Procjena biokompatibilnosti četiriju vrsta dentinskih adheziva kao sredstava za indirektno prekrivanje pulpe

Biocompatibility Evaluation of Four Dentin Adhesives Used as Indirect Pulp Capping Materials

Stomatološka klinika Sveučilišta u Murciji, Bolnica Morales Meseguer, Španjolska
Clínica Odontológica, Universidad de Murcia, Hospital Morales Meseguer, Spain

Sažetak
U mnogim slučajevima indirektno prekrivanje pulpe (IPP) prihvatljiva je terapija za trajne zube u slučaju njezine reverzibilne upale. Za IPP koriste se različiti lijekovi – od kalcijeva hidroksida i staklenog ionomera do dentinskih adheziva. Svrha istraživanja: Svrha ovog istraživanja in vitro bila je izmjeriti citotoksičnost u staničnoj kulturi, uspoređujući četiri adheziva: Xeno® V (XE), Excite® F DSC (EX), Adhese® Onef (AD) i Prime & Bond NT (PB). Materijali i metode: Adhezivi su primijenjeni u skladu s uputama proizvođača. Nakon 24-satne izloženosti procijenjena je vijabilnost stanica s pomoću foto-metrijskog testa (MTT test). Podatci su podvrgnuti analizi varijance (ANOVA). Rezultati: Adhezivi čija je glavna komponenta bila 2-hidroksietil metakrilat (HEMA) pokazali su se manje citotoksičnima, a oni koji su u svojem sastavu imali monomer uretan-dimetakrilat (UDMA) bili su najcitotoksičnijii. Učinak na vijabilnost statistički su između adheziva značajno varirali. Zaključak: Rezultati pokazuju da je Adhese® Onef najmanje citotoksičan od ispitanih adheziva i može se koristiti kao sredstvo za indirektno prekrivanje pulpe. No Prime & Bond NT u istim je uvjetima pokazao smanjenu biokompatibilnost.

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Adresa za dopisivanje  
Mª Antonia Alcaina  
Clínica Odontológica, Universidad de Murcia  
Hospital Morales Meseguer- 2º Pl  
Avd, Marques d elos Velez s/n  
30008 Murcia, Spain

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Uvod
Regeneracija pulpe moguća je u slučaju reverzibilnog pulpitisa, bilo da je uzrokovano karijessom, jatrogeno ili traumom (1). Svrha indirektnog prekrivanja pulpe (IPP) jest očuvanje vitalnosti zuba na kojemu se pojavio reverzibilni pulpitis ili duboki karijes bez izloženosti pulpe (2). Kako bi se osigurao uspjeh IPP-a, važno je ukloniti karijes s caklinsko-dentinskog spojišta i bočnih stijenki kaviteta da bi se osiguralo najbolje moguće brtvljenje između zuba i ispuna te tako spriječila mikropropusnost (3–5). U tom postupku kalcijev hidroksid i staklenoionomerni cementi koriste se kao podloge s dobrim rezultatima.

Adhezivi se također mogu koristiti za IPP. Jekanje prije nanošenja adheziva olakšava otapanje dentina jer oslobađa čimbenike rasta kojima se dobija reverzibilni pulpitis ili duboki karijes bez izloženosti pulpe (2). Kako bi se osigurao uspjeh IPP-a, važno je ukloniti karijes s caklinsko-dentinskog spojišta i bočnih stijenki kaviteta da bi se osiguralo najbolje moguće brtvljenje između zuba i ispuna te tako spriječila mikropropusnost (3–5). U tom postupku kalcijev hidroksid i staklenoionomerni cementi koriste se kao podloge s dobrim rezultatima.

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Korištenje samojetkih adhezivnih sustava ili adheziva s prethodnom jekanjem kiselom smanjuje rubnu mi
Procjena biokompatibilnosti četiriju vrsta dentalnih adheziva

Kropopusnost i tako snižava mogućnost stvaranja rekurentnog karijesa (8,9).

