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Utjecaj duljine primjene antioksidanta na deproteinizirani dentin: istraživanje SEM/EDS-om

Influence of Application Time of Antioxidant on the Deproteinized Dentin: A SEM/EDS Study

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Sažetak

Svrha: U uvjetima *in vitro* željelo se procijeniti utječe li i kako primjena 20-postotnog natrijeva askorbata na deproteinizirani dentin, a za to se rabio elektronski mikroskop/rendgenska spektroskopija raspršivanja energije (SEM/EDS). **Materijal i metode:** Sedam ekstrahiranih humanih trećih kutnjaka odabrano je za istodobnu analizu sastava i dentinske površine uz pomoć SEM/EDS-a. Bilo je odabrano i poduzeto sljedeće: G1 – zdrav dentinski supsrat; G2 – demineralizirani dentinski supstrat demineraliziran 15 sekundi 37-postotnom ortofosfornom kiselinom prema uputi proizvođača; G3 – deproteinizirani dentinski supstrat deproteiniziran 60 sekundi uz neprekidno miješanje u 10-postotnoj vodenoj otopini natrijeva hipoklorida; G4, G5, G6 i G7 – demineralizirani i deproteinizirani dentinski supstrat primjenom 10-postotne vodene otopine natrijeva askorbata tijekom 15, 30, 60 sekundi te jednu minutu prema redoslijedu navedenih grupa. Uzorci su pregledani elektronskim mikroskopom (Quanta 2000 – Fei Company) opremljenim spektrometrom raspršujuće energije i naponom ubrzanja od 10 KV pri povećanju od 2000 X u vakuumu. **Rezultati:** Analiza SEM/EDS-om pokazala je progresivno odlaganje kristala natrijeva askorbata usporedno s povećanjem antioksidacijskog sredstva, stvarajući koru koja može prouzročiti zatvaranje pojedinih dentinskih tubula. Nije bilo statistički značajne razlike između G2 i G3 ($p>0,05$). Primjena 10-postotnog natrijva askorbata 60 sekundi smanjila je razinu kisika ($P=0,029$), magnezija ($P=0,019$) i natrija ($P=0,029$). **Zaključak:** Nakon primjene 10-postotnog natrijeva askorbata, progresivno se talože njegovi kristali i stvaraju koru na dentinskom supstratu koja začepljuje pojedine dentinske kanaliće.

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Ključne riječi
dentin; antioksidansi; askorbinska kiselina; mikroskopija, elektronska, scintigrafska; spektrometrija, radiografska

Uvod

Prodiranje monomera smole u dentinske tubule i njihove ogranke te impregnacija tankog sloja demineraliziranog eksponiranog intertubularnog kolagenskog matriksa, kao rezultat jetkanja kiselinom, osnovna je komponenta vezivanja ispuna na bazi smola za dentin (1). Prepreke koje mogu spriječiti adheziju za dentin uključuju kemijski sastav (organski i tekući sadržaj); strukturalno-topografske varijacije (promjer i broj dentinskih tubulusa); prisutnost i odsutnost smear-layera, što je rezultat preparacije zuba. Polimerizacijsko skupljanje, razlike u koeficijentu termalne i hidroskopske ekspanzije kompozita mogu također pridonijeti neuspjehu adhezije i nastanku rubne pukotine te mikrocurenja (2). Stvaranje hibridnog sloja koji se sastoji od mješavine adhezivnih monomera i dentinskoga matriksa, preduvjet je za osiguranje vezivanja i sposobnosti brtvljenja (3). Zna se da na kvalitetu veze

Introduction

The penetration of resin monomer into dentin tubules and their branches and its impregnation of the thin layer of demineralized intertubular collagen matrix exposed as a result of acid etching are essential components in bonding resin based restorations to dentin (1). The barriers that may challenge dentin adhesion include chemical composition (organic and aqueous content); structural topographic variations (number and 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). The formation of a hybrid layer consisting of mixture of adhesive monomers and dentin matrix is paramount to ensure imme-

smola–dentin neposredno utječe infiltracija smole u eksponirani kolagen (4). Potpuno uklanjanje kolagenskog matriksa natrijevim hipokloridom (NaOCl) prije postupka vezivanja predloženo je kao način za sprječavanje kasnije degradacije koja može ugroziti dugotrajnost veze smola–dentin povećanjem osjetljivosti i stvaranjem veće hidrofilne površine jer kolagen ima nisku površinsku energiju (5,6). Otopine NaOCl-a koriste se diljem svijeta zbog antimikrobnog svojstva (7). Kao dodatak uklanjanju izloženih kolagenskih vlakana jetkanjem, NaOCl otapa organsku komponentu dentinske površine (8). Mogući nedostatak proteolitičkog kondicioniranja odnosi se na preostale slobodne radikale, zato što degeradacija NaOCl-a u dentinu rezultira nepotpunom polimerizacijom zbog završavanja polimerizacijskog lanca (9). Tretiranje dentina, nakon što je natrijev hidroklorid ispran askorbinskom kiselinom ili natrijevim askorbatom, vraća čvrstoću vezivanja na prihvatljive vrijednosti (10, 11).

