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IZVORNI ZNANSTVENI RAD

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Effects of Different Over – the - Counter Whitening Products on the Microhardness, Surface Roughness, Color and Shear Bond Strength of Enamel

Učinak komercijalnih proizvoda za izbjeljivanje na mikrotvrdoću, hrapavost površine, boju i smičnu čvrstoću veze cakline i ispuna

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Abstract
Objective: The purpose of this *in vitro* study was to evaluate the effects of four over-the-counter (OTC) whitening products on the microhardness, surface roughness, color, shear bond strength (SBS) and surface charecteristics of human enamel compared with a product used for dentist-supervised home whitening. **Material and methods:** Seventy eight enamel specimens allocated into 6 groups (n=13): 1-Opalescence PF 10% (OP) dentist prescribed home whitening product, 2-Opalescence Go prefilled tray (PT), 3-Opalescence Whitening Toothpaste (WT), 4-Listerine Healthy White whitening mouth rinse (WMR), 5-Cavex Bite&White whitening pen (WP) and 6- no treatment (Con). The microhardness (VHN), surface roughness (Ra) and color of the specimens were measured (T_0). The specimens were then subjected to whitening protocols for 14 days (T_1). followed by artificial saliva storage for 14 days (T_2). The measurements were repeated at T_1 and T_2. The SBS test was done after the application of 35% phosphoric acid (Scotchbond Universal Etchant), followed by a universal adhesive (G-Premio Bond) and a micro hybrid/universal resin composite (Essentia) into a Teflon tube attached to the enamel surface ($p<0.05$). Surface morphologies of the enamel surfaces were examined by SEM. p value was set at 0.05 **Results** Application of OP, PT and WP decrased the microhardness of enamel specimens ($p<0.05$) whereas, no significant changes were seen in the microhardness of enamel specimens treated with WT and WMR ($p>0.05$). Ra values of enamel specimens increased with the application of OP, PT and WT ($p<0.05$); whereas no changes were observed after the applications of WMR and WP ($p>0.05$). OP, PT, WMR, and WP changed the color of the enamel($p<0.05$). There were not any significant differences among the SBSs of the groups, apart from OP applied enamel specimens. OP showed the least SBS values ($p=0.001$). SEM observations revealed smooth enamel surfaces. **Conclusions:** The whitening products affected the microhardness, surface roughness, color of enamel differently. Only OP decreased the SBS of the enamel.

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Introduction

Over the last decade, an increased interest has been observed in the field of tooth whitening since esthetic dentistry received more attention. Tooth whitening is gained growing popularity among the patients since it has been considered a conservative, safe, effective and minimally invasive method (1-4).

Today, tooth whitening methods range from professionally applied in-office whitening, professionally prescribed home whitening, nonprescription over-the-counter (OTC) whitening to the do-it-yourself (DIY) application (5-9).

Professionally prescribed home whitening is the most preferred whitening method (4). Dispensed to the patients

Uvod

Posljednjih deset godina poraslo je zanimanje za izbjeljivanje zuba usporedo s pojačanim zanimanjem za estetiku u dentalnoj medicini. Budući da se smatralo konzervativnim, sigurnim, učinkovitim i minimalno invazivnim postupkom, izbjeljivanje zuba postajalo je sve popularnije među pacijentima (1 – 4).

Danas je raspon metoda izbjeljivanja zuba velik – od profesionalnih koje se obavljaju u ordinaciji, onih koje se provode kod kuće, zatim komercijalnih proizvoda koji se mogu kupiti u ljekarnama (engl. *over-the-counter* – OTC) pa sve do metoda *uradi-sam* (5 – 9).

and closely monitored by the dentists, this technique is done by using 10% carbamide peroxide (CP) in a tray that is worn for at least two weeks. This concentration is well accepted due to its safety, excellent esthetic results, low incidence of side effects and increased effectiveness of the whitening (9, 10).

The satisfactory results achieved with professionally prescribed home whitening systems have led the developments of OTC products. OTC products were first marketed by the year 2000, with their lower cost, availability, easy access and application (9, 11, 12).

Currently, there have been a huge number of OTC products available with capability of whitening within 1-4 weeks. These whitening products are in the form of gels, mouth washes, pens, gums, toothpastes, films or paint-ons with lower concentrations of hydrogen peroxide (HP), and are sold at pharmacies, supermarkets and over the Internet (9, 12-15). These products may have potential side effects (14-16). The influence of the whitening agent to the oral tissues is important due to the oxidizing process which occurs during the whitening procedure. Several studies have reported an increase in the porosity, over-etched appearance, loss of prismatic structure and calcium and alterations in the organic content of enamel (3, 4, 6, 8, 16, 17).

However, there have not been a sufficient number of reports providing a scientific background for these whitening products. Since there has been a huge variety of new products and lack of evidence about their efficacy, the aim of this *in vitro* investigation was to evaluate the effects of four OTC products, that is, a prefilled tray, a whitening tooth paste, a whitening mouth rinse and a whitening pen, on the microhardness, surface roughness, color, shear bond strength (SBS), and surface charecteristics of human enamel as compared with a whitening gel (10% CP) used for dentist-supervised home whitening. The null hypothesis was that there would be no significant differences among the tested whitening products with regard to (1) microhardness, (2) surface roughness, (3) color, and (4) SBS to enamel.

Material and methods

The whitening products tested are shown in Table 1 and the experimental procedure is illustrated in Figure 1.

Ethical aspects

The study protocol was approved by the non- interventional Ethics Committee of the University (2020/08-41).

Sample size calculation:

One-way ANOVA-type power analyses were done to calculate the estimated sample size using G*Power package (version 3.1, Heinrich-Heine Dusseldorf University, Dusseldorf, Germany). The selected parameters were; 95% confidence interval, 80% power and 0.50 effect size. Twelve specimens per group were calculated.

Specimen Preparation

One hundred and twenty human permanent maxillary central incisors obtained from the pool of extracted teeth at

Izbjeljivanje kod kuće koje kontroliraju doktori dentalne medicine najpopularniji je način izbjeljivanja (4). Sredstvo se daje pacijentu koji se njime koristi uz profesionalnu kontrolu. Ta tehnika koristi se 10-postotnim karbamidnim peroksidom (CP) u udlazi koja se nosi najmanje dva tjedna. Koncentracija je prihvatljiva jer je sigurna, estetski rezultati su odlični, incidencija nuspojava je niska i povećana je učinkovitost izbjeljivanja (9, 10).

Zadovoljavajući rezultati koji se postižu tim načinom izbjeljivanja potaknuli su komercijalnu proizvodnju, pa se OTC proizvodi mogu kupiti u ljekarnama. Počeli su se reklamirati 2000. godine, a prednost im je niska cijena, dostupnost, jednostavna i pristupačnost u primjeni (9, 11, 12).

Trenutačno se može nabaviti niz OTC proizvoda koji izbjeljuju zube između jednoga i četiri tjedna. Dostupni su u obliku gelova, tekućina za ispiranje, olovaka, žvakaćih guma, zubnih pasti, premaza te lakova s niskim koncentracijama vodikova peroksida (HP), a prodaju se u ljekarnama, supermarketima ili na internetu (9, 12 – 15). Svi ti proizvodi imaju potencijalne nuspojave (14 – 16). Utjecaj sredstva za izbjeljivanje na oralna tkiva važan je zbog procesa oksidacije tijekom izbjeljivanja. U nekoliko istraživanja opisano je povećanje poroznosti cakline, pretjerano najetkani izgled cakline, gubitak prizmatske strukture i kalcija te promjene u organskome sastavu cakline (3, 4, 6, 8, 16, 17).

Nema dovoljno tekstova koji bi dali znanstvenu podlogu svim tim proizvodima za izbjeljivanje. Budući da ih je mnogo, a nedostaju podatci o učinkovitosti, cilj ovog istraživanja *in vitro* bio je procijeniti učinke četiriju OTC proizvoda – unaprijed pripremljene udlage, zubne paste za izbjeljivanje, tekućine za ispiranje koja izbjeljuje i olovke za izbjeljivanje – na mikrotvrdoću, hrapavost površine, boju i snagu vezivanja (engl. *shear bond strength* – SBS) te površinska obilježja ljudske cakline u usporedbi s djelovanjem gela za izbjeljivanje (10 % CP) koji se koristi pod nadzorom doktora dentalne medicine. Nulta hipoteza glasila je da neće biti značajnih razlika između ispitivanih proizvoda kada je riječ o (1) mikrotvrdoći, (2) hrapavosti površine, (3) boji te (4) SBS-u cakline.