No kad je riječ o IPP-u, povoljni klinički ishodi ne ovise samo o fizičkim i kemijskim svojstvima korištenog proizvođa, nego i o biokompatibilnosti adhezivnog sustava (10). Biološka kompatibilnost mora biti osnovno svojstvo bilo kojeg materijala koji se koristi u ustima, a to je osobito važno za adhezive koji se primjenjuju u neposrednoj blizini Zubne pulpe.

Klinička istraživanja otkrila su razmjerno malo nepovoljnih bioloških učinaka pri primjeni adheziva izravno na dentin. No mnogobrojna istraživanja (11, 12) pokazuju da komponente adhezivnih smola mogu citotoksično djelovati na fibroblaste. Tkiva pulpe mogu se patološki promijeniti kad dođu u doticaj s adhezivima jer nepolimerizirani monomeri mogu prodrijeti kroz dentin i ući u pulpu (13). Smolasti kompozitni materijali u organskoj matrici sadržavaju citotoksične komponente kao što su monomeri i konomoneri (14). Restauracijski materijali obično sadržavaju hidroksietil-metakrilat (HEMA), trietilenglikol-dimetakrilat (TEGDMA), bisfenol-A-glicidil-metakrilat (Bis-GMA) i uretan-dimetakrilat (UDMA), a svi su pronađeni u vodenim ekstraktima dobivenim iz polimeriziranih materijala (15, 16). Polimerizirane dentinske smole u vodeni medij oslobađaju camphoroquinone (CQ) – is a photoinitiator that was released following the polymerization (26, 27). CQ is not a constituent of the polymer chain; hence a proportion of the unreacted double bonds can remain in the cured resin. This may have harmful effects on odontoblast vitality, as well as physiological activity of the pulp (22).

Polymerized dental resin release TEGDMA into aqueous media in large quantities causing a high proportion of their unreacted double bonds (23). TEGDMA is a frequent constituent of dental adhesive agents, and is present at concentrations that vary between 30 and 55%, playing a key role in the process of dentin impregnation (21). Due to its low molecular weight and relative hydrophilicity, HEMA can spread through residual dentin, which may have harmful effects on odontoblast vitality, as well as physiological activity of the pulp (22).

In IPT, however, good clinical outcomes depend not only on the physical and chemical properties of the product used but also on the biocompatibility of the adhesive system (10). Biological compatibility must be a basic property of any dental material, and this is particularly relevant for adhesives used in cases involving proximity to dental pulp.

Clinical research has revealed relatively few adverse biological effects derived from applying adhesives directly to dentin. But numerous in vitro studies (11, 12) have found that the components of adhesive resins can have cytotoxic effects on fibroblasts. The pulp tissues may suffer pathological alteration when they come into contact with resin-composite adhesives, since uncured monomers can penetrate the dentinal tubules and thus reach the pulp (13). Resin composite materials contain cytotoxic components such as monomers and co-monomers in their organic matrix (14). Restoration materials commonly include two-hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), bisphenol A-glycidyl-methacrylate (Bis-GMA), and urethane dimethacrylate (UDMA), all of which have been found in aqueous extracts taken from the cured restoration materials (15, 16). It has been shown that dentin adhesives present differing levels of cytotoxicity after exposure times of 24 and 72 hours as follows (from most to least toxic): Bis-GMA>UDMA>TEGDMA>HEMA (17). HEMA and TEGDMA would appear to present less cytotoxicity in vitro than Bis-GMA or UDMA, which are more hydrophobic (18, 19). Adhesive systems contain a range of components, hence interactions between these may lead to varying levels of cytotoxicity that may be higher or lower than the individual substances alone (17,20).

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Commercial dental resin release TEGDMA into aqueous media in large quantities causing a high proportion of their unreacted double bonds (23). TEGDMA makes up 25-50% of the content of dentin adhesives (24). Because of its lipophilic characteristics, TEGDMA has a capacity of penetrating the cytosol and membrane lipid compartments of mammalian cells with a number of cytotoxic effects (25).

Another common component of dentinal adhesives – camphoroquinone (CQ) – is a photoinitiator that was released following the polymerization (26, 27). CQ is not a constituent of the polymer chain; hence a proportion of the component not involved in polymerization can provoke oxidative stress, DNA damage, and cytotoxicity (27).

