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Utjecaj antioksidacijskih sredstava na rubno brtvljenje ispuna V. razreda

Influence of Antioxidant Agents on the Marginal Seal of Class V Restorations

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Sažetak

Svrha: Ovim se istraživanjem željelo ocijeniti *in vitro* učinak primjene antioksidacijskog sredstva – 10-postotnog natrijeva askorbata na deproteinizirani dentin stijenke kaviteta kompozitnih ispuna te na rubno brtvljenje. **Materijali i postupak:** Odabrano je 30 humanih ekstrahiranih trećih kutnjaka kod kojih su na bukalnim i lingvalnim plohama bili preparirani kaviteti V. razreda. Šezdeset kaviteta nasumce je bilo podijeljeno u šest skupina po deset uzoraka. U prvoj skupini na dentin je bila 15 sekundi primijenjena samo 37-postotna fosforna kiselina (demineralizacija); u drugoj se skupini nakon demineralizacije od 60 sekundi koristio 10-postotni natrijev hipoklorit (deproteinizacija); od treće do šeste skupine, nakon demineralizacije i deproteinizacije, bilo je upotrijebljeno antioksidacijsko sredstvo – 10-postotni natrijev askorbat i to u trajanju od 15 i 30 sekundi te nakon jedne minute i deset minuta. Zatim je bio primijenjen adhezivni sustav s jetkanjem i ispiranjem (Adper Single Bond 2; 3M/ESPE), a svi su kaviteti restaurirani kompozitom (Filtek Z250; 3M/ESPE). Nakon 24-satnog pohranjivanja u destiliranoj vodi na temperaturi od 37ºC, uzorci su bili podvrgnuti termocikliranju (500 ciklusa) i uronjeni 24 sata u 2-postotno metilensko modrilo. Nakon toga su prerezani bukolingvalno te se određivala rubna mikropropusnost na spoju dentina i smole. **Rezultati:** Statistički značajna razlika zabilježena je između druge i šeste skupine ($p=0,002$) te treće i šeste ($p=0,007$). U mikropropusnosti između deproteiniziranih i nedeproteiniziranih skupina nije pronađena statistički velika razlika ($p=0,300$). Ipak, usporedbom srednjih vrijednosti mikropropusnosti dobivenih u skupinama nakon što je bilo primijenjeno antioksidacijsko sredstvo, zabilježena je znatna razlika između druge skupine i ostalih skupina ($p=0,018$). **Zaključak:** Primjena 10-postotnog natrijeva askorbata nakon deproteinizacije dentinskog supstrata omogućila je bolje rubno brtvljenje ispuna V. razreda, iako se navedeno antioksidacijsko sredstvo taložilo na površini dentina.

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Uvod

Ako adhezija ne uspije, mogu se pojaviti rubna mikropropuštanja i sekundarni karijes zbog složenosti bezbrojnih varijabli uključenih u adhezijski protokol (1). Prepreke koje mogu kompromitirati adheziju na dentin uključuju kemijski sastav (organski sadržaj i sadržaj vode), strukturne topografske varijacije (broj i promjer dentinskih tubulusa) i zaostali sloj kao rezultat preparacije zuba. Polimerizacijsko skupljanje, razlike u koeficijentima termalne te higroskopske ekspanzije kompozita također mogu pridonijeti neuspjehu adhezije i formiranju rubnih pukotina te posljedično i mikropropuštanju (2).

Introduction

Adhesive failures may lead to the occurrence of marginal microleakage and secondary caries lesions due to the complexity of the innumerable variables involved in the adhesive protocol (1). The barriers that may challenge dentin adhesion include chemical composition (organic and aqueous content); structural topographic variations (number and e diameter of the dentinal tubules); and the presence of smear layer resulting from tooth preparation. Polymerization shrinkage, differences in the coefficients of thermal and hygroscopic expansion of the composite resins may also contribute to the failure of adhesion, with the formation of marginal gaps and consequent microleakage (2).

