In vitro biocompatibility of preheated giomer and microfilled-hybrid composite

Introduction

Composite materials have been successfully used for many years as dental restorative materials due to their mechanical and excellent esthetic properties. They are used in everyday clinical practice not only as restorative materials but also as liners or as luting agents for cementation of inlays, onlays, crowns, veneers, and orthodontic brackets. With CAD/CAM appearance on the market, the use of composite materials as a luting material becomes more popular. Besek et al. first introduced the use of composite materials for CAD/CAM bonding at the time when CAD/CAM system was not so accurate. Due to mechanical properties of composite materials and extended curing time Besek et al. introduced composite materials as a luting agent with the aim to prevent microleakage, postoperative sensitivity, recurrent caries and to improve overall aesthetic appearance of a cemented restora-

Uvod

Kompozitni materijali uspješno se koriste kao stomatološki restaurativni materijal već godinama zbog svojih mehaničkih i estetskih svojstava. U svakodnevnoj kliničkoj praksi nisu samo materijal za nadomjestak izgubljenog tvrdog zubničkog materijala nego i svojevrsna podloga te sredstvo za adhezijsko cementiranje inleja, onleja, krunica, ljuskica te ortodontskih tlova nego i svojevrsna podloga te sredstvo za adhezijsko cementiranje. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018. Prihvaćen: 30. studenoga 2018.

Sažetak

Svrha: Svrha rada bila je izpitati citotoksičnost dvaju svjetlosno stvrdnjavajućih kompozitnih materijala nakon zagrijavanja na različitim temperaturama te osvjetljanja izravno i preko CAD/CAM cedera. Materijali i postupci: Kompozitni materijali (Gradia Direct Posterior i Beautifill II) zagrijani su preuredaji za zagrijavanje Calset na tri različite temperature (T1: 57 °C, T2: 54 °C, T3: 68 °C). Primarno je mala količina postavljena na okrugli kalup (promjera 6 mm; debljine 0,65 mm), prekrivena Mylar folijom, te sprešana i polimerizirana LED uređajem Bluephase. Jedna skupina uzoraka polimerizirana je izravno, a ostale preko CAD/CAM polimersa pojačanim keramikom (CRP) i CAD/CAM litijevog disilicija sredstvom za adhezijsko cementiranje postaje sve bravc (1).

Zaključak: Osim sastavom kompozitnog materijala, citotoksičnost je uvjetovana načinom polimerizacije i vremenom osvjetljanja te temperaturom prethodnog zagrijavanja. U ovom istraživanju je uočeno grijanje na 54 °C (T3), a uzorci polimerizirani preko CAD/CAM cedera pokazali su manju citotoksičnost negoli oni izravno polimerizirani. Završetak: Osim sastavom kompozitnog materijala, citotoksičnost je uvjetovana načinom polimerizacije i vremenom osvjetljanja te temperaturom prethodnog zagrijavanja kompozitnog materijala.
1. Materials heated on lower temperature will cause less cytotoxicity regardless of the curing time and polymerization pattern (directly polymerized or through CAD/CAM overlay).

2. Longer curing time will cause less cytotoxicity regardless of the curing time and polymerization pattern (directly polymerized or through CAD/CAM overlay).

3. Samples polymerized directly will show less cytotoxicity of the polymerized material (3 – 7). However, some studies reported that pre-heating of a composite material may cause negative effects on the composite restoration margins due to the increased polymerization shrinkage in heated composite resin (7). On the contrary, some studies showed that pre-heating of a composite material before light-curing did not alter mechanical properties and monomer conversion but did provide enhanced adaptation of composite materials to the cavity walls (4). Deb et al. (8) confirmed in their study that a pre-heated composite material produced more shrinkage than non-heated composite, but still less than flowable composite materials. The same authors also concluded that there is no difference between cytotoxicity of heated and non-heated composite materials. Reactive components released from unpolymerized or underpolymerized composite resins may induce toxicity or inflammatory tissue responses depending on their aggressiveness and the thickness of the remaining dentin on the cavity floor (9, 10).

The aim of the present investigation has been to evaluate and compare cytotoxic potencies of one micro hybrid composite material (Gradia Direct Posterior) and one glass composite material (Beautifil II) after heating at different temperatures and cured directly and through CAD/CAM overlay for 20 and 40 seconds. Isolated human peripheral lymphocytes were used as a model system. For the purpose of the study following null hypotheses were established:

1. Materials heated on lower temperature will cause less cytotoxicity regardless of the curing time and polymerization pattern (directly polymerized or through CAD/CAM overlay).

2. Longer curing time will cause less cytotoxicity regardless of the curing time and polymerization pattern (directly polymerized or through CAD/CAM overlay).

3. Samples polymerized directly will show less cytotoxicity than the samples polymerized through CAD/CAM overlay regardless of the temperature used.

**Materijali i postupci**

Preparation of CAD/CAM ceramic-reinforced polymer (CRP), CAD/CAM lithium disilicate ceramic (LDC), gionomer and composite samples

Two blocks of the CRP CAD/CAM (shade A2, block size 14L, 3M ESPE, LAVA Ultimate, St. Paul, MN, USA), and LDC CAD/CAM (shade A2, block size 14L, e.max, Ivoclar

**Materials and methods**
Veličina bloka 14L, e.max, Ivoclar Vivadent, Amherst, NY, USA), CRP i blokovi LDC CAD/CAM-a izrezani su dijamantnom pilom na uzorke debljine 2 mm. Uzorci su zatim polirani pod vodenim hlađenjem s obje strane u uređaju za poliranje (11). Svaki LDC CAD/CAM uzorak popraskan je sprejem za glazuru (Crystall/Glaze Spray; Ivoclar/Vivadent, Schaan, Liechtenstein) te stavljen u uređaj Pro 100 (Whip-Mix; Louisville, KY) prema uputama proizvođača.

Beautifil II (SHOFU Dental GBH; Ratingen, Germany) jest nano-hibridni kompozitni materijal koji je uvršten u skupinu Giomer. Prema podacima proizvođača, sadržava tvz. površinske čestice stakla koje su već reagirale i mogu o primati fluoridne, natrijeve, stroncijeve, aluminijanske, silikatne i boratne ione, te bisfenol glicidil dimetakrilat (Bis-GMA) i trietilen glikol dimetakrilat (TEGDMA).

Gradia Direct Posterior (GC, Europe N.V.; Leuven, Belgium) mikropunjeni je hibridni kompozitni materijal. Prema podacima proizvođača sadržava mikrofone prepolidirane čestice punila (silika 19 % težinskog udjela, prosječna veličina čestica 0,85 µm; prepolidirano punilo 20 % težinskog udjela). Organski matriks mješavina je uretan-dimetakrilata (UDMA) i dimetakrilatnih konomomera (23 % težinskog udjela). Zbog radiopaknosti dodano je fluoro-aluminosili catno staklo (38 % težinskog udjela).