Svrha ovog istraživanja bila je procijeniti in vitro utjecaj vremena u primjeni antioksidacijskog sredstva (10-postotnog natrijeva askorbata) na deproteinizirani dentin pomoću elektronskog mikroskopa/rendgenskom spektroskopijom raspršivanja energije (SEM/EDS).

Materijali i metode

Sedam tek ekstrahiranih humanih trećih kutnjaka očišćeno je i pohranjeno u 1-postotnu otopinu timola na temperaturi od 4°C, ne dulje od šest mjeseci. Nakon vizualnog pregleda ima li nepravilnih površina, preparirani zubi nasumce su podijeljeni u grupe te su se njihove površine različito tretirale (tablica 1.). Okluzalna caklina uklonjena je rezom okomitim na uzdužnu osovinu zuba, približno jedan milimetar od vrška kvržice, paralelno sa centralnom fisurom, a to je učinjeno obostrano prekrivenim dijamantnim diskom (SSWhite, Rio de Janeiro, Brazil) s niskom rotacijom (200 rpm) i neprekidnim hlađenjem površine vodom. Uz pomoć stroja za

diate bonding and sealing stability (3). It is well known that the quality of resin–dentin bonds is affected by the extent of resin infiltration into the exposed collagen (4).

Complete removal of the collagen matrix with sodium hypochlorite (NaOCl) prior to dentin bonding procedures has been proposed as a strategy to prevent later degradation, which may jeopardize the longevity of resin-dentin bonds (5) by increasing the wettability and producing a more hydrophilic surface, since collagen has low surface energy (6). Sodium hypochlorite solutions are used all over the world due to their antimicrobial characteristics (7). In addition to removing the exposed collagen fibers by acid etching, the application of NaOCl dissolves the exposed organic component from the dentin surface (8).

A probable inconvenience of proteolytic conditioning referred to existence of remaining free radicals resultant since NaOCl degradation in dentin would result in deficient polymerization due to precipitate termination of the polymer chain (9). Nevertheless, treating NaOCl rinsed dentin with ascorbic acid or sodium ascorbate returned bond strengths to acceptable levels (10,11).

The purpose of this study was to evaluate *in vitro* the influence of application time of an antioxidant agent (10% sodium ascorbate) on the deproteinized dentin through scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM/EDS).

Materials and methods

Seven freshly extracted human third molars were debrided and stored in a 1% thymol solution at 4°C for no longer than 6 months. After visual inspection for imperfect finish lines, the prepared teeth were randomly assigned into seven groups, corresponding to different surface treatments used (Table 1).

The occlusal enamel was removed by a cut perpendicular to the long axis of the tooth, approximately 1 mm from the cusp tip, parallel to the central sulcus, using a double-sided diamond disc (SSWhite, Rio de Janeiro, Brazil) rotating at low speed (200rpm) with constant irrigation, to expose the

Tablica 1. Raspored grupa u skladu s odabirom sredstava za deproteinizaciju i antioksidacijskih sredstava
Table 1 Distribution of groups according to the use of deproteinizing and antioxidant agents.

