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IZVORNI ZNANSTVENI RAD

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Effect of Ozone on Adhesion of Bioactive Restorative Materials *in Vitro*

Učinak ozona na adheziju bioaktivnih restaurativnih materijala in vitro

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Abstract

Objective: This *in vitro* preliminary study investigated how different ozone formulations affect the adhesion, surface topography, and interfacial ion release of two pediatric bioactive restorative materials. **Materials and Methods:** Standardized enamel and dentin surfaces (n=96) from extracted human teeth were randomized into 8 groups which differ by the treatment protocol (no treatment, ozone gas, ozonated water, ozonated gel) and restorative material used (Fuji II LC, ACTIVA Bioactive Restorative). Adhesive performance was evaluated after 7 days of storage (HBSS, 37 °C) by shear bond strength (SBS) test and microscopic fracture mode analysis. Surface roughness (Sa) was measured by optical profilometry; interfacial elemental composition was analyzed by Raman spectroscopy and scanning electron microscopy with energy dispersive X-ray detectors (SEM-EDX). **Results:** Ozone treatment did not significantly affect SBS (mean 3.59±3.23 MPa [min 0.05 MPa; max 11.69 MPa]), fracture modes, or surface roughness (p>0.05 for all domains). Chemical analyses revealed no appreciable difference, with the possible exception of minimal fluoride release. **Conclusions:** Regardless of the vector agent, ozone treatments showed no significant effect on the short-term adhesion capability of either restorative material, or on the bonding quality with enamel and dentin. Additionally, it seemed that they they neither affected the surface topography nor the possible bioactivity at the interface of the tested materials. However, these preliminary results are restricted to the specific experimental conditions adopted, and further studies are required before any definitive clinical recommendations can be made.

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Introduction

Medical ozone applications (oxygen–ozone therapy, OOT) have been introduced to various medical and dental fields, including pediatric restorative dentistry (1–3). Thanks to its antibacterial and disinfectant properties, ozone has shown promising results for cavity decontamination before restorative procedures or sealant placement, as an alternative antiseptic in selective caries removal, and as a non-restorative caries treatment for anxious or uncooperative children (4–7).

Uvod

Primjena medicinskog ozona (oxygen-ozone therapy – OOT) uvedena je u različita područja medicine i dentalne medicine, uključujući pedijatrijsku restaurativnu dentalnu medicinu (1 – 3). Zahvaljujući antibakterijskim i dezinfekcijskim svojstvima, ozon je pokazao obećavajuće rezultate u dekontaminaciji kaviteta prije restaurativnih postupaka ili postavljanja pečatnih ispuna kao alternativni antiseptik u selektivnom uklanjanju karijesa te kao nerestaurativni tretman

It has also been used to prevent or reverse early carious lesions by promoting remineralization (8).

In minimally invasive pediatric dentistry, understanding treatment interactions is essential to develop synergistic protocols that improve restorative outcomes. Minimally invasive techniques modify the residual dental surface, thus altering chemical composition, porosity, hardness, and roughness, which in turn affects material adhesion and bond durability (9–14). Consequently, the caries removal method and resulting surface characteristics are key factors influencing bond strength and mineral exchange at the tooth–restoration interface (11, 15–18).

However, limited evidence exists on how minimally invasive procedures and surface conditioning affect bond strength in primary teeth (11, 19). Clear recommendations for selecting restorative materials after specific caries treatment protocols are still lacking (20).

When ozone is applied before restoration, its strong oxidizing properties may interfere with adhesive polymerization, thus potentially reducing bond strength and altering ion release from bioactive materials (21, 22). In the context of restorative dentistry, "bioactivity" refers to the ability of a material to interact with dental tissues by releasing ions and promoting interfacial mineral changes (e.g., formation or stabilization of apatite-like phases) (23,24). Because ozone is a strong oxidizing agent, it could theoretically influence this process by modifying surface chemistry and microstructure, such as altering surface acidity, dentin tubule patency, or enamel microporosity, thereby affecting ion exchange and interfacial mineralization (6,11,17,18,25,26)

Since enamel adhesion is critical to prevent microleakage and restoration failure, assessing the effects of ozone on both enamel and dentin bonds is particularly important (27). Only few studies have explored the impact of ozone on adhesion and its interactions with bioactive materials, with heterogeneous protocols and conflicting results. While several authors have reported no significant effects of ozone application on bond strength to either enamel or dentin (5, 21, 28–30), others have observed a reduction in bond strength (31) or, conversely, an improvement when ozone was applied to caries-affected dentin (32). Additionally, direct comparisons among ozone formulations are lacking.

This *in vitro* study aimed to address these gaps by evaluating and comparing the adhesive performance of two pediatric bioactive restorative materials on non-carious enamel and dentin after different ozone application protocols used for final cavity decontamination. Fuji II LC was selected as representative resin-modified glass ionomer cement with long-standing clinical use in pediatric dentistry, while ACTIVA Bioactive Restorative was chosen as a newer hybrid material combining resin-based and glass-ionomer–like features with claimed bioactive behavior. Together, these materials represent two clinically relevant and commonly used approaches to bioactive restorations in pediatric patients.

The secondary objective was to assess the resulting surface topography and interface mineral composition to identify possible ozone-induced effects on bioactivity. The null hypotheses (H_0) were as follows: (i) bond strength and failure

karijesa kod anksiozne ili nesuradljive djece (4 – 7). Također je primjenjivan u prevenciji ili reverziji ranih karijesnih lezija poticanjem remineralizacije (8).