Za zagrijavanje kompozitnih materijala korišten je uređaj Calase (AdDent Inc., Danbury, Connecticut, USA). Proizvođač preporučuje zagrijavanje kompozita na temperaturu iznad 68 °C. U ovom radu korištene su tri različite temperature - T1: 37 °C, T2: 54 °C i T3: 68 °C. Ampule kompozitnih materijala stavljaju se u kućište s poklopcem te je potrebno optrijepiti za 20 i 40 sekundi, odnosno za 10 minuta da se kompozit zagrije na željenu temperaturu.

Uzorci za ispitivanje citotoksičnosti pripremljeni su na sljedeći način: mala količina kompozitnog materijala, prije tog zagrijana na T1, T2 ili T3, stavljen je u kulup u obliku prstena promjera 6 mm i debljine 0,65 mm. Kulup je zatim stavljena na okrutku pločicu od plemenitog čelika debljine 5 mm prekrivenu Mylar folijom. Nakon toga ispunjen je kompozitnim materijalom odnosno te prekriven Mylar folijom. Zatim je uzorak pritisknut drugom pločicom od plemenitog čelika istih dimen sija kao i prva pločica kako bi se dobio homogeni uzorak iste debljine kao i kulup (0,65 mm) (11). Mylar folija korištena je između ostalog i da bi se spriječila nastajanje sloja inhibiranog kisikom na površini polimeriziranoga kompozitnog materijala. Nakon što je uzorak kompozitnog materijala spojen, polimeriziran je uređajem za polimerizaciju Bluephase (Vivadent, Schaan, Liechtenstein) upotrebom programa visokog intenziteta (1180 mW/cm²) u trajanju 20 i 40 sekunda. Pripremljeni su po sedam uzoraka za svaku ispitivanu skupinu. Korištena su tri načina polimerizacije uzoraka kompozitnog materijala: (1) izravna polimerizacija preko Mylar folije (2) polimerizacija preko Mylar folije iznad koje je postavljen CAD/CAM CRP overlap debljine 2 mm (3) polimerizacija preko Mylar folije iznad koje je postavljen CAD/CAM LDC overlap debljine 2 mm.

Tako pripremljeni uzorci nakon polimerizacije i usklađenja Mylar folije urojene su izravno u staničnu kulturu limfo cica.

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Kultura ljudskih limfocita izoliranih iz periferne krvi

Ovo istraživanje odobrilo je Etičko povjerenstvo Stomatološkog fakulteta u Zagrebu, Hrvatska. Kako bi se izbjegle moguće interindividualne razlike u odgovoru na tretman, u istraživanju je korišten uzorak venske krvi jednog zdravog muškog donora (39 godina, nepušač) bez povijesti kronične ali akutne bolesti. Prije uzimanja uzorka krvi, donor je bio obaviješten o postupku i svrhi uzimanja krvi, zatim o svrhi testiranja uzetoga uzorka te je potpisao informirani pristanak.

Venska krva izvijedena je sterilnim priborom za jednokratnu upotrebu u spreminik s litijevim heparinom (Becton Dickinson, UK). Odmah je obavljena izolacija limfocita u skladištu s uputama proizvođača reagensa Histopaque-1077 (Sigma Chemical Co., St. Louis, MO, USA).

Suspensija izoliranih limfocita podijeljena je na manje volumene koji su prebačeni u sterline epruvete (Nange Nunc Int, Naperville, IL, USA) napunjene hranjivim medijem za stanične kulture RPMI 1640 (Gibco Invitrogen, UK) kako bi se postigla gustoća od 50 000 limfocita po kulturi. Ukupni volumen takoj pripremljenih kultura iznosio je 7 ml. U kulturi nije dodavan ni mitogen ni teleći serum.

Kulture limfocita tretirane su testiranim materijalom u polimeriziranom i nepolimeriziranom stanju. Ovi testirani materijali su stupnjevano prebačeni u sterilne epruvete i držani 24 sata u inkubatoru za uzgoj staničnih kulture (Heraeus Hera Cell 240 Incubator, Langenselbold, Germany) na temperaturi od 37 °C i 5 % CO₂. Isti postupak korišten je i u ranijim istraživanjima (11 – 13).

Kvantitativna fluorescencijska metoda za procjenu preživljenja stanica, apoptoze i nekrose

Nakon 24-satnog tretmana kulture su centrikugirane 10 minuta brzinom od 600 okr./min. Supernatant je uklonjen, a zatim je korištena supernatantna limfocita. Aliquots of lymphocyte suspension (V=20 µl) were pipetted, put on the microscope slide and mixed with the same volume of ethidium bromide and acridine orange dyes (Sigma-Aldrich, USA), prepared in final concentrations of 100 µg/ml (1:1; v/v). After covering the preparation with a coverslip, lymphocyte viability was immediately evaluated under a fluorescence microscope (Olympus BX 51; 400 x magnification; Olympus, Tokyo, Japan), analyzing the appearance of nuclei with an intact structure.

S pomoću mikropipete uzorci stanične suspenzije (V=20 µl) preneseni su na predmetno skaklo i pomiješani s jednakim volumenom boja – etidijeva bromida i akridinske narančaste boje (Sigma-Aldrich, USA) koje su pripremljene u koncentracijama od 100 µg/ml (1 : 1 ; v/v). Neposredno nakon bojenja, na fluorescencijskom mikroskopu (Olympus BX 51; 400 x magnification; Olympus, Tokyo, Japan) analizirano je preživljenje limfocita primjenom metode dvojnom bojenja (14). Kvantitativna procjena obavljena je određivanjem postotka živih, apoptotičnih i nekrotičnih stanica. Kako žive stanice u svoj DNK ne ugrađuju etidijev bromid, jezgra im je nakon bojenja po dvije boje (17). apoptotične su izrazito zelene i imaju visoko koncentrirana ili fragmentirana jezgra.

Za svaki uzorak obavljena su tri uzastopna testa, te je ukupno pregledano 300 stanica po uzorku. Usporedo s testiranim uzorcima, u istim je uvjetima držan i kontrolni uzorak, (3) Polymerization through the Mylar sheet overlaid with 2 mm thick CAD/CAM LDC overlay.

So prepared samples were removed from the mold and the Mylar sheet and placed directly into the lymphocyte cell cultures.

