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IZVORNI ZNANSTVENI RAD

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Assessment of Cytotoxic and Genotoxic Effect of Modern Dental Materials *in vivo*

Procjena citotoksičnog i genotoksičnog utjecaja suvremenih dentalnih materijala in vivo

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Abstract

Objectives: The aim of the study was to assess the biocompatibility of modern composite and amalgam dental fillings. **Material and Methods:** The research was conducted on 150 healthy patients between the ages of 10 and 20 who had amalgam and composite fillings between 6 and 12 months. Under *in vivo* conditions, a swab of buccal cells near the fillings was taken, and the cytotoxic and genotoxic impact of composite and amalgam fillings on these cells was analyzed using the extended micronucleus test (cytomeassay). **Results:** The results showed statistically significant differences between the groups of subjects with amalgam and composite fillings and subjects without fillings for the following parameters: number of micronuclei ($p=0.006$), number of buds ($p<0.001$), number of binuclear cells ($p<0.001$), number of nucleoplasmic bridges ($p<0.001$).The number of micronuclei was statistically significantly higher in the group of subjects with amalgam and composite fillings compared to the group without fillings. The results for nuclear buds, for the number of binuclear cells and the number of nucleoplasmic bridges showed that the group with amalgam fillings had a statistically significantly higher number of these changes compared to other groups.The results of the analysis of the relationship between the parameters of the micronucleus test and the number of amalgam and composite surfaces did not show statistically significant values. Parameters indicating cell cytotoxicity were not statistically significantly elevated in subjects with fillings.The results of the analysis of the influence of the patients' lifestyle on the results of the micronucleus test showed statistically significant results for certain predictors (diagnostic X-ray radiation, coffee consumption, consumption of cooked, dried meat and baked food). **Conclusion:** Based on the results, it can be concluded that the buccal cells of subjects with amalgam fillings showed the highest degree of genotoxic changes, followed by those with composite fillings and the least buccal cells of patients without fillings.

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Introduction

Composite materials and dental amalgams, as materials for dental fillings, come into direct contact with oral tissues and, due to this close and permanent contact, must have the highest degree of biocompatibility. Biocompatibility is defined as the ability of a material to stimulate a favorable host response after application within the host organism (1-3). Before receiving a license for use, biomaterials, including dental materials, must pass a series of tests and regulations (1). The biocompatibility of materials is evaluated through numerous parameters: (a) cytotoxicity (systemic and local), (b) genotoxicity, (c) mutagenicity, (d) carcinogenicity and (d) immunogenicity (1, 4). Cytotoxicity is a term that describes how toxic an agent is to cells; hence it can cause cell damage or death, mostly through necrosis or apoptosis (5). The term genotoxicity refers to harmful effects of a certain substance (genotoxin) on the genetic material of cells (DNA, RNA), that is, chromosomes, causing mutations. To assess the level of toxicity of materials that lead to DNA damage, numerous sophisticated techniques, i.e. *in vitro* and *in vivo/ex vivo* tests, have been developed. The most important *in vivo* tests include three cytogenetic procedures: the comet test, the chromosomal aberration test, and various types of micronucleus (MN) tests (1, 6). When the toxicity of dental amalgams is discussed, the toxicity of mercury (Hg) is mainly thought of. The dominant forms of Hg include: elemental Hg (Hg0); the ionic form of Hg also called inorganic Hg (II) or Hg^{2+} and the organic form of mercury, such as methylmercury (MeHg) (7).

Mercury in the body can come from food, air, industry, dental medicine, some medicines and other products. Dental amalgam contains elemental mercury, which is lipophilic, but as soon as it enters the body, as a result of the action of the enzyme hydrogen peroxide catalase, it changes to an inorganic form, which is not lipophilic and is more difficult to resorb into cells (7, 8). If mercury enters the body, it has affinity towards sulfhydryl groups and damages DNA (9), especially in people with certain genetic variants (10). The genotoxicity of Hg and its derivatives is mainly due to their ability to generate ROS (reactive oxygen species), which are formed when Hg enters the cell through the plasma membrane or via protein transporters (11, 12). Some scientific papers stated that the tripeptide glutathione is decreased in the population exposed to Hg (12, 13). Possible toxicity of dental amalgam was the reason for constant doubts about its danger to the health of amalgam filling holders, which stimulated numerous researches in *in vitro* and *in vivo* conditions (14,15) and numerous articles in scientific and popular magazines (16,17). Although the World Health Organization (WHO) and FDI, as an international dental organization, have a plan to reduce and gradually withdraw dental amalgam from use (18), the use of amalgam in dental medicine is still not completely prohibited (19). However, as all dental materials in the oral cavity are subject to mechanical, chemical, thermal, microbiological, enzymatic and other influences, their biocompatibility may change over time due to the release of ingredients from the material (20-22). After polymerization, composite fillings

Uvod

Kompozitni materijali i dentalni amalgami, kao materijali za izradu zubnih ispuna, dolaze u izravni doticaj s oralnim tkivima te zbog toga bliskog i trajnog kontakta moraju imati visok stupanj biokompatibilnosti. Biokompatibilnost se definira kao svojstvo materijala da, poslije primjene unutar organizma domaćina, obavlja određenu funkciju i stimulira povoljan odgovor domaćina (1 – 3). Prije nego što dobiju dozvolu za uporabu, biomaterijali, u koje se ubrajaju i dentalni materijali, moraju zadovoljiti niz testova i propisa (1). Biokompatibilnost materijala procjenjuje se na temelju mnogobrojnih parametara: (a) citotoksičnosti (sistemska i lokalna), (b) genotoksičnosti, (c) mutagenosti, (d) kancerogenosti i (d) imunogenosti (1, 4). *Citotoksičnost* je izraz koji opisuje koliko je neki agens otrovan za stanice te može prouzročiti njihovo oštećenje ili smrt, uglavnom putem nekroze ili apoptoze (5). Pojam *genotoksičnost* odnosi se na štetan utjecaj određene supstancije (genotoksina) na genetski materijal stanica (DNK, RNK), odnosno, kromosoma, uzrokujući mutacije. Za procjenu razine toksičnosti materijala koji oštećuju DNK, postoje mnogobrojne sofisticirane tehnike, odnosno testovi *in vitro* i *in vivo/ex vivo* (1, 6). Najvažniji testovi in *vivo* tri su citogenetska postupka: komet-test, test kromosomskih aberacija i različite vrste mikronukleusnog (MN) testa (1, 6).

Kad se raspravlja o toksičnosti dentalnih amalgama, uglavnom se misli na živu (Hg). Njezini dominantni oblici uključuju elementarnu živu (Hg^0); ionski oblik žive koji se naziva i anorganski [Hg (II) ili Hg^{2+}] i organski oblik žive, kao što je metil-živa (MeHg) (7).

Živa u organizmu može potjecati iz hrane, zraka, industrije, dentalne medicine, nekih lijekova i drugih proizvoda. Dentalni amalgam sadržava elementarnu živu koja je lipofilna, no čim uđe u organizam, zbog djelovanja enzima vodikperoksidne katalaze prelazi u anorganski oblik koji nije lipofilan i teže se resorbira u stanice (7,8). Ako uđe u organizam, živa ima afinitet prema sulfihidrilnim skupinama te oštećuje DNK (9), osobito kod osoba s pojedinim genetskim varijantama (10). Genotoksičnost žive i njezinih derivata uglavnom je posljedica njihova svojstva da stvaraju ROS vrste (reaktivne vrste kisika) koje nastaju kada živa uđe u stanicu kroz plazmatsku membranu ili putem proteinskih transportera (11, 12). U nekim radovima autori navode da je tripeptid glutation snižen u populaciji izloženoj živi (12, 13).

Moguća toksičnost dentalnoga amalgama bila je razlog za stalne sumnje u njegovu opasnost kad je riječ o zdravlju nositelja amalgamskih ispuna, što je potaknulo mnogobrojna istraživanja u uvjetima *in vitro* i *in vivo* (14, 15) te objavu mnogih članaka u znanstvenim i popularnim časopisima (16, 17). Iako Svjetska zdravstvena organizacija (WHO) i FDI, kao međunarodna dentalna organizacija, imaju plan za smanjenje i postupno povlačenje dentalnoga amalgama iz uporabe, (18) njegova primjena još uvijek nije potpuno zabranjena (19).