U minimalno invazivnoj pedijatrijskoj dentalnoj medicini razumijevanje međudjelovanja tretmana ključno je za razvoj sinergističkih protokola koji poboljšavaju ishod restauracije. Minimalno invazivne tehnike djeluju na rezidualnu dentalnu površinu tako da mijenjaju kemijski sastav, poroznost, tvrdoću i hrapavost, što posljedično utječe na adheziju materijala i trajnost veze (9 – 14). Zato su metoda uklanjanja karijesa i rezultirajuće značajke površine ključni čimbenici koji utječu na čvrstoću veze i mineralnu razmjenu na sučelju zub - restauracija (11,15 – 18).

No postoje ograničeni dokazi o tomu kako minimalno invazivni postupci i kondicioniranje površine utječu na čvrstoću veze u mliječnim zubima (11, 19). Jasne preporuke za odabir restaurativnih materijala poslije specifičnih protokola tretmana karijesa još uvijek nedostaju (20).

Kada se ozon primjenjuje prije restauracije, njegova snažna oksidacijska svojstva mogu interferirati s polimerizacijom adheziva, potencijalno smanjujući čvrstoću veze i mijenjajući oslobađanje iona iz bioaktivnih materijala (21, 22). U kontekstu restaurativne dentalne medicine, *bioaktivnost* se odnosi na sposobnost materijala za interakciju s dentalnim tkivima oslobađanjem iona i poticanjem mineralnih promjena na sučelju (npr., stvaranje ili stabilizacija faza sličnih apatitu) (23, 24). Budući da je ozon snažan oksidans, teorijski bi mogao utjecati na taj proces modificiranjem površinske kemije i mikrostrukture, primjerice, promjenom površinske kiselosti, prohodnosti dentinskih tubula ili mikroporoznosti cakline, čime bi se mogao promijeniti ionski izmjenjivački proces i mineralizacija na sučelju (6, 11, 17, 18, 25, 26).

S obzirom na to da je adhezija na caklinu ključna za sprječavanje mikropropuštanja i neuspjeha restauracije, osobito je važno procijeniti učinke ozona na vezu s caklinom i dentinom (27). Tek je u nekoliko studija istražen utjecaj ozona na adheziju i njegovo međudjelovanje s bioaktivnim materijalima, uz heterogene protokole i proturječne rezultate. Dok su pojedini autori izvijestili da primjena ozona ne utječe značajno na čvrstoću veze ni na caklini ni na dentinu (5, 21, 28 – 30), drugi su uočili smanjenje čvrstoće veze (31) ili, suprotno, poboljšanje kada je ozon primijenjen na dentinu zahvaćenom karijesom (32). Nadalje, nedostaju izravne usporedbe različitih formulacija ozona.

Ovim istraživanjem *in vitro* nastojalo se adresirati navedene praznine procjenom i usporedbom adhezivne učinkovitosti dvaju pedijatrijskih bioaktivnih restaurativnih materijala na nekarioznoj caklini i dentinu nakon različitih protokola primjene ozona koji se koriste za završnu dekontaminaciju kaviteta. Fuji II LC odabran je kao predstavnik staklenoionomernog cementa modificiranog smolom s dugogodišnjom kliničkom primjenom u pedijatrijskoj dentalnoj medicini, a ACTIVA Bioactive Restorative odabran kao noviji hibridni materijal u kojemu su kombinirane značajke kompozita na bazi smole i značajke slične staklenoionomeru, uz deklarirano bioaktivno ponašanje. Zajedno, ti su materijali dva klinički relevantna i često korištena pristupa bioaktivnim restauracijama kad je riječ o pedijatrijskim pacijentima.

modes do not differ among groups regardless of ozone formulation, and (ii) both materials perform similarly in bond strength. Ultimately, this study is expected to help guide the selection of treatment-material combinations that optimize restorative outcomes through synergistic interactions.

Materials and Methods

Sample selection and preparation

With regard to the shear bond strength evaluation, the sample size determination was performed using G*Power software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), setting a moderate effect size (i.e. 0.4), a power of 80%, and an alpha error of 0.05. The resulting minimum required sample size for each group was 5 samples, which was similar to another analogous study (33). The sample size was increased by 10% to compensate for possible sample damage during procedures.

A pool of human sound molars extracted for periodontal reasons in patients aged 18 to 40 years was selected for this study. Ethical review board and approval were waived because the sample teeth were obtained from a pooled biobank and categorized as irreversibly anonymized. In accordance with the guidelines and regulations of the local ethics committee, all the patients involved were informed about the use of their extracted teeth in research, and their oral consent was obtained.

After extraction, the teeth were disinfected in 3% hydrogen peroxide for ten minutes, cleaned of soft tissue debris by gently scraping the external surface with periodontal instruments (Hu-Friedy, Chicago, IL, USA), then rinsed with sterile water, gently dried with an air syringe, and finally stored at -20 °C until further use.

To conduct the experimental procedures, a pool of eligible teeth was collected from the biobank and stored in 0.02% thymol solution (Baxter Inc., Rome, Italy) at 4 °C for no longer than 1 month. Before any procedure, specimens were fully thawed at room temperature, and kept moist prior to bonding and testing to avoid dehydration and potential microcracking and impact on bond strength (34,35). Teeth were inspected under 10x optical microscope (OPMI Pico, Carl Zeiss Meditec Inc., Jena, Germany) to exclude crown caries, cracks, fractures, or restorations. A size 701 high-speed fissure bur (Komet USA LLC, Rock Hill, SC, USA) was used to separate the crown from the root portion below the level of the cement-enamel junction. The sample teeth were then transversally and longitudinally sectioned using a rotary microtome (Micromet Digital, Remet S.A.S., Bologna, Italy) at a speed of 3000 rpm under continuous water spray to obtain respectively buccal and lingual sections as enamel surfaces and occlusal sections as dentin surfaces. The specimen surfaces were polished with a lapping machine (LS2, Remet S.A.S.) with a 600-grit silicon-carbide (SiC) paper for 10 seconds.