In this way, the cytotoxicity of adhesives may vary depending on the proportions of these components and their potential to penetrate the dentin.

This in vitro study used indirect contact testing to evaluate the potential cytotoxic effects of four recently developed adhesives in different cell culture dilutions.
Materijali i metode

U istraživanju je korištena linija fibroblasta L929 (European Collection of Cell Cultures) u mediju za kultiviranje [Dulbecco's Modified Eagle's Medium (DMEM)] u kombinaciji s 10% fetalnoga telećeg seruma (FCS) i antibioticima (penicillin 100 i.j./ml i streptomycin 100 µg/ml).

Materijal 1: Xeno® V

Materijal 2: Excite® F DSC

Materijal 3: Adhese® One F

Materijal 4: Prime & Bond® NT

Korišteni materijali, njihov sastav i proizvođači naveđeni su u tablici 1. Svaki materijal stavljen je na Petriju, inkubiran u 7,5% C0 2. Izmjerena je raspona od 0,45 ml.

Indirektna metoda (na temelju ekstrakata) korištena je prema ISO standardu 10993-5 (28). Pilot-istraživanjem potvrđena je prikladnost korištene metodologije i valjanost prototipova. Nakon odmrzavanja stanične linije provedeno je centrifugiranje 200 g tijekom 10 minuta, nakon čega su izbrišene stanice i usadene u posudu za kultiviranje od 75 cm², koja je zatim inkubirana u 7,5% C0 2. Izmjeren je pH ekstrakta svakog materijala asimetrijskih rezultata, odlučeno je da se kultivira 5000 stanica po stanici tijekom 24 sata.

Adhezivni postupak

- Materijal 1: Xeno® V
- Materijal 2: Excite® F DSC
- Materijal 3: Adhese® One F
- Materijal 4: Prime & Bond® NT

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Adhesive procedures

Experimental procedures were carried out in triplicate with 6 wells per variable. Materials were used following the manufacturer’s instructions:

- Material 1: Xeno® V
- Material 2: Excite® F DSC
- Material 3: Adhese® One F
- Material 4: Prime & Bond® NT

These materials, their compositions, and the manufacturer of each material are listed in Table 1. Each material was placed on a Petri plate, light-cured, and allowed to set for two hours. The samples were covered with 2-8 ml of culture medium, without phenol red, at a surface-to-unit volume ratio of 64 mm²/200 ml, and were kept in the CO2 incubator for 24 hours. After this period of time, the pH of the extracts was determined; all yielded a pH of 8.5. Afterwards, the extract of pH was determined.