Kako su svi dentalni materijali u usnoj šupljini podložni mehaničkim, kemijskim, termičkim, mikrobiološkim, enzimatskim i drugim utjecajima, njihova se biokompatibilnost može tijekom godina mijenjati zbog oslobađanja sastojaka iz materijala (20 – 22).

are never completely polymerized (23). The cytotoxicity and genotoxicity of the composite mainly depends on the chemical composition of the organic component and is, most often, the result of the release of free, residual monomers of HEMA, TEGDMA, UDMA and Bis-GMA from the filling due to the action of the aforementioned factors (20). Free monomers can generate ROS compounds and reduce glutathione levels, which can promote oxidative stress (22, 24). They can stimulate the formation of inflammatory factors (25), increase the number of micronuclei with a clastogenic effect (26), cause cell necrosis (22) and can have numerous other toxic effects (16, 17). In the case of modern nanofilled or nanohybrid composites, in addition to the toxicity of free monomers, the potential toxicity of nanofillers from such fillings is also analyzed (27, 28). For this reason, constant monitoring and *ex vivo* and *in vivo* research of all dental materials is needed, even though they have passed all tests and received permission for official use (29-32). Since numerous studies have reported a correlation between the age of fillings and the degree of cell damage (13, 33, 34), in this study a swab of buccal cells was taken near composite and amalgam fillings aged six to twelve months. The goal was to evaluate the potential cytotoxic and genotoxic effects of fillings on the cells of the oral mucosa using the extended micronucleus (MN) test (cytomeassay). The cells of the oral mucosa are excellent indicators of the cytotoxic and genotoxic effects of dental materials and other factors within the oral cavity because they are directly and permanently exposed to them. An analysis of micronuclei on human buccal cells using the MN test is an effective and minimally invasive procedure for assessing the cytotoxic and genotoxic impact of dental materials and other factors on these cells. The aforementioned impact is measured by evaluating the findings of MN and other parameters for monitoring genetic damage (number of cells with micronuclei, with binuclear cells, with nucleoplasmic bridges and with nuclear buds („broken egg")) and cell death (number of cells with pyknosis, with condensed chromatin, with karyolysis and with karyorrehxis) (35-37). Numerous papers have described the assessment of cytotoxic and genotoxic effects on oral epithelial cells using the MN test (38-41).

Material and Methods

Composite materials 3M™ Filtek™ Z550 (3M) and dental amalgam Amalgam ANA 2000 (Nordiska Dental AB, Ängelholm, Sweden) were used in this research.

In the production of composite fillings, in addition to the composite material Filtek Z 550, an appropriate adhesive system from the same manufacturer was used (Scotchbond™ Universal Adhesive, 3M Espe), and for etching hard dental tissues a 37% orthophosphoric acid (Total Etch, Ivoclar Vivadent, Schaan, Liechtenstein) was used

Subjects

The research was carried out on 150 voluntary respondents aged between 10 and 20, who are patients of the Dental Practice of the Health Center Vrgorac, School of Dental Medicine University of Zagreb

Nakon polimerizacije kompozitni ispuni nisu nikada potpuno polimerizirani (23). Citotoksičnost i genotoksičnost kompozita uglavnom ovisi o kemijskom sastavu organske komponente te je najčešće posljedica oslobađanja slobodnih, zaostatnih monomera HEMA-e, TEGDMA-e, UDMA-e i Bis-GMA-e iz ispuna zbog djelovanja navedenih čimbenika (20). Slobodni monomeri mogu stvarati ROS spojeve i smanjiti razinu glutationa, što može potaknuti stvaranje oksidacijskog stresa (22, 24). Mogu poticati stvaranje upalnih čimbenika (25) povećati broj mikronukleusa s klastogenim učinkom (26), izazvati nekrozu stanica (22) i mnogobrojna druga toksična djelovanja (16, 17). Kod suvremenih nanopunjenih, odnosno, nanohibridnih kompozita, osim toksičnosti slobodnih monomera, analizira se i moguća toksičnost nanopunila iz takvih ispuna (27, 28).

Zato je potrebno stalno pratiti istraživanja svih dentalnih materijala i *ex vivo* i *in vivo* iako su prošli sva testiranja i dobili dozvolu za službenu uporabu (29 – 32). Kako se u mnogim dosadašnjim radovima navodi korelacija između starosti ispuna i stupnja oštećenja stanica (13, 33 – 34), u ovom istraživanju uzimao se bris bukalnih stanica u blizini kompozitnih i amalgamskih ispuna starosti od šest do dvanaest mjeseci. Cilj je bio, primjenom proširenoga mikronuklusog (MN) testa (cytomeassay), procijeniti mogući citotoksični i genotoksični utjecaj ispuna na stanice oralne sluznice. Stanice oralne sluznice smatraju se izvrsnim pokazateljima citotoksičnoga i genotoksičnoga utjecaja dentalnih materijala i drugih čimbenika unutar usne šupljine jer su im izravno i trajno izložene. Analiza mikronukleusa na humanim bukalnim stanicama primjenom MN testa učinkovit je i minimalno invazivni postupak za procjenu citotoksičnoga i genotoksičnoga utjecaja dentalnih materijala i drugih čimbenika na te stanice. Navedeni utjecaj mjeri se procjenom nalaza MN-a i ostalih parametara za praćenje genetskih oštećenja (broj mikronukleusa, broj pupova, broj morfoloških promjena tipa *broken egg*, broj binuklearnih stanica, broj nukleoplazmatskih mostova) i smrti stanica (broj piknoza, broj karioliza, broj karioreksija i broj morfoloških promjena tipa kondenzirani kromatin) (35 – 37). U mnogobrojnim radovima opisana je procjena citotoksičnoga i genotoksičnoga utjecaja na oralnim epitelnim stanicama primjenom MN testa (38 – 41).

Materijali

U ovom radu rabljeni su kompozitni materijali 3M™ Filtek™ Z550 (3M) i dentalni amalgam Amalgam ANA 2000 (Nordiska Dental AB, Ängelholm, Švedska).

U izradi kompozitnih ispuna, uz kompozitni materijal Filtek Z 550, korišten je i odgovarajući adhezivni sustav istog proizvođača (Scotchbond™ Universal Adhesive, 3M Espe). Za jetkanje tvrdih zubnih tkiva koristila se 37-postotna ortofosforna kiselina (Total Etch, Ivoclar Vivadent, Schaan, Lihtenštajn).

Ispitanici

Istraživanje je provedeno na 150 dobrovoljnih ispitanika u dobi između 10 i 20 godina koji su pacijenti Stomatološke ordinacije Doma zdravlja Vrgorac te Stomatološkog fakulteta Sveučilišta u Zagrebu. U istraživanje su uključeni samo pot-

Only completely healthy subjects were included in the research. Individuals who consumed two or more units of alcohol three or more times per week were excluded from the study, as well as the patients who smoke, patients with oral lesions, patients with a history of malignancy, and those exposed to materials used in orthodontics and/or mobile and fixed prosthetics.

After the patient's or parent's/guardian's consent, a swab of the buccal mucosa was taken from each patient in order to assess the biocompatibility of modern restorative materials. The subjects were divided according to the number of filling areas and the age of the filling.

Group 1 consisted of 50 subjects aged between 10-20 years, who had amalgam fillings aged 6 to 12 months, and the number of amalgam surfaces was counted for each patient. Group 2 consisted of 50 subjects aged between 10-20 years, who only had composite fillings between 6 and 12 months old, and the number of composite surfaces was counted for each patient. Group 3 consisted of 50 subjects aged between 10-20 years who had healthy teeth and did not have a single dental filling.