Sekundarni cilj bio je procijeniti rezultirajuću površinsku topografiju i mineralni sastav na sučelju kako bi se identificirali mogući učinci na bioaktivnost induciranu ozonom. Nulte hipoteze (H_0) glasile su: (i) čvrstoća veze i načini loma ne razlikuju se među skupinama neovisno o formulaciji ozona, te (ii) oba materijala pokazuju slične vrijednosti kad je riječ o čvrstoći veze. U konačnici, očekuje se da će ovo istraživanje pomoći u odabiru kombinacija tretman-materijal koje optimiziraju restaurativne ishode putem sinergijskih interakcija.

Materijali i metode

Odabir i priprema uzoraka

Kad je riječ o procjeni smične čvrstoće veze, određivanje veličine uzorka provedeno je u programu G*Power (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Njemačka), uz postavljanje umjerene veličine učinka (0,4), snage testa od 80 % i alfa pogreške od 0,05. Dobivena minimalna potrebna veličina uzorka za svaku skupinu iznosila je 5 uzoraka, što je bilo usporedivo s drugim analognim istraživanjem (33). Veličina uzorka povećana je za 10 % radi kompenzacije mogućih oštećenja uzoraka tijekom postupaka.

Za ovo istraživanje odabran je skup humanih intaktnih kutnjaka ekstrahiranih iz parodontoloških razloga, a pacijenti su bili u dobi od 18 do 40 godina. Etičko povjerenstvo i odobrenje nisu bili potrebni jer su uzorci zuba dobiveni iz objedinjene biobanke i kategorizirani kao nepovratno anonimizirani. U skladu sa smjernicama i propisima lokalnoga Etičkoga povjerenstva, svi uključeni pacijenti bili su obaviješteni o uporabi njihovih ekstrahiranih zuba u istraživanju te je pribavljena njihova usmena suglasnost.

Nakon ekstrakcije zubi su 10 minuta dezinficirani u 3-postotnom vodikovu peroksidu, očišćeni od ostataka mekih tkiva nježnim struganjem vanjske površine parodontološkim instrumentima (Hu-Friedy, Chicago, IL, SAD), zatim su isprani sterilnom vodom i nježno posušeni zračnom štrcaljkom te pohranjeni na -20 °C do daljnje uporabe.

Za provedbu eksperimentalnih postupaka iz biobanke je dobiven skup prihvatljivih zuba i pohranjen u 0,02-postotnoj otopini timola (Baxter S.p.a, Rim, Italija) na 4 °C, ne dulje od 1 mjeseca. Prije bilo kojeg postupka uzorci su potpuno odmrznuti na sobnoj temperaturi te održavani vlažnima prije aplikacije adhezijskog sustava i testiranja da bi se izbjegla dehidracija i moguće mikropukotine te njihov utjecaj na čvrstoću veze (34, 35). Zubi su pregledani optičkim mikroskopom pri povećanju od 10 puta (OPMI Pico, Carl Zeiss Meditec Inc., Jena, Njemačka) radi isključenja koronarnog karijesa, pukotina, fraktura ili postojećih restauracija. Visokobrzinskim fisurnim svrdlom veličine 701 (Komet USA LLC, Rock Hill, SC, SAD) odvojena je kruna od korijena ispod razine caklinsko-cementnoga spojišta. Zatim su uzorci transverzalno i longitudinalno izrezani rotacijskim mikrotomom (Micromet Digital, Remet S.A.S., Bologna, Italija) pri brzini od 3000 o/min, uz kontinuirano hlađenje vodenim sprejom da bi se dobile bukalne i lingvalne sekcije kao površine cakline, te okluzalne sekcije kao površine dentina. Površine uzoraka polirane su 10 sekunda na uređaju za lapiranje (LS2, Remet S.A.S.) abrazivnim papirom od silicijeva karbida (SiC) granulacije 600.

Table 1 Subgroup analysis by material and treatment for the mean ± standard deviation of the shear bond strength (MPa) of restorative materials on enamel and dentin, and related pairwise comparison.
Tablica 1. Analiza podskupina prema materijalu i tretmanu za srednju vrijednost ± standardnu devijaciju smične čvrstoće veze (MPa) restaurativnih materijala na caklini i dentinu te pripadajuće usporedbe parova. a: Wilcoxonov test ranga-zbroja za dva uzorka (Mann-Whitneyjev test); b: Kruskal-Wallisov test i Dunnov post hoc test za višestruke usporedbe parova

	Enamel		Dentin	
	Mean ± SD	*p*	*Mean ± SD*	*p*
FUJI	2.04 ± 2.94 (n=21)	$p<0.0001^a$	1.62 ± 1.85 (n=21)	$p=0.01^a$
ACTIVA	7.45 ± 2.29 (n=22)		3.05 ± 1.70 (n=21)	
Control	4.41 ± 3.89 (n=12)	$p=0.94^b$	2.44 ± 2.37 (n=12)	$p=0.55^b$
Gas	4.79 ± 3.15 (n=14)		3.02 ± 1.81 (n=12)	
Water	4.16 ± 4.13 (n=12)		1.93 ± 1.36 (n=12)	
Gel	5.32 ± 3.99 (n=12)		1.71 ± 1.39 (n=14)	

[a]: Two-sample Wilcoxon rank-sum (Mann-Whitney) test
[b]: Kruskal-Wallis and Dunn's post-hoc test for multiple pairwise comparison

Table 2 Semi-quantitative analysis of the elemental composition of the tooth-restoration interface and the sub-interfacial portions of enamel and dentin based on the mean stoichiometric ratios acquired through SEM-EDX analysis (mean ± standard deviation).
Tablica 2. Polukvantitativna analiza elementarnog sastava sučelja zub-restauracija i subinterfacijalnih dijelova cakline i dentina na temelju srednjih stehiometrijskih omjera dobivenih SEM-EDX analizom (srednja vrijednost ± standardna devijacija).