| Table 1. Materijali, proizvođači i sastav | Table 1. Materials, manufacturer and composition. |
| Dentinski adheziv • Dentin adhesive | Proizvođač • Manufactured | Sastav • Components |
| Xeno V (XE) | DENTSPLY De Trey GmbH (Konstanz, Baden-Württemberg, Njemačka • Germany) | Bifunkcijski akralati, kiseli akralati, esteri fosforne kiseline, akralna kiselina, voda, dl-kamforkinon, tercijarni butan, stabilizatori • Bifunkcional acrylate, acidic acrylate, functionalized phosphoric acid ester, acryl acid, water, dl-camphorquinone, tertiary butan, stabilizer. |
| Excite F DSC | Ivoclar Vivadent, Schaan, Lichtenstein • Liechtenstein dvokomponentni adheziv • Two-step adhesive | HEMA, akral fosfonске kiseline, Bis-GMA, dimetakrilati, silicijev dioksid, etanol, katalizatori, stabilizatori • HEMA, phosphonic acid acrylate, Bis-GMA, dirue thacrylates, silica, ethanol, catalysts, stabilizers. |
| Adhese One F | Ivoclar Vivadent, Schaan, Lichtenstein • Liechtenstein samojetkajući • Self-adhesive | Primer: akralni etet fosfonске kiseline, bisakrilamid, voda, kamforkinon, stabilizatori. Bond: Bis-GMA, GDMA, HEA, pirogeni silicijev dioksid, CQ, tercijarni amin, stabilizatori • Primer: acryl ether phosphonic acid, bisacrylamide, water, Camphorquinone, stabilizers. Bonding: Bis-GMA, GDMA, HEA, fumedsilica, CQ, tertiary amine, stabilizers. |
| Prime & Bond NT/ NRC | DENTSPLY De Trey (Konstanz, Njemačka • Germany) dvokomponentni adheziv • Two-step adhesive | Adheziv: PENTAl, UDMA, cetilamid hidrofluorid, acetone, nanopunila (amorfni silicijev dioksid 8 nm), stabilizatori • Adhesive: PENTA, UDMA, cetilamine hydrofluoride, acetone, nanofiller (amorphous silicon dioxide 8 nm), stabilizers. |
S medijem za kultiviranje bez fenolnog crvenila i odgovarajućim ekstraktom, pripremljene su otopine 1/1 (100 % ekstrakta), 1/2, 1/4, 1/8 i 1/16 za svaki materijal i izmjerenja je osmrtnost otopina. Te su otopine dodane stanicama 24 sata nakon kultiviranja. Također su dodane otopine metil-metakrilata od 10 %, 5 %, 2,5 % i 1,25 % koje su korištene kao pozitivne kontrole. Da bi se procijenio utjecaj pH na vijabilnost stanica, uključene su jažice s medijem za kultiviranje bez fenolnog crvenila koje su služile kao negativna kontrola zajedno s drugim jažicama s medijem za kultiviranje pripravljenim na pH 8. Ploče su zatim 24 sata inkubirane u 7,5 % CO₂. Provedeno je ispitivanje citotoksičnosti metil-tetrazolija (MTT) (MTT, Sigma Chemical Co. St. Louis, MO, SAD), mjerenjem apsorbancije u čitaču na 570 nm, koristeći se valnom duljinom od 690 nm kao referencijom. Nakon 24 sata izmjeren je pH ekstrakta – kod svih je vrijednost pH iznosio 8.

Rezultate je tumačio tehničar koji nije znao koji su materijali bili uključeni u različite uzorke. Citotoksičnost je analizirana i kvantitativno (postotak preživljavanja u odnosu na kontrolu) i kvalitativno (morfološka stanica i sposobnost preživljavanja).

Statistička analiza

Podaci su podvrgnuti dvosmjernoj univarijantnoj analizi varijance (ANOVA), dopunjenoj ispitivanjem podudaranja parova uz korištenje metode najmanje statistički značajne razlike (LSD) s Bonferroniijevom korekcijom.

Rezultati

Na slici 1. su postotci vijabilnosti pulpnih fibroblasta. Kvantitativni rezultati citotoksičnosti (postotak vijabilnosti u usporedbi s kontrolom) dobiveni su za svaki materijal. Za sve materijale se vijabilnost stanica smanjivala kako se povećava usporedbi s kontrolom) dobiveni za svaki materijal. Za sve materijal sadržavali su zaobljene stanice kod kojih se dogodila degeneracija (slike 3. i 4.). Each material was aspirated with a sterile syringe and filtered through a pore diameter of 0.45 μm.

With culture medium without phenol red and the corresponding extract, 1/1 (100% extract), 1/2, 1/4, 1/8 and 1/16 dilutions were prepared for each material, and the osmolarity of the dilutions was measured. These dilutions in turn were added to the cells 24 hours after seeding of the latter in 96-well culture plates. Methyl methacrylate dilutions of 10%, 5%, 2.5% and 1.25% were also added and used as positive controls. To assess the influence of pH upon cell viability, wells containing culture medium without phenol red were included, which served as negative controls, together with other wells containing culture medium prepared at pH 8. The plates were then incubated under 7.5% CO₂ for 24 hours, and methyl thiazol tetrazolium (MTT) cytotoxicity assay was performed (MTT; Sigma Chemical Co. St. Louis, MO, USA), measuring absorbance in a plate reader at 570 nm, using a wavelength of 690 nm as reference. After 24 hours, the extracts’ pH was measured: all presented a pH of 8.