Sample collection

Each patient was asked not to drink alcohol, smoke or eat for 1 hour before sampling. Before taking the swab, the subjects rinsed their mouths three times with water, then the superficial, dead layer of cells was removed with sterile gauze, and a swab of buccal cells was gently taken with a sterile cytological brush (Cytobrush Plus, GmbH, Dietramszell-Linden, Germany). The cell suspension was carefully applied to the slide, then fixed with methanol (80% v/v) at 40C for 20 minutes and air-dried. After that, the cells were stained with Giemsa solution (Sigma) for 10 minutes, washed with distilled water and air-dried and analyzed with a light microscope. In order to evaluate the cytotoxic and genotoxic effect of the material, an expanded micronucleus test (cytomeassay) was used.

Micronucleus assay in buccal epithelial cells

As a measure of cytotoxicity and genotoxicity, using the MN test, the number of micronuclei and other morphological changes in the nucleus was determined in the cells. The analysis was carried out with an Olympus CX 40 light microscope (Olympus, Tokyo, Japan) under a magnification of 400x, whereby each micronucleus and other chromatin anomalies were additionally checked under a magnification of 1000x. For each subject, one thousand of epithelial cells were analyzed. The frequency of occurrence of certain parameters of the micronucleus test (number of micronuclei, buds, morphological changes of the broken egg type, binuclear cells, nucleoplasmic bridges, pyknosis, karyolysis, karyorexia and morphological changes of the condensed chromatin type) was evaluated and systematized according to Tolbert et al. (37).

Statistical analysis

The obtained results were processed with the Shapiro-Wilk and Kolmogorov-Smirnov test to assess the normality of the data distribution, while the statistical analysis of the obtained data was carried out by Kruskal-Wallis non-para-

puno zdravi pacijenti. Isključeni su pojedinci koji su trošili dvije ili više jedinica alkohola tri ili više puta tjedno te pacijenti s oralnim lezijama, poviješću maligne bolesti te oni koji su bili izloženi materijalima koji se upotrebljavaju u ortodonciji i/ili u mobilnoj i fiksnoj protetici.

Nakon pacijentova ili roditeljeva/skrbnikova pristanka svakom se pacijentu, u svrhu procjene biokompatibilnosti suvremenih restaurativnih materijala, uzimao bris bukalne sluznice. Ispitanike se podijelilo prema broju ploha ispuna i prema starosti ispuna.

Skupinu 1. činilo je 50 ispitanika u dobi između 10 i 20 godina koji su imali amalgamske ispune starosti od 6 do 12 mjeseci te se svakom pacijentu izbrojio broj amalgamskih ploha.

Skupinu 2. činilo je 50 ispitanika u dobi između 10 i 20 godina koji su imali samo kompozitne ispune čija je starost između 6 i 12 mjeseci te se svakom pacijentu izbrojio broj kompozitnih ploha.

Skupinu 3. činilo je 50 ispitanika u dobi između 10 i 20 godina koji su imali zdrave zube te nisu imali ni jedan zubni ispun.

Uzorkovanje stanica

Svakog se pacijenta zamolilo da jedan sat prije uzorkovanja ne pije alkohol, ne puši i ne jede. Prije uzimanja brisa ispitanici su tri puta isprali usta vodom, zatim se sterilnom gazom uklonio površinski, odumrli sloj stanica te se nježno sterilnom citološkom četkicom (Cytobrush Plus, GmbH, Dietramszell-Linden, Njemačka) uzimao bris bukalnih stanica. Stanična suspenzija pažljivo je nanesena na predmetno stakalce, fiksirana je metanolom (80 % v/v) temperature 4 ^{0}C tijekom 20 minuta i osušena na zraku. Nakon toga stanice su obojene otopinom Giemsae (Sigma) u trajanju od 10 minuta, isprane destiliranom vodom i osušene na zraku te analizirane svjetlosnim mikroskopom. Kako bi se procijenio citotoksični i genotoksični učinak materijala, koristio se prošireni mikronukleusni test (cytomeassay).

Mikronukleusni test na bukalnim epitelnim stanicama

Kao mjera citotoksičnosti i genotoksičnosti, primjenom MN testa, u stanicama se utvrdio broj mikronukleusa te drugih morfoloških promjena jezgre. Analiza se obavljala svjetlosnim mikroskopom Olympus CX 40 (Olympus, Tokio, Japan) pod povećanjem od 400 puta, pri čemu su svaki mikronukleus i ostale kromatinske anomalije dodatno provjerene pod povećanjem od 1000 puta. Za svakog ispitanika analizirano je 1000 epitelnih stanica. Učestalost pojavljivanja pojedinih parametara mikronukleusnog testa (broj mikronukleusa, pupova, morfoloških promjena tipa *broken egg*, binuklearnih stanica, nukleoplazmatskih mostova, piknoza, karioliza, karioreksija i morfoloških promjena tipa kondenzirani kromatin) procijenjena je i sistematizirana prema Tolbertu i suradnicima (37).

Statistička obrada podataka

Dobiveni rezultati obrađeni su Shapiro-Wilkovim i Kolmogorov-Smirnovljevim testom za procjenu normalnosti distribucije podataka, a za statističku analizu dobivenih podataka primijenjena je Kruskal-Wallisova neparametrijska analiza

Table 1 Results of the regression analysis of the dependence of the parameters of the micronucleus test as an independent variable and the number of amalgam and composite surfaces as predictor variables.
Tablica 1. Rezultati regresijske analize ovisnosti parametara mikronukleus testa kao nezavisne varijable i broja amalgamskih i kompozitnih površina kao prediktorske varijable.

Independent variable •	R^2	Predictor variables	β	t	p-value
MN	0.066	Number of amalgam surfaces	0.113	1.396	0.165
		Number of composite surfaces	0.233	2.864	0.005
Broken egg	0.007	Number of amalgam surfaces	0.068	0.803	0.423
		Number of composite surfaces	0.038	0.455	0.650
Binucleated cells	0.183	Number of amalgam surfaces	0.394	5.181	<0.001
		Number of composite surfaces	0.127	1.675	0.096
Nucleoplasmic bridges	0.199	Number of amalgam surfaces	0.451	6.000	<0.001
		Number of composite surfaces	0.026	0.352	0.726
Pyknosis	0.004	Number of amalgam surfaces	-0.124	-1.483	0.140
		Number of composite surfaces	0.078	0.932	0.353
Karyolysis	0.013	Number of amalgam surfaces	-0.060	-0.714	0.476
		Number of composite surfaces	-0.086	-1.028	0.306
Karyorrexis	0.033	Number of amalgam surfaces	-0.035	-0.427	0.670
		Number of composite surfaces	0.219	2.647	0.009
Condensed Chromatin	0.034	Number of amalgam surfaces	-0.016	-0.198	0.843
		Number of composite surfaces	0.220	2.659	0.009

metric analysis using Kruskal-Wallis one-way analysis of variance (ANOVA) with Bonferroni adjustment for multiple comparisons (Table 1). A multivariate regression analysis was performed for the dependence of the parameters of the micronucleus test. Statistical analysis was performed in the SPSS 25.0 software package (IBM, Armonk, NY, USA) with a significance level of 0.05.

An analysis for multiple comparisons was not required in cases where the omnibus Kruskal-Wallis one-way ANOVA result was not statistically significant in subjects with fillings.

Results

In accordance with the deviations from the normal distribution, the results are presented using boxplots, which better emphasize the features of non-normal distributions compared to the display of mean values and standard deviations.

The results in Figure 1 show that the number of micronuclei was statistically significantly higher in the group of subjects with amalgam fillings compared to the group without fillings (p=0.006). A marginally significant increase in the number of micronuclei was also observed in the group with composite fillings compared to the group without fillings (p=0.050). The groups with amalgam fillings and composite fillings did not statistically significantly differ from each other.

The results for nuclear buds in Figure 2 show that the group with amalgam fillings had a statistically significantly higher number of these morphological changes compared to the group without fillings (p<0.001) and the group with composite fillings (p=0.003). The group with composite fillings did not differ statistically significantly from the group without fillings.

The results of the number of binuclear cells, shown in Figure 3, showed statistically significant differences between

s pomoću jednosmjerne analize varijance (ANOVA) uz Bonferronijevu prilagodbu za višestruke usporedbe (tablica 1.). Za ovisnost parametara mikronukleusnog testa provedena je multivarijatna regresijska analiza. Statistička analiza obavljena je u softverskom paketu SPSS 25.0 (IBM, Armonk, NY, SAD) uz razinu značajnosti od 0,05.