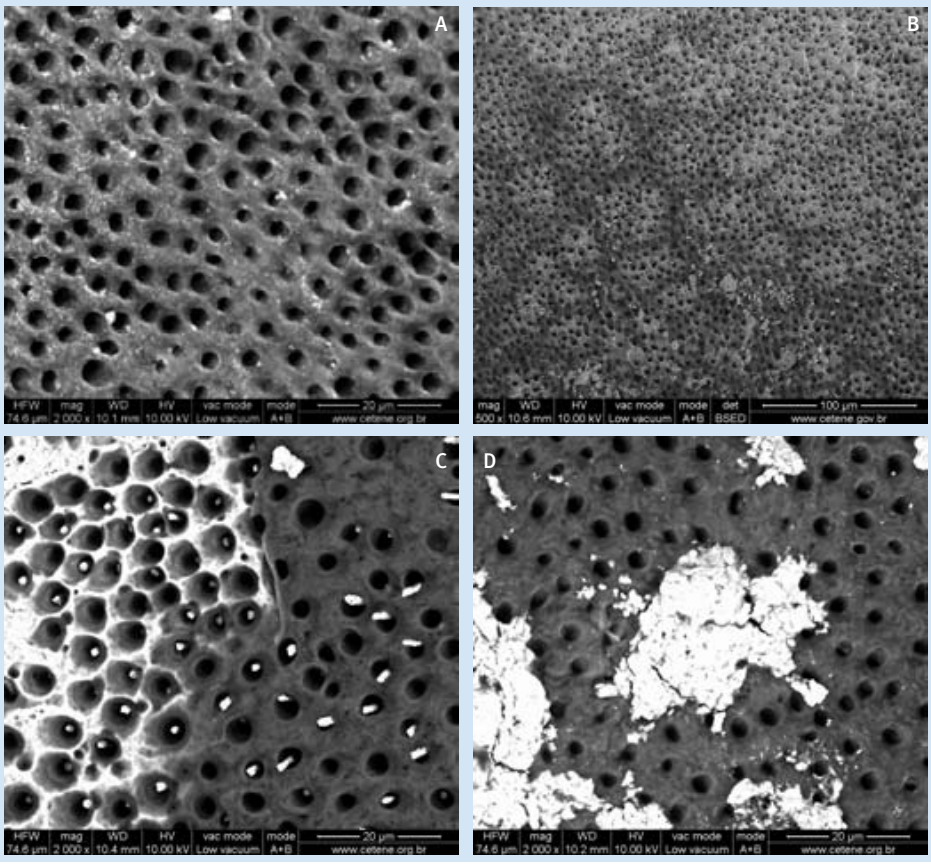
Grupa • Groups	Demineralizirajući predtretman • Demineralizer Pretreatment	NaOCl–predtretman • NaOCl Pretreatment	Antioksidant–predtretman • Antioxidant Pretreatment
G1	Bez 37% H3 PO4 • Without **37%** H_3PO_4	Bez 10% NaOCl • Without 10% NaOCl	Bez natrijeva askorbata • Without Sodium Ascorbate
G2	37% H_3PO_4	Without 10% NaOCl	Bez natrijeva askorbata • Without Sodium Ascorbate
G3	37% H_3PO_4	10% NaOCl	Bez natrijeva askorbata • Without 10% Sodium Ascorbate
G4	37% H_3PO_4	10% NaOCl	10% natrijev askorbat 15 sekundi • 10% Sodium Ascorbate 15s
G5	37% H_3PO_4	10% NaOCl	10% natrijev askorbat 30 sekundi • 10% Sodium Ascorbate 30s
G6	37% H_3PO_4	10% NaOCl	10% natrijev askorbat 1 minutu • 10% Sodium Ascorbate 1 min
G7	37% H_3PO_4	10% NaOCl	10% natrijev askorbat 10 minuta • 10% Sodium Ascorbate 10 min

poliranje (Panambra, São Paulo, Brazil), dentinska je površina obrađena silikonsko-karbidnim abrazivima sa sve finijim zrnima – 180, 240, 320 i 600, uz hlađenje vodom i obostrano dijamantnim diskovima (SSWhite, Rio de Janeiro, Brazil) do debljine dentina od jednog milimetra.

Za analizu sastava dentinske površine SEM/EDS-om, odabrano je istodobno sedam uzoraka prema sljedećem protokolu: G1 – zdrav dentinski supstrat; G2 – dentin demineraliziran 15 sekundi 37-ortofosfornom kiselinom (Attaque gel, Biodinâmica, Ibiporã, PR, Brazil) prema uputama proizvođača; G3 – demineraliziran 37-postotnom ortofosfornom kiselinom i deproteiniziran dentin 60 sekundi 10-postotnim NaOCl-om (Phormula Ativa, Recife, PE, Brazil) uz neprekidno miješanje (12); G4, G5, G6 i G7 – demineraliziran i deproteiniziran dentin tretiran 10-postotnim natrijevim askorbatom (Phormula Ativa, Recife, PE, Brazil) 5 sekundi, 30 sekundi, 60 sekundi i 10 minuta prema redoslijedu navedenih grupa. Uzorci su obostrano bili fiksirani za držače ugljičnom trakom te pregledani elektronskim mikroskopom (Quanta 2000 – Fei Company) opremljenim spektrometrom s disperziranom energijom i naponom ubrzanja od 10 KV pri ×2,000 povećanju u vakuumu. U svakom uzorku određeno je pet područja – sjeverno, južno, istočno, zapadno i centralno te su ona kvantificirana EDS-om (13). Središnje vrijednosti i standardne devijacije dobivene su i statistički analizirane Mann–Whitneyjevim i Kruskal–Wallisovim testom. Razina statističke značajnosti bila je postavljena na 5 posto kod svih testova.

surface. By using a polishing machine (Panambra, São Paulo, Brazil), the dentin surface was abraded by silicon carbide abrasives with decreasing grits 180, 240, 320 and 600, respectively, under water cooling and the use of a double-sided diamond disc (SSWhite, Rio de Janeiro, Brazil), a disc of dentin thickness of 1 mm was obtained.