Primary lymphocyte cultures

This study was approved by the Ethical Committee, School of Dental Medicine, University of Zagreb, Croatia. To overcome possible inter-individual differences in response to the treatment, a blood sample was obtained from one healthy male donor (age 39 years, non-smoker), with no medical records of chronic or acute adverse health conditions. Prior to blood sampling, the donor was acquainted with the procedure, purpose of blood donation, and the aim of blood testing. He signed an informed consent. Venous blood (40 ml) was collected under sterile conditions in heparinized vacutainer tubes (Becton Dickinson, UK) containing lithium heparin as anticoagulant. Lymphocytes were freshly isolated using the Histopaque-1077 reagent (Sigma Chemical Co., St. Louis, MO, USA) according to the manufacturer’s instructions. Following the isolation, 50,000 lymphocytes were seeded in sterile tubes (Nange Nunc Int, Naperville, IL, USA) in RPMI 1640 culture medium with penicillin and streptomycin (Gibco Invitrogen, Paisley, UK). The final culture volume was 7 ml. No newborn calf serum or mitogen was added. Each culture was treated for 24 h with 0.06 g of unpolymerized or polymerized tested material at 37 °C in a 5 % CO₂ atmosphere (Heraeus Hera Cell 240 incubator, Langenselbold, Germany). The same study design has been proved in our previous investigation (11 – 13).

Quantitative fluorescent assay for the assessment of cell viability, apoptosis and necrosis

After 24 hours of treatment, the cultures were centrifuged at 600 rpm for 10 minutes, supernatant was removed and the remaining pellet was gently re-suspended. Aliquots of lymphocyte suspension (V=20 µl) were pipetted, put on the microscope slide and mixed with the same volume of ethidium bromide and acridine orange dyes (Sigma-Aldrich, USA), prepared in final concentrations of 100 µg/ml (1:1; v/v). After covering the preparation with a coverslip, lymphocyte viability was immediately evaluated under a fluorescence microscope (Olympus BX 51; 400 x magnification; Olympus, Tokyo, Japan), applying a dye exclusion method (14). Quantitative assessments were made by determination of the percentage of viable, apoptotic and necrotic cells. Viable cells with intact plasma membrane excluded ethidium bromide and the appearance of their nuclei with an intact structure was bright green. Non-viable necrotic cells had orange to red colored chromatin with organized structure, while apoptotic cells were bright green with highly condensed or fragmentated nuclei. Three tests with aliquots of the same sample were performed and a total of 300 cells per sample were counted. The untreated lymphocyte culture was studied in parallel as a control group.
Rezultati

Rezultate kvantitativne fluorescencijske metode za procjenu preživljenja stanica, apoptoze i nekroze u uzorcima limfocita inkubiranih nepolimeriziranim i polimeriziranim kompozitnim materijalima Beautifil II i Gradia Direct Posterior, vidi u tablicama 1. i 2.

Polimerizacija 20 sekunda

Profil citotoksičnosti za oba ispitivana materijala prikazan je na slici 1. Nepolimerizirani Beautifil II ima značajno veću citotoksičnost negoli Gradia Direct Posterior (P < 0,0001). Nakon izravne polimerizacije na temperaturama T1 i T3, Beautifil II pokazuje nižu citotoksičnost negoli Gradia Direct Posterior, no razlika je bila statistički značajna samo za T3 (P = 0,0013). Na toj temperaturi za Gradia Direct Posterior uočena je statistički značajna veća učestalost nekroze limfocita.

Polimerizirani 40 sekunda

D – izravna polimerizacija • directly polymerized; T1, T2, T3 – temperature zagrijavanja • polymerization temperatures; CRP - CRP CAD/CAM overlej • overlay; LDC - LDC CAD/CAM e.max overlej • e.max overlay

Analizirano je 300 stanica po uzorku za svaku ispitivanu točku. • 300 cells per sample per each experimental point were analysed.

Statističke značajnosti utvrđene su primjenom Pearsonova $\chi^2$-testa • Statistical significance of data was evaluated using Pearson $\chi^2$-test.

Results

The results of the quantitative fluorescent assay for simultaneous identification of apoptotic and necrotic cells in lymphocyte samples incubated with non-polymerized and polymerized Beautifil II are shown in Table 1 and 2.

Polymerization time 20 seconds

Cytotoxicity profiles of both tested materials are shown in Figure 1. Unpolymerized Beautifil II had a significantly higher cytotoxicity than Gradia Direct Posterior (P<0.0001). After direct polymerization at T1 and T3, Beautifil II had lower cytotoxicity than Gradia Direct Posterior, but the difference was statistically significant only at T3 (P=0.0013). At this polymerization temperature, Gradia Direct Posterior caused a significantly higher frequency of lymphocyte necrosis than...

Tablica 1. Rezultati kvantitativne fluorescencijske metode za procjenu preživljenja stanica, apoptoze i nekroze; limfociti su tretirani u uvjetima in vitro 24 sata nepolimeriziranim i polimeriziranim kompozitnim materijalim Beautifill II; usporedo je promatrana kontrolna skupina netretiranih stanica

| Materijal • Material – Beautifil II | Žive stanice • Viable cells (%) | Mrteve stanice • Non-viable cells (%) |
|-----------------------------------|---------------------------------|--------------------------------------|
|                                   | Σ                               | Apoptozo • Apoptosis | Nekroza • Necrosis |
| Kontrola • Control                | 97.3±1.5                        | 2.7±1.5                           | 2.0±1.0 | 0.7±1.2 |
| Nepolimerizirani • Unpolymerized  | 16.0±6.6                        | 84.0±6.6                         | 24.3±3.5 | 59.7±8.7 |
| Polimerizirani 20 sekunda • Polymerized for 20 seconds |
| D–T1                              | 87.7±2.1                        | 12.3±2.1                          | 8.0±1.0 | 4.3±1.2 |
| D–T2                              | 56.7±10.7                       | 43.0±11.1                        | 22.3±11.1 | 20.7±0.6 |
| D–T3                              | 78.7±4.2                        | 21.3±4.2                          | 17.0±4.6 | 4.3±2.5 |
| CRP–T1                            | 92.0±1.0                        | 8.0±1.0                           | 5.7±2.1 | 2.3±1.2 |
| CRP–T2                            | 82.7±8.6                        | 17.3±8.6                         | 14.0±6.2 | 3.3±2.5 |
| CRP–T3                            | 68.3±8.0                        | 31.8±8.0                         | 15.0±9.5 | 16.7±3.8 |
| LDC–T1                            | 90.3±1.5                        | 9.7±1.5                           | 4.3±3.2 | 5.3±2.1 |
| LDC–T2                            | 64.3±3.2                        | 39.0±8.2                         | 22.7±1.5 | 13.0±2.0 |
| LDC–T3                            | 81.0±7.5                        | 19.0±7.5                         | 13.3±7.5 | 5.7±1.2 |
| Polimerizirani 40 sekunda • Polymerized for 40 seconds |
| D–T1                              | 83.3±2.1                        | 16.7±2.1                          | 9.7±2.3 | 7.0±1.7 |
| D–T2                              | 67.0±6.6                        | 33.0±6.6                         | 11.7±4.7 | 21.3±8.4 |
| D–T3                              | 72.7±5.0                        | 27.3±5.0                         | 17.3±6.5 | 10.0±6.2 |
| CRP–T1                            | 75.7±3.8                        | 24.3±3.8                         | 14.3±1.5 | 10.0±3.0 |
| CRP–T2                            | 81.0±1.0                        | 19.0±1.0                          | 11.3±3.5 | 7.7±4.2 |
| CRP–T3                            | 74.0±7.8                        | 26.0±7.8                         | 11.0±3.6 | 15.0±4.4 |
| LDC–T1                            | 75.7±2.5                        | 24.3±2.5                         | 12.3±0.6 | 12.0±2.0 |
| LDC–T2                            | 84.3±1.5                        | 15.7±1.5                         | 11.3±0.6 | 4.3±1.5 |
| LDC–T3                            | 77.0±4.6                        | 23.0±4.6                         | 12.0±1.7 | 11.0±4.6 |