Analiza za višestruke usporedbe nije bila potrebna u slučajevima kada omnibus-rezultat Kruskal-Wallisove jednosmjerne ANOVA-e nije bio statistički značajan kod ispitanika s ispunima.

Rezultati

U skladu s odstupanjima od normalne distribucije, rezultati su prikazani s pomoću okvirnih dijagrama (*boxplots*) kojima se bolje ističu značajke nenormalnih distribucija u usporedbi s prikazom srednjih vrijednosti i standardnih devijacija.

Iz rezultata na slici 1. jasno je da je broj mikronukleusa bio statistički značajno veći u skupini ispitanika s amalgamskim ispunima u usporedbi sa skupinom bez ispuna (p = 0,006). Marginalno značajno povišenje broja mikronukleusa također je opaženo u skupini s kompozitnim ispunima u usporedbi sa skupinom bez ispuna (p = 0,050). Pritom se skupine s amalgamskim ispunima i kompozitnim ispunima uzajamno nisu statistički značajno razlikovale.

Rezultati za jezgrene pupove na slici 2. pokazuju da je skupina s amalgamskim ispunima imala statistički značajno više tih morfoloških promjena u usporedbi sa skupinom bez ispuna (p < 0,001) i skupinom s kompozitnim ispunima (p = 0,003). Skupina s kompozitnim ispunima nije se statistički značajno razlikovala od skupine bez ispuna.

Rezultati broja binuklearnih stanica prikazani na slici 3. pokazali su statistički značajne razlike između svih triju skupina ispitanika. Dobivene p-vrijednosti bile su visoko značajne za usporedbu skupine s amalgamskim ispunima i sku-

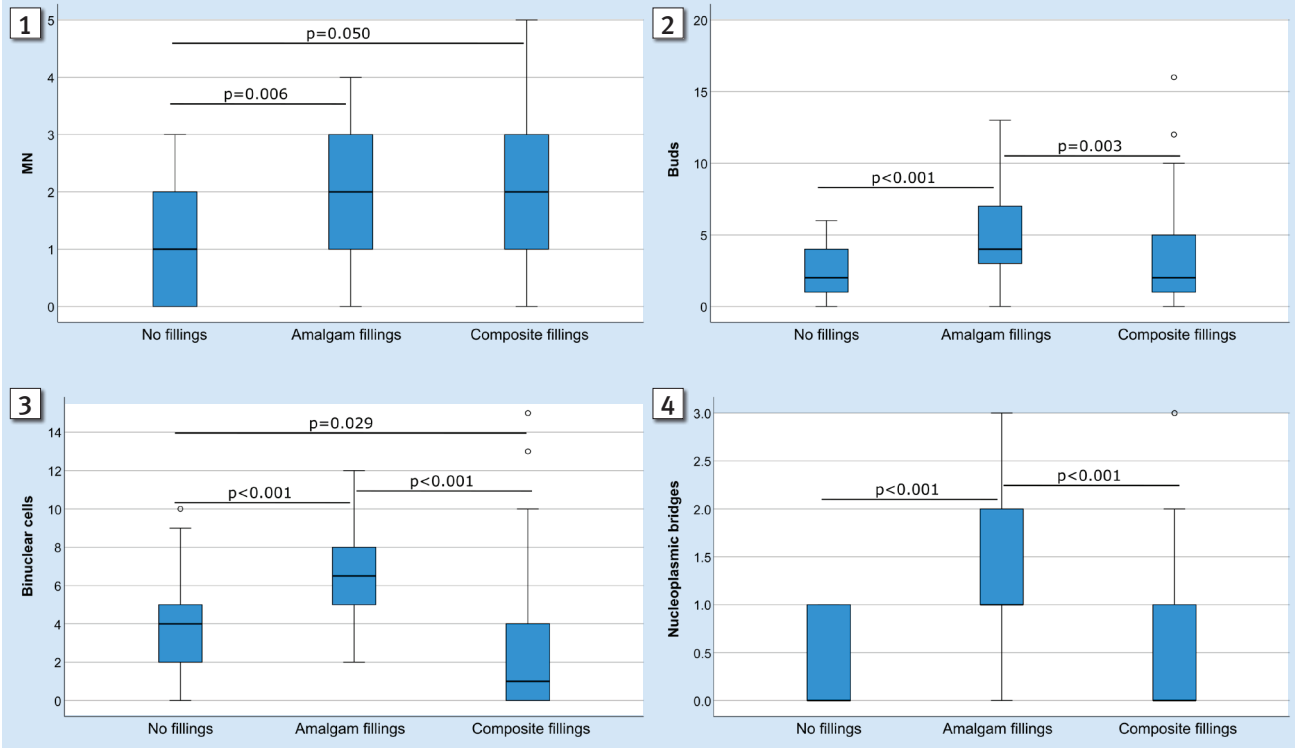

Figure 1 Boxplots for the number of micronuclei. Boxes represent 25% and 75% quartiles, black lines in boxes represent medians, and upper and lower horizontal lines represent 1.5 x interquartile range. Statistically significant differences and corresponding p-values are shown by horizontal lines above the box.

Slika 1 Okvirni dijagrami za broj mikronukleusa. Okviri predstavljaju 25% i 75% kvartila, crne linije u okvirima predstavljaju medijane, a gornje i donje horizontalne linije predstavljaju 1,5 x interkvartilni raspon. Statistički značajne razlike i odgovarajuće p-vrijednosti prikazane su horizontalnim linijama iznad okvira.

Figure 2 Boxplots for the number of buds.Boxes represent 25% and 75% quartiles, black lines in boxes represent medians, and upper and lower horizontal lines represent 1.5 x interquartile range. Statistically significant differences and corresponding p-values are shown by horizontal lines above the box.

Slika 2 Okviri za broj pupova. Okviri predstavljaju 25% i 75% kvartila, crne linije u okvirima predstavljaju medijane, a gornje i donje horizontalne linije predstavljaju 1,5 x interkvartilni raspon. Statistički značajne razlike i odgovarajuće p-vrijednosti prikazane su horizontalnim linijama iznad okvira.

Figure 3 Boxplots for the number of binuclear cells. Boxes represent 25% and 75% quartiles, black lines in boxes represent medians, and upper and lower horizontal lines represent 1.5 x interquartile range. Statistically significant differences and corresponding p-values are shown by horizontal lines above the box.

Slika 3 Okvirni dijagrami za broj binuklearnih stanica. Okviri predstavljaju 25% i 75% kvartila, crne linije u okvirima predstavljaju medijane, a gornje i donje horizontalne linije predstavljaju 1,5 x interkvartilni raspon. Statistički značajne razlike i odgovarajuće p-vrijednosti prikazane su horizontalnim linijama iznad okvira.

Figure 4 Boxplots for the number of nucleoplasmic bridges.Boxes represent 25% and 75% quartiles, black lines in boxes represent medians, and upper and lower horizontal lines represent 1.5 x interquartile range. Statistically significant differences and corresponding p-values are shown by horizontal lines above the box.

Slika 4 Okviri za broj nukleoplazmatskih mostova. Okviri predstavljaju 25% i 75% kvartila, crne linije u okvirima predstavljaju medijane, a gornje i donje horizontalne linije predstavljaju 1,5 x interkvartilni raspon. Statistički značajne razlike i odgovarajuće p-vrijednosti prikazane su horizontalnim linijama iznad okvira.

all three groups of subjects. The obtained p-values were highly significant for the comparison of the group with amalgam fillings and the group without fillings (p<0.001), that is, for the comparison of the group with amalgam fillings and the group with composite fillings (p<0.001). A significant difference was also observed between the group without fillings and the group with composite fillings (p=0.029).

Figure 4 shows a statistically significantly higher number of nucleoplasmic bridges in the group of subjects with amalgam fillings compared to the group without fillings (p<0.001) and the group with composite fillings (p<0.001).