For analysis of the composition of the dentin surface by SEM/EDS, 7 samples were analyzed simultaneously as follows: G1: Healthy dentin substrate, G2: Demineralized dentin substrate with 37% phosphoric acid (Attaque gel, Biodinâmica, Ibiporã, PR, Brazil) for 15 s (demineralization), strictly following the manufacturer's recommendations, G3: Deproteinized dentin substrate after demineralization, 10% sodium hypochlorite aqueous solution (Phormula Ativa, Recife, PE, Brazil) for 60 s under constant agitation (deproteinization), (12), G4, G5, G6 and G7: demineralized, deproteinized dentin substrate after application of 10% sodium ascorbate (Phormula Ativa, Recife, PE, Brazil) for 15 s, 30 s, 60 s, 10 min, respectively. The specimens were fixed on stubs using double-faced carbon tape and were examined by a scanning electron microscope (Quanta 2000 - Fei Company) equipped with an energy dispersive spectrometer with acceleration voltage 10 KV at ×2,000 magnification under vacuum. In each specimen, 5 areas located north, south, east, west and center were assessed quantitatively by EDS (13). The mean EDS values and standard deviations were obtained and analyzed statistically by the Mann-Whitney and Kruskal-Wallis tests with pairwise comparisons. A significance level of 5% was used in all statistical tests.



Slika 1. (A) Izgled dentina nakon deproteinizacije i primjene 10-postotnog natrijva askorbata 15 sekundi; (B) 30 sekundi; (C) 60 sekundi; (D) i 10 minuta

Figure 1 (A) Dentin aspect after deproteinization and after application of sodium ascorbate for 15s; (B) 30s; (C) 60s; (D) 10 min.

Rezultati

Analiza SEM-om pokazala je da nakon primjene 10-postotnog natrijeva askorbata 15, 30 i 60 sekundi te 10 minuta (slika 1 a–d) nastaju naslage natrijeva askorbata progresivno s povećanjem vremena aplikacije te stvaraju koru na dentinskom supstratu koja uzrokuje zatvaranje dentinskih tubula. Kvantitativna analiza SEM/EDS-om pokazala je da se znatno povećava količina ugljičnih (p=0,016) i dušičnih (p=0,008) iona u uzorcima, a jako smanjuju ioni kisika (p=0,008), fosfora (p=0,016) i natrija (p=0,008) tijekom demineralizacije dentinskog supstrata (slika 2.). Nije uočena statistički značajna razlika između G2 i G3 (p>0,05) (slika 3.). Primjena 10-postotnog natrijeva askorbata 60 sekundi smanjila je broj iona kisika (P=0,029), magnezija (P=0,019) i natrija (P=0,029) (slika 4.).

Results

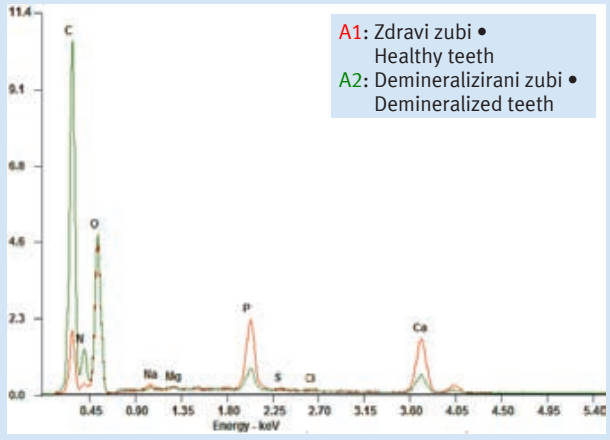
The SEM analysis showed that after application of 10% sodium ascorbate for 15 s, 30 s, 60 s and 10 min (Figure 1 A-D), the deposition of sodium ascorbate crystals occurred progressively as the application time increased, forming crusts on dentin substrate, which causes occlusion of some dentinal tubules. The quantitative analysis by SEM/EDS showed a significant increase in the carbon (p=0.016) and nitrogen (p=0.008) ions in the sample together with a significant decrease in the oxygen (p=0.008), phosphorus (p=0.016), sodium (p=0.008) ions during demineralization of dentin substrate (Figure 2). There was no statistically significant difference between G2 and G3 (P>0.05) (Figure 3). However, the application of 10% sodium ascorbate for 60 s resulted in a decrease in the oxygen (P=0.029), magnesium (P=0.019) and sodium (P=0.029) levels (Figure 4).

Rasprava

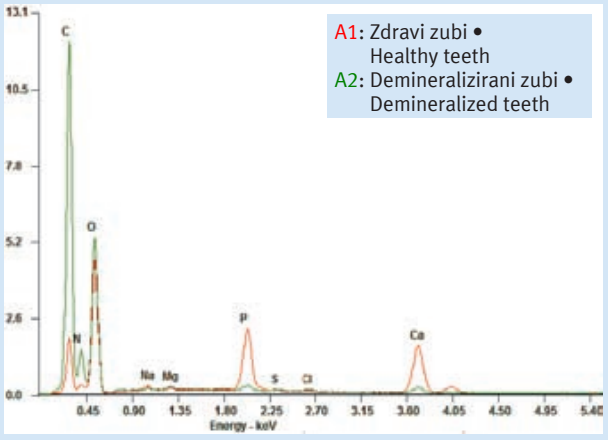
Stabilnost veze smola–dentin prijeko je potrebna za dugotrajnost adhezivnog postupka u restaurativnoj dentalnoj medicini zato što mikropropuštanje vodi prema dentinskoj