Materijal i metode

Ispitivana sredstva za izbjeljivanje prikazana su u tablici 1., a eksperimentalni postupak na slici 1.

Etički aspekti

Protokol istraživanja odobrilo je Sveučilišno etičko povjerenstvo za neintervencijska klinička istraživanja (2020/08-41).

Izračun veličine uzorka

Za izračunavanje procijenjene veličine uzorka primijenjena je jednosmerna ANOVA analiza snage s pomoću G*Power paketa (verzija 3.1, Heinrich-Heine Universität, Düsseldorf, SR Njemačka). Odabrani parametri bili su: 95-postotni interval pouzdanosti, 80-postotna snaga i učinak veličine 0,50. Dobiven je rezultat od 12 uzoraka po skupini.

Priprema uzoraka

Stotinu dvadeset gornjih trajnih središnjih sjekutića (inciziva), dobivenih od Zavoda za oralnu kirurgiju Fakulteta

Table 1 Whitening products used in the study
Tablica 1. Proizvodi za izbjeljivanje korišteni u istraživanju

Whitening Product/Proizvod za izbjeljivanje	Manufacturer/Proizvođač	Composition/Sastav	Daily Use/Total Number of Treatment Days/ Dnevno korištenje/broj dana korištenja
Opalescence PF 10 % (Dentist-supervised home whitening (OP)/Izbjeljivanje kod kuće koje kontrolira doktor	Ultradent Products, South Jordan UT, USA	10 % Carbamide Peroxide, Polyacrylic acid, 0.3 % Sodium fluoride, 3 % Sodium hydroxide	8 hours a day/14 days/8 sati dnevno/14 dana
Opalescence Go (Prefilled Tray) (PT)/Unaprijed pripremljena udlaga	Ultradent Products, South Jordan UT, USA	6 % Hydrogen Peroxide, sodium hydroxide, potassium nitrate, sodium fluoride	30 minutes a day/14 days/30 minuta dnevno/14 dana
Opalescence (Whitening Toothpaste) (WT)/zubna pasta za izbjeljivanje	Ultradent Products, South Jordan UT, USA	Sodium fluoride, glycerin, water (aqua), silica, sorbitol, xylitol, flavor, poloxamer, sodium lauryl sulfate, carbomer, FD&C Blue # 1 (Cl 42090), FD&C Yellow # 5 (Cl 19140), sodium benzoate, sodium hydroxide, Sparkle (CI 77019, CI 77891), sucralose, xanthan gum.	2 minutes twice daily/14 days/2 minute 2 puta dnevno/14 dana
Cavex Bite&White (Whitening Pen) (WP)/olovka za izbjeljivanje	Cavex, Haarlem, Holland	6 % Hydrogen Peroxide, polyethylene glycol, PVP peroxide, glycerin, Peppermint oil	30 minutes a day/14 days/30 minuta dnevno/14 dana
Listerine Healty White (Whitening Mouth Rinse) (WMR)/tekućina za izbjeljivanje	Johnson & Johnson Consumer Inc., New Jersey, USA	Water, 8 % alcohol, 2% Hydrogen Peroxide, sodium phosphate, poloxamer 407, sodium lauryl sulfate, sodium citrate, mint aroma, menthol, eucalyptol, Sodium saccharin, sucralose	2 minutes twice daily/14 days/2 minute 2 puta dnevno/14 dana

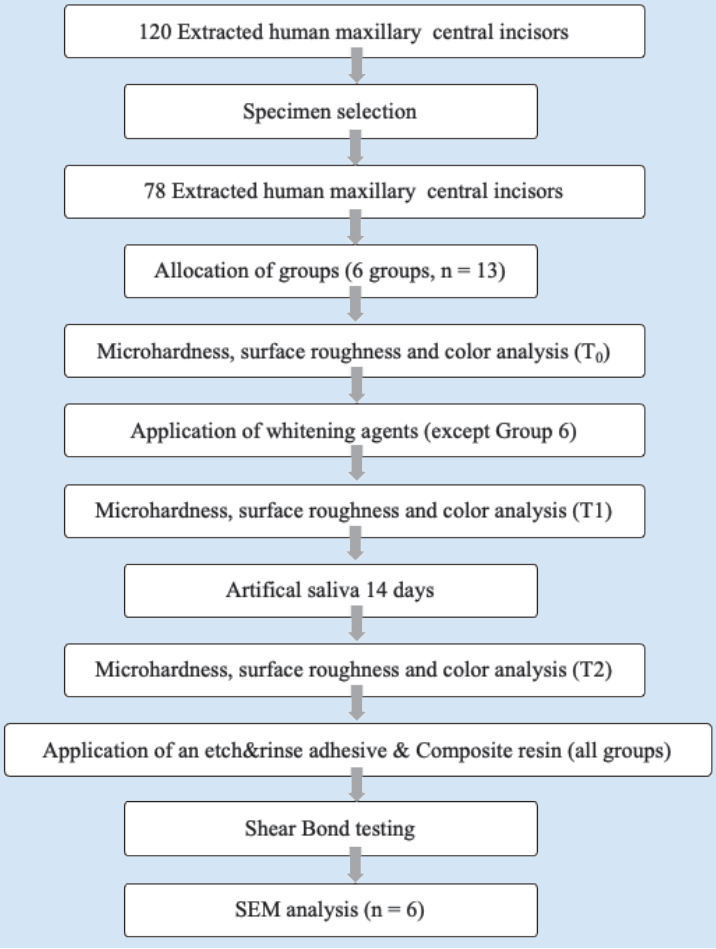

Figure 1 Study protocol
Slika 1. Protokol istraživanja

Oral Surgery Department of the Dental School were examined by a stereomicroscope at 10 X magnification (American Optical, Buffalo, NY, USA), and 78 teeth were selected for the study in line with the Human Tissue Act procedures. The teeth with similar shade, size and surface texture were employed. The roots were cut 1 mm below the cemento-enamel junction with a diamond saw attached to a sectioning machine (Isomet 1000 Precision Diamond Saw, Buehler Ltd, Illinois, USA). Debris was removed by a curette and air / water jet from the crowns and then stored in 0.1% thymol solution at 5^{O}C. The crowns were embedded in acrylic resin molds (Integra, Ankara, Turkey) leaving the buccal surfaces exposed and then the enamel surfaces were polished by silicon carbide papers (600, 800, 1000, 1200 and 2000 grit) (English Abrasives, London, UK). Subsequently, the specimens were allocated into 6 groups (n = 13) randomly for whitening purposes as follows:

1- Opalescence PF 10% (OP)/dentist prescribed at- home whitening product
2- Opalescence Go (PT) / prefilled tray
3- Opalescence (WT) /Whitening Toothpaste
4- Listerine Healthy White (WMR)/ whitening mouth rinse
5- Cavex Bite&White (WP)/whitening pen
6- No treatment (Con)

Representative specimens (1; per group) were allocated for SEM. All the specimens were prepared for microhardness (VHN), surface roughness (Ra) and color testing before the application of whitening products.

Microhardness measurement

The baseline microhardness (VHN) of the specimens were measured with a Vicker's microhardness tester (HMV-2, Shimadzu Corp., Kyoto, Japan) with a load of 980 g for 15s. Five indentations were taken at 100 mm intervals and averaged. Vicker's microhardness tester was calibrated after each reading.

Surface roughness measurement

To measure the baseline surface roughness (Ra) of the specimens, a contact-type profilometer (Perthometer M2, Mahr GmbH, Gottingen, Germany) was used. Each specimen was placed on a custom-made jig to ensure its position, and then the needle of the device was inserted on the surface of the specimen. Five Ra measurements were recorded from the center of each specimen's surface and averaged. The profilometer was calibrated after every 3 readings.