Element (wt. %)	Enamel (n=32)		Dentin (n=32)	
	Interface	Control (below the interface)	Interface	Control (below the interface)
O	42.23 ± 1.65	44.07 ± 1.97	39.57 ± 2.54	40.00 ± 1.57
Ca	36.22 ± 1.39	35.33 ± 1.82	28.06 ± 1.96	27.85 ± 1.47
P	18.88 ± 0.80	17.76 ± 0.96	16.76 ± 1.22	16.39 ± 1.04
C	0.78 ± 0.30	1.05 ± 0.98	13.45 ± 1.31	13.14 ± 1.86
Mg	0.46 ± 0.13	0.56 ± 0.26	0.93 ± 0.34	1.42 ± 0.49
Na	0.30 ± 0.14	0.50 ± 0.29	0.48 ± 0.15	0.49 ± 0.18
F	0.35 ± 0.15	0.04 ± 0.05	0.06 ± 0.06	0.01 ± 0.01
N	0.17 ± 0.03	0.03 ± 0.08	0.33 ± 0.18	0.31 ± 0.14
Si	0.30 ± 0.19	0.08 ± 0.12	0.04 ± 0.02	0.01 ± 0.02
Ca/P	1.92 ± 0.08	1.99 ± 0.08	1.68 ± 0.08	1.70 ± 0.09

Table 3 Mean fluoride content (F, wt. %) and Ca/P ratios at the interface and below the interface with restorations.
Tablica 3. Srednji udio fluorida (F, mas.%) i omjeri Ca/P na sučelju i ispod sučelja s restauracijama

FUJI	Enamel				Dentin			
	Interface		Control (below the interface)		Interface		Control (below the interface)	
Element	F (wt. %)	Ca/P	F (wt. %)	Ca/P	F (wt. %)	Ca/P	F (wt.%)	Ca/P
Control	0.35 ± 0.13	1.92 ± 0.10	0.03 ± 0.05	2.01 ± 0.12	0.05 ± 0.04	1.69 ± 0.07	0 ± 0.01	1.71 ± 0.11
Gas	0.35 ± 0.14	1.93 ± 0.12	0.03 ± 0.05	1.98 ± 0.08	0.06 ± 0.05	1.70 ± 0.05	0 ± 0.02	1.69 ± 0.12
Water	0.30 ± 0.08	1.93 ± 0.11	0.01 ± 0.01	2.00 ± 0.08	0.02 ± 0.01	1.64 ± 0.12	0.01 ± 0.01	1.72 ± 0.08
Gel	0.33 ± 0.13	1.91 ± 0.10	0.08 ± 0.09	2.02 ± 0.07	0.04 ± 0.04	1.67 ± 0.06	0.01 ± 0.01	1.68 ± 0.11
ACTIVA	**Enamel**				**Dentin**			
	Interface		Control (below the interface)		Interface		Control (below the interface)	
Element	F (wt. %)	Ca/P	F (wt. %)	Ca/P	F (wt. %)	Ca/P	F (wt. %)	Ca/P
Control	0.45 ± 0.21	1.90 ± 0.06	0.06 ± 0.05	1.93 ± 0.06	0.07 ± 0.09	1.65 ± 0.63	0.01 ± 0.01	1.70 ± 0.07
Gas	0.48 ± 0.25	1.91± 0.10	0.04 ± 0.04	1.97 ± 0.12	0.01 ± 0.08	1.70 ± 0.12	0.01 ± 0.01	1.71 ± 0.11
Water	0.35 ± 0.13	1.92 ± 0.06	0.01 ± 0.01	2.00 ± 0.09	0.05 ± 0.04	1.67 ± 0.07	0.02 ± 0.01	1.71 ± 0.06
Gel	0.23 ± 0.15	1.95 ± 0.06	0.04 ± 0.04	1.99 ± 0.08	0.09 ± 0.08	1.70 ± 0.07	0.01 ± 0.01	1.69 ± 0.10

After discarding the specimens that were damaged during sectioning, 96 enamel and dentin samples, respectively, were finally included. The specimens obtained were then immersed for 7 days in Hank's balanced salt solution (HBSS – Sigma Aldrich, St. Louis, MO, USA).

Nakon isključenja uzoraka oštećenih tijekom rezanja, uključeno je ukupno 96 uzoraka cakline i 96 uzoraka dentina. Dobiveni uzorci uronjeni su zatim tijekom 7 dana u Hankovu uravnoteženu otopinu soli (HBSS; Sigma Aldrich, St. Louis, MO, SAD).

Ozone treatment

Enamel and dentin samples were randomly divided by computer sequence (random.org) into 4 experimental groups (n=24 each), based on the different protocols of surface conditioning with ozone (33, 36). The specimens were dried with an air syringe and provisionally fixed with wax on resin stubs for subsequent procedures. Prior to ozone application, the samples were disinfected with 70 % ethyl alcohol using a micro-brush, rinsed with distilled water for 10 s and gently dried with an air syringe for 10 s.

Samples from groups indicated by the number 1 served as control groups and were not treated with ozone.