The results of the regression analysis of the relationship between the parameters of the micronucleus test as an independent variable and the number of amalgam and composite fillings (p < 0,001), odnosno za usporedbu skupine s amalgamskim ispunima i skupine s kompozitnim ispunima (p < 0,001). Značajna razlika opažena je i između skupine bez ispuna i skupine s kompozitnim ispunima (p = 0,029).

Slika 4. prikazuje statistički značajno veći broj nukleoplazmatskih mostova u skupini ispitanika s amalgamskim ispunima u usporedbi sa skupinom bez ispuna (p < 0,001) i skupinom s kompozitnim ispunima (p < 0,001).

Rezultati regresijske analize povezanosti parametara mikronukleusnog testa kao nezavisne varijable i broja amalgamskih te kompozitnih ploha kao prediktorskih varijabli, prikazani u tablici 1. općenito su pokazali niske vrijednosti R^2 što upućuje na činjenicu da se razmjerno mali udio ukupne varijance može objasniti prediktorskim varijablama, odnosno

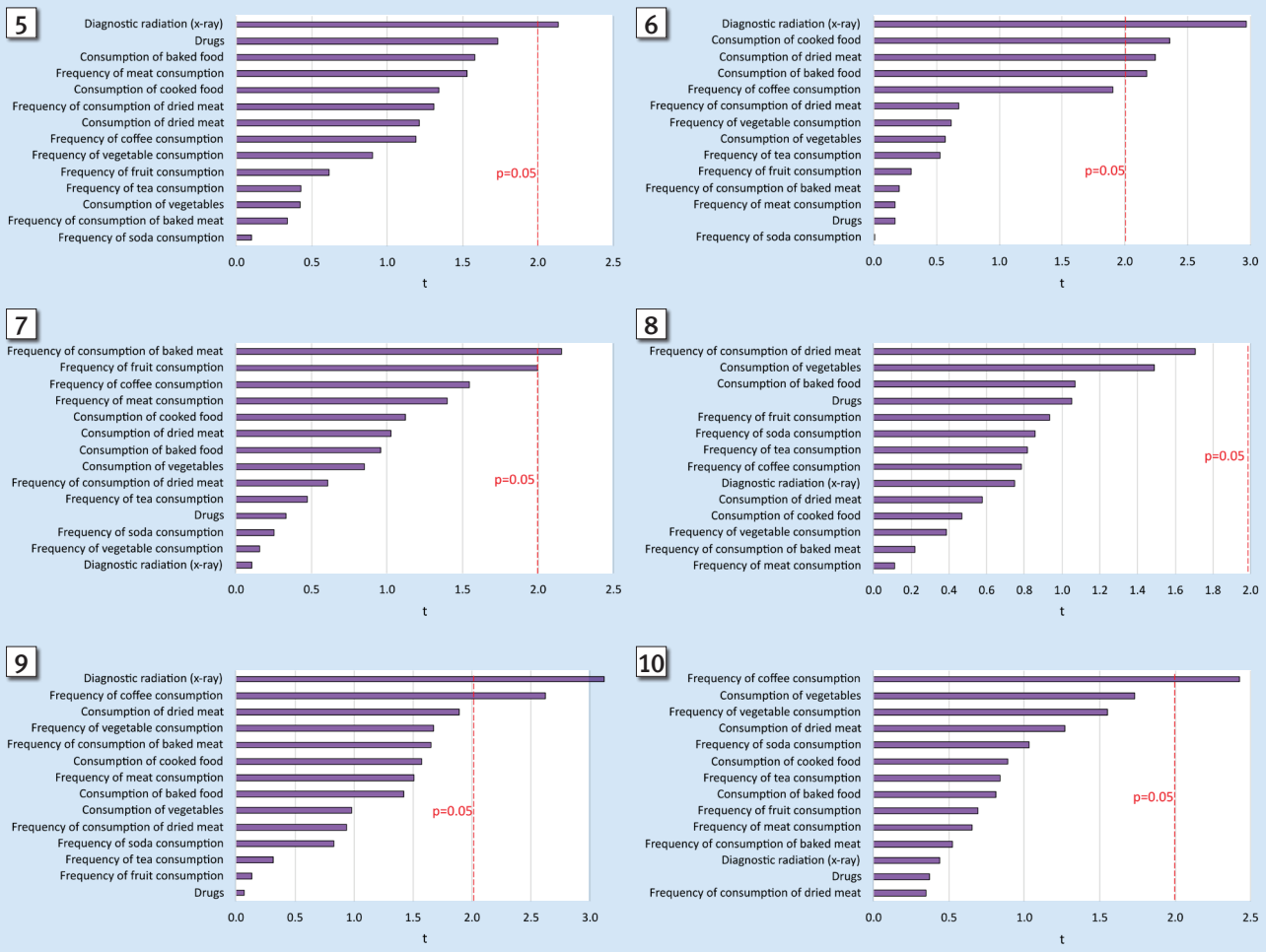

Figure 5 Pareto diagram of the dependence of the number of micronuclei on the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 5. Pareto dijagram ovisnosti broja mikronukleusa o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

Figure 6 Pareto diagram of the dependence of the number of buds on the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 6. Pareto dijagram ovisnosti broja pupova o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

Figure 7 Pareto diagram of the dependence of the number of micronuclei broken eggon the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 7. Pareto dijagram ovisnosti broja razbijenih mikronukleusa o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

Figure 8 Pareto diagram of the dependence of the number of binuclear cells on the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 8. Pareto dijagram ovisnosti broja binuklearnih stanica o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

Figure 9 Pareto diagram of the dependence of the number of nucleoplasmic bridgeson the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 9 Pareto dijagram ovisnosti broja nukleoplazmatskih mostova o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

Figure 10 Pareto diagram of the dependence of the number of pyknosis on the predictor variables. The dashed red line marks the significance limit. Predictor variables with t-values greater than this limit have a statistically significant effect in the regression model.

Slika 10. Pareto dijagram ovisnosti broja piknoza o prediktorskim varijablama. Isprekidana crvena linija označava granicu značajnosti. Prediktorske varijable s t-vrijednostima većim od ove granice imaju statistički značajan učinak u regresijskom modelu.

ite surfaces as predictor variables shown in Table 1 generally showed low R2 values, which points to the fact that a relatively small proportion of the total variance can be explained by the predictor variables, i.e. the number of amalgam/composite surfaces surface.

In order to determine the connection between potential genotoxic factors related to the patients' lifestyle and the parameters of the micronucleus test, a multivariate regression analysis was performed. For each of nine parameters of the micronucleus test, the following variables were examined as predictors: diagnostic radiation (x-ray), drugs, consumption of dried meat, consumption of cooked food, consumption of baked food, frequency of meat consumption, frequency of consumption of baked meat, frequency of consumption of dried meat, consumption of vegetables, frequency consumption of vegetables, frequency of fruit consumption, frequency of coffee consumption, frequency of tea consumption and frequency of soda consumption. By reorganizing the order of individual predictors according to the decreasing values of the test statistic t, i.e. the corresponding increase in the p-value, Pareto diagrams (figures 5-10) were obtained that facilitate the visualization of the relative influence of predictor variables on the parameters of the micronucleus test. Only predictors with t-values higher than the limit values defined at the significance level of 0.05 can be considered statistically significant. They are shown as dashed red vertical lines on the Pareto diagrams.

Discussion

Numerous papers (13, 31-34) stated the influence of time on the cytotoxicity and genotoxicity of dental materials. The purpose of the current *in vivo* study was to use the MN test to analyze buccal epithelial cells from the vicinity of amalgam and composite fillings that were exposed to the conditions of the oral cavity for longer than 6 months but not longer than 12 months. The MN test is very important for the monitoring, diagnosis and timely treatment of diseases caused by genetic damage because it can detect the activity of clastogenic (chromosomal breakage) and aneugenic (loss of chromosomes) genotoxic factors (2). There are numerous articles in scientific and popular magazines dealing with the harmful effects of amalgam fillings on the entire body, mostly referring to the toxicity of mercury from amalgam (14-17). However, the findings of some studies of cytogenetic effects in humans exposed to Hg and its compounds from various sources have been negative, controversial or uncertain as to the actual role of Hg in some positive results; therefore standardization of cytotoxicity and genotoxicity tests is recommended (12). Composite fillings, due to the action of various factors inside the oral cavity, can release monomers and various compounds which can have a toxic effect on the surrounding tissues and the entire body of the composite filling wearer (20-26).