Discussion

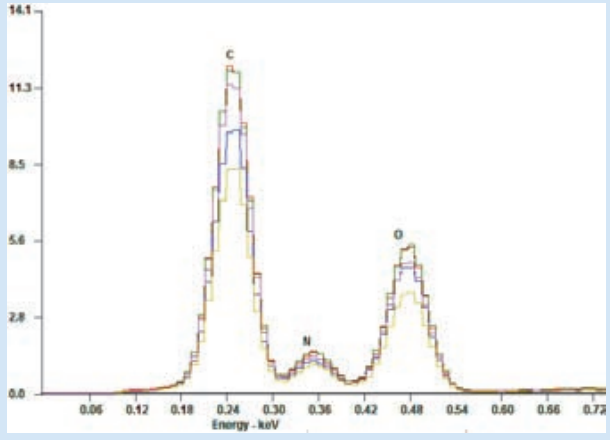
The stability of the resin-dentin bond is of utmost importance for the durability of adhesive procedures in restorative dentistry (14) because microleakage usually leads to dentin



Slika 2. Kvantitativna analiza razina ugljika, dušika, kisika, magnezija, fosfora, sumpora, klorida i kalcija tijekom demineralizacije
Figure 2 Quantitative analysis of the levels of carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, sulfur, chloride and calcium during demineralization.



Slika 3. Kvantitativna analiza razina ugljika, dušika, kisika, magnezija, fosfora, sumpora, klorida i kalcija tijekom deproteinizacije
Figure 3 Quantitative analysis of the levels of carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, sulfur, chloride and calcium during deproteinization.



Slika 4. Kvantitativna analiza razina ugljika, dušika i kisika tijekom primjene 10-postotnog natrijeva askorbata 15 sekundi, 30 sekundi, 60 sekundi i 10 minuta
Figure 4 Quantitative analysis of the levels of carbon, nitrogen and oxygen during application of 10% sodium ascorbate for 15 s, 30s, 60 s and 10 min.

preosjetljivosti, sekundarnom karijesu, rubnom obojenju i oštećenju pulpe (15).

Adheziju smole za dentin određuju mnogobrojni čimbenici, kao što su stvaranje hibridnog sloja, propagacija hidrofilnog monomera kroz cijeli intertubularni dentin, stvaranje smolastih produžetaka u dentinskim tubulama te kemijska veza s organskim i anorganskim komponentama supstrata (16).

Konvencionalni adhezivni sustavi uključuju odvojeno jetkanje i ispiranje, nakon kojih slijedi postavljanje primera i adhezivne smole, upotrebljava se 37-postotna ortofosforna kiselina za jetkanje 15 sekundi za uklanjanje *smear layera*, otvore se dentinske tubule, demineraliziraju intertubularni i peritubularni dentin, povećavajući mu propusnost i otkrivajući kolagenska vlakna koja su bila "zarobljena" kristalima hidroksilapatita. To što je sačuvana prostorna konfiguracija mreže kolagenskih vlakana tijekom postupka hibridizacije dentina, pridonosi difuziji smolastog monomera (17).

S druge strane, NaOCl se ponaša kao oksidirajuća komponenta u dentinskom matriksu koja interferira s propagacijom slobodnih radikala na dodirnoj površini smole i dentina te smanjuje čvrstoću vezivanja (18-20). Tretman s natrijevim askorbatom mijenja oksidacijski supstrat na sniženi supstrat, što vraća redoksni potencijal dentina i vjerojatno pomaže polimerizaciji methylmethacrylat/polymethylmethacrylatne smole (21–23).

Otopina natrijeva askorbata neutralna je i biokompatibilna antioksidacijska otopina sigurna za oralnu uporabu jer je sastavljena od netoksičnih tvari. Smatra se također da dobro i sustavno štiti od degenerativnih bolesti i procesa koji su rezultat oksidacijskog stresa, te se uspješno primjenjuje u različitim područjima zdravstva, kao što su dermatologija, medicina i prehrana (24, 25). U dentalnoj medicini izvrsno sprječava razvoj bakterijskoga biofilma kod terapije parodontnih bolesti i onemogućuje obojenje zuba tijekom primjene minociklina. Sa mogućnošću čišćenja superoksida hipokloričnе kiseline i hidroksilnih radikala (26, 27).