Bilješka • Note:

Knezevic i sur. Kompatibilnost prethodno zagrijanog kompozita

tj. netretirana limfocitna kultura.

Procjena statističke značajnosti rezultata dobivenih za preživljenje stanica, apoptozu i nekrozu u tretiranim i kontrolnim uzorcima učinjena je Pearsonovim hi-kvadrat testom. Kao prag statističke značajnosti koristen je $p < 0.05$. Comparisons between values obtained for the cell viability treated and control samples were made by the Pearson’s $\chi^2$ test for two-by-two contingency tables. Statistical decisions were made at a significance level of $p < 0.05$.
Beautifil II (P < 0,0001). Suprotno je ustanovljeno za temperaturu zagrijavanja T2, kada je Gradia Direct Posterior pokazao veću citotoksiknost negoli Beautifil II (P = 0,0003). Nakon polimerizacije preko CRP CAD/CAM overlay na temperaturi T1, Gradia Direct Posterior pokazao je veću citotoksiknost negoli Beautifil II (P = 0,0015). Nakon polimerizacije preko LDC CAD/CAM overlay na temperaturi T1, Gradia Direct Posterior pokazao je veću citotoksiknost negoli Beautifil II (P < 0,0001). Slično je uočeno i pri temperaturi zagrijavanja T2 – Gradia Direct Posterior pokazao je veću citotoksiknost negoli Beautifil II (P = 0,0037, zbog većeg udjela nekrotičnih stanica – P = 0,0001). Na temperaturi zagrijavanja T3, Gradia Direct Posterior pokazao je veću citotoksiknost negoli Gradia Direct Posterior, no razlika nije bila statistički značajna. Analizirano je 300 stanica prema uzorku za svaku ispitivanu točku. Bilješka • Note: D – izravna polimerizacija • directly polymerized; T1, T2, T3 – temperature zagrijavanja • polymerization temperatures; CRP - CRP CAD/CAM overlay • overlay; LDC - LDC CAD/CAM e.max overlay • e.max overlay; Analizirano je 300 stanica prema uzorku za svaku ispitivanu točku. • 300 cells per sample per each experimental point were analysed. Statističke značajnosti utvrđene su primjenom Pearsonova χ²-testa • Statistical significance of data was evaluated using Pearson χ² test.
Polimerizacija 40 sekunda

Citotoksičnost obaju ispitivanih materijala nakon 40-sekundne polimerizacije prikazana je na slici 2. Nepolimerizirani Beautifil II ima značajno veću citotoksičnost negoli Gradia Direct Posterior (P < 0,0001). Nakon izravne polimerizacije na temperaturi zagrijavanja T1, Beautifil II pokazao je značajno nižu citotoksičnost negoli Gradia Direct Posterior (P < 0,0001). To je većinom uzrokovano porastom udjela limfocita u nekrozii. Na ostalim temperaturama zagrijavanja Gradia Direct Posterior pokazao je nižu citotoksičnost negoli Beautifil II, uz statističku značajnost na temperaturi T2 (P = 0,0013, zbog porasta udjela nekrotičnih stanica – P < 0,0001). Polimerizacija preko obaju overleja smanjila je citotoksičnost, s tim da su bolji rezultati u oba slučaja zabilježeni za Gradia Direct Posterior. Polimerizacija Beautifil II i Gradia Direct Posterior preko CRP CAD/CAM overleja na temperaturama T1-T3; CRP-CRP CAD/CAM overlej; LDC-LDC CAD/CAM overlej, pokazala je veću citotoksičnost negoli Gradia Direct Posterior (P < 0,0001). Nakon izravne polimerizacije preko obaju overleja, utjecao je na značajnu smanjenje citotoksičnosti u oba materijala, ali je statistički značajno veća značajnost u Gradia Direct Posterioru (P < 0,0001). Nakon simultane polimerizacije na temperaturama T1 i T2, rezultirala je većim preživljjenjem limfocita, u usporedbi s rezultatima pri zagrijavanju na temperaturi T3. Statistički značajna razlika zabilježena je između ispitivanih materijala jedino na temperaturi T2 (P = 0,0017). U ovom slučaju značajno je veću smrtnost limfocita u odnosu prema Gradia Direct Posterioru (P = 0,0082). Nakon polimerizacije preko LDC CAD/CAM overleja pri temperaturi zagrijavanja T1, Beautifil II pokazao je veću citotoksičnost negoli Gradia Direct Posterior (P = 0,0265, zbog porasta udjela nekrotičnih stanica – P = 0,0006). Na temperaturi zagrijavanja T2 citotoksičnost je bila gotovo podjednaka za oba materijala. Pri temperaturi T3 Beautifil II bio je citotoksičniji negoli Gradia Direct Posterior, ali razlika nije bila statistički značajna.

Slika 1. Usporedba citotoksičnosti materijala Beautifil II i Gradia Direct Posterior na temperaturama T1-T3.

Slika 2. Usporedba citotoksičnosti materijala Beautifil II i Gradia Direct Posterior na temperaturama T1-T3.

Figure 1 Comparison of cytotoxicity between Beautifil II and Gradia Direct Posterior for 20 seconds.

Figure 2 Comparison of cytotoxicity between Beautifil II and Gradia Direct Posterior for 40 seconds.