Stvaranje kompozitno-dentinske zone interdifuzije (hibridnog sloja) glavna je strategija za adhezivne restorativne postupke (3). Adhezija na hibridizirani dentin obavlja se uglavnom zahvaljujući djelomice demineraliziranoj mineralnoj dentinskoj površini lokaliziranoj u bazi zone eksponiranog kolagena, prije negoli mikromehaničkoj retenciji u kolagenu dentina (4).

Uporaba natrijeva hipoklorita (NaOCl) smatrala se jednom od mogućnosti za poboljšanje dentinske adhezije jer bi uklanjanje kolagenih vlakana pojačalo vezu (5) povećavajući vlaženje i stvarajući više hidrofilnu površinu, jer kolagen ima malu površinsku energiju (6). Deproteinizirana sposobnost te supstancije može se pripisati oksidacijskoj reakciji na organski sadržaj (7). Kao dodatak kod uklanjanja eksponiranih kolagenih vlakana jetkanjem kiselinom, primjena hipoklorita uzrokuje otapanje nekih kolagenih vlakana u susjednom mineraliziranom dentinskom matriksu, ostavljajući pore u mineralnom dijelu dentina. Prema tome, adhezivni bi sustav zauzeo područje u kojem su prije toga bila kolagena vlakna, formirajući obrnuti hibridni sloj koji je odgovoran za kompozitnu mikroretenciju (8).

Mogući nedostatak deproteinizacije odnosi se na činjenicu da bi prisutnost rezidualnih slobodnih radikala, kojom rezultira degradacija NaOCl-a u dentinu, dovela do nepotpune polimerizacije zbog preranog završetka polimerizacijskog lanca (9). No, djelovanje antioksidacijskog sredstva pomoglo bi neutralizirati i preokrenuti nepoželjne učinke NaOCl-a u biološkim sustavima (10,11).

Svrha istraživanja bila je ocijeniti *in vitro* učinak primjene antioksidacijskog sredstva (10-postotnog natrijeva askorbata) na deproteinizirani dentin stijenke kaviteta kompozitnih ispuna te na rubno brtvljenje. Hipoteza koja se ispitivala glasila je da deproteinizacija, zajedno s primjenom antioksidacijskog sredstva, omogućuje slično ili bolje rubno brtvljenje kompozitnih ispuna u usporedbi s jetkanjem fosfornom kiselinom.

Materijal i postupak

Trideset tek ekstrahiranih humanih trećih kutnjaka bilo je očišćeno i pohranjeno u 1-postotnu otopinu timola na temperaturi od 4ºC na razdoblje ne dulje od šest mjeseci. Na bukalnim i lingvalnim plohama svakog zuba preparirani su kaviteti V. razreda (dva milimetra duboki, dva milimetra široki i tri milimetra dugi) ravnim #330 karbidnim fisurnim svrdlom (SSWhite, Rio de Janeiro, RJ, Brazil) pri visokom broju okretaja i sa zračno-vodenim hlađenjem. Preparacije su bile centrirane na caklinsko-cementnom spojištu.

Nakon vizualnog pregleda kako bi se pronašli nepravilni rubovi, preparirani zubi su nasumce bili podijeljeni u šest skupina (G) prema različitim postupcima tretiranja površine (Tablica 1.).

Ukupno 60 kaviteta nasumce je bilo podijeljeno u šest skupina po deset uzoraka. U prvoj je 15 sekundi na dentin bila primijenjena samo 37-postotna fosforna kiselina (demineralizacija) točno prema uputama proizvođača (Attaque gel, Biodinâmica, Ibiporã, PR, Brazil).

U drugoj je skupini nakon demineralizacije bila upotrijebljena 10-postotna vodena otopina natrijeva hipoklorita

The formation of resin-dentin interdiffusion zone (hybrid layer) is the main strategy for adhesive restorative procedures (3). Adhesion to hybridized dentin is mostly due to the partially demineralized mineral dentin surface, localized on the base of the zone of exposed collagen, rather than to the micromechanical retention with dentin collagen (4).