The results were interpreted by a technician blinded as to which materials were involved in different samples. Cytotoxicity was analyzed both quantitatively (% viability with respect to control) and qualitatively (cell morphology and viability).

Statistical analysis

Data underwent two-way univariate analysis of variance (ANOVA), supplemented by equality of matched pairs testing, using the least significant difference (LSD) method, with Bonferroni correction.

Results

Figure 1 shows percentages of pulp fibroblast cell viability. Quantitative cytotoxicity results (% viability in comparison with the control) obtained for each material are expressed in Figure 1. For all materials, cell viability decreased as the concentration of extracts was increased. No significant differences were identified between concentrations 1, 2, and 3. Nor were differences obtained between 4 and 5, with the exception of Prime and Bond.

The least cytotoxic of the adhesives tested was Adhese, followed by Excite, Xeno and Prime and Bond (the most cytotoxic). Their effects on cell viability varied with statistically significant differences (p<0.001).

It was observed that pH 8 reduced cell viability, which was reduced by 40% in comparison with the control (Figure 2).

In the qualitative evaluation of cytotoxicity (compared with controls), methyl methacrylate had an effect evidenced by cell rounding and the disappearance of the cell nucleus. In the case of the negative control (culture medium), the cells were seen to maintain their characteristic elongated shape and the nucleus remained intact. In general, the materials tested included some rounded cells undergoing degeneration. (Figures 3 and 4).
Rasprava

Biocompatibilnost je osnovno svojstvo svakoga dentinskih materijala, a posebno je značajna u slučaju dentinskih adheziva koji se nalaze u neposrednoj blizini pulpe. Smola- 

stoni i druge komponente adhezivnih sustava mogu uzrokovati različite razine oštćenja stanica zbog razlika u kemijskom sastavu (29). Interakcije između tih komponen-
ti i dentina potiču različite reakcije pulpnog tkiva (30). Tako

testirani adhezivi imaju različite razine citotoksičnosti, vjerojatno zbog varijacija u kemijskom sastavu, fizičkim svojstvi-

ma i načinu primjene.

Pri procjeni citotoksičnosti u istraživanjima se koriste raz- 

ličite metode kontakta stanica i materijala (31). Za ispitiva-

nje citotoksičnosti Međunarodna organizacija za normiranje 
(ISO) (28) preporučuje uporabu etabliranih staničnih linija, uključujući L-929, Balb/3T3 i WI-38. One imaju homoge-

nu morfolingu i svojstvo rasta te tako olakšavaju ponovljivost 
ispitivanja citotoksičnosti in vitro (32). U ovom istraživa-
nju odabrana je stanična linija L-929 jer je dostupna, često se 
upotrebljava u sličnim istraživanjima i ima povoljna svojstva 
in vitro situacijama.

Materijali za indirektno prekrivanje pulpe nisu u izravnom doticaju s pulmom pa se primjenjuje ispitivanje u indirektom kontaktu jer omogućuje realnije in vitro uvjete analize citotoksičnosti adheziva. Toksčini učinci na stanice procijenjeni su MTT testom. Ta analiza reducira metil-tia-
zol-tetrazolij u obojeni formazan. Boja reagira na čimbenike koji inhibiraju aktivnost dehidrogenaze (33, 34).

Chen i suradnici (35) utvrdili su da su u pulnim sta-
icama u kontaktu s adhezivima nastali različiti citotoksični učinci tijekom 24 sata, te da je to ovisilo o razrijedenosti adheziva, tako da se citotoksičnost povećava proporcional-

Discussion

Biocompatibility must be a fundamental property of any dental material and this is of particular relevance in the case of dentin adhesives in close proximity to the pulp. Resinous monomers and other components of adhesive systems can cause varying levels of cell damage due to differences in chemical composition (29). Interactions between these components and dentin will lead to varying pulp tissue responses (30). In this way, the evaluated adhesives produced different levels of cytotoxicity, probably due to variations in chemical composition, physical properties, and the method of application.