The results of this research showed statistically significant differences between the groups of subjects with amalgam fillings, with composite fillings and without fillings, for the following parameters of the micronucleus test: number of micronuclei, number of binucleated cells with micronuclei, number of cells with nuclear buds, number of binucleated cells with nuclear buds, number of karyolitic cells, number of condensed chromatin cells, number of karyorrhectic cells, number of pyknotic cells and number of cells in mitosis.

brojem amalgamskih/kompozitnih ploha.

Multivarijatna regresijska analiza provedena je da bi se utvrdile povezanosti potencijalnih genotoksičnih čimbenika vezanih uz način života pacijenata i parametara mikronukleusnog testa.

Za svaki od devet parametara mikronukleusnog testa kao prediktori ispitani su sljedeći podatci iz upitnika: dijagnostičko zračenje (rtg), lijekovi, konzumacija suhomesnatih proizvoda, konzumacija kuhanih i pečenih jela, učestalost konzumacije mesa, pečenoga mesa i suhomesnatih proizvoda, konzumacija povrća, učestalost konzumacije povrća i voća, učestalost konzumacije kave, čaja te gaziranih pića. Reorganizacijom redoslijeda pojedinih prediktora prema padajućim vrijednostima test-statistike t, odnosno odgovarajućim porastom p-vrijednosti, dobiveni su Paretovi dijagrami (slike 5. – 10.) koji olakšavaju vizualizaciju relativnih utjecaja prediktorskih varijabli na parametre mikronukleusnog testa. Pritom se statistički značajnima mogu smatrati samo prediktori s t-vrijednostima većima od graničnih vrijednosti definiranih na razini značajnosti od 0,05, a koje su na Paretovim dijagramima prikazane kao isprekidane crvene okomite crte.

Rasprava

U mnogobrojnim radovima autori (13, 31 – 34) navode utjecaj vremena na citotoksičnost i genotoksičnost dentalnih materijala. Svrha ovoga istraživanja *in vivo* bila je s pomoću MN testa analizirati bukalne epitelne stanice iz blizine amalgamskih i kompozitnih ispuna koji su uvjetima usne šupljine bili izloženi dulje od 6 mjeseci, ali ne dulje od 12 mjeseci.

MN test vrlo je važan za praćenje, dijagnostiku i pravodobno liječenje bolesti nastalih zbog genetskih oštećenja jer može otkriti aktivnost klastogenenih (kromosomski lom) i aneugenih (gubitak kromosoma) genotoksičnih čimbenika (2). U znanstvenim i popularnim časopisima objavljeni su mnogi članci o štetnom djelovanju amalgamskih ispuna na cijeli organizam, pri čemu se uglavnom misli na toksičnost žive iz amalgama (14 – 17). Ipak, nalazi nekih studija o citogenetskim učincima kod ljudi izloženih živi i njezinim spojevima iz raznih izvora bili su negativni, kontroverzni, dvojbeni ili nesigurni kad je riječ stvarnoj ulozi žive u nekim pozitivnim rezultatima i zato se preporučuje standardiziranje testova citotoksičnosti i genotoksičnosti (12). Kompozitni ispuni nisu nikada potpuno polimerizirani te tijekom vremena, zbog djelovanja različitih čimbenika unutar usne šupljine, mogu otpustiti monomere, različite spojeve dodane radi postizanja karakteristika kompozitnih materijala i čestice punila te toksično djelovati na okolna tkiva i cijeli organizam nositelja kompozitnih ispuna (20 – 26).

Rezultati ovog istraživanja pokazali su statistički značajne razlike između skupina ispitanika s amalgamskim ispunima, s kompozitnim ispunima i bez ispuna, za sljedeće parametre

cronuclei (p=0.006), number of buds (p<0.001), number of binuclear cells (p< 0.001), the number of nucleoplasmic bridges (p<0.001). For the other parameters of the micronucleus test (morphological changes of the broken egg type, pyknosis, karyorexia, karyolysis, condensed chromatin), no statistically significant differences were observed between the groups with the mentioned fillings.

The results show that the number of micronuclei was statistically significantly higher in the group of subjects with amalgam fillings compared to the group without fillings (p=0.006). A marginally significant increase in the number of micronuclei was also observed in the group with composite fillings compared to the group without fillings (p=0.050). The groups with amalgam fillings and composite fillings did not statistically significantly differ from each other. Despite the high variability within the groups, the results showed statistically significant effects of amalgam and composite fillings on the morphological changes of cells of the oral mucosa indicative of genome damage.

The results for nuclear buds showed that the group with amalgam fillings had a statistically significantly higher number of this morphological change compared to the group without fillings (p<0.001) and the group with composite fillings (p=0.003). The results of the number of binuclear cells showed statistically significant differences between all three groups of subjects. The number of nucleoplasmic bridges was significantly higher in the group of subjects with amalgam fillings compared to the group without fillings (p<0.001) and the group with composite fillings (p<0.001). The appearance of this morphological characteristic indicates a significant damage to the genome. Parameters indicating cell cytotoxicity (number of pyknosis, number of karyolysis, number of karyorrehxis and number of morphological changes of the condensed chromatin type) show that the presence of amalgam and composite fillings did not lead to a measurable increase in these morphological anomalies. Reichl et al. (42) based on their *in vitro* study on the cytotoxicity of dental composite monomers and amalgam component Hg^2 in human gingival fibroblasts concluded that Hg from amalgam is more toxic than composite components. Visalli et al. (13) monitored the genotoxic effect of amalgam and composite fillings on the cells of the buccal mucosa. They observed that the frequency of MN in the cells of the oral mucosa was significantly higher in subjects with restorative fillings compared to that in subjects without fillings. Ahmed et al. (33) stated that in subjects with composite fillings, cytotoxic changes on human buccal and labial cells become more pronounced the longer the filling is in the oral cavity, while in amalgam fillings the greatest toxic damage was observed in the first few hours after the filling was placed. Mary et al. (34) reported that in their study that the average number of MNs in the cells of amalgam filling carriers was statistically significantly higher than that of composite fillings. Likewise, the average number of MNs in the cells of subjects with amalgam and composite fillings was statistically significantly higher compared to the cells of participants without fillings. In addition to the aforementioned studies, there are numerous other studies on the toxicity, that is, the biocompatibility of restor-

mikronukelusnog testa: broj mikronukleusa (p = 0,006), broj pupova (p < 0,001), broj binuklearnih stanica (p < 0,001) i broj nukleoplazmatskih mostova (p < 0,001). Za ostale parametre mikronukleusnog testa (morfološke promjene tipa *broken egg*, piknoza, karioreksija, karioliza, kondenzirani kromatin) nisu opažene statistički značajne razlike među skupinama s navedenim ispunima.

Iz rezultata je vidljivo da je broj mikronukleusa bio statistički značajno veći u skupini ispitanika s amalgamskim ispunima u usporedbi sa skupinom bez ispuna (p = 0,006). Marginalno značajno povišenje broja mikronuklusa opaženo je također u skupini s kompozitnim ispunima u usporedbi sa skupinom bez ispuna (p = 0,050). Pritom se skupine s amalgamskim i kompozitnim ispunima uzajamno nisu statistički značajno razlikovale. Unatoč visokoj varijabilnosti unutar skupina, rezultati su pokazali statistički značajne učinke amalgamskih i kompozitnih ispuna na morfološke promjene stanica oralne sluznice indikativne za oštećenje genoma.

Rezultati za jezgrene pupove, morfološku promjenu koja služi kao indikator oštećenja genetskoga materijala koji je zbog težih oštećenja izdvojen iz genoma jezgre i putem egzocitoze izbacuje se iz stanice, pokazuju da je skupina s amalgamskim ispunima imala statistički značajno veći broj ove morfološke promjene u usporedbi sa skupinom bez ispuna (p < 0,001) i skupinom s kompozitnim ispunima (p = 0,003).