Kad je riječ o kemijskom sastavu dentinskog supstrata nakon tretmana površine, rezultati u ovom istraživanju ne slažu se s rezultatima prijašnjih istraživanja jer analiza SEM/EDS-om nije pokazala povećanje iona magnezija, za razliku od iona ugljika čija se količina znatno povećala nakon primjene antioksidacijskog sredstva (28). Neki autori istaknuli su da je u njihovim istraživanjima tretiranje dentina NaOCl-om smanjilo organski matriks, ali ne i količinu karbonatnih i fosfatnih iona, a to smo ustanovili i mi u našem istraživanju (18).

U literaturi se pretpostavlja moguća kemijska interakcija adhezivnog sustava s ionima kalcija u dentinu (29). Analiza EDS-om pokazala je da se koncentracija kalcija smanjila s 28,62 na 12,77 posto, te nakon deproteinizacije na 8,68 posto, a povećala se na 15,99 posto nakon desetominutne primjene natrijeva askorbata. Na takav način ova kemijska interakcija s kalcijem može također pridonijeti rubnom mikropropuštanju, što se razlikuje od rezultata u prijašnjim istraživanjima (30, 31). Podpovršinski sloj deproteiniziranog dentina bogat je kristalima hidroksilapatita, a oni pridono-

hypersensitivity, secondary caries, marginal pigmentation and ultimately, pulpal damage (15).

The device of adhesion of resins to dentin has been defined through numerous factors that incorporate the development of a hybrid layer, propagation of hydrophilic monomers throughout the intertubular dentin, occurrence of resin *tags* within the dentinal tubules, otherwise still chemical bonds such as organic and inorganic components of the substrate (16).

Conventional adhesive systems involve a separate etch-and-rinse step, followed by priming and resin adhesive application, using 37% phosphoric acid etching for 15 s to remove smear layer, open the dentinal tubules, and demineralize the intertubular and peritubular dentin, increasing its permeability, and revealing the collagen fiber arrangement that was permeated by hydroxyapatite crystals. The preservation of the spatial configuration of the collagen mesh network for the period of dentin hybridization contributes to the resin monomer diffusion (17).

The management with NaOCl produces larger porosity on the mineralized dentin surface (18) that promotes enhanced micromechanical retention (19) owing to the increase of dentin permeability and adhesive system wettability (20).

On the other hand, NaOCl acts to oxidize a component in the dentinal matrix that interferes with free radical propagation at the resin– dentin interface, leading to lower bond strength. Treatment with sodium ascorbate changes the oxidized substrate to a reduced substrate which restores the redox potential of the dentin and is believed to aid the polymerization of the methylmethacrylate/polymethylmethacrylate resin (21-23).

Sodium ascorbate solution is a neutral, biocompatible antioxidant and safe product for oral use that is composed of non-toxic substances, which is also considered the most significant systemic protector against degenerative diseases and processes resulting from oxidative stress (24), and has been effectively used in different health fields such as dermatology, medicine and nutrition (25). This substance has been used in dental medicine to reduce the development of biofilm bacteria in the treatment of periodontal diseases (26) and to avoid the formation of stains on tooth surface caused by the use of minocycline (27) with regard to its ability of scavenging the superoxides, hypochlorous acid and hydroxyl radicals.

Regarding the chemical composition of dentin substrate after the surface treatments, the findings of this study do not agree with those of previous investigations (28), since the SEM/EDS analysis showed no increase of magnesium ions unlike the carbon ions, which increased significantly after application of the antioxidant agent. Some authors reported that the treatment of demineralized dentin with NaOCl reduces the organic matrix (18), but does not affect the amount of carbonate and phosphate ions, in the same way as observed in the present study.

The literature has suggested a possible chemical interaction of adhesive systems with calcium ions of dentin (29). The EDS analysis showed that calcium concentration after demineralization dropped from 28.62 to 12.77%, remaining at 8.68% after deproteinization, and increasing to 15.99%

se velikoj površinskoj energiji i usporedno povećavaju koncentraciju kalcija, što potvrđuju i rezultati u ovom istraživanju (32).

Potrebna su dodatna istraživanja kako bi se procijenila stabilnost veze smola–dentin i utjecaj primjene NaOCl-a na degradaciju adhezivne površine. Osim toga treba istražiti moguće kemijske veze za dentin i korištenje proteolitičkog kondicioniranja kod uporabe samojetkajućih adhezivnih sustava, zbog potpuno suprotnih vrijednosti rubnog mikrocurenja (33, 34). Nadalje, potrebno je detaljno istražiti učinak antioksidacijskih sredstava na citotoksičnost nekoliko materijala za dentalnu medicinu kada im prođe rok valjanosti za neutralizaciju metakrilatnog monomera redoksnim učinkom (35).

Zaključak

Nakon primjene 10-postotnog natrijeva askorbata nastaju naslage njegovih kristala i progresivno se stvaraju ljuske na dentinu koje zatvaraju pojedine dentinske kanaliće.

Zahvale

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after application of sodium ascorbate for 10 min, in such a way that this chemical interaction with calcium might also have contributed to the decrease of marginal microleakage, unlike the results of previous studies (30,31). The subsurface of the deproteinized dentin layer is rich in hydroxyapatite crystals, which favors a high surface energy as the calcium concentration increases (32), confirming the findings of the present study.