The use of sodium hypochlorite (NaOCl) has been considered one of the possible resources to improve dentin adhesion because the removal of collagen fibers would increase the bond strength by increasing the wettability and producing a more hydrophilic surface, since collagen has low surface energy (5,6). The desproteinizing capacity of this substance is due to an oxidation reaction of the organic matter (7). In addition to removing the exposed collagen fibers by acid etching, the application of hypochlorite causes solubilization of some collagen fibers in the adjacent mineralized dentin matrix, leaving pores on the mineral phase. Therefore, the adhesive system would occupy the region that was previously occupied by collagen fibers, forming a reverse hybrid layer, which is responsible for the composite resin micro-retention (8).

A possible disadvantage of deproteinization refers to the fact that presence of residual free radicals resulting from NaOCl degradation in dentin would result in incomplete polymerization due to premature termination of the polymer chain (9). However, the action of an antioxidant agent would help neutralizing and reverting the adverse effects of NaOCl in biological systems (10,11).

The purpose of this study was to evaluate in vitro the effect of the application of an antioxidant agent (10% sodium ascorbate) to deproteinized dentin on the marginal seal of composite resin restorations. The tested hypothesis was that deproteinization associated with the application of an antioxidant agent promotes similar or better marginal seal of composite restorations compared to phosphoric acid etching.

Methods and Materials

Thirty six freshly extracted human third molars were debrided and stored in a 1% thymol solution at 4ºC for no longer than 6 months. Class V cavities (2 mm deep, 2 mm wide, 3 mm long) were prepared on the buccal and lingual surfaces of each tooth with straight #330 carbide fissure burs (SS-White, Rio de Janeiro, RJ, Brazil) at high speed under air/water coolant. Preparations were centered on the cementoenamel junction.

After visual inspection for imperfect finish lines, the prepared teeth were randomly assigned in six groups, corresponding to different surface treatments used (Table 1).

The 60 cavities were randomly assigned to 6 groups of 10 specimens each. In G1, only 37% phosphoric acid (Attaque gel, Biodinâmica, Ibiporã, PR, Brazil) was applied to dentin for 15 s (demineralization), strictly following the manufacturer's recommendations. In G2, after demineralization, 10% sodium hypochlorite aqueous solution (Phormula Ativa, Recife, PE, Brazil) for 60 s under constant agitation (deproteinization). (12); In G3, G4, G5 and G6, after demineralization and deproteinization, the antioxidant agent

Tablica 1. Raspodjela skupina (G) prema uporabi deproteiniziranih i antioksidacijskih sredstava.
Table 1 Distribution of groups according to the use of deprotenizing and antioxidant agents.

Skupine • Groups	NaOCl predtretman • NaOCl pretreatment	Antioksidacijski predtretman • Antioxidant pretreatment
G1	bez • without 10% NaOCl	bez natrijeva askorbata • without sodium ascorbate
G2	10% NaOCl	bez natrijeva askorbata • without sodium ascorbate
G3	10% NaOCl	10-postotni natrijev askorbat • sodium ascorbate 15 sek.
G4	10% NaOCl	10-postotni natrijev askorbat • sodium ascorbate 30 sek.
G5	10% NaOCl	10-postotni natrijev askorbat • sodium ascorbate 1 min.
G6	10% NaOCl	10-postotni natrijev askorbat • sodium ascorbate 10 min.

(Phormula Ativa, Recife, PE, Brazil) i to 60 sekundi uz stalno miješanje (deproteinizacija) (12). U trećoj, četvrtoj, petoj i šestoj skupini, nakon demineralizacije i deproteinizacije, bilo je primijenjeno antioksidacijko sredstvo – 10-postotni natrijev askorbat (Phormula Ativa, Recife, PE, Brazil) u trajanju 15 i 30 sekundi te 1 minutu i 10 minuta. Kaviteti su zatim isprani vodom, posušeni zrakom i odmah prekriveni slojem Adper Single Bonda 2 (3M ESPE; St. Paul, MN, SAD) te restaurirani hibridnom kompozitnom smolom (Filtek Z 250, 3M ESPE; St. Paul, MN, SAD) u dva sloja, od kojih je prvi bio postavljen uz gingivnu stijenku te svaki stvrdnut osvjetljavanjem od 20 sekundi (Poly 600S, Kavo, Joinvile, SC, Brazil; snage 500 nw/cm^2).