Za morfološku promjenu tipa *broken egg* nisu opažene statistički značajne razlike među skupinama ispitanika bez ispuna te s amalgamskim i kompozitnim ispunima. Rezultati broja binuklearnih stanica pokazali su statistički značajne razlike između svih triju skupina ispitanika.

Broj nukleoplazmatskih mostova bio je značajno veći u skupini ispitanika s amalgamskim ispunima u usporedbi sa skupinom bez ispuna (p < 0,001) i skupinom s kompozitnim ispunima (p < 0,001). Pojava te morfološke karakteristike upućuje na značajnija oštećenja genoma.

Parametri koji upozoravaju na citotoksičnost stanica (broj piknoza, broj karioliza, broj karioreksija i broj morfoloških promjena tipa kondenzirani kromatin) pokazuju da prisutnost amalgamskih i kompozitnih ispuna nije potaknula mjerljivi porast tih morfoloških anomalija. Reichl i suradnici (42) zaključili su na temelju svoje studije *in vitro* o citotoksičnosti dentalnih kompozitnih monomera i amalgamske komponente Hg^2 u ljudskim gingivalnim fibroblastima da je živa iz amalgama toksičnija od kompozitnih komponenti. Visalli i suradnici (13) pratili su genotoksični učinak amalgamskih i kompozitnih ispuna na stanice bukalne sluznice. Uočili su da je učestalost MN-a u stanicama oralne sluznice značajno viša kod ispitanika s restaurativnim ispunima nego kod onih bez ispuna. Ahmed i suradnici (33), na temelju rezultata svojih istraživanja, ističu da kod ispitanika s kompozitnim ispunima citotoksične promjene na humanim bukalnim i labijalnim stanicama postaju izražajnije što je ispun dulje u usnoj šupljini, za razliku od istih stanica nositelja amalgamskih ispuna kod kojih su najveća toksična oštećenja uočena u prvih nekoliko sati poslije postavljanja ispuna. Mary i suradnici (34) u svojoj su studiji procjenjivali genotoksični utjecaj amalgamskih i kompozitnih ispuna na oralne epitelne stanice ispitanika primjenom MN testa. Autori navode da je u njiho-

ative dental materials, especially dental amalgams and composite materials, in relation to local tissues and the entire organism of the dental filling holder (43, 44).

The results of the regression analysis of the relationship between the parameters of the MN test as an independent variable and the number of amalgam and composite surfaces as predictor variables generally showed low R2 values. Despite the low values of R2, the regression results were statistically significant for certain parameters of the micronucleus test. Among all examined parameters, the number of buds stands out, for which the regression showed statistical significance with the number of surfaces for both types of fillings, i.e. amalgam (p=0.003) and composite (p=0.006) with beta-coefficients of 0.237 and 0.221. The above mentioned results are consistent with the findings of previous research (13, 33,34), which stated that a higher level of DNA damage in the cells was correlated with a higher number of fillings. A multivariate regression analysis was performed to determine the associations between potential genotoxic factors related to patients' lifestyle and micronucleus test parameters. The goal of such an analysis was to examine which of the subjects' dietary habits and other factors could influence the results of the MN test, in addition to the influence of the previously discussed main factors related to the presence of amalgam and composite fillings.A number of biological, environmental and demographic factors can interfere with *in vivo* research. Lifestyle factors most often associated with genetic damage include smoking, alcohol consumption, diet, lack of vitamins and supplements (35). In this paper, the effect of some habits such as smoking and alcohol consumption could not be estimated by regression model, since the vast majority of subjects did not smoke (98%), nor did they consume alcohol (96%), which is expected, since the research was conducted on subjects aged between 10 and 20 years.The results of some studies (13,31,32,35) did not find any effects of smoking and alcohol on the appearance of micronuclei in the cells of the oral cavity, while some other studies (35,45) describe the influence of the synergistic interaction of alcohol consumption and smoking on buccal cell damage.

In this research, the Pareto diagram for the number of micronuclei showed a statistically significant effect of diagnostic radiation, while other predictors did not show a significant effect.The regression model for the number of buds showed a statistically significant effect of a number of predictors: diagnostic radiation, consumption of cooked food, consumption of dried meat and consumption of baked food.The regression model for morphological changes of the broken egg type showed the frequency of consumption of roasted meat and the frequency of fruit consumption as statistically significant predictors for the appearance of this morphological anomaly. The opposite signs of the beta coefficients (0.275 for the frequency of consumption of baked meat, i.e. -0.191 for the frequency of consumption of fruit) indicate that these two factors worked in opposite directions.A positive sign of the beta coefficient for the frequency of consumption of roasted meat is consistent with the known genotoxic effect of this type of food, while a negative sign of the beta coefficient for the frequency of fruit consumption indicates a possible protective

vu istraživanju prosječan broj MN-a u stanicama sudionika s amalgamskim ispunima bio statistički značajno viši u usporedbi s kompozitnim ispunima. Isto tako je prosječni broj MN-a u stanicama ispitanika s amalgamskim i kompozitnim ispunima bio statistički značajno viši u odnosu prema stanicama bez ispuna. Uz navedene radove postoje i mnogobrojna druga istraživanja o toksičnosti, odnosno o biokompatibilnosti restaurativnih dentalnih materijala, osobito dentalnih amalgama i kompozitnih materijala u odnosu na lokalna tkiva i cijeli organizam nositelja zubnih ispuna (43, 44).

Rezultati regresijske analize povezanosti parametara MN testa, kao nezavisne varijable i broja amalgamskih te kompozitnih ploha kao prediktorskih varijabli, općenito su pokazali niske vrijednosti R^2. Unatoč niskim vrijednostima R^2, regresijski rezultati su bili statistički značajni za pojedine parametre mikronukleusnog testa. Među svim ispitivanim parametrima ističe se broj pupova za koji je regresija pokazala statističku značajnost s brojem ploha za obje vrste ispuna, tj. amalgamske (p = 0,003) i kompozitne (p = 0,006) s beta-koeficijentima od 0,237 i 0,221. Navedeni rezultati u skladu su s nalazima dosadašnjih istraživanja (13, 33, 34) u kojima se navodi da je veća razina oštećenja DNK u stanicama bila u korelaciji s većim brojem ispuna.

Multivarijatna regresijska analiza provedena je kako bi se utvrdile povezanosti potencijalnih genotoksičnih čimbenika vezanih uz način života pacijenata i parametara mikronukleusnoga testa. Svrha takve analize bila je ispitati koje su prehrambene navike i drugi čimbenici kod ispitanika mogli utjecati na ishode MN testa, osim utjecaja već istaknutih glavnih čimbenika vezanih za prisutnost amalgamskih i kompozitnih ispuna. Mnogobrojni biološki, ekološki i demografskih čimbenici mogu ometati istraživanje *in vivo*. Čimbenici načina života koji se najčešće povezuju s genetskim oštećenjima obuhvaćaju pušenje, konzumaciju alkohola, način prehrane te nedostatak vitamina i suplemenata (35).

U ovom radu učinak nekih navika, poput pušenja i konzumacije alkohola, nije mogao biti procijenjen regresijskim modelom zato što većina ispitanika nije pušila (98 %), niti je pila alkohol (96 %), što je i očekivano jer je istraživanje provedeno na ispitanicima u dobi između 10 i 20 godina. Rezultati nekih istraživanja (13, 31, 32, 35) o utjecaju pušenja i alkohola na učestalost pojavljivanja stanica s mikronukleusom nisu otkrili značajan učinak pušenja i alkohola na pojavu mikronukleusa u stanicama sluznice usne šupljine, no u nekim drugim istraživanjima (35, 45) autori opisuju utjecaj sinergističke interakcije konzumacije alkohola i pušenja na oštećenje bukalnih stanica.