Color Evaluation

The baseline color evaluations were assessed by a spectrophotometer (CM-700d, Konica Minolta, Tokyo, Japan) with software (Spectra Magic NX, Konica Minolta). CIE L*a*b* recordings were obtained as L* is lightness, from white to black (100 - 0), a* is red – green and b* is yellow – blue chromatic coordinates. For each specimen, three readings were obtained and averaged. D65 standart light was used for irradiation, and a white reflectance standard (CM-A117, Konica Minolta) and a black box (CM-A182, Konica, Minolta) were used for calibration. Color change was calculated as follows:

dentalne medicine, analizirano je stereomikroskopom na deseterostrukom povećanju (American Optical, Buffalo, SAD) (18) te je njih 78 odabrano za istraživanje u skladu s procedurama Zakona o ljudskom tkivu. Zubi su bili slične nijanse, veličine i površinske teksture. Korijeni su odrezani 1 mm ispod caklinsko-cementnoga spojišta dijamantnom pilom pričvršćenom na sekcijski aparat (Isomet 1000 Precision Diamond Saw, Buehler Ltd, Illinois, SAD). Otpad s kruna uklonjen je kiretom i mješavinom zraka i vode te su uronjene u 0,1-postotnu otopinu timola temperature 5 °C. Krune su zatim uronjene u akrilatne kalupe (Integra, Ankara, Turska) tako da su bukalne strane ostale izvan kalupa. Površina cakline ispolirana je silikonskim karbidnim papirima (grubosti 600, 800, 1000, 1200 i 2000, English Abrasives, London, Ujedinjeno Kraljevstvo). Poslije toga uzorci su podijeljeni u šest skupina (n = 13) slučajnim odabirom u svrhu izbjeljivanja i to na sljedeći način:

1. Opalescence PF 10 % – proizvod za korištenje kod kuće pod nadzorom doktora dentalne medicine
2. Opalescence Go (PT) – unaprijed ispunjeni kalup
3. Opalescence (WT) – zubna pasta za izbjeljivanje
4. Listerine Healthy White (WMR) – tekućina za ispiranje koja izbjeljuje
5. Cavex Bite&White (WP) – olovka za izbjeljivanje
6. Con – bez intervencije.

Reprezentativni uzorci (1 po skupini) odabrani su za SEM. Svi uzorci testirani su za mikrotvrdoću (VHN), hrapavost površine (Ra) te boju prije primjene sredstava za izbjeljivanje.

Mjerenje mikrotvrdoće

Početna vrijednost Vickersove tvrdoće (VHN) uzoraka mjerena je Vickersovim ispitivačem mikrotvrdoće (HMV-2, Shimadzu Corp., Kyoto, Japan) opterećenim s 980 g tijekom 15 sekunda. Na udaljenosti od 100 mm napravljeno je pet udubina te su zaokružene njihove vrijednosti. Ispitivač je provjeravan nakon svakoga testiranja.

Mjerenje hrapavosti površine

Za mjerenje početne hrapavosti površine uzoraka (Ra) korišten je kontaktni profilometar (Perthometer M2, Mahr GmbH, Göttingen, SR Njemačka). Svaki uzorak postavljen je na njemu prilagođeno postolje te je igla naprave uronjena u površinu uzorka. Iz sredine svakoga uzorka obavljeno je pet mjerenja i izračunata je srednja vrijednost. Profilometar je ponovno kalibriran nakon triju mjerenja.

Procjena boje

Početna procjena boje uzoraka procjenjena je spektrofotometrom (CM-700d, Konica Minolta, Tokyo, Japan) i softverom (Spectra Magic NX, Konica Minolta). Dobivene su vrijednosti mjerenja CIE L*a*b, u kojem je L* bila svjetlina, od bijeloga do crnoga (100-0), a* je crveno-zelena, a b* žutoplava kromatska koordinata. Svaki uzorak mjeren je tri puta te je izračunata srednja vrijednost. Za obasjavanje je korišteno standardno svjetlo D65, a za kalibraciju je upotrijebljen bijeli reflektirajući standard (CM-A117, Konica Minolta) i crna kutija (CM-A182, Konica Minolta). Promjena boje izračuna-

$\Delta E=[(L1-L0)^2+(a1-a0)^2+(b1-b0)^2]^{1/2}$ and $\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T * (\Delta C'/K_C S_C) * (\Delta H'/K_H S_H)]^{1/2}$ (19-21).

After surface hardness, roughness and color evaluations, all goups were subjected to whitening protocols according to the manufacturers' instructions.

Whitening protocols

Whitening agents were used with reference to the the manufacturer's instructions. Between whitening procedures, artificial saliva at 4 °C (22) was used as storage media.

OP (n:12): Opalescence PF 10% (Ultradent, Inc., South Jordan UT, USA) was applied with a brush onto the enamel surfaces approximately 1 mm thick for 8 hours a day and the specimens were kept in humid enviroment at 37°C for 14 consecutive days. At the end of each whitening procedure, the whitening gel was removed and the specimens were thoroughly rinsed and dried. The specimens were then transferred into the artificial saliva which was renewed daily.

PT (n:12): After the prefilled whitening tray (Opalescence Go, Ultradent, Inc., South Jordan UT, USA) had been taken out from its packaging, the colored outer tray was removed leaving the white inner tray. Then, this layer was cut according to the size of enamel specimen and enamel surface was covered with the whitening strip for 30 min at 37°C for 14 days. After each whitening protocol, the specimens were thoroughly rinsed and dried, and then transferred into the artificial saliva.

WT (n:12): A soft electric toothbrush in daily mouth cleaning mode (Triumph 5000 D34; Oral B, Braun GmbH, Kronberg Germany) with a whitening toothpaste (Opalescence, Ultradent Inc., South Jordan, Utah,USA) which was dilued by distilled water at a 1:3 (w/v) ratio (23) was used for brushing purposes for 2 min twice/ a day during 14 days (24). Following the brushing procedure, the specimens were thoroughly rinsed and dried and then transferred into the artificial saliva.

WMR (n:12): The specimens in this group were immersed in whitening mouth rinse (Listerine Healthy White, Johnson & Johnson Consumer Inc., New Jersey, USA) for 2 min twice a day for 14 days in humid atmosphere at 37°C. The specimens were thoroughly rinsed and dried and then kept in artificial saliva following each immersion.

WP (n:12): After the cap of the whitening pen (Cavex Bite&White, Cavex, Haarlem, Nederland) had been taken off, the pen button was turned until a small droplet of whitening gel appeared on the tip brush. The enamel surface was covered with a thin layer of gel for 30 min/ a day for 14 days. At the end of each treatment, specimens were thoroughly rinsed and dried and then transfered into articial saliva.

After 14 days of whitening protocols, surface hardness, roughness and color measurements were repeated (T_1) and then the specimens were kept in artificial saliva at 37^{0}C for another 14 days. The measurements were again repeated (T_2).

SBS test

The specimens including the CON group, underwent a SBS test. After the application of 35% phosphoric acid (Scotchbond Universal Etchant, 3M ESPE, St. Paul, MN,

ta je prema formulama $\Delta E=[(L1-L0)^2+(a1-a0)^2+(b1-b0)^2]^{1/2}$ i $\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T * (\Delta C'/K_C S_C) * (\Delta H'/K_H S_H)]^{1/2}$ (19-21). Nakon procjene tvrdoće i hrapavosti površine te boje, sve su skupine podvrgnute postupku izbjeljivanja prema uputama proizvođača.

Postupci izbjeljivanja

Sredstva za izbjeljivanje korištena su u skladu s uputama proizvođača. Između postupaka uzorci su čuvani u umjetnoj slini na temperaturi od 4 °C (22).

OP (n:12): Opalescence PF 10 % (Ultradent Inc., South Jordan, UT, SAD) primijenjen je četkicom na caklinsku površinu u debljini od otprilike 1 mm tijekom 8 sati na dan. Uzorci su se 14 dana u kontinuitetu nalazili u vlažnoj okolini na temperaturi od 37 °C. Na kraju svakog postupka izbjeljivanja gel je uklonjen, a uzorci su detaljno isprani i posušeni. Poslije toga vraćeni su u umjetnu slinu koja se obnavljala svaki dan.

PT (n:12): Nakon što je pripremljena udlaga izvađena iz pakiranja (Opalescence GO, Ultradent Inc., South Jordan, UT, SAD), uklonjena je obojena vanjska udlaga, a ostavljena je unutarnja udlaga za izbjeljivanje. Taj je sloj izrezan na veličinu cakline i držan na njezinoj površini 30 minuta pri 37 °C tijekom 14 dana. Nakon svakog izbjeljivanja, uzorci su isprani i posušeni, a zatim vraćeni u umjetnu slinu.