Samples from the groups indicated by the number 2 were treated with gaseous ozone produced from pure medical oxygen (Humadent Unit, Humares Gmbh, Bruchsal, Germany). The samples on resin stubs were positioned in a glass container covered with parafilm, and gaseous ozone (40 µg/mL, 300 mL/min flow) was applied via a dental handpiece placed perpendicularly to dental surface at 1 mm of distance, for 60 s, with suction in close proximity.

Samples from groups indicated by the number 3 were rinsed with ozonized water (Ozonsan Cytozon, Hänsler Medical GmbH, Iffezheim, Germany) for 60 s (0.2 L/min). Ozonized water was delivered via a dedicated water syringe. Ozonized water (20 µg/mL was obtained by bubbling ozone at a fixed concentration of 85 µg/mL at 0.3-1.00 L/min O_3 output, 5.1 g O_3/h) for 6 mins into 1 L of double-distilled. The samples were then gently dried for 10 s using an air syringe.

Samples from groups indicated by the number 4 were treated with 1 mL of ozonized gel (Ozoral, Innovares, Reggio Emilia, Italy) containing 15% of ozonized sunflower oil (Ozonia 3000), vitamin E and ascorbyl palmitate, with a pH of 6.4 and a peroxide index of 450 meqO2/kg. The gel was applied onto the surface with a micro-brush and left in place for 60 s. The samples were thoroughly rinsed with a distilled water/air spray for 10 s and gently dried with an air spray for 10 s.

Surface roughness

Four samples of enamel and dentin from each group were randomly selected immediately after ozone application and analyzed to detect any possible modifications to surface roughness induced by ozone.

Surface roughness was assessed using an optical microscope (Eclipse LV150N, Nikon, Tokyo, Japan) at 10x magnification, with structured illumination camera (Confovis, Jena, Germany), and elaborated using the software Mountains Map (Digital Surf, Besançon, France). The profiles were filtered using a robust Gaussian filter with 25 µm cut-off length to separate the large-scale waviness of the tooth surface from the small-scale roughness. The arithmetic mean of the heights in the experimental area A (Sa) was considered as the roughness value, according to ISO 25178 for Geometrical Product Specifications (GPS). Additionally, the Sdr (Developed interfacial area ratio) parameter was recorded, which expresses the percentage of the additional surface area contributed by the texture as compared to the related planar area. Two measurements were taken on each sample and then averaged.

Tretman ozonom

Uzorci cakline i dentina nasumično su podijeljeni računalnom sekvencijom (random.org) u 4 eksperimentalne skupine (n = 24 u svakoj), prema različitim protokolima kondicioniranja površine ozonom (33, 36). Uzorci su posušeni zračnom štrcaljkom i privremeno fiksirani voskom na smolne nosače radi daljnjih postupaka. Prije primjene ozona uzorci su dezinficirani su 70-postotnim etilnim alkoholom mikročetkicom, 10 sekunda ispirani destiliranom vodom te nježno posušeni zračnom štrcaljkom.

Uzorci iz skupina označenih brojem 1 služili su kao kontrola i nisu tretirani ozonom.

Uzorci iz skupina označenih brojem 2 tretirani su plinovitim ozonom proizvedenim iz čistoga medicinskog kisika (Humadent Unit, Humares Gmbh, Bruchsal, Njemačka). Uzorci na smolnim nosačima postavljeni su u staklenu posudu prekrivenu parafilmom, a plinoviti ozon (40 µg/mL, protok 300 mL/min) primijenjen je namjenskim dentalnim nastavkom postavljenim okomito na zubnu površinu na udaljenosti od 1 mm tijekom 60 sekunda, uz aspiraciju u neposrednoj blizini.

Uzorci iz skupina označenih brojem 3 ispirani su ozoniranom vodom (Ozonsan Cytozon, Hänsler Medical GmbH, Iffezheim, Njemačka) 60 sekunda (0,2 L/min). Ozonirana voda aplicirana je namjenskom vodenom štrcaljkom. Ozonirana voda (20 µg/mL) dobivena je uvođenjem ozona u fiksnoj koncentraciji od 85 µg/mL pri izlazu O_3 od 0,3 do 1,00 L/min (5,1 g O_3/h) tijekom 6 minuta u 1 L dvostruko destilirane vode. Uzorci su zatim nježno sušeni 10 sekunda zračnom štrcaljkom.

Uzorci iz skupina označenih brojem 4 tretirani su s 1 mL ozoniranoga gela (Ozoral, Innovares, Reggio Emilia, Italija) koji sadržava 15 % ozoniranoga suncokretova ulja (Ozonia 3000), vitamin E i askorbil-palmitat, pH 6,4 te peroksidni indeks 450 meqO_2/kg. Gel je nanesen na površinu mikročetkicom i ostavljen 60 sekunda. Uzorci su zatim temeljito isprani destiliranom vodom uz zračni sprej 10 sekunda te nježno posušeni zračnim sprejom.

Hrapavost površine

Četiri uzorka cakline i dentina iz svake skupine nasumično su odabrana neposredno poslije primjene ozona te analizirana zbog detekcije mogućih promjena u hrapavosti površine induciranih ozonom.