Cytotoxicity profiles of both tested materials are shown in Figure 2. Unpolymerized Beautifil II had significantly higher cytotoxicity than Gradia Direct Posterior (P<0.0001). After direct polymerization at T1, Beautifil II had a significantly lower cytotoxicity than Gradia Direct Posterior (P<0.0001). This was mostly influenced by an increased frequency of lymphocyte necrosis. At two higher polymerization temperatures, Gradia Direct Posterior showed lower cytotoxicity than Beautifil II, which was statistically significant at T2 (P=0.0013) due to an increased frequency of necrotic cells, P<0.0001). Polymerization through both overlays contributed to lowering of cytotoxicity, and better results in both cases were obtained for Gradia Direct Posterior. Polymerization of Beautifil II and Gradia Direct Posterior through CRP CAD/CAM overlay at T1 and T2 resulted with higher lymphocyte viability, as compared with T3. A statistically significant difference was recorded only between two tested materials at T2 (P=0.0017). In this case, Beautifil II caused more lymphocyte necrosis as compared to Gradia Direct Posterior (P=0.0082). After polymerization through LDC CAD/CAM overlay at T1, Beautifil II was more cytotoxic than Gradia Direct Posterior (P=0.0265) due to increased frequency of necrotic cells P=0.0006). At T2 their cytotoxicity was similar. At T3 Beautifil II was again more cytotoxic than Gradia Direct Posterior, but the difference was not statistically significant.
Rasprava

Ovo istraživanje provedeno u uvjetima in vitro procjenjuje citotoksičnost mikrohitrinog kompozitnog materijala Gradia Direct Posterior te gomera Beautifill II, prethodno zagrijanih na tri različite temperature te polimeriziranih tijekom 20 i 40 sekundi izravnom polimerizacijom ili polimerizacijom preko CAD/CAM overleja. Kao modelni sustav košteni su izolirani limfociti periferne krvi. Taj se sustav često primjenjuje u toksikološkim ispitivanjima u uvjetima in vitro. Limfociti su lako dostupni i dobar su model surogatnih stanica u različitim uvjetima testiranja. Ipak, najveća je prednost što su primarne stanice. Takav eksperimentalni postav na primarnim kulturama limfocita prihvatljiv je za procjenu biokompatibilnosti kompozitnih materijala, a na osnovi njegove uspješne primjenjivosti u drugim studijama (11, 14, 15) korišten je i u ovom istraživanju.

Iako oba ispitivana materijala imaju određeni citotoksični potencijal, naši rezultati pokazuju da u određenim eksperimentalnim uvjetima Gradia Direct Posterior ima bolju biokompatibilnost negoli Beautifill II. Dobiveni rezultati također pokazuju da, uz sastav materijala, na preživljenje stanica značajno utječe vrijeme polimerizacije, temperatura na koju je kompozit prethodno zagrijan te način na koji je obavljena polimerizacija. Treba istaknuti da je veće preživljenje stanica zabilježeno nakon polimerizacije uzoraka preko CAD/CAM overleja kod obaju ispitivanih materijala.

Kako bismo procijenili preživljenje limfocita, primijenili smo brzi test dvojnjog bojenja etidijevim bromidom i akridinemskom narancastom bojom koji omogućuje određivanje udjele živih, apoptotičnih i nekrotičnih stanica na osnovi stanične morfologije te dezintegracije jezgre i kromatina. Dok je u kontrolnim limfocitima morfologija jezgre sačuvana, stanice u kasnoj apoptosis imaju znake promjene na membranama, fragmentacije jezgre i stvaranja apoptotičkih tijela. Propada-nje kromatina u apoptotičnim stanicama većinom je neorganizirano i praćeno nastankom vakuloa u citoplazmi. Zbog narušavanja integriteta membrane, u nekrotičnim se stanica-ma napuklja etidijev bromid i zato je njihov kromatin obojen crveno (14). Gledano u cjelini, nalazi dobiveni analizama provedenima s pomoću fluorescencijskog mikroskopa upućuju na to da su citotoksični učinci nakon tretmana s oba ispitivana materijala u polimeriziranom stanju većinom posredovani apoptozom. Takva su zapažanja važna za kliničkih gledišta jer je apoptosis dobro kontroliran i visoko usklađen sredovani apoptozom. Such results are important from the clinical point of view as they were also influenced by curing time, temperature of pre-heating and the polymerization pattern.

Iz perspektive kliničara, te kao što je dokumentirano u nekim kliničkim studijama, postupak upotrebe prethodno zagrijanih kompozitnih materijala kao sredstva za adhezijsko cementiranje inelj i onelj restauracija, ima značajne prednosti kao što su produženo vrijeme rukovanja, odnosno obavljanja postupaka adhezijskog cementiranja, lakše uklanjanje viška materijala nakon cementiranja te bolje rubno bavljenje i režim pružaju snažne rezultaje.

Discussion

The present study reports results regarding the in vitro assessment of cytotoxic potencies of one micro hybrid composite material (Gradia Direct Posterior) and one goimeric composite material (Beautifill II) polymerized at three temperatures for two time periods. Peripheral blood lymphocytes were used as a model system in the present study. This test system is well-established in in vitro toxicology. Lymphocytes are easily available and proven to be good surrogate cells in different testing conditions. The most important fact is that lymphocytes are primary cells. Since an in vivo situation is generally better-simulated by primary cultures (11, 14, 15), such experimental design seems to be appropriate for the assessment of biocompatibility of composite materials as it was performed in the present study.

Although both studied materials possessed certain degrees of cytotoxicity, our results suggest that in the experimental conditions as applied here, Gradia Direct Posterior was more biocompatible material than Beautifill II. The obtained results have also shown that, apart from material composition, cell viability was also influenced by curing time, temperature of pre-heating and the polymerization pattern. It has to be stressed that greater cell viability was observed after polymerization of both materials through CAD/CAM overlays, as compared to direct polymerization.

To assess the lymphocyte viability in this study, we applied a rapid viability assay with acridine orange and ethidium bromide, which allowed for counting the fractions of viable, apoptotic and necrotic cells based on the cell morphology, nuclear and chromatin disintegration. While control lymphocytes showed intact morphology, in late apoptotic cells we observed membrane blebbing, fragmentation of nuclei and formation of apoptotic bodies. In necrotic cells, on the other hand, more irregular chromatin destruction was noticed, along with vacuole formation in the cytoplasm. Due to breakdown of the plasma membrane, necrotic cells accumulated ethidium bromide and their chromatin thus was stained bright red, as reported in the literature (14). On the whole, our fluorescent microscopic findings suggest that the cytotoxic effects of both tested materials in their polymerized forms have been predominantly mediated by apoptosis. Such results are important from the clinical point of view as apoptosis represents a well-controlled, and tightly-regulated physiological process, which does not result in inflammation around the dying cell, in contrast to necrosis (16 – 19). The highest percentage of necrotic cells (about 60%) was found in the sample incubated with unpolymerized Beautifill II, which resulted in 84 % of dead cells after 24 hours of in vitro exposure. The most important are induction of lipid peroxidation, glutathione depletion, downregulation of glutathione peroxidase levels and increase of intracellular calcium levels.