When evaluating cytotoxicity, research has employed different methods of cell-to-material contact (31). For cytotoxicity testing, The International Organization for Standardization (ISO), (28) recommends the use of established cell lines including L-929, Balb/3T3 and WI-38. These offer homogeneous morphology and growth characteristics and so facilitate reproducibility in in vitro cytotoxicity testing (32). The present study selected the L-929 cell line as it is readily available, has been widely used in similar research, and behaves efficiently in in vitro situations.

Indirect pulp capping materials do not enter into direct contact with the pulp; therefore, indirect contact testing was used, since it could provide more realistic in vitro conditions for testing the cytotoxicity of the adhesives. The toxic effects on cells were evaluated using the MTT assay. This assay reduces methyl thiazol tetrazolium metabolically to colored formazan; the color reacts to the factors inhibiting dehydrogenase activity (33, 34).

Chen et al. (35) observed that adhesives might cause cyto-
toxicity in pulp cells when they came into close contact for 24 hours, which depended on their dilution, hence cytotox-
no njihovoj koncentraciji. Citotoksični učinci smanjivali su se razrjeđenjem materijala zbog sve manje koncentracije toksičnih sastojaka.

Dentinski adhezivi sadržavaju različite kombinacije i različite koncentracije metakrilatnih monomera: Bis-GMA-e, HEMA-e, UDMA-e i PENTA-e. Varijacije u njihovoj koncentraciji utječu na toksičnost svakog materijala. Analize citotoksičnosti akralita i metakrilata u dentalnim materijalima pokazuju različite vrijednosti koje ovise o strukturi (36). TEGDMA, Bis-GMA i UDMA imaju umjerenu razinu citotoksičnosti (36, 27). Ratanasathien i suradnici (17) ispitivali su citotoksičnost sastojaka dentinskih adheziva i rangirali su toksičnost od najviše do najniže kako slijedi: Bis-GMA > UDMA > TEGDMA > HEMA nakon 24 sata i 72 sata izloženosti. Kusdemir i suradnici (10) također su izvijestili da je primer korišten s dvokomponentnim samojetkajućim adhezivom temeljenim na HEMA-i imao nižu razinu citotoksičnosti od jednokomponentnog bonda koji sadržava monomerije veće molekularne težine.

Dosađena istraživanja pokazala su da se tipične komponente adheziva i materijala za ispune, kao što su HEMA i TEGDMA, mogu isiriti kroz dentinske tubule i prodirjeti u pulp u koncentracijama milimila (13, 18). In vitro se pokazalo da čak i pri netoksičnoj razini ti monomeri mogu potremiti normalne postupke diferencijacije pulpcnih fibroblasta (13, 18). To otkriće u skladu je s rezultatima još jednog istraživanja koje je potvrdilo da su HEMA i TEGDMA štetne za diferencijaciju matičnih/progenitorskih stanica u odontogene, što negativno utječe na homeostazu i regeneraciju pulpne tkive (37 – 40). U dubokim kavitetima rezidualni monomeri mogu stići do pulp difuzijom i lakše prodirjeti kada je dentin najetkan. U određenim koncentracijama oni toksično djeluju na stanice pulpne, što rezultira upalom i disorganizacijom tkiva. Pulpcne reakcije variraju ovisno o dodatnim čimbenicima, uključujući sastav materijala i primijenjene kliničke tehnike (41).

U ovom istraživanju Adhese je imao nisku toksičnost, što je u skladu s rezultatima drugih istraživanja (42). To je zbog HEMA-e u sastavu. Bis-GMA ima najveću toksičnost među Adheseovim komponentama, ali i manji kapacitet prodiranja u dentin zbog veće molekularne težine (28, 29). No, Bis-GMA podliježe hidrolizi, stvarajući metakrilin kiselinu kao metabolit topljiv u vodi (MAA). MAA je izvor citotoksičnosti jer može stimulirati otpuštanje TNF-α, ili mijenjati lipidni sloj staničnih membrana, a to utječe na propusnost membrane (43). UDMA je toksičnija za stanice od HEMA-e. Huang i Chang (29) utvrdili su veću citotoksičnost Prime & Bond, što objašnjavaju prisutnošću UDMA-e u sastavu. I u ovom istraživanju adhezivi koji sadržavaju UDMA-u pokazali su se više citotoksičnijima, pri čemu je Prime & Bond najcitotoksičniji. Ovaj rezultat slaže se s drugim istraživanjima koja su pokazala da je Prime & Bond početno vrlo citotoksičan (10).