Hrapavost površine procijenjena je optičkim mikroskopom (Eclipse LV150N, Nikon, Tokio, Japan) pri povećanju od 10 puta, sa strukturno osvijetljenom kamerom (Confovis, Jena, Njemačka), a obrada je obavljena u softveru MountainsMap (Digital Surf, Besançon, Francuska). Profili su filtrirani robusnim Gaussovim filtrom s graničnom duljinom 25 µm radi odvajanja valovitosti većeg mjerila od hrapavosti manjeg mjerila. Kao vrijednost hrapavosti uzeta je aritmetička sredina visina u eksperimentalnom području A (Sa), u skladu s normom ISO 25178 za geometrijske specifikacije proizvoda (GPS). Dodatno je zabilježen parametar Sdr (omjer razvijene spojne površine) koji izražava postotak dodatne površine prouzročene teksturom u odnosu prema pripadnoj planarnoj površini. Na svakom uzorku obavljena su dva mjerenja te su poslije toga izračunate prosječne vrijednosti.

Build-up of restorative materials

The remaining enamel and dentin samples from each group were further randomized into two groups, defined by the restorative material used to create the build-up. Ultimately, we obtained a total of 8 groups for each substrate (i.e. enamel and dentin, n=10 each). Custom-made silicon molds were used to create standardized build-ups of approximately 3 mm thickness and 4x4 mm area. The build-up of the materials was conducted in accordance with the respective manufacturers' recommendations.

The build-up from groups A were made with a resin-modified glass-ionomer cement (RMGIC) material (FUJI II LC A3, GC Dental, Luzern, Switzerland) as follows: 20% polyacrylic acid (GC Cavity Conditioner, GC Corp, Tokyo, Japan) was applied to the dental surface for 10 s, then gently rinsed and dried with an air syringe for 10 s respectively, without desiccating the tooth, as recommended in the manufacturer's instructions. GC Cavity Conditioner was used to condition dentin surfaces because it effectively removes the smear layer while maintaining partial tubule occlusion, creating a mildly demineralized surface favorable for the ionic exchange and chemical bonding mechanisms of resin-modified glass ionomer cements (37). The RMGIC material was mixed for 10 s and extruded in a single bulk increment in the silicon mold firmly placed on the dental surface. Light curing through a Mylar foil (Starlight Pro, Mectron Inc., Carasco, Genova, Italy; l = 440-465 nm, 5 W, power density >1400 mW/cm^2) was performed for 20 s. The samples were left resting for 10 minutes to allow further self-curing before removing the silicon molds, and then a second light-curing phase of 40 s was performed to allow the light to reach the areas previously covered by the silicon walls and ensure a complete curing process. A layer of resin coating (GC FUJI Coat LC, GC Corp) was then applied using a micro-brush on the build-up surface as per the manufacturer's instructions and light-cured for 10 s.

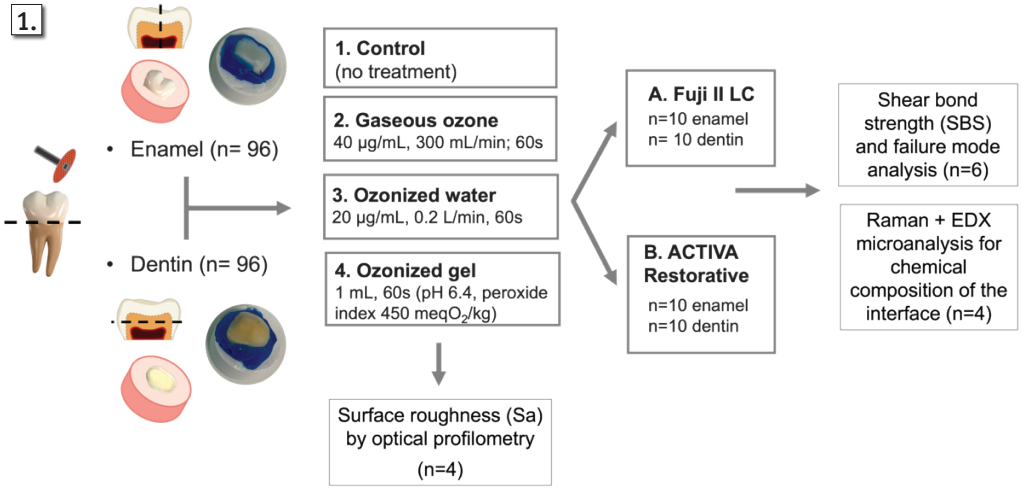
The build-up from groups B was made with the hybrid composite ACTIVA Bioactive Restorative A3 (Pulpdent, Watertown, MA, USA) as follows: etching with 37% orthophosphoric acid was performed for 15 s, then the surface was rinsed and dried with an air syringe to remove water from the surface, without desiccating the tooth. An etch-only protocol without a separate bonding agent was used for ACTIVA, in line with the manufacturer's instructions for specific clinical indications, in order to assess the material's intrinsic self-adhesive and bioactive interaction with dental tissues and to avoid the confounding influence of an intermediate adhesive layer when evaluating the effect of ozone pretreatment. The self-mixing material was extruded in a single bulk increment in the silicon mold firmly placed on the dental surface and allowed to initially self-cure for 20 s and then light cured (Starlight Pro, Mectron Inc., Carasco, Genova, Italy; l = 440-465 nm, 5 W, power density >1400 mW/cm^2) through a Mylar foil for 20 s. The samples were allowed to further self-cure for 10 minutes before removing the silicon molds, and a second light-curing phase of 40 s was performed to allow the light to reach the areas previously covered by the silicon walls and ensure a complete curing process.