WT (n:12) Za četkanje je korištena mekana električna četkica kao i svaki dan (Triumph 5000 D34, Oral B, Braun GmbH, Kronberg, SR Njemačka) s pastom za izbjeljivanje (Opalescence, Ultradent Inc., South Jordan, SAD) razrijeđenom destiliranom vodom u omjeru 1 : 3 (w/v) (23). Četkanje je trajalo dvije minute dva puta na dan tijekom 14 dana (24). Nakon četkanja uzorci su detaljno ispirani i ponovno odloženi u umjetnu slinu.

WMR (n:12): Uzorci u ovoj skupini uronjeni su u tekućinu za ispiranje usne šupljine koja izbjeljuje (Listerine Healthy White, Johnson & Johnson Consumer Inc., New Jersey, SAD) po dvije minute na dan tijekom 14 dana u vlažnom okolišu na temperaturi od 37 °C. Nakon toga su temeljito isprani i osušeni te vraćeni u umjetnu slinu.

WP (n:12): Nakon otvaranja olovke za izbjeljivanje (Cavex Bite&White, Cavex, Haarlem, Nizozemska) pritišće se gumb dok se na vrhu olovke ne pojavi kap sredstva za izbjeljivanje. Površina cakline premazana je gelom koji je ostavljen da djeluje 30 minuta na dan tijekom 14 dana. Na kraju svakog tretmana uzorci su temeljito isprani i osušeni te vraćeni u umjetnu slinu.

Nakon 14-dnevnoga protokola za izbjeljivanje, ponovljena su mjerenja tvrdoće, hrapavosti i boje (T_1) te su uzorci vraćeni u umjetnu slinu gdje su ostali sljedećih 14 dana, nakon čega su mjerenja ponovljena (T_2).

SBS test

Svi uzorci, čak i oni u skupini CON, podvrgnuti su SBS testu. Nakon nanošenja 35-postotne fosforne kiseline (Scotchbond Universal Etchant, 3M ESPE, St. Paul, SAD)

USA) onto the enamel surfaces for 15s, rinsing with water for 5s and gentlty air-drying, a universal adhesive (G-Premio Bond, GC Corporation, Tokyo, Japan) was applied over the enamel surface according to the manufacturer's instruction for 10 s by a micro brush, air blown for 5s and light-irradiated for 10s using an LED light curing device (440-465 nm, 1.400 mW/cm^2, Starligt S, Mectron s.p.a., Carasco, Italy). Then, a Teflon tube (4mm diameter X 3mm height) was attached to the enamel surface and a micro hybrid/universal resin composite (Essentia, GC, Tokyo, Japan) was incrementally inserted, polymerized for 40s and then the tube was removed. Having been stored in distilled water at 37°C for 24h, the specimens were transferred to a universal testing machine (LR50K, Lloyd Instruments Ltd., Fareham, Hants, UK) in shear mode with a knife-edge testing apparatus at a crosshead speed of 1 mm/min. SBS was calculated as the ratio of fracture load and bonding area, expressed in megapascals (MPa) (25).

Scanning Electron Microscope (SEM) Analysis

Six specimens (1; per group) were gently air dried, dehydrated with alcohol, gold coated and then analyzed by SEM (JSM6400, Jeol, Tokyo, Japan) at X400 magnification (25).

Statistical Analysis

SPSS software 23.0 (The Statistical Package for The Social Sciences) was used to analyze the data. Surface microhardness, roughness, color change and SBS of groups were compared by Two-way ANOVA. Mean values were analyzed by ANOVA and Turkey's test. $P < 0.05$ was considered as statistically significant.

Results

The average VHN values of enamel specimens and standard deviations (± SD) at T_0, T_1 and T_2 are presented in Figure 2. No significant differences were found among VHN values of the enamel specimens before the application of whitening products ($p>0.05$). The application of OP, PT and WP decrased the microhardness of enamel specimens ($p<0.001$, $p<0.001$, $p=0.010$, respectively) whereas, there were no significant changes in the microhardness of enamel specimens treated with WT and WMR ($p=0,058$; $p=0,052$). After they had been stored in artificial saliva for 14 days, VHN values of the enamel specimens did not change ($p>0.05$).

The average Ra values and standard deviations (± SD) of enamel specimens at T_0, T_1 and T_2 are shown in Figure 3. There were no significant differences among Ra values of the enamel specimens before the apllication of whitening products ($p>0.05$). Ra values of enamel specimens increased with the application of OP, PT and WT ($p=0.002$, $p=0.033$, $p=0.002$ respectively); whereas there were no changes after the applications of WMR and WP ($p=0.747$, $p=0.174$, respectively). After artificial saliva immersion for 14 days, no signicant changes were observed in the Ra of enamel specimens ($p>0.05$). Neither the application of WMR and WP nor the storage in artificial saliva for 14 days caused any changes in the Ra of the enamel specimens ($p>0.05$).

na caklinsku površinu gdje je djelovala 15 sekunda i ispiranja vodom tijekom pet sekunda te sušenja komprimiranim zrakom, na površinu je nanesen univerzalni adheziv (G-Premio Bond, GC Corporation, Tokio, Japan) prema uputi proizvođača –10 sekunda razmazivati mikročetkicom, sušiti zrakom 5 sekunda i osvjetljavati 10 sekunda s pomoću LED izvora svjetlosti (440 – 465 nm, 1400 mW/cm^2, Starlight S, Mectron s.p.a., Carasco, Italija). Zatim je na jetkanu površinu postavljena teflonska tuba (4 mm u promjeru i 3 mm visine) te je u nju u slojevima dodavana kompozitna masa koja je polimerizirana 40 sekunda, poslije čega je tuba uklonjena. Nakon 24-satnoga čuvanja u destiliranoj vodi na temperaturi od 37 °C, uzorci su postavljeni na univerzalni uređaj za testiranje (LR50K, Lloyd Instruments Ltd., Fareham, Ujedinjeno Kraljevstvo) gdje je ispitivana snaga vezanja brzinom od 1 mm/min. SBS je izračunat kao odnos frakturnoga opterećenja i područja vezivanja te izražen u megapaskalima (MPa) (25).

Elektronska mikroskopska (SEM) analiza

Šest uzoraka (po jedan iz skupine) nježno je posušeno zrakom, dehidrirano alkoholom i prekriveno zlatom te je primijenjena SEM analiza (JSM6400, Jeol, Tokio, Japan) pod povećanjem od 400 puta (25).

Statistička analiza

Za analizu podataka korišten je softver SPSS 23.0 (The Statistical Package for Social Sciences). Mikrotvrdoća, hrapavost, promjena boje i SBS skupina uspoređivani su dvosmjernom ANOVA-om. Srednje vrijednosti analizirane su ANOVA-om i Tukeyjevim testom. Razina značajnosti postavljena je na p 0,05.

Rezultati

Prosječne VNH vrijednosti caklinskih uzoraka i standardne devijacije (± SD) na T_0, T_1 i T_2 prikazane su na slici 2. Nije bilo statistički značajnih razlika između VNH vrijednosti caklinskih uzoraka prije primjene sredstava za izbjeljivanje ($p > 0,05$). Primjena OP-a, PT-a i WP-a smanjila je mikrotvrdoću caklinskih uzoraka ($p < 0,001$, $p < 0,001$ i $p = 0,010$), ali nije bilo statistički značajne razlike u mikrotvrdoći cakline nakon primjene WT-a i WMR-a ($p = 0,058$ i $p = 0,052$). Nakon 14-dnevnoga čuvanja u umjetnoj slini, vrijednosti VNH-a nisu se promijenile ($p > 0,05$).

Prosječne vrijednosti Ra i standardne devijacije (± SD) na T_0, T_1 i T_2 prikazane su na slici 3. Nije bilo statistički značajnih razlika u vrijednostima Ra caklinskih uzorka prije primjene sredstava za izbjeljivanje ($p > 0,05$). Njihove vrijednosti porasle su poslije primjene OP-a, PT-a i WT-a ($p = 0,002$, $p = 0,033$ i $p = 0,002$), no promjena nije bilo poslije primjene WMR-a i WP-a ($p = 0,747$ i $p = 0,174$). Nakon 14-dnevnoga čuvanja u umjetnoj slini nije bilo značajnih promjena u vrijednostima Ra caklinskih uzoraka ($p > 0,05$). Ni primjena WMR-a i WP-a, ni čuvanje u umjetnoj slini tijekom 14 dana nije rezultiralo promjenama u vrijednostima R caklinskih uzoraka ($p > 0,05$).