From the dentists' point of view, using heated composite material as a luting agents for inlay and onlay restorations has advantage regarding prolonged handling time, easier removal of excess of the material, better sealing of unideal fitting of the restoration as it is documented in some clinical studies (9, 20 – 22). Dab et al. (8) measured shrinkage in pre-heated and
Kompatibilnost prethodno zagrijanog kompozita

Knezevic i sur.

restauracija koje ne priličenje idealno uz rubove kvitetova (9, 20 – 22). Dabi i suradnici (8) ispitivali su skupljanje prethodno zagrijanih i nezagrijanih kompozita te zaključili da, bez obzi- ra na porast skupljanja, ono još ne mora biti značajno u kli- ničkim uvjetima. Razlog za to može biti i pad temperature materijala tijekom prijenosa iz uređaja za zagrijavanje u kavi- tet. Daronch i suradnici (22) promatrali su ponašanje kom- pozitnih materijala zagrijanih u uređaju za zagrijavanje Calset na različitim temperaturama te su uočili negli pad tempera- ture kompozita nakon što je izvažen iz uređaja za zagriava- nje. U rezultatima njihove studije navodi se pad temperature materijala i do 50 % samo dvije minute nakon što je izva- den iz uređaja za zagrijavanje (22). Bez obzira na to je li ko- risten prethodno zagrijan ili nezagrijan kompozitni materijal, iznimno je važno da je kompozit odgovarajuće polimeriziran. Ranija studija (3) pokazala je da prethodno zagrijani kompo- zit dopušta kraću polimerizaciju, uz isti stupanj konverzi- ja kao kompozit polimeriziran dulje na sobnoj temperaturi. Prva radna hipoteza da će kompozitni materijali zagrija- ni na nižoj temperaturi uzrokovati manju citotoksičnost bez obzira na vrijeme osvjetljanja i način polimerizacije (izrav- na polimerizacija ili polimerizacija preko CAD/CAM overle- ja), prihvaćena je za sve slučajeve, osim za 20-sekundnu polimerizaciju kompozita Gradia Direct Posterior preko CRP overleja. Za kompozit Beautifil II najbolje preživljenje stani- ca zabilježeno je u slučaju polimerizacije od 20 sekunda i pri temperaturi zagrijavanja T1 tijekom izravne polimerizacije i polimerizacije preko obaju CAD/CAM overleja te tijekom izravne polimerizacije u trajanju od 40 sekunda. Tijekom poli- merizacije materijala Beautifil II preko CAD/CAM overleja u trajanju od 40 sekunda, uzorci zagrijani na temperaturi T2 pokazuju najveći broj živih stanica. Kod kompozitnog ma- terijala Gradi Direct Posterior svi uzorci polimerizirani 40 sekunda pokazuju najveći broj živih stanica kada je materij- al zagrijan na temperaturi T2. Tijekom polimerizacije u tra- janju od 20 sekunda za isti spomenuti materijal zabilježen je najveći broj živih stanica kada je materijal prethodno bio za- grijan na temperaturi T1, osim u slučaju polimerizacije preko CRP CAD/CAM overleja, kao što je već navedeno. Druga hipoteza, da dulje osvjetljanje uzrokuje manju citotoksičnost bez obzira na temperature osvjetljanja i po- limerizacijski postupak (izravna polimerizacija ili polimeriza- cija preko CAD/CAM overleja), prihvaćena je za kompozit Gradia Direct Posterior. Za Beautifil II ta je hipoteza prihva- ćena u slučaju zagrijavanja materijala na najvišu temperatu- ru – T3 (68 °C), ali odbijena je za dvije niže temperature jer je uočen veći broj živih stanica u slučaju osvjetljanja uzor- ka 20 sekunda. Treća radna hipoteza da će izravno polimerizirani uzorci pokazati manju citotoksičnost negoli oni polimerizirani pre- ko CAD/CAM overleja bez obzira na temperature zagrijava- nja, odbijena je za oba materijala i za oba vremena osvjetlji- vanja. Moglo se očekivati da će materijal osvijetljen izravnim postupkom biti bolje polimeriziran te da će nakon završetka polimerizacije ostati manje monomerova koji je već reagirao. No naši rezultati ne idu u prilog spomenutoj tvrdnji. Očito postoji drugi razlog koji bi mogao utjecati na broj živih stani- ca u staničnoj kulturi inkubiranoj uzorcima polimeriziranim non-heated composite and concluded that despite a shrink- age increase, this increase may not be significant in clinical scenarios. This may also occur due to drop of the materi- al temperature, while the material is taken from the heating unit and placed in the cavity. Daronch et al. (22) observed the behavior of composite materials heated in Calset heating unit at different temperatures and noticed a rapid decrease in composite temperature after the removal from the heating unit. They reported a drop of 50 % in material temperature 2 minutes after the removal from the heating unit (22). Regard- less of whether the pre-heated or non-heated composite materials are used, it is essential to cure composite material proper- ly. A previous study (3) demonstrated that pre-heated composite allows for a shorter time of light exposure with a similar degree of conversion rate than when the composite is irradiated for a longer exposure time at a room-temperature.

The first working hypothesis of this study stating that ma- terials heated on lower temperature will cause less cytotoxicity regardless of the curing time and polymerization pattern (directly polymerized or through CAD/CAM overlay) was accepted in all cases except in the case of polymerization of Gradia Direct Posterior composite through CRP overlay for 20 seconds. For Beautifil II composite, the highest number of viable cells was recorded in the case of 20 seconds polymeri- zation and heating temperature T1 for direct polymerization and polymerization through both CAD/CAM overlays and for 40 seconds direct polymerization. For 40 seconds, Beau- tifil II polymerization through CAD/CAM overlays samples heated at temperature T2 showed the highest number of vi- able cells. For Gradia Direct Posterior composite material, all samples polymerized for 40 seconds showed the highest number of viable cells when the material was heated at tempera- ture T2, while for 20 seconds polymerization, the highest number of viable cells were recorded when the materi- al was heated at temperature T1, apart from polymerization through CRP CAD/CAM overlay as stated before.

The second hypothesis stating that longer curing time causes less cytotoxicity regardless of the curing time and po- limerization pattern (directly polymerized or through CAD/ CAM overlay) was accepted for Gradia Direct Posterior com- posite. For Beautifil II, this hypothesis was accepted in the case of material heating at the highest temperature, T3 (68 °C), but it was rejected at two lower temperatures where higher numbers of viable cells were recorded when the sam- ples were polymerized for 20 seconds.