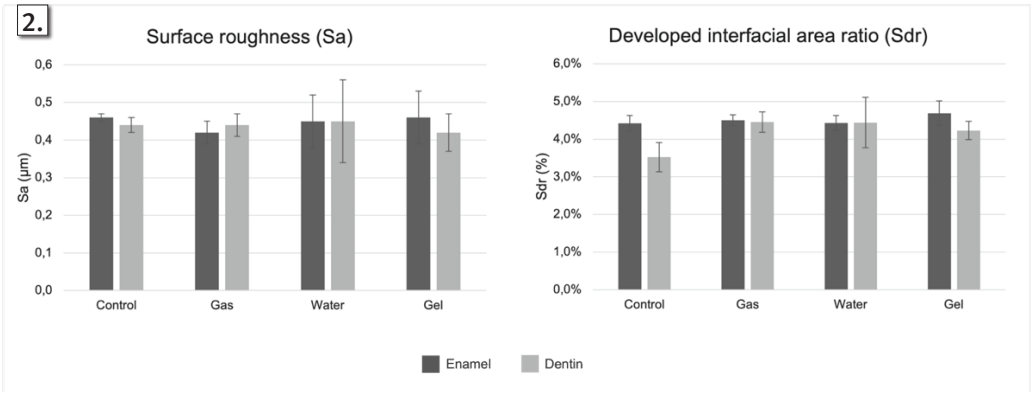
Izrada nadogradnji restaurativnim materijalima

Preostali uzorci cakline i dentina iz svake skupine dodatno su randomizirani u dvije skupine definirane prema restaurativnom materijalu upotrijebljenom za izradu nadogradnje. Konačno je dobiveno ukupno 8 skupina za svaki supstrat (caklina i dentin, n = 10 u svakoj). Za izradu standardiziranih nadogradnji debljine približno 3 mm i površine 4 × 4 mm korišteni su posebno izrađeni silikonski kalupi. Izrada nadogradnji provedena je u skladu s preporukama proizvođača.

Nadogradnje u skupinama A izrađene su od staklenoionomernog cementa modificiranog smolom (RMGIC) (FUJI II LC A3, GC Dental, Luzern, Švicarska) na sljedeći način: 20-postotna poliakrilna kiselina (GC Cavity Conditioner, GC Corp, Tokio, Japan) nanesena je 10 sekunda na zubnu površinu, zatim je nježno isprana i sušena zračnom štrcaljkom 10 sekunda bez isušivanja zuba, u skladu s uputama proizvođača. GC Cavity Conditioner korišten je za kondicioniranje dentinskih površina jer učinkovito uklanja zaostatni sloj uza zadržavanje djelomične okluzije tubula, stvarajući blago demineraliziranu površinu povoljnu za ionsku razmjenu i mehanizme kemijskog vezivanja staklenoionomernih cemenata modificiranih smolom (37). RMGIC materijal miješan je 10 sekunda te istisnut u jednom *bulk* inkrementu u silikonski kalup čvrsto postavljen na zubnu površinu. Svjetlosna polimerizacija kroz foliju *mylar* (Starlight Pro, Meactron s.p.a., Carasco, Genova, Italija; λ = 440 – 465 nm, 5 W, gustoća snage > 1400 mW/cm^2) trajala je 20 sekunda. Uzorci su ostavljeni mirovati 10 minuta radi dodatnoga samopolimeriziranja prije uklanjanja silikonskih kalupa, nakon čega je provedena druga faza svjetlosne polimerizacije od 40 sekunda da bi svjetlo dosegnulo područja prethodno prekrivena silikonskim stijenkama i osiguralo potpuno stvrdnjavanje. Zatim je na površinu nadogradnje mikročetkicom nanesen sloj smolastoga premaza (GC FUJI Coat LC, GC Corp) prema uputama proizvođača te je svjetlosno polimeriziran 10 sekunda.

Nadogradnje u skupinama B izrađene su hibridnim kompozitom ACTIVA Bioactive Restorative A3 (Pulpdent, Watertown, MA, SAD) na sljedeći način: jetkanje 37-postotnom ortofosfornom kiselinom provedeno je 15 sekunda, zatim je površina isprana i posušena zračnom štrcaljkom radi uklanjanja vode s površine, bez isušivanja zuba. Za ACTIVA-u je korišten protokol *etch-only* bez zasebnoga adhezivnog sustava, u skladu s uputama proizvođača za specifične kliničke indikacije kako bi se procijenila intrinzična samoadhezivna i bioaktivna interakcija materijala sa zubnim tkivima te izbjegao zbunjujući utjecaj posrednoga adhezivnog sloja pri procjeni učinka ozonske prijetretmanske obrade. Samomiješajući materijal istisnut je u jednom *bulk* inkrementu u silikonski kalup čvrsto postavljen na zubnu površinu, ostavljen za početno samopolimeriziranje 20 sekunda te zatim svjetlosno polimeriziran (Starlight Pro, Mectron s.p.a., Carasco, Genova, Italija; λ = 440–465 nm, 5 W, gustoća snage > 1400 mW/cm^2) kroz foliju *mylar* 20 sekunda. Uzorci su nakon toga ostavljeni još 10 minuta radi daljnjeg samopolimeriziranja prije uklanjanja silikonskih kalupa, a druga faza svjetlosne polimerizacije od 40 sekunda provedena je zato da bi svjetlo dosegnulo područja prethodno prekrivena silikonskim stijenkama i osiguralo potpuno očvršćivanje.