U ovom radu nije pronadena značajna razlika između adheziva Xeno i Excite. Takav nalaz ne podrudava se s drugim autorima (11) koji su uočili da Xeno pokazuje manju citotoksičnost jer ima manju tendenciju degradacije, više stabilnih molekula i ne sadržava HEMA-u ili Bis-GMA-u.
Kamferokinon, as reported by other authors (11), has a lesser tendency to degrade, and contains HEMA or bis-GMA.

Camphorquinone (CQ) may be another cause of dentin adhesive cytotoxicity, being the most frequently used photoinitiator (44). This substance was present in all the materials evaluated. Camphorquinone (CQ) may be another cause of dentin adhesive cytotoxicity, being the most frequently used photoinitiator (44). This substance was present in all the materials evaluated (Table 1) and could affect cell metabolism, a possible mechanism provoking negative clinical and subclinical responses (42).

With regard to control samples, pH was seen to be a non-specific variable that influenced the total cell viability. Thus, viability was seen to reduce due (partly) to this factor rather than the specific toxicity of the material; for this reason, the results obtained could achieve greater reliability by controlling this variable.

The thickness of the Dentin can have an effect on both the concentration and quantity of the adhesive reaching the pulp area. Hamid and Hume (45) investigated the influence of dentin thickness on the level of penetration by the resin monomers in bonding agents after 24 hours incubation, testing dentin slices of 0.4-3.6 mm thickness. The diffusion rate was inversely proportional to the area of dentin consisting of dentinal tubules. Toxicity decreases as dentin thickness increases; if it is greater than 0.5 mm, toxicity is reduced by 75%, and if greater than 1 mm, toxicity falls by 90% (46). Therefore, dentin thickness in IPC is a determining factor for controlling the toxicity of adhesive systems.

Conclusions

Both self-etching and two-step adhesive systems show high cytotoxicity, which decreases as dilution increases. Adhesives presented the highest biocompatibility among the adhesives that were evaluated, and the lowest cytotoxicity. Next in order was Excite, found to present moderate cytotoxicity. Xeno presented high cytotoxicity, and the lowest cytotoxicity, as UDMA is its main component. Further studies are needed to determine which of the components of the material are responsible for harmful effects on cells. Such studies will need to take into account other physical and chemical properties of adhesives, which could affect the successful treatment.

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Conflict of Interest

None declared
Abstract
In many cases, the indirect pulp treatment (IPT) is an acceptable treatment for deciduous teeth with reversible pulp inflammation. Various medicaments have been used for IPT, ranging from calcium hydroxide and glass ionomers to dentin adhesives. Objective: This in vitro trial aimed to measure cytotoxicity in a cell culture, comparing the following four adhesives: Xeno® V (XE), ExciGal® F DSC (EX), Adhese® OneF (AD) and Prime & Bond NT (PB). Materials and methods: The adhesives were prepared according to the manufacturer’s instructions. After 24 hours of exposure, the cell viability was evaluated using a photometrical test (MTT test). Data were subjected to analysis of variance (ANOVA). Results: Adhesives, the main component of which was 2-hydroxyethyl methacrylate (HEMA), were found to be less cytotoxic, while those that included the monomer urethane dimethacrylate (UDMA) were the most cytotoxic in their composition. The effects on cell viability assay varied between the adhesives assayed with statistically significant differences. Conclusions: The results may support the argument that Adhese® OneF is the least cytotoxic of the adhesives assayed, and may be considered as an adhesive agent for indirect pulp treatment. However, Prime and Bond NT showed a reduced biocompatibility under the same conditions.

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