sterile glass slide to a mean distance of 0.6 ± 0.1 mm. Vertically sectioned bars (ff1mmx 1mm) were submitted to tensile tests at constant crosshead speed (1mm/min) using a universal testing machine. Fractured surfaces were inspected to determine the mode of fracture. Regarding statistical analysis, One-way variance, Tukey and Chi-square tests were applied. **Results:** The micro-tensile bond strengths of group C (34.63±15.59 MPa) was significantly higher than group A (19.86±7.08 MPa) (p:0.0001), group B (24.49±9.38 MPa) (*p*:0.002) and group D (19.84±9.92 MPa) (*p*:0.0001). Failures were predominantly adhesive in nature; where there were no statistically significant differences in fracture patterns between groups. (*p*:0,343). **Conclusion:** The micro-tensile bond strength of a self-etching adhesive system previously treated with chlorhexidine cavity disinfectant was significantly higher than an antibacterial self-etching adhesive system alone, a self-etching adhesive system and a conventional acid etching system.

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Key words
Tensile Strength; Pit and Fissure Sealants; Dental Enamel; Anti-Bacterial Agents; Adhesives

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