Za završnu obradbu i poliranje korišteni su diskovi *soft-lex* (3M Dental Products Division; St. Paul, MN, SAD). Postupke vezivanja obavljao je samo jedan operater. Restaurirani zubi pohranjeni su zatim 24 sata u destiliranu vodu (temperatura 37ºC). Nakon toga bili su podvrgnuti termocikliranju u 500 ciklusa vodenih kupelji od 5ºC ± 2ºC i 55ºC ± 2ºC s vremenom urona od 30 sekundi. Vrhovi korjenova zuba zapečaćeni su kompozitnom smolom (Filtek Z 250, 3M ESPE; St. Paul, MN, SAD) a sve zubne površine, osim jedan milimetar širokog područja oko rubova svakog ispuna, izolirane su lakom za nokte. Nakon toga zubi su bili uronjeni 24 sata u 2-postotnu puferiranu otopinu metilenskog modrila (13), zatim temeljito 10 minuta isprani vodom iz vodovoda i kroz centar ispuna uzdužno razrezani dijamantnim diskom s niskim brojem okretaja (KG Sorensen, São Paulo, SP, Brazil) kako bi se ispitao prodor boje. Sekcije su razdvojene, a rezane površine koje odgovaraju mezijalnom, središnjem i distalnom dijelu dodirne površine zuba i ispuna, pregledane su na caklinskim i gingivnim rubovima pod stereomikroskopom NSZ 606 LED (Coleman, Santo André, SP, Brazil) s povećanjem od 30 puta.

Obojenje duž ruba cakline i gingive zabilježeno je prema ranije definiranom sustavu bodovanja od četiri točke (14): zbroj bodova 0 = nema prodora boje; zbroj bodova 1 = prodor boje duž granice do polovice dubine stijenke kaviteta; zbroj bodova 2 = prodor boje cijelom dubinom stijenke kaviteta, ali ne uključuje aksijalnu stijenku; zbroj bodova 3 = prodor do aksijalne stijenke i uzduž nje.

Uzorke su pregledavala dva istraživača koja nisu bila uključena u restorativne postupke na način "slijepog pokusa". U graničnim slučajevima odlučivalo se dogovorom, a za analizu je bio odabran najlošiji rezultat (maksimalno propuštanje) zabilježen za svaki rub. Zbroj bodova za propušta-

10% sodium ascorbate (Phormula Ativa, Recife, PE, Brazil) was applied for 15 s, 30 s, 1 min and 10 min, respectively. The cavities were rinsed with water, air dried, immediately coated with Adper Single Bond 2 (3M ESPE; St. Paul, MN, USA), and restored with a hybrid composite resin (Filtek Z 250, 3M ESPE; St. Paul, MN, USA) in two increments, the first being accommodated against the gingival wall, which were light cured for 20 s each (Poly 600S, Kavo, Joinvile, SC, Brazil; output: 500 nw/cm^2).

Soft-lex disks (3M Dental Products Division; St. Paul, MN, USA) were used for finishing and polishing. The bonding procedures were performed by a single operator. The restored teeth were stored in distilled water at 37ºC for 24 h. After the storage time, the teeth were subjected to 500 thermal cycles between 5ºC ± 2ºC and 55ºC ± 2ºC water baths with dwell time of 30 s. The tooth apices were sealed with composite resin (Filtek Z 250, 3M ESPE; St. Paul, MN, USA), and all tooth surfaces except a 1-mm-wide zone around the margins of each restoration were sealed with nail polish. Subsequently, the teeth were immersed in a 2% buffered solution of methylene blue for 24 h (13), and thereafter thoroughly rinsed under tap water for 10 min and sectioned longitudinally through the center of the restorations with slow-speed diamond disk (KG Sorensen, São Paulo, SP, Brazil) to evaluate the dye penetration. The sections were then separated, and the cut surfaces corresponding to the mesial, central, and distal portion of the tooth/restoration interface were examined at the enamel and gingival margins with a stereomicroscope NSZ 606 LED (Coleman, Santo André, SP, Brazil) at ×30 magnification.