U ovom se istraživanju na Paretovu dijagramu za broj mikronukleusa opaža statistički značajan učinak dijagnostičkog zračenja, a ostali prediktori nisu pokazali značajan učinak. Regresijski model za broj pupova pokazao je statistički značajan učinak većeg broja prediktora, naime, u tom su modelu osim dijagnostičkog zračenja značajni prediktori bili i konzumacija kuhanih i pečenih jela te suhomesnatih proizvoda. Regresijski model za morfološke promjene tipa *broken egg* pokazao je učestalost konzumacije pečenoga mesa i učestalost konzumacije voća kao statistički značajne prediktore za pojavu te morfološke anomalije. Suprotni predznaci koeficijenata

effect, probably mediated by antioxidants from fruits that protect against genome damage. Some studies (35, 46,47) indicate that a number of micronutrients, including beta-carotene and some other vitamins and N-acetylcysteine, significantly reduce MN levels in healthy smokers, as well as in people with precancerous lesions. The regression model for the dependence of the number of binuclear cells on the subjects' habits did not show statistical significance for any of the predictor variables. The high discriminatory value of the number of binuclear cells on exposure to potential harmful substances from restorative materials and at the same time relative insensitivity to variability in the habits and lifestyle of the test subjects could enable high sensitivity and specificity of this parameter in future research on the genotoxicity of restorative materials.The number of nucleoplasmic bridges according to the results of the regression model showed a statistically significant effect of the predictor diagnostic radiation and the frequency of coffee consumption. The frequency of coffee consumption was also a statistically significant predictor in the regression model for the number of pyknosis. Exposure to diagnostic radiation (X-radiation) was a significant predictor of three parameters of the micronucleus test: the number of micronuclei, the number of buds and the number of nucleoplasmic bridges. Ionizing radiation plays an important role in diagnosis and treatment, but it can also cause DNA damage. Some authors (41) did not find a statistically significant increase in MN in patients exposed to X-rays and CBCT (Cone-beam computed tomography) with an effective dose of 12mSv, however, they reported an increase in MN parameters that indicate cytotoxicity (karyorrehxis, pyknosis, karyolysis). In this study, for the number of karyolysis and for morphological changes of the condensed chromatin type, none of the predictor variables showed a statistically significant effect, while in the case of karyorrehxis, the variable of frequency of fruit consumption showed a protective effect.

Conclusions

Amalgam fillings showed a genotoxic effect on buccal mucosa cells, composite fillings showed a limited genotoxic effect, while the number of surfaces of amalgam and composite fillings in the oral cavity did not significantly affect their genotoxic effect on buccal cells. Cytotoxic effects have not been proven for either amalgam or composite fillings.

Due to the limited number of respondents who voluntarily participated in this research, the obtained effects of the material are indicative values and should be confirmed on a larger study group over a longer period of time.

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beta (0,275 za učestalost konzumacije pečenoga mesa, odnosno -0,191 za učestalost konzumacije voća) označavaju da su ta dva čimbenika djelovala u suprotnim smjerovima. Pozitivan predznak koeficijenta beta za učestalost konzumacije pečenoga mesa u skladu je s poznatim genotoksičnim učinkom te vrste hrane, a negativni predznak koeficijenta beta za učestalost konzumacije voća upućuje na mogući protektivni učinak, vjerojatno posredovan antioksidansima iz voća koji štite od oštećenja genoma. U pojedinim istraživanjima (35, 46, 47) ističe se da niz mikronutrijenata, uključujući beta-karoten i neke druge vitamine te N-acetilcistein znatno smanjuju razine MN-a kod zdravih pušača te osoba s prekanceroznim lezijama.

Regresijski model za ovisnost broja binuklearnih stanica o navikama ispitanika nije pokazao statističku značajnost ni za jednu prediktorsku varijablu. Visoka diskriminatorna vrijednost broja binuklearnih stanica na izloženost potencijalnim štetnim tvarima iz restaurativnih materijala i istodobno relativna neosjetljivost na varijabilnost u navikama i načinu života ispitanika mogli bi omogućiti visoku osjetljivost i specifičnost toga parametra u budućim istraživanima genotoksičnosti restaurativnih materijala.

Broj nukleoplazmatskih mostova, prema rezultatima regresijskog modela, pokazao je statistički značajan učinak prediktora dijagnostičko zračenje i učestalost konzumacije kave. Učestalost pijenja kave također je bio statistički značajni prediktor u regresijskom modelu za broj piknoza. Izlaganje dijagnostičkom zračenju (rendgensko zračenje) bilo je značajni prediktor kod triju parametara mikronukleusnog testa: broja mikronukleusa, broja pupova i broja nukleoplazmatskih mostova. Ionizirajuće zračenje važno je u dijagnostici i liječenju, no može prouzročiti i oštećenja DNK. U nekim istraživanjima autori (41) ne nalaze statistički znatno povećanje MN-a kod pacijenata izloženih x-zrakama i CBCT-u (Cone-beam computed tomography) uz efektivnu dozu od 12mSv, no navode povećanje MN parametara koji upozoravaju na citotoksičnost (karioreksija, piknoza, karioliza).

U ovom radu za broj karioliza i za morfološke promjene tipa kondenzirani kromatin ni jedna od prediktorskih varijabli nije pokazala statistički značajan učinak, a kod karioreksije varijabla učestalosti konzumacije voća pokazala je protektivni učinak.

Zaključak

Amalgamski ispuni pokazali su genotoksični učinak na bukalne stanice, kompozitni ispuni pokazali su ograničeni genotoksični učinak, a stupanj genotoksičnosti nije bio značajno povezan s brojem ploha amalgamskih i kompozitnih ispuna u usnoj šupljini. Citotoksični učinak nije dokazan ni za amalgamske, ni za kompozitne ispune.

Zbog ograničenog broja ispitanika koji su dragovoljno sudjelovali u ovom istraživanju, dobiveni učinci materijala indikativne su vrijednosti i treba ih potvrditi na većoj studijskoj skupini tijekom duljeg razdoblja.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Podatci koji podupiru nalaze iz ove studije dostupni su kod odgovarajućeg autora na standardni zahtjev.

Conflict of interest

The authors declare no conflict of interest related to this study.

Sukob interesa

Autori nisu bili u sukobu interesa.

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Sažetak

Svrha rada: Željela se procijeniti biokompatibilnost suvremenih kompozitnih i amalgamskih zubnih ispuna. **Materijali i postupci:** Istraživanje je provedeno na skupini od 150 zdravih pacijenata u dobi između 10 i 20 godina koji su imali amalgamske i kompozitne ispune starosti između 6 i 12 mjeseci. U uvjetima *in vivo* uzimao se bris bukalnih stanica u blizini ispuna te se primjenom proširenoga mikronukleusnoga testa (cytomeassay) analizirao citotoksični i genotoksični utjecaj kompozitnih i amalgamskih ispuna na te stanice. **Rezultati:** Rezultati su pokazali statistički značajne razlike između skupina ispitanika s amalgamskim i kompozitnim ispunima te ispitanika bez ispuna za sljedeće parametre: broj mikronukleusa ($p = 0,006$), broj pupova ($p < 0,001$), broj binuklearnih stanica ($p < 0,001$) i broj nukleoplazmatskih mostova ($p < 0,001$). Broj mikronukleusa bio je statistički značajno veći u skupini ispitanika s amalgamskim i kompozitnim ispunima u usporedbi sa skupinom bez ispuna. Rezultati za jezgrene pupove, za broj binuklearnih stanica i broj nukleoplazmatskih mostova pokazali su da je skupina s amalgamskim ispunima imala statistički značajno veći broj tih promjena u usporedbi s ostalim skupinama. Rezultati analize povezanosti parametara mikronukleusnog testa i broja amalgamskih te kompozitnih ploha nisu pokazali statistički značajne vrijednosti. Parametri koji upozoravaju na citotoksičnost stanica nisu bili statistički znatno povišeni kod ispitanika s ispunima. Rezultati analize utjecaja načina života pacijenata na ishode mikronukleusnog testa pokazali su statistički značajne rezultate za određene prediktore (dijagnostičko rendgensko zračenje, konzumacija kave, kuhanih i pečenih jela te suhomesnatih proizvoda). **Zaključak:** Na temelju rezultata može se zaključiti da su bukalne stanice ispitanika s amalgamskim ispunima pokazale najviši stupanj genotoksičnih promjena, zatim slijede one s kompozitnim ispunima, a najmanje su promjene zabilježene kod pacijenata bez ispuna.

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