Additional studies should be conducted to estimate the stability of the resin-dentin bonds and the influence of the treatment with NaOCl on the degradation of the adhesive interface. Similarly, further research is needed to advance the perception of the probable chemical bonding to dentin and the use of proteolytic conditioning among self-etch adhesives, which has revealed discrepant values of marginal microleakage (33,34). Furthermore, the action of antioxidant agents in reducing the cytotoxicity of several dental materials due to the neutralization of methacrylate monomers by a redox effect should also be explored (35).

Conclusion

After application of 10% sodium ascorbate, the deposition of sodium ascorbate crystals occurred progressively forming crusts on dentin substrate, which causes occlusion of some dentinal tubules.

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Abstract

Objective: To evaluate *in vitro* the influence of application time of sodium ascorbate to 10% on the deproteinized by scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM/EDS). **Methods:** Seven extracted human third molars were selected. For analysis of the composition of the dentin surface by SEM/EDS, 7 samples were analyzed simultaneously as follows: G1: Healthy dentin substrate, G2: Demineralized dentin substrate with 37% phosphoric acid for 15 s (demineralization), strictly following the manufacturer's recommendations, G3: Deproteinized dentin substrate after demineralization, 10% sodium hypochlorite aqueous solution for 60 s under constant agitation (deproteinization), G4, G5, G6 and G7: demineralized, deproteinized dentin substrate after application of 10% sodium ascorbate for 15 s, 30 s, 60 s, 10 min, respectively. The samples were examined by a scanning electron microscope (Quanta 2000 - Fei Company) equipped with an energy dispersive spectrometer with acceleration voltage 10 KV at ×2,000 magnification under vacuum. **Results:** The SEM/EDS analysis showed progressive deposition of sodium ascorbate crystals as the application time of the antioxidant agent increased, forming crusts which can cause occlusion of some dentinal tubules. There was no statistically significant difference between G2 and G3 (P>0.05). However, the application of 10% sodium ascorbate for 60 s resulted in a decrease in the oxygen (P=0.029), magnesium (P=0.019) and sodium (P=0.029) levels. **Conclusion:** After application of 10% sodium ascorbate, deposition of sodium ascorbate crystals occurred progressively forming crusts on dentin substrate, which causes occlusion of some dentinal tubules.

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Key words
Dentin; Antioxidants; Ascorbic Acid; Microscopy, Electron, Scanning; Spectrometry, X-Ray Emission

References

1. Mjör IA. Dentin permeability: the basis for understanding pulp reactions and adhesive technology. Braz Dent J. 2009;20(1):3-16.
2. Pioch T, Kobaslija S, Huseinbegović A, Müller K, Dörfer CE. The effect of NaOCl dentin treatment on nanoleakage formation. J Biomed Mater Res. 2001 Sep 15;56(4):578-83.
3. Carrilho MR, Tay FR, Pashley DH, Tjäderhane L, Carvalho RM. Mechanical stability of resin-dentin bond components. Dent Mater. 2005 Mar;21(3):232-41.
4. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H et al. In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM. Biomaterials. 2004 Nov;25(25):5565-74.
5. Sauro S, Mannocci F, Tay FR, Pashley DH, Cook R, Carpenter GH et al. Deproteinization effects of NaOCl on acid-etched dentin in clinically-relevant vs prolonged periods of application. A confocal and environmental scanning electron microscopy study. Oper