The staining along both enamel and gingival margins was recorded according to the following pre-defined four-point scoring system[14]: score 0 = no dye penetration; score 1 = dye penetration along the interface to ½ the depth of the cavity wall; score 2 = dye penetration the full depth of the cavity wall, but not including the axial wall; score 3 = penetration to and along the axial wall.

Two observers who were not previously involved in the restorative procedures undertook examination of the specimens in a blind fashion. Borderline cases were decided by consensus among the observers, and the worst value (maximum amount of leakage) recorded for each margin was selected for the analysis. The scores of leakage at the coronal and gingival margins of each experimental group were analyzed with the ANOVA ($p<0.05$). If a significant difference was observed at either margin location, the Tukey's test was used ($p<0.05$).

nje na krunskoj i gingivnoj stijenki svake istraživane skupine analiziran je metodom ANOVA (p<0,05). Ako je bila pronađena značajna razlika za bilo koju lokaciju ruba, koristio se Tukeyev test (p<0,05).

Rezultati

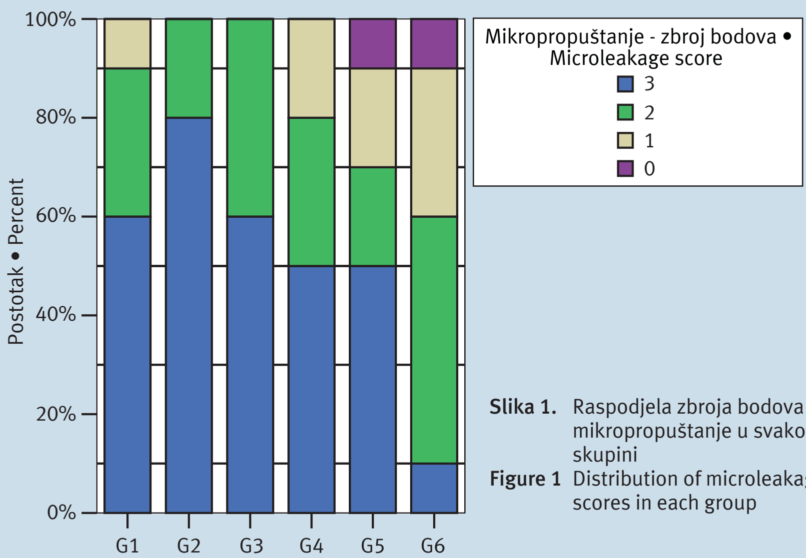
Na Slici 1. je distribucija zbroja bodova za mikropropuštanje u svakoj skupini (G). Zabilježena je statistički značajna razlika između druge i šeste skupine (p=0,002) te treće i šeste skupine (p=0,007).

Nije bilo statistički značajne razlike u rubnom mikropropuštanju između deproteiniziranih i nedeproteiniziranih skupina (p=0,300). Ipak, uspoređujući srednje vrijednosti mikropropuštanja u skupinama u kojima je bilo primijenjeno antioksidacijsko sredstvo, značajna razlika (p=0,018) pronađena je između druge skupine i ostalih skupina – treće, četvrte, pete i šeste koje se međusobno nisu znatno razlikovale (p>0,05).

Results

Figure 1 shows the distribution of microleakage scores in each group. There was statistically significant difference between G2 x G6 (p=0.002) and G3 x G6 (p=0.007).