Srednje vrijednosti i standardne devijacije (± SD) razlika u CIELab parametrima (ΔL, Δa i Δb) prikazane su u

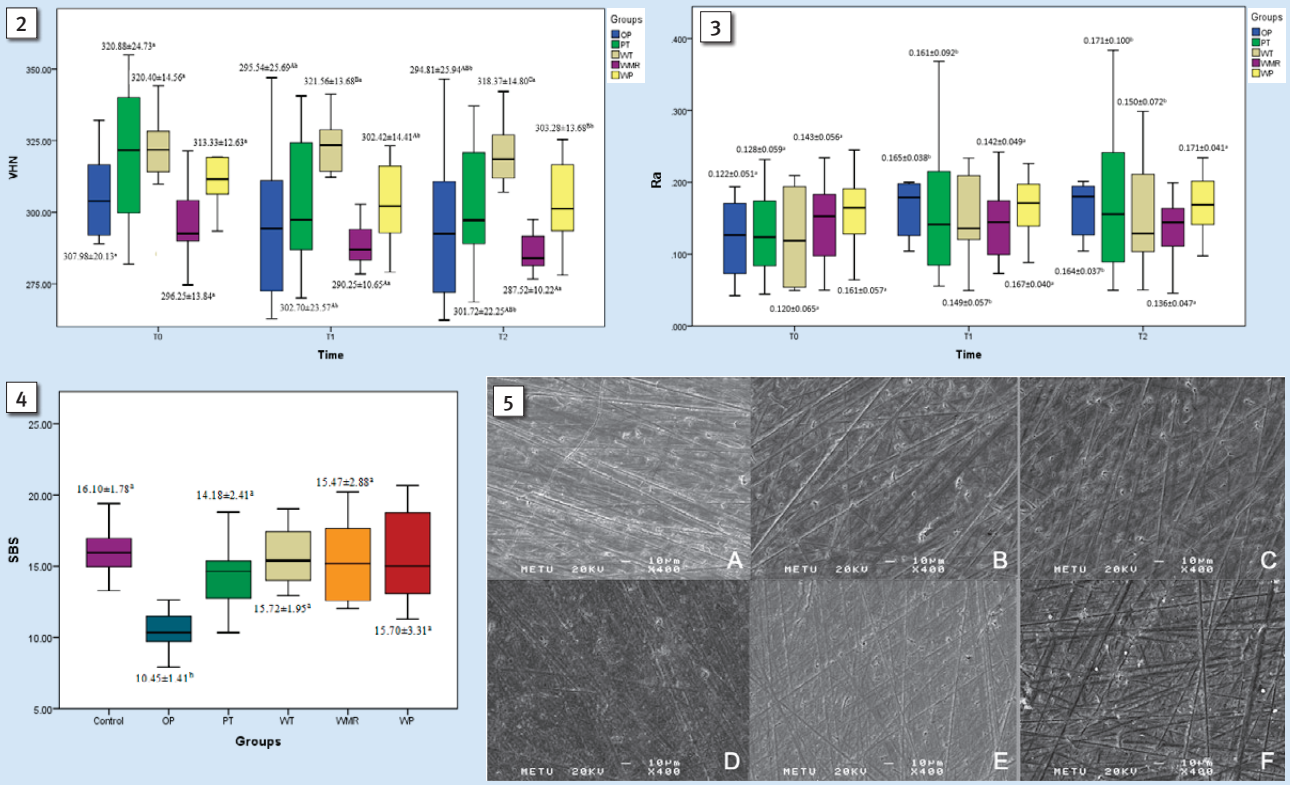

Figure 2 Microhardness (VHN) values (mean ± SD) of the tested groups. Different lowercase letters indicate significant differences among times and different uppercase letters indicate significant differences among groups ($p < 0.05$).
Slika 2. Vrijednosti mikrotvrdoće (VHN, sr. vrijednost ± SD) ispitivanih skupina. Različita mala slova upozoravaju na značajne razlike između razdoblja, a različita velika slova na značajne razlike između skupina ($p < 0,05$).
Figure 3 Surface roughness (Ra) (μm) values (mean ± SD) of the tested groups. Different lowercase letters indicate significant differences among times and different uppercase letters indicate significant differences among groups ($p < 0.05$).
Slika 3. Vrijednosti hrapavosti površine (Ra) (μm) (sr. vrijednost ± SD) ispitivanih skupina. Različita mala slova upozoravaju na značajne razlike između razdoblja, a različita velika slova na značajne razlike između skupina ($p < 0,05$).
Figure 4 Shear bond strength (SBS) values (MpA) (mean ± SD) of the tested groups. Different letters indicate differences among whitening products ($p < 0.05$).
Slika 4. Vrijednosti snage vezivanja (SBS) (MpA) (sr. vrijednost ± SD) ispitivanih skupina. Različita slova upozoravaju na razlike između sredstava za izbjeljivanje ($p < 0,05$).
Figure 5 (A-F): Representative SEM images of enamel surfaces subjected to the tested products; A- untreated, intact enamel, B-after application of OP, C- after application of PT, D- after application of WT, E- after application of WMR, F after application of WP (X400 magnifications)
Slika 5 (A-F). Reprezentativni SEM prikazi površina cakline podvrgnutih ispitivanim proizvodima: A – netretirana intaktna caklina, B – nakon primjene OP-a, C – nakon primjene PT-a, D – nakon primjene WT-a, E – nakon primjene WMR-a, F – nakon primjene WP-a (povećanje 400 puta).

Mean and standard deviation (± SD) of difference of CIELab parameters (ΔL, Δa and Δb) are shown in Table 2 and ΔE0 and $\Delta E0_{00}$ (color difference between T_0-T_1), ΔE1 and $\Delta E1_{00}$ (color difference between T_1-T_2) and ΔE2 and $\Delta E2_{00}$ (color difference between T_0-T_2) values are shown in Tables 3 and 4. There were significant differences in ΔE0 and ΔE2 of the enamel specimens treated with the whitening products ($p<0.001$), however, no differences were found among the groups in terms of ΔE1 ($p=0.870$). OP, PT, WMR and WP caused significant color differences ($p<0.001$, $p=0.010$, $p=0.018$, $p<0.001$ respectively); whereas no difference was observed with WT ($p=0.221$).

Significant differences among the $\Delta E0_{00}$ and $\Delta E2_{00}$ of the enamel specimens treated with whitening products were found ($p<0.001$) but there were no differences among the groups in terms of $\Delta E1_{00}$ ($p=0.095$). Significant differences were also found among the $\Delta E0_{00}$, $\Delta E1_{00}$ and $\Delta E2_{00}$ of the groups except WT.

tablici 2., a vrijednosti ΔE0 i $\Delta E0_{00}$ (razlika u boji između T_0 i T_1), ΔE1 i $\Delta E1_{00}$ (razlika u boji između T_1 i T_2) te ΔE2 i $\Delta E2_{00}$ (razlika u boji između T_0 i T_2) prikazane su u tablicama 3. i 4. Bilo je značajnih razlika između ΔE0 i ΔE2 caklinskih uzoraka tretiranih sredstvima za izbjeljivanje ($p < 0,001$), no nije bilo razlika između skupina kada je riječ o ΔE1 ($p = 0,870$). OP, PT, WMR i WP potaknuli su značajne promjene boje ($p < 0,001$, $p = 0,010$, $p = 0,018$ i $p<0,001$), no nije bilo nikakve razlike pri primjeni WT-a ($p = 0,221$).

Otkrivene su značajne razlike između $\Delta E0_{00}$ i $\Delta E2_{00}$ caklinskih uzoraka tretiranih sredstvima za izbjeljivanje ($p < 0,001$), ali nije bilo razlika između skupina kada je riječ o $\Delta E1_{00}$ ($p = 0,095$). Značajne razlike ustanovljene su između $\Delta E0_{00}$, $\Delta E1_{00}$ i $\Delta E2_{00}$ u svim skupinama osim u WT-u.