Dent. 2009 Mar-Apr;34(2):166-73.
6. Montes MA, de Goes MF, Ambrosano GM, Duarte RM, Sobrinho LC. The effect of collagen removal and the use of a low-viscosity resin liner on marginal adaptation of resin composite restorations with margins in dentin. Oper Dent. 2003 Jul-Aug;28(4):378-87.
7. Nicoletti MA, Siqueira EL, Bombana AC, Oliveira GG. Shelf-life of a 2.5% sodium hypochlorite solution as determined by Arrhenius equation. Braz Dent J. 2009;20(1):27-31.
8. Soeno K, Taira Y, Ito S, Atsuta M, Pashley DH. Effects of a ferric chloride primer on collagen-depleted dentin bonding between tri-n-butylborane initiated self-curing resin and dentin. J Biomed Mater Res B Appl Biomater. 2007 Nov;83(2):359-63.
9. Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM et al. Reversal of compromised bonding to oxidized etched dentin. J Dent Res. 2001 Oct;80(10):1919-24.
10. Nagpal R, Tewari S, Gupta R. Effect of various surface treatments on the microleakage and ultrastructure of resin-tooth interface. Oper Dent. 2007 Jan-Feb;32(1):16-23.
11. Yiu CK, García-Godoy F, Tay FR, Pashley DH, Imazato S, King NM et al. A nanoleakage perspective on bonding to oxidized dentin. J Dent Res. 2002 Sep;81(9):628-32.
12. Toledano M, Osorio R, Perdigao J, Rosales JI, Thompson JY, Cabrerizo-Vilchez MA. Effect of acid etching and collagen removal on dentin wettability and roughness. J Biomed Mater Res. 1999 Nov;47(2):198-203.
13. Weerasinghe DD, Nikaido T, Ichinose S, Waidyasekara KG, Tagami J. Scanning electron microscopy and energy-dispersive X-ray analysis of self-etching adhesive systems to ground and unground enamel. J Mater Sci Mater Med. 2007 Jun;18(6):1111-6.
14. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008 Jan;24(1):90-101.
15. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005 Feb;84(2):118-32.
16. Nakabayashi N, Pashley DH. Hybridization of dental hard tissues. Tokyo: Quintessence Publishing; 2000.
17. Haller B. Recent developments in dentin bonding. Am J Dent. 2000 Feb;13(1):44-50.
18. Mountouris G, Silikas N, Eliades G. Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin. J Adhes Dent. 2004 Autumn;6(3):175-82.
19. Maior JR, Da Figueira MA, Netto AB, de Souza FB, da Silva CH, Tredwin CJ. The importance of dentin collagen fibrils on the marginal sealing of adhesive restorations. Oper Dent. 2007 May-Jun;32(3):261-5.
20. Correr GM, Alonso RC, Grando MF, Borges AF, Puppin-Rontani RM. Effect of sodium hypochlorite on primary dentin--a scanning electron microscopy (SEM) evaluation. J Dent. 2006 Aug;34(7):454-9.
21. Pamir T, Türkün M, Kaya AD, Sevgican F. Effect of antioxidant on coronal seal of dentin following sodium-hypochlorite and hydrogen-peroxide irrigation. Am J Dent. 2006 Dec;19(6):348-52.
22. Kimyai S, Valizadeh H. Comparison of the effect of hydrogel and a solution of sodium ascorbate on dentin-composite bond strength after bleaching. J Contemp Dent Pract. 2008 Feb 1;9(2):105-12.
23. Weston CH, Ito S, Wadgaonkar B, Pashley DH. Effects of time and concentration of sodium ascorbate on reversal of NaOCl-induced reduction in bond strengths. J Endod. 2007 Jul;33(7):879-81.
24. Gutteridge JM. Biological origin of free radicals, and mechanisms of antioxidant protection. Chem Biol Interact. 1994 Jun;91(2-3):133-40.
25. Naidu KA. Vitamin C in human health and disease is still a mystery? An overview. Nutr J. 2003 Aug 21;2:7.
26. Väänänen MK, Markkanen HA, Tuovinen VJ, Kullaa AM, Karinpää AM, Luoma H et al. Dental caries and mutans streptococci in relation to plasma ascorbic acid. Scand J Dent Res. 1994 Apr;102(2):103-8.
27. Bowles WH. Protection against minocycline pigment formation by ascorbic acid (vitamin C). J Esthet Dent. 1998;10(4):182-6.
28. Inaba D, Ruben J, Takagi O, Arends J. Effect of sodium hypochlorite treatment on remineralization of human root dentine in vitro. Caries Res. 1996;30(3):218-24.
29. Abo T, Asmussen E, Uno S, Tagami J. Short- and long-term in vitro study of the bonding of eight commercial adhesives to normal and deproteinized dentin. Acta Odontol Scand. 2006 Aug;64(4):237-43.
30. Andia-Merlin RY, Garone-Netto N, Arana-Chavez VE. SEM evaluation of the interaction between a three-step adhesive and dentin. Oper Dent. 2001 Sep-Oct;26(5):440-4.
31. Meira Ribeiro AI, Tenório Guenes GM, Dantas DC, da Silva JW, Cavalcanti AL, Braz R. Influence of antioxidant agents on the marginal seal of class V restorations. Acta Stomatol Croat. 2010;44(4):225-31.
32. Panighi M, G'Sell C. Influence of calcium concentration on the dentin wettability by an adhesive. J Biomed Mater Res. 1992 Aug;26(8):1081-9.
33. Erhardt MC, Osorio E, Aguilera FS, Proença JP, Osorio R, Toledano M. Influence of dentin acid-etching and NaOCl-treatment on bond strengths of self-etch adhesives. Am J Dent. 2008 Feb;21(1):44-8.
34. Fawzy AS, Amer MA, El-Askary FS. Sodium hypochlorite as dentin pretreatment for etch-and-rinse single-bottle and two-step self-etching adhesives: atomic force microscope and tensile bond strength evaluation. J Adhes Dent. 2008 Feb;10(2):135-44.
35. Soheili Majd E, Goldberg M, Stanislawski L. In vitro effects of ascorbate and Trolox on the biocompatibility of dental restorative materials. Biomaterials. 2003 Jan;24(1):3-9.