The third working hypothesis was that samples polymer- ized directly would show less cytotoxicity than the samples polymerized through CAD/CAM overlay regardless of the temperature used was rejected for both materials and both curing times. It would be expected that the material which is cured directly will possess better curing quality and less un- reacted components left after curing. Our results, however, did not speak in favor of this assumption. Obviously, there is another reason that might influence the number of viable cells in cultures that were incubated with directly polymer- ized material, and that is most likely the temperature. If this was the case, a higher temperature produced from the curing unit in direct contact with the Mylar sheet which was cover-
izravnim postupkom, a to je najvjerojatnije temperatura. Ako je to točno, više temperatura emitirana tijekom osvjetljavanja iz uređaja za polimerizaciju iz izravnog kontaktu s Mylar folijom koja prekriva materijal, može pridonijeti povećanju citotoksčnosti.

Rezultati dobiveni u našim ranijim ispitivanjima o utjecaju intenziteta programa polimerizacijskog uređaja na citotoksičnost kompozitnog materijala (12, 13) u skladu su s rezultatima ove studije. Rasvjetljanje uzroka smrtnosti limfocita nakon inkubacije s uzorcima materijala polimeriziranih različitim polimerizacijskim postupcima svakako bi trebalo biti predmet budućih istraživanja.

Rezultati su također pokazali da polimerizacija preko oba CAD/CAM overlaya pridonosi smanjenju citotoksčnosti ispitivanog materijala te su bolji rezultati u oba slučaja zabilježeni za kompozitni materijal Gradia Direct Posterior pri polimerizaciji od 40 sekunda.

Objašnjenje za to može biti u različitom organskom stavljanju svakog materijala, kompozitnih materijala korištenih u studiji, a i u CAD/CAM overlaya. Zbog svojeg različitog sastava oba CAD/CAM materijala posjeduju različita svojstva loma i propuštanja svjetla iz uređaja za polimerizaciju, što uvelike utječe na stupanj konverzije materijala koji se polimerizira preko CAD/CAM overlaya. Uz to, Gradia Direct Posterior u svojem sustavu ima UDMA-u koja je najbolja izuzetno visoki rizik toksičnosti u usporedbi s Bis-GMA-om koja je u sastavu materijala Beautifil II i, prema navodima u literaturi, uzrokuju veću citotoksčnost (23). Tadin i suradnici (24) ispitivali su genotoksičnost Gradia Direct Posteriora i ustanovili su da taj materijal pokazuje veću toksičnost nakon pet dana negoli nakon prvog dana, što objašnjavaju kao postupno otpuštanje i biodegradaciju UDMA-e.

Prema podatcima proizvođača, Beautifil II sadržava površinsko stakleno (S-PRG) punilo koje je već reagiralo i pokazalo sve da ima svojstvo neutraliziranja kiseline i inhibicije formiranja glaoka. Glasionomeri i komпозite zahtijevaju apsorpciju vode nakon osvjetljavanja kako bi mogli otpuštati fluoridne ione. Suprotno tomu, kompoziti sadržavaju multikomponentnu jezgru stakla koja podiže acido-baznu reakciju tijekom postupka proizvodnje te je zaštićena površinskim modificiranim slojem. Restaurativni materijal sa svojstvom otpuštanja fluorida nakon što je postavljen u kavitetu, može služiti kao pričuva fluorida te potaknuti lagano otupljanje fluorida, povećavajući tako njihovu razinu u oralnim tečinama i sprječavajući nastanak zubnih karijesa. Madhyastha i suradnici (25) ispitivali su otpuštanje fluorida Gradia Direct Posteriora i ustanovili su da taj materijal pokazuje veću toksičnost nakon pet dana negoli nakon prvog dana, što objašnjavaju kao postupno otpuštanje i biodegradaciju UDMA-e.

Beautifil II contains a surface pre-reacted glass (S-PRG) filler that has been shown to possess acid neutralization capabilities and inhibition of plaque formation according to the manufacturer data. Glass-ionomers and compomers require water absorption after light curing in order to release fluoride ions. Conversely, gomers contain a multifunctional glass core which undergoes an acid-base reaction during manufacturing procedure and is protected by surface modified layer. Restorative materials with potential fluoride release when placed in a cavity may serve as a fluoride reservoir and lead to low fluoride release, thus increasing the fluoride level in oral fluids and preventing the dental caries. Madhyastha et al. (25) tested the fluoride release at different temperatures and concluded that it is highest at the temperature of 55 °C. It is interesting that Beautifil II in this study showed the highest number of viable cells at temperature T1 (37 °C) and T2 (54 °C). The same authors also concluded that the highest amount of fluoride release occurs the first day after application followed by the days 7 and 14 with least release after 28 days. Some other studies also showed that the cytotoxicity level drops with time in the same manner (25). The findings of Madhyastha et al. (25) suggest that pre-heating of this material prior to placement in the cavity will accelerate and facilitate the fluoride release. Potentially, the use of this material for luting of CAD/CAM may be beneficial due to fluoride release of this material. However, although the results of this study have clinical implications, further clinical research is needed to implement this material as a potential luting agent for CAD/CAM restorations.
jenke kaviteta, što će pak ubrzati otpuštanje fluorida. Upotreba ovog materijala za vezivanje CAD/CAM restauracija može biti korisna upravo zbog otpuštanja fluorida. Iako rezultati ovog ispitivanja imaju moguću kliničku primjenu, u budućnosti je potrebno nastaviti s kliničkim ispitivanjima, a trebalo bi se usredotočiti na to kako najbolje primijeniti materijal te potencijalno sredstvo adhezijskog cementiranja CAD/CAM restauracija.

Zaključak
Uzimajući u obzir da dosad nije bilo mnogo studija sličnih ovoj, naše spoznaje daju preliminarni uvid u citotoks-
sičnost mikrohibridnoga kompozitnog materijala (Gradia Direct Posterior) i giernog kompozitnog materijala (Beau-
tifill II) prema ljudskim neciljnim stanicama. Budući da su rezultati ovog istraživanja dobiveni na sustavu stanične kultu- re, ne mogu se izravno preslikati na uvjete in vivo. No do-
beni rezultati čvrst su okvir za iduća istraživanja istih ma-
terijala kako bi se objasnili mehanizmi uključeni u njihovu
citotoksičnost.

Sukob interesa
Autori nisu bili u sukobu interesa.

Conclusion
Bearing in mind the fact that similar studies on this topic
have been rare, our findings provide a preliminary insight in-
to the cytotoxicity of micro hybrid composite material (Grad-
ia Direct Posterior) and giem composite material (Beau-
tifill II) toward human non-target cells. Since these find-
ings are observed on a cell culture system, they cannot be directly
extrapolated to in vivo situations. However, our results make a
solid frame for designing future studies with the same ma-
terials aiming to further clarify mechanisms involved in their
cytotoxic action.