Srednje vrijednosti i standardne devijacije (± SD) vrijednosti SBS-a prikazane su na slici 4. Nije bilo statistički značajnih razlika između skupina, osim kod uzoraka na koje je

Table 2. ΔL, Δa and Δb values (mean ± SD) of the tested groups
Tablica 2. ΔL, Δa and Δb values (sr. vrijednost ± SD) ispitivanih skupina

	OP	PT	WT	WMR	WP
ΔL_{0-1}	0.47 ±2.86	-0.13 ±2.30	-0.38 ±1.58	-0.63 ±1.69	-0.01 ±2.52
ΔL_{1-2}	-0.88 ±1.75	0.45 ±2.77	0.05 ±1.19	0.21 ±1.23	0.90 ±1.55
ΔL_{0-2}	-0.42 ±2.03	0.33 ±2.63	-0.33 ±0.96	-0.43 ±1.59	0.89 ±3.16
Δa_{0-1}	-0.83 ±0.69	-0.73 ±0.92	-0.35 ±0.57	-0.70 ±0.60	-0.58 ±1.06
Δa_{1-2}	-0.11 ±0.48	-0.43 ±0.89	-0.39 ±0.52	-0.22 ±0.34	-0.22 ±0.64
Δa_{0-2}	-0.94 ±0.78	-1.17 ±0.77	-0.74 ±0.46	-0.92 ±0.79	-0.80 ±0.96
Δb_{0-1}	-5.53 ±4.26	-2.19 ±2.27	-0.69 ±1.68	-1.30 ±1.67	-2.84 ±2.39
Δb_{1-2}	-1.07 ±1.74	-2.18 ±1.94	-1.47 ±1.13	-1.56 ±1.43	-0.84 ±1.93
Δb_{0-2}	-6.60 ±4.23	-4.37 ±2.02	-2.16 ±1.30	-2.86 ±1.20	-3.68 ±1.80

Table 3 ΔE0, ΔE1 and ΔE2 values (mean ± SD) of the tested groups
Tablica 3 ΔE0, ΔE1 and ΔE2 values (sr. vrijednost ± SD) ispitivanih skupina

Groups/Skupina	ΔE0	ΔE1	ΔE2	p+
OP	7.90 ± 1.78^{Aa}	2.56 ± 1.13^{Ab}	7.35 ± 1.50^{Aa}	<0.001*
PT	5.37 ± 1.59^{Ba}	3.49 ± 2.12^{Ab}	3.89 ± 0.82^{Bb}	0.010*
WT	2.67 ± 0.92^{Ca}	1.95 ± 1.15^{Aa}	2.36 ± 0.61^{Ca}	0.221
WMR	3.52 ± 1.08^{Ca}	2.14 ± 1.20^{Ab}	2.69 ± 0.96^{Cb}	0.018*
WP	5.11 ± 1.44^{Ba}	2.60 ± 0.93^{Ab}	4.49 ± 0.63^{Ba}	<0.001*
p	<0.001	0.870	<0.001	

Different letters, lowercase in rows for different times and uppercase in columns for different whitening products indicate significant difference ($p < 0.05$). • Različita slova, mala slova u redovima u različitim vremenskim intervalima te velika slova u stupcima različitih sredstava označavaju značajne razlike ($p < 0,05$).

Table 4 ΔE_{00}, $\Delta E1_{00}$ and $\Delta E2_{00}$ values (mean ±SD) of the tested groups
Tablica 4. ΔE_{00}, $\Delta E1_{00}$ and $\Delta E2_{00}$ values (sr. vrijednost ±SD) ispitivanih skupina

Groups/Skupina	$\Delta E0_{00}$	$\Delta E1_{00}$	$\Delta E2_{00}$	p+
OP	3.59 ± 0.86^{aA}	1.50 ± 0.71^{bA}	3.87 ± 0.97^{aA}	<0.001
PT	2.27 ± 0.54^{aB}	2.20 ± 1.25^{aA}	3.08 ± 0.80^{bA}	0.035
WT	1.39 ± 0.35^{aC}	1.10 ± 0.65^{aA}	1.48 ± 0.43^{aC}	0.187
WMR	1.63 ± 0.59^{aC}	1.21 ± 0.56^{aA}	2.08 ± 0.68^{bB}	0.005
WP	2.58 ± 0.44^{aB}	1.54 ± 0.63^{bA}	2.98 ± 0.81^{aAB}	<0.001
p	$<0.001^{++}$	0.095^{+++}	$<0.001^{++}$	

+: One-way repeated measures ANOVA results/rezultati jednosmernog ponovljenog ANOVA testa; ++: One-way ANOVA results/rezultati jednosmernog ANOVA testa, +++: Welch's test results/rezultati Welchovog testa. Different letters, lowercase in rows for different times and uppercase in columns for different whitening products indicate significant difference ($p < 0.05$)/Različita slova, mala slova u redovima u različitim vremenskim intervalima te velika slova u stupcima različitih sredstava označavaju značajne razlike ($p < 0,05$).

Mean and standard deviations (± SD) of SBS values are illustrated in Figure 4. There were no significant differences among the groups, except OP applied enamel specimens that showed the lowest SBS value (p=0.001).

SEM micrographs are illustrated in Figure 5. SEM observations revealed smooth enamel surfaces without any deleterious effects of whitening products. Only few scratches, due to the grinding procedure, were observed on the enamel surfaces.

Discussion

The results of studies evaluating enamel microhardness after whitening with OTC products are quite different. Zantner et al. (26) tested different OTC and home whitening products in terms of the changes they created on the surface of enamel, and reported that all materials used significantly

primijenjen OP koji su imali najniže vrijednosti SBS-a (p = 0,001).

Mikrofotografije SEM-a prikazane su na slici 5. Uočena je glatka površina cakline bez ozbiljnijih razornih učinaka sredstava za izbjeljivanje. Na površini cakline vidjelo se samo nekoliko ogrebotina nastalih zbog rezanja.

Rasprava

Rezultati studija o procjeni mikrotvrdoće cakline poslije izbjeljivanja OTC proizvodima dosta su različiti. Zantner i suradnici (26) testirali su različite OTC proizvode i proizvode za kućno izbjeljivanje zbog promjena koje su stvarali na površini cakline te su izvijestili da svi korišteni materijali znatno

affected the surface microhardness. Azer et al. (17) examined the microhardness of enamel specimens treated with 3 whitening tray and 2 whitening strip systems and observed that all products decreased the microhardness of the enamel. Greenwall-Cohen et al. (13) also reported that the tested non-HP OTC whitening products available in UK resulted in reduction in Vickers microhardness.

In the present study, OP, PT and WP decreased the microhardness of the enamel, while WMR and WT did not cause any changes in the microhardness of the enamel. Therefore, the first hypothesis was rejected. A shorter application time of WMR and WT compared to other tested materials could affect the results. The storage in artificial saliva 14 days after the whitening was completed did cause no change in the microhardness values of all specimens.

The change in enamel surface roughness has also been considered as a problem in tooth whitening. Several studies have reported enamel surface roughness increases after whitening with high concentrations of HP or CP (27-29). However, Sasaki et al. (30) examined the surface morphology of the enamel specimens whitened with Colgate Platinum (10% CP) and Day White 2Z (7,5% HP) and reported some micro changes on the surfaces of enamel treated with both materials. On the other hand, Kwon et al.(27) have observed no changes in the enamel surface roughness after the application of professionally prescribed or OTC whitening agents. In the present study, a significant increase in the enamel surface roughness was observed in OP, PT and WT; whereas no changes were found in WMR and WP. Therefore, the second hypothesis was rejected. WMR had the lowest concentration of peroxide (2% HP) among products examined. Although WP had higher HP concentration (6%) than WMR, no change was observed on enamel surfaces roughness treated with this whitening product, either. The storage in artificial saliva 14 days after the whitening had been completed did not change the values of surface roughness of all specimens too.