There was no statistically significant difference in the marginal microleakage between the deproteinized and the non-deproteinized groups (p=0.300). However, comparing the mean microleakage values obtained in the groups the received application of the antioxidant agent, significant difference (p=0.018) was found between G2 and the other groups (G3, G4, G5 and G6), which did not differ significantly (p>0.05) from each other.



Slika 1. Raspodjela zbroja bodova za mikropropuštanje u svakoj skupini
Figure 1 Distribution of microleakage scores in each group

Rasprava

Trajnost veze smola-dentin najvažnija je za trajnost adhezivnih postupaka u restaurativnoj stomatologiji (15) jer mikropropuštanje neizbježno vodi prema rubnoj pigmentaciji, sekundarnom karijesu, dentinskoj preosjetljivosti i na kraju završava upalom pulpe (16).

Mehanizam adhezije smole na dentin objašnjava nekoliko čimbenika – stvaranje hibridnog sloja, prisutnost smolastih zubaca unutar dentinskih tubulusa, difuzija hidrofilnih monomera kroz intertubularni dentin ili čak kemijska povezanost s organskom ili anorganskom komponentom supstrata (17).

Tehnika jetkanja i ispiranja upotrebljava se za jetkanje 37-postotnom fosfornom kiselinom tijekom pet sekundi kako bi se uklonio zaostali sloj, otvorili dentinski tubulusi, demineralizirao intertubularni i peritubularni dentin povećavajući svoju propusnost i izlažući mrežu kolagenih vlakana koju prožimaju kristali hidroksilapatita. Održavanje prostor-

Discussion

The durability of the resin-dentin bond is of paramount importance for the longevity of adhesive procedures in restorative dentistry because microleakage invariably leads to marginal pigmentation, secondary caries, dentin hypersensitivity and eventually pulpal damage (14-16)

The mechanism of adhesion of resins to dentin has been explained by several factors that include the formation of a hybrid layer, presence resin *tags* inside the dentinal tubules, diffusion of hydrophilic monomers through the intertubular dentin or even chemical bonds such as organic and inorganic components of the substrate (17)

The etch-and-rinse technique uses 37% phosphoric acid etching for 15 s to remove smear layer, open the dentinal tubules demineralize the intertubular and peritubular dentin, increasing its permeability, and exposing the collagen fiber network that was permeated with hydroxyapatite crystals. The maintenance of the spatial structure of the collagen ne strukture kolagene mreže tijekom hibridizacije dentina pogoduje difuziji smolastog monomera (18).

Tretman s NaOCl-om rezultira većom poroznošću mineralizirane dentinske površine (19) koja pomaže boljoj mikromehaničkoj retenciji (20) zato što povećava dentinsku propusnost i vlaži adhezivni sustav (21).

Pokazalo se da deproteinizirana dentinska površina s tankim hibridnim slojem ili bez njega, ima bolja svojstva kad se ocjenjuje nakon različitog trajanja pohrane (22), što smanjuje rubno mikropropuštanje (23). Ipak, deproteinizacija ne mora biti korisna te može čak povećati rubno mikropropuštanje, ovisno o adhezivnom sustavu (22). To pokazuje da na rezultate ne utječe samo vrsta otapala, nego i vrsta monomera (24).

U ovom istraživanju deproteinizacija dentina s NaOCl-om nije utjecala na smanjenje rubnog propuštanja, što upućuje na to da se rezultati ne mogu smatrati odlučujućima za kliničku uporabu toga postupka (25). Osim toga niži modul elastičnosti hibridnog sloja u odnosu prema podliježućem dentinu ponašao bi se kao elastični sloj koji može apsorbirati stres zbog polimerizacijskog skupljanja kompozitne smole (26).