Conflict of interest
None declared

Abstract
Objective: The aim of this study was to evaluate cytotoxic potencies of two light cured composite ma-
terials after heating on different temperatures and cured directly and through CAD/CAM overlay. Ma-
terials and methods: Composite materials (microfilled-hybrid Gradia Direct Posterior and Beauti-
tifill II) were heated in a Calset warming unit at three different temperatures (T1:37°C, T2:54°C, T3:68°C).
A small amount of heated composite material was placed in a round mold (diameter 6mm; 0.65mm
thick), covered with Mylar sheet, pressed and polymerized with Bluephase LED unit. One group of
samples were polymerized directly, and the other group through 2mm thick CAD/CAM ceramic-rein-
forced polymer (CRP) and CAD/CAM lithium disilicate ceramic (LDO) overlay for 20 and 40 seconds.
The polymerized samples were placed immediately after curing in a lymphocyte cell culture. The via-
bility of peripheral blood lymphocytes was evaluated using a dye exclusion technique by simultane-
ous staining with ethidium bromide and acridine orange. Quantitative assessments were made by de-
termination of the percentage of viable, apoptotic and necrotic cells. The Pearson chi-square test was
used for statistical analysis. Results: In case of 20 seconds polymerization, the highest number of vi-
able cells polymerization were recorded when materials were heated at 37°C (T1), while in case of 40
seconds polymerization, the highest number of viable cells were recorded when the materials were
heated at 54°C (T2). The samples polymerized through CAD/CAM overlays showed less cytotoxicity
than samples polymerized directly. Conclusion: Apart from composite material composition, the cell
viability was also influenced by curing time, temperature of pre-heating and polymerization pattern.

References
1. Darmani H, Al-Hiyasat AS, Milhem MM. Cytotoxicity of dental composites and their leached compo-
sites. Quintessence Int. 2007 Oct;38(9):789-95.
2. Schweiz Monatsschr Zahnmed. 1995;105(9):1123-8. The curing of composites under Cerec inlays. Schweiz Monatsschr Zahnmed. 1995;105(9):1123-1128.
3. Daronch M, Rueggeberg FA, De Goes MF. Monomer conversion of pre-heated composite. J Dent Res. 2005 Jul;84(7):663-7.
4. Froes-Salgado NR, Silva LM, Kawano Y, Franci Ca, Reis A, Lougherico AD. Composite pre-heating: Effects on marginal adaptation, degree of conversion and mechanical properties. Dent Mater. 2010 Sep;26(9):908-16.
5. Daronch M, Rueggeberg FA, De Goes MF. Polymerization kinetics of pre-heated composite. J Dent Res. 2006 Jan;85(1):38-43.
6. Asmussen E, Peutzfeldt A. Influence of pulse-delay curing on soft-
ening of polymer structures. J Dent Res. 2001 Jun;80(6):1570-3.
7. Lohbauer U, Zinelis S, Rahiotis C, Petschell A, Eliades G. The ef-
effect of resin composite pre-heating of monomer conversion and polymerization shrinkage. Dent Mater. 2009 Apr;25(4):514-9.
8. Deb S, Di Silvio L, Mackler HE, Millar BJ. Pre-warming of dental composites. Dent Mater. 2011 Apr;27(4):e51-9.
9. Tabatabaea MH, Mahdavi H, Zandi S, Kharrazi MJ. HPLC analysis of eluted monomers from two composite resins cured with LED and halogen curing lights. J Biomed Mater Res B Appl Biomater. 2009 Jan;88(1):191-6.
10. Ruix-de-Castaneda E, Gaton-Hernandez P, Rodriguez EG, Silva RAB, Nelson-Filho P, Silva LAB. Pulpal and periapical response after restoration of deep cavities in dogs’ teeth with Filtek Silorane and Filtek Supreme XT systems. Oper Dent. 2013 Jan-Feb;38(1):73-81.
11. Knežević A, Želježić D, Kopjar N, Duarte S Jr, Par M, Tarle Z. Toxicity of Pre-heated Composites Polymerized Directly and Through CAD/CAM Overlay. Acta Stomatol Croat 2018;52(3):203-217.
12. Knežević A, Želježić D, Kopjar N, Tarle Z. Cytotoxicity of compos-
ite materials polymerized with LED curing units. Oper Dent. 2008 Jan-Feb;33(1):23-30.
13. Knežević A, Želježić D, Kopjar N, Tarle Z. Influence of curing mode intensity on cell culture cytotoxicity/genotoxicity. Am J Dent. 2009 Feb;22(1):43-8.
14. Duke, RC; Cohen, JJ. Morphological and biochemical assays of apoptosis. In: Coligan, JE; Kruisbeek, AM – editors. Current Protocols in Immunology. John Willey and Sons: New York; 1992. p. 1–3.

15. Geurtsen W. Biocompatibility of Resin-Modified Filling Materials. Crit Rev Oral Biol Med. 2000;11(3):333-55.

16. Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol. 2004 Dec;16(6):663-9.

17. Pinna L, Brackett MG, Lockwood PE, Huffman BP, Mai S, Cotti E et al. In vitro cytotoxicity evaluation of a self-adhesive, methacrylate resin-based root canal sealer. J Endod. 2008 Sep;34(9):1085-8.

18. Matsui S, Takahashi C, Tsujimoto Y, Matsushima K. Simulatory effects of low-concentration reactive oxygen species on calcification ability of human dental pulp cells. J Endod. 2009 Jan;35(1):67-72.

19. Camargo Se, Camargo CH, Hiller KA, Rode SM, Schweikl H, Schmalz G. Cytotoxicity and genotoxicity of pulp capping materials in two cell lines. Int Endod J. 2009 Mar;42(3):227-37.

20. Krämer N, Lohbauer U, Frankenberger R. Adhesive luting of indirect restorations. Am J Dent. 2000 Nov;13(Spec No):60D-76D.

21. Krämer N, Frankenberger R. Clinical performance of bonded leucite-reinforced glass ceramic inlays and onlays after 8 years. Dent Mater. 2005 Mar;21(3):262-71.

22. Daronch M, Rueggeberg FA, Moss L, de Goes MF. Clinically relevant issues related to preheating composites. J Esthet Restor Dent. 2006;18(6):340-50; discussion 351.

23. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater. 2006 Mar;22(3):211-22.

24. Tadin A, Marovic D, Galic N, Milevoj A, Medvedec Mikic I, Zeljezic D. Genotoxic biomonitoring of flowable and non-flowable composite resins in peripheral blood leukocytes. Acta Odontol Scand. 2013 May-Jul;71(3-4):923-9.

25. Madhyastha P, Kotain R, Vivekananda P, Khader AMA. Fluoride release from glass-ionomer cements: Effect of temperature, time interval and storage condition. J Contemp Dent. 2013;3:68-73.