In this study, the profilometer readings were also supported by the SEM evaluations. SEM investigations have been largely preferred for the evaluation of surface morphology of whitened teeth. However; the results of the SEM studies were different and conflicting. Most of them show little or no change of whitened enamel surfaces.(26, 27, 30-32) Auschill et al. (32) examined the changes in enamel morphology treated with two different whitening products; a tray based (5% HP) and a whitening strip (5.3% HP) by SEM and reported that both of the whitening products caused no changes on the enamel surface structure. In the present study, SEM examinations also revealed no changes of the enamel surfaces after applying whitening products.

The behavior of enamel regarding its color change (ΔE and ΔE_{00}) was addressed as well. The clinical acceptance threshold considered for ΔE is 2.7 and for $E\Delta_{00}$ is 1.8 (19). Any color difference value higher than these thresholds can be distinguished by an unskilled individual and cannot be considered clinically acceptable (19). OP, PT, WMR and WP created significant ΔE and ΔE_{00} values but no difference was observed with WT. Therefore, the third hypothesis was also rejected. The highest ΔE value after 14 days was in OP fol-

utječu na mikrotvrdoću površine. Azer i suradnici (17) ispitivali su mikrotvrdoću uzoraka cakline tretiranih trima udlagama za izbjeljivanje i dvama sustavima traka za izbjeljivanje i istaknuli da svi proizvodi smanjuju mikrotvrdoću cakline. Greenwall-Cohen i suradnici (13) također su izvijestili da su testirani OTC proizvodi za izbjeljivanje koji ne sadrže vodikov peroksid i dostupni su u Ujedinjenome Kraljevstvu, rezultirali smanjenjem Vickersove mikrotvrdoće.

U ovom istraživanju su OP, PT i WP smanjili mikrotvrdoću cakline, a WMR i WT nisu prouzročili promjene. Dakle, prva hipoteza je odbačena. Kraća primjena WMR-a i WT-a od ostalih testiranih materijala mogla bi utjecati na rezultate. Pohranjivanje u umjetnu slinu tijekom 14 dana nakon završetka izbjeljivanja nije promijenilo vrijednosti mikrotvrdoće svih uzoraka.

Promjena hrapavosti površine cakline također se smatra problemom pri izbjeljivanju zuba. Autori nekoliko studija izvijestili su da se hrapavost površine cakline povećava nakon izbjeljivanja s visokim koncentracijama HP-a i CP-a (27 – 29). Međutim, Sasaki i suradnici (30) ispitali su površinsku morfologiju uzoraka cakline izbjeljivanih Colgate Platinumom (10 % CP) i Day Whiteom 2Z (7,5 % HP) i uočili mikropromjene na površinama cakline tretirane s oba materijala. S druge strane, Kwon i suradnici (27) nisu zabilježili promjene u hrapavosti površine cakline poslije primjene profesionalno propisanih sredstava ili OTC sredstava za izbjeljivanje. U ovom istraživanju uočeno je značajno povećanje hrapavosti površine cakline pri primjeni OP-a, PT-a i WT-a, a kod WMR-a i WP-a nisu uočene nikakve promjene. Zato je i druga hipoteza odbačena. WMR ima najnižu koncentraciju peroksida (2 % HP) među ispitivanim proizvodima. Iako WP ima veću koncentraciju HP-a (6 %) od WMR-a, nije uočena promjena ni u hrapavosti površina cakline tretirane tim proizvodom za izbjeljivanje. Skladištenje u umjetnoj slini 14 dana nakon završetka izbjeljivanja također nije promijenilo vrijednosti hrapavosti površine svih uzoraka.

U ovom istraživanju su mjerenja profilometrom također potkrijepljena SEM evaluacijama. SEM istraživanja uglavnom su bila korištena za ocjenu morfologije površine izbijeljenih zuba. No rezultati SEM istraživanja različiti su i proturječni. U većini se ističe mala ili nikakva promjena na izbijeljenim površinama cakline (26, 27, 30 – 32). Auschil i suradnici (32) ispitali su promjene u morfologiji cakline tretirane dvama različitim proizvodima za izbjeljivanje, jednim na načelu udlage (5 % HP) i drugim na načelu traka za izbjeljivanje (5,3 % HP). Pokazali su da ni jedan proizvod nije prouzročio promjene u strukturi površine cakline. U ovom istraživanju SEM analize nisu otkrile nikakve promjene na površini cakline poslije nanošenja proizvoda za izbjeljivanje.

Također je obrađeno djelovanje cakline kada je riječ o promjeni boje (ΔE i ΔE00). Klinički prag prihvatljivosti za ΔE iznosi 2,7, a za EΔ00 1,8 (19). Bilo koju vrijednost razlike u boji, ako je viša od ovih razina, može razlikovati nestručni pojedinac i ne može se smatrati klinički prihvatljivom (19). OP, PT, WMR i WP postigli su značajne vrijednosti ΔE i ΔE00, ali nije uočena razlika u odnosu na WT. Dakle, i treća hipoteza je odbačena. Najviša vrijednost ΔE nakon 14 dana bila je kod OP-a, a zatim WP-a, PT-a i WMR-a. Najniža

lowed by WP, PT and WMR. The lowest ΔE value was seen in WT. However, the highest ΔE_{00} value after 14days was in OP followed by PT, WP and WMR respectively. Additionally, the ΔE and ΔE_{00} values presented by WT were lower than 2.7 and 1.8, respectively. This may be due to the ingredients and and short application time of the product. Although WT contains silica; an abrasive which was considered to be a whitening agent, no color difference was seen after using this product.

It has been stated that whitening products with lower HP concentration show less whitening effect compared to products having higher peroxide concentrations (30, 31). However, other studies have reported that low or high concentrations do not make significant differences in terms of whitening when the application time is 1-2 weeks (30-32). Successful results were obtained with tray systems (30-32). Dietschi et al. (33) compared various OTC products and they obtained best whitening results with tray systems. Kielbassa et al. (34) examined 5 different OTC products including a tray system, a whitening strip and varnishes and concluded that whitening occured in the first few minutes and did not change with the extended time. In this study, the highest color change was also observed immediately after whitening, and color change decreased in all products after 14 days of bleaching. These results supported the recommendation of a delayed color determination (9).

It has been reported in several studies that whitening agents negatively affect the bond strength of resin composites (32, 35). When a subsequent esthetic resin composite restoration was planned after the whitening procedure, it has been recommended to wait for 2-3 weeks to ensure adequate bond strength (36, 37). Nevertheless, Zu et al. (25) reported that the amount of oxygen in the enamel either whitened or not did not differ and the reason for low bonding strength was not the residual oxygen, but structural micromorphological deterioration in the tooth tissues. A decrease in microhardness, calcium loss and organic structure changes have also been associated with weakening in bond strength (38). In the current study, a SBS test was done after the artificial saliva storage for 14 days. Only SBS values of OP applied specimens were different and lower than the specimens treated with other whitening products. This may be the due to the long application time of this product. The SBS values of the enamel surfaces treated with PT, WT, WMR and WP were not different from the values of the intact enamel surfaces. Hence, the fourth hypothesis was rejected.

Tooth surface undergoes a special interaction with the saliva that involves the interchange of various ions and regulates the re- and demineralization process in the oral cavity. Since it is not easy to imitate the actual oral conditions which could vary in each person, the *in vitro* changes of human enamel after whitening may not be relevant. Therefore, more clinical studies are required to understand the effects of whitening products, especially the effects of new products with different whitening mechanisms. Despite an increase in the variety of OTC products in the market, there is no long-term proof of safety and durability of whitening of these products. Therefore, it should be mandatory to observe the long-term results and their potential detrimental effects on the enamel.

vrijednost ΔE uočena je kod WT-a. Međutim, najviša vrijednost ΔE00 nakon 14 dana bila je kod OP-a, a slijede PT, WP i WMR. Dodatno, vrijednosti ΔE i ΔE00 koje su dobivene kod WT-a bile su manje od 2,7, odnosno 1,8. Razlog može biti sastav, ili kratka primjena proizvoda. Iako WT sadržava silicij, abraziv za koji se smatralo da izbjeljuje, kod toga proizvoda nije uočena razlika u boji.