Ipak, rubno brtvljenje moglo bi se poboljšati primjenom natrijeva askorbata (27) nakon deproteinizacije s NaOCl-om jer bi mogući nedostatak proteolitičkog kondicioniranja bio da se rezidualni slobodni radikali, nastali degradacijom NaOCl-a, ponašaju kao spremnik kisika u dentinskom supstratu (28) potičući nepotpunu polimerizaciju zbog preranog završetka polimerizacijskog lanca (11). Na taj bi način antioksidacijsko sredstvo neutraliziralo redoks-potencijal i preokrenulo nepoželjne učinke NaOCl-a u biološkim sustavima (29).

Natrijev askorbat je biokompatibilan i siguran proizvod za oralnu uporabu. Sastoji od neotrovnih tvari, a smatra se najvažnijim sustavnim zaštitnikom od degenerativnih bolesti i procesa uzrokovanih oksidacijskim stresom (30). Uspješno se rabio u različitim područjima zdravstva, primjerice, u nutricionizmu, dermatologiji i medicini (31). U stomatologiji se natrijev askorbat upotrebljavao kako bi se spriječio rast bakterija biofilma u liječenju parodontne bolesti (32) te u prevenciji stvaranja mrlja na zubnoj površini pri uporabi minociklina (33) zbog toga što čisti superoksid, hipoklornu kiselinu i hidroksilne radikale.

Unatoč tomu, u ovom istraživanju opaženo je da vrijeme primjene natrijeva askorbata nije znatno utjecalo na sprječavanje rubnog mikropropuštanja, a to je opisano i u jednom ranijem istraživanju (34). Svojstvo natrijeva askorbata da djeluje kao antioksidacijsko sredstvo na dentinsku površinu moglo se provjeriti u trenutku njegova djelovanja i nije bilo ovisno o vremenu. Jednominutna primjena 10-postotnog natrijeva askorbata smanjila je pojavu mikropropusnosti, što se slaže s podacima iz literature (10, 27). Primjena natrijeva askorbata 30 i 60 sekundi te 10 minuta pomogla je taloženju soli koje su zatvorile neke tubuluse, što je možda poremetilo prodor adhezivnog sustava.

Potrebna su daljnja istraživanja kako bi se procijenila trajnost veze smola-dentin te utjecaj tretmana s NaOCl-om na propadanje adhezivnog povezivanja. Također je nužno po-

mesh network during dentin hybridization favors the resin monomer diffusion (18).

The treatment with NaOCl produces greater porosity on the mineralized dentin surface (19), which promotes better micromechanical retention (20) due to the increase of dentin permeability and adhesive system wettability (21).

The deproteinized dentin surface with thin or absent hybrid layer has been shown to have a better behavior when evaluated after different storage times (22), which lessens the occurrence of marginal microleakage (23). However, deproteinization might not be beneficial (14) or even increase the occurrence of marginal microleakage, depending on the adhesive system (22). This suggests that not only the type of solvent, but also the type of monomer influences the results (24).

In the present study, dentin deproteinization with NaOCl did not influence the decrease of marginal microleakage, which indicates that these results cannot be considered conclusive for the clinical use of this procedure (25). In addition, the lower module of elasticity of the hybrid layer than that of the subjacent dentin would act as an elastic layer capable of absorbing the stresses generated from the composite resin polymerization shrinkage (26).

However, the marginal seal could be improved by the application of sodium ascorbate (27) after deproteinization with NaOCl because a possible disadvantage of the proteolytic conditioning would be that residual free radicals from NaOCl degradation would act as a oxygen reservoir in the dentin substrate (28), leading to incomplete polymerization due to the premature termination of the polymer chain (11). In this way, an antioxidant agent would neutralize the redox potential and reverse the adverse effects of NaOCl in biological systems (29).

Sodium ascorbate is a biocompatible and safe product for oral use that is composed of non-toxic substances, which is also considered as the most important systemic protector against degenerative diseases and processes caused by oxidative stress (30), and has been successfully used in different health fields such as nutrition, dermatology and medicine (31). In dentistry, sodium ascorbate has been used to inhibit the growth of biofilm bacteria in the treatment of periodontal disease (32) and to prevent the formation of stains on tooth surface caused by the use of minocycline (33) due to its capacity scavenging of superoxides, hypochlorous acid and hydroxyl radicals.