Smatra se da proizvodi za izbjeljivanje s niskim koncentracijama HP-a imaju manji učinak izbjeljivanja u odnosu prema proizvodima s višim koncentracijama peroksida (30, 31). No druga istraživanja pokazala su da se visokim ili niskim koncentracijama ne postižu značajne razlike u izbjeljivanju ako je vrijeme primjene od 1 do 2 tjedna (30 – 32). Uspješni rezultati postignuti su sustavima s udlagama (30 – 32). Dietschi i suradnici (33) uspoređivali su različite OTC proizvode te su najbolje rezultate postigli sustavom s udlagama. Kielbassa i suradnici (34) ispitivali su pet različitih OTC proizvoda – sustav s udlagama, trake za izbjeljivanje i lakove te su zaključili da se izbjeljivanje događa u prvih nekoliko minuta te da se ono ne mijenja s duljinom postupka. U našem istraživanju najveća promjena boje opažena je neposredno nakon postupka, a promjena boje smanjivala se tijekom 14 dana izbjeljivanja. Taj rezultat podupire preporuku o kasnijem određivanju finalne boje (9).

U nekoliko istraživanja autori su istaknuli da sredstva za izbjeljivanje negativno utječu na snagu vezivanja kompozitnih materijala (32, 35). Ako se planiraju adhezivni ispuni poslije postupka izbjeljivanja, preporučuje se pričekati od dva do tri tjedna da bi se osigurala adekvatna snaga vezivanja materijala na caklinu (36, 37). No, Zu i suradnici (25) ustanovili su da je količina kisika u caklini koja nije bila izbjeljivana i one koja jest jednaka te da mala snaga vezivanja ne ovisi o rezidualnom kisiku, nego o mikromorfološkom razaranju strukture tvrdih zubnih tkiva. Smanjenje u mikrotvrdoći, gubitak kalcija i promjene u organskoj strukturi također se povezuju sa slabljenjem snage vezivanja (38). U našem istraživanju je SBS test primijenjen nakon što su uzorci 14 dana bili u umjetnoj slini. Samo su vrijednosti SBS testa nakon primjene OP-a imali drukčije vrijednosti, tj. bile su niže nego kod ostalih uzoraka tretiranih drugim sredstvima. Uzrok može biti duljina primjene toga proizvoda. Vrijednosti SBS testa na caklinskoj površini tretiranoj PT-om, WT-om, WMR-om i WP-om nisu bile drukčije od vrijednosti intaktne cakline. Zato smo odbacili i četvrtu hipotezu.

Površina cakline ima posebnu interakciju sa slinom koja uključuje izmjenu različitih iona i regulira remineralizacijski i demineralizacijski proces u usnoj šupljini. Budući da nije jednostavno oponašati stvarne uvjete u usnoj šupljini, te da se oni razlikuju od osobe do osobe, promjene *in vitro* na ljudskoj caklini nakon izbjeljivanja mogle bi biti nebitne. Zato je potrebno više kliničkih istraživanja da bismo bolje razumjeli učinke postupaka izbjeljivanja, posebno novih proizvoda koji sadržavaju drukčije mehanizme izbjeljivanja. Unatoč porastu u raznolikosti OTC proizvoda za izbjeljivanje na tržištu, nema dugotrajnih dokaza o sigurnosti i trajnosti izbjeljivanja tim proizvodima. Trebalo bi biti obvezno podastrijeti dugoročne rezultate i potencijalne štetne učinke na caklinu.

Conclusions

The results of this *in vitro* study indicate that whitening for the 14 days with Opalescence PF 10%, dentist prescribed at-home whitening product (OP), Opalescence Go, prefilled tray (PT) and Cavex Bite&White, whitening pen (WP) decreased the microhardness of the enamel, whereas Opalescence, whitening toothpaste (WT) and Listerine Healthy White, whitening mouth rinse (WMR) did not make any changes.

The application of OP, PT and WT increased the surface roughness of the enamel, while WMR and WP did not cause any changes.

The most evident colour difference was found after application of OP, then PT, WMR and WP, respectively.

Only the application of OP decreased the shear bond strength of enamel to resin composite bonded with universal adhesive. Other teeth whitening products had no effects on shear bond strength.

The SEM analysis revealed that the tested whitening products cause no deleterious effects on tooth enamel.

Conflict of interest

Authors declare no conflict of interest

Author's contribution: **E. Y.** – investigation; **U. K. V.** - Validation, visualization, formal analysis; **F. Y. C.** - Methodology, resources, data curation; **S. G.** - writing original manuscript, conceptualization, project administration, review and editing.

Zaključak

Rezultati ovoga istraživanja *in vitro* pokazuju da 14-dnevno izbjeljivanje sredstvima Opalescence PF 10 %, proizvodom koji propisuje doktor dentalne medicine za upotrebu kod kuće, Opalescence Go (OP), unaprijed pripremljenom udlagom te Cavex Bite&White (WP), olovkom za izbjeljivanje, smanjuje mikrotvrdoću cakline, a upotreba Opalescence paste za izbjeljivanje (WT) i Listerine Healthy White, tekućine za izbjeljivanje (WMR) ne rezultira nikakvim promjenama.

Primjena OP-a, PT-a i WT-a povećala je hrapavost površine cakline, a WMR i WP nisu je promijenili.

Najveća promjena boje uočena je pri primjeni OP-a, zatim PT-a, WMR-a pa WP-a.

Jedino je primjena OP-a smanjila snagu vezivanja na caklinu pri primjeni univerzalnoga adheziva. Drugi proizvodi nisu utjecali na snagu vezivanja.

SEM analiza otkrila je da testirani proizvodi ne oštećuju caklinu.

Sukob interesa

Autori nisu bili u sukobu interesa.

Doprinos autora: **E. Y.** – istraživanje; **U. K. V.** - Validacija, pisanje originalnog rukopisa, vizualizacija, formalna analiza; **F. Y. C.** - Metodologija, resursi, prikupljanje podataka; **S. G.** - Konceptualizacija, administracija projekta, pregled i uređivanje.

Sažetak

Cilj: Svrha ovoga istraživanja *in vitro* bila je procijeniti učinak četiriju komercijalnih proizvoda za izbjeljivanje na mikrotvrdoću, hrapavost površine, boju i snagu vezivanja (SBS) te svojstva površine ljudske cakline u usporedbi s proizvodom koji se koristi uz nadzor doktora dentalne medicine. **Materijal i metode:** U šest skupina podijeljeno je 78 uzoraka cakline (n=13): 1. Opalescence PF 10 % (OP) koji propisuje doktor dentalne medicine za upotrebu kod kuće; 2. Opalescence GO unaprijed pripremljena udlaga (PT); 3. zubna pasta Opalescence Whitening; 4. tekućina za ispiranje usne šupljine Listerine Healthy White (WMR); 5. olovka za izbjeljivanje Cavex Bite&White (WP; 6. bez tretmana (Con). Izmjereni su mikrotvrdoća (VHN), hrapavost površine (Ra) i boja uzoraka (T_0) te su uzorci povrgnuti 14-dnevnim protokolima izbjeljivanja (T_1) nakon čega su uskladišteni u umjetnoj slini također 14 dana (T_2). Mjerenja su ponovljena na T_1 i T_2. SBS test proveden je primjenom 35-postotne fosforne kiseline (Scotchbond Universal Etchant) te je na uzorke nanesen univerzalni adheziv (G-Premio Bond) i mikrohibridni/univerzalni kompozitni smolasti materijal (Essentia) u teflonskoj tubi pričvršćenoj na površinu cakline ($p < 0,05$). Morfologija površine cakline analizirana je SEM-om. Vrijednost **p** postavljena je na 0,05. **Rezultati:** Primjena OP-a, PT-a i WP-a smanjila je mikrotvrdoću uzoraka cakline ($p < 0,05$), a u mikrotvrdoći cakline nakon primjene WT-a i WMR-a ($p > 0,05$) nije bilo značajnih promjena. Vrijednosti Ra porasle su nakon primjene OP-a, PT-a i WT-a ($p < 0,05$), no nakon primjene WMR-a i WP-a nije bilo promjene ($p > 0,05$). OP, PT, WMR i WP promijenili su boju cakline ($p < 0,05$). Nije bilo značajnih razlika u SBS-ovima između skupina, osim kod uzoraka na koje je primijenjen OP i imali su najniže vrijednosti SBS-a (p=0.001). SEM analizom ustanovljena je glatka površina cakline. **Zaključak:** Proizvodi za izbjeljivanje različito utječu na mikrotvrdoću, hrapavost površine i boju cakline. Samo je OP smanjio SBS cakline.

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