Nevertheless, in the present study, it was observed that the application time of the sodium ascorbate did not influence significantly the prevention of marginal microleakage, which has been described in a previous study (34). The capacity of the sodium ascorbate acting as an antioxidant agent on dentin surface could be verified at the moment of its action, and was not time-dependent. However, the application of 10% sodium ascorbate for 1 min reduced the occurrence of microleakage, which in agreement with the reports in the literature (10,27). The application of sodium ascorbate for 30 s, 60 s and 10 min promoted sedimentation of salts that closed some tubules, which might have hindered the penetration of the adhesive system.

boljšati razumijevanje mogućih kemijskih veza s dentinom i uporabe proteolitičkog kondicioniranja zajedno sa samojetkajućim adhezivima jer je pokazalo proturječne vrijednosti rubnog mikropropuštanja (35,36). Trebalo bi istražiti i djelovanje antioksidacijskih sredstava na smanjenje citotoksičnosti nekih dentalnih materijala zbog neutralizacije metakrilatnih monomera redoks-reakcijom (37).

Zaključak

Primjena deproteiniziranog sredstva na dentin nije smanjila rubno mikropropuštanje; raspon mikropropuštanja smanjio se nakon primjene antioksidacijskog sredstva; vrijeme primjene antioksidacijskog sredstva nije utjecalo na rubno mikropropuštanje.

Further research should be performed to evaluate the durability of the resin-dentin bonds and the influence of the treatment with NaOCl on the degradation of the adhesive interface. Likewise, future studies are needed to improve the understanding on the possible chemical union to dentin and the use proteolytic conditioning in association with self-etch adhesives, which has shown discrepant values of marginal microleakage (35,36). Moreover, the action of antioxidant agents in reducing the cytotoxicity of some dental materials due to the neutralization of methacrylate monomers by a redox reaction should also be investigated (37).

Conclusion

The application of the deproteinizing agent on dentin did not lessen the occurrence of marginal microleakage; The extension of microleakage decreased with the application of the antioxidant agent; The application time of the antioxidant agent did not interfere in the marginal microleakage.

Abstract

Objective: To evaluate *in vitro* the effect of the application of the antioxidant agent 10% sodium ascorbate to deproteinized dentin on the marginal seal of composite resin restorations. **Methods**: Thirty extracted human third molars were selected and had class V cavities prepared on the buccal and lingual surfaces. The 60 cavities were randomly assigned to 6 groups of 10 specimens each. In G1, only 37% phosphoric acid was applied to dentin for 15 s (demineralization); In G2, after demineralization, 10% sodium hypochlorite was applied for 60 s (deproteinization); In G3-G6, after demineralization and deproteinization, the antioxidant agent 10% sodium ascorbate was applied for 15 s, 30 s, 1 min and 10 min, respectively. An etch-and-rinse adhesive system (Adper Single Bond 2; 3M/ESPE) was applied and all cavities were restored with composite resin (Filtek Z250; 3M/ESPE). After storage in distilled water at 37°C for 24 h, the specimens were subjected to a thermal cycling regimen (500 cycles) and immersed in 2% methylene blue for 24 h. The specimens were then sectioned in a buccolingual direction and the occurrence of marginal microleakage at the dentin/resin interface was assessed. **Results**: There was statistically significant difference between G2 x G6 (p=0.002) and G3 x G6 (p=0.007). No statistically significant difference (p=0.300) was found regarding microleakage between the deproteinized and the non-deproteinized groups. However, comparing the mean microleakage values obtained in the groups that received application of the antioxidant agent, significant difference was found between G2 and the other groups (p=0.018). **Conclusion**: The application of 10% sodium ascorbate after deproteinization of dentin substrate promoted a better marginal seal of class V restorations, although precipitation of this antioxidant agent occurred on dentin surface.

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Key words
Dental Leakage, Dentin, Dental Restoration Failure, Composite Resins.

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