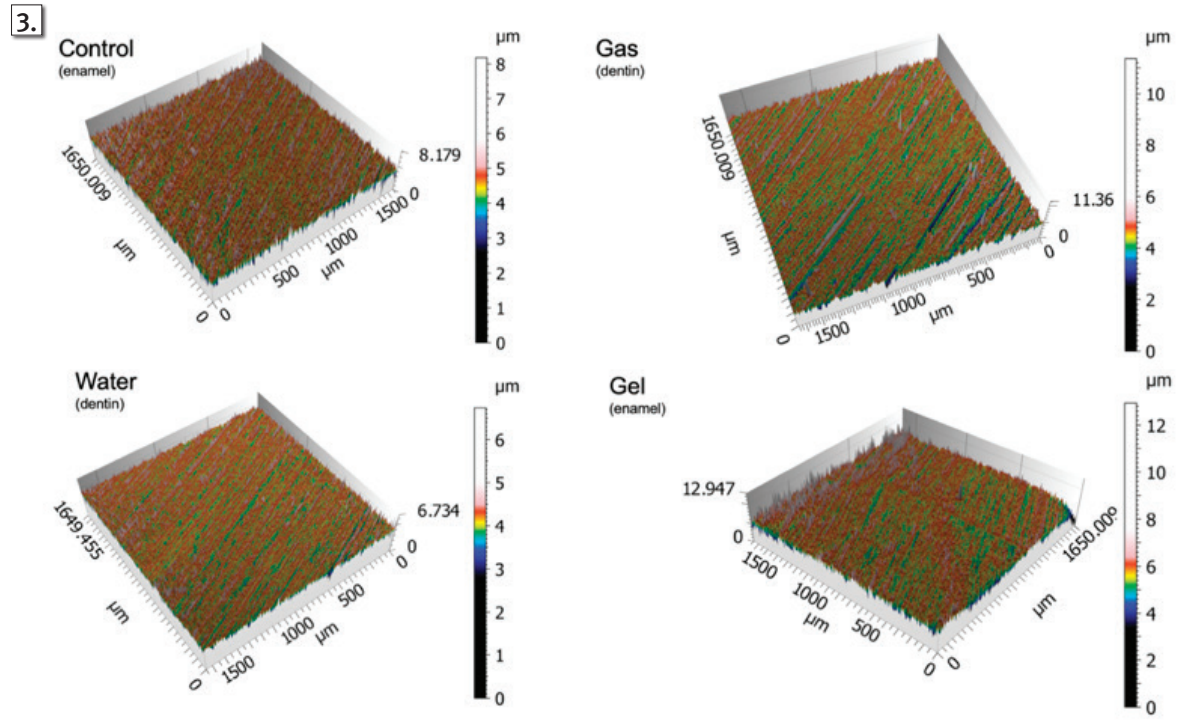
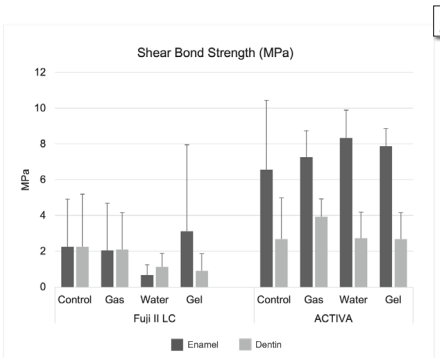
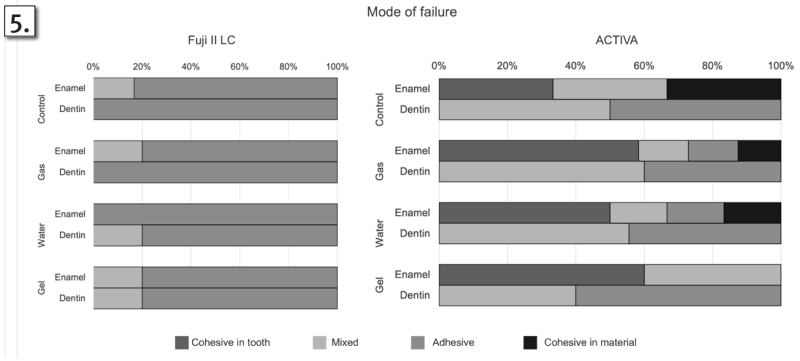
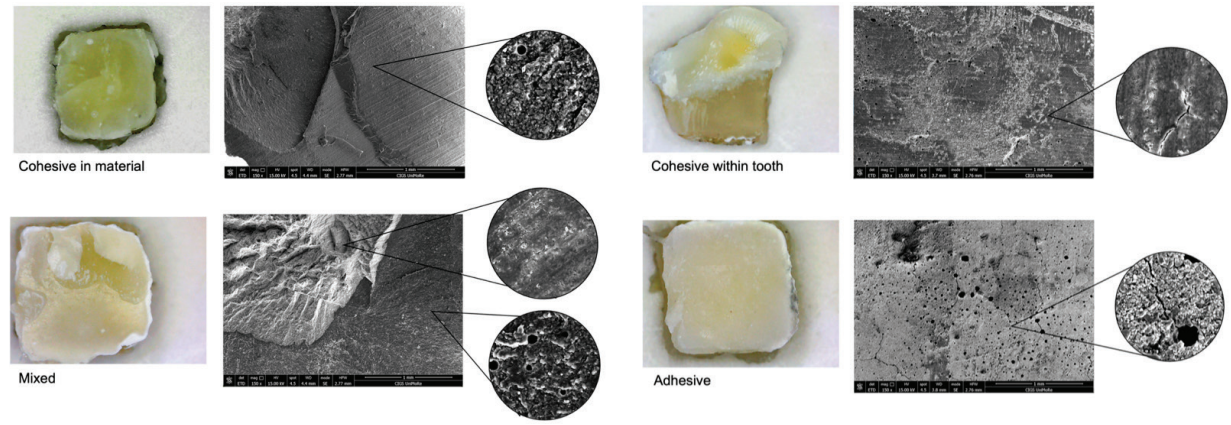


Figure 1 Schematic of the experimental protocol
Slika 1. Shematski prikaz eksperimentalnog protokola
Figure 2 Bar chart representing mean ± standard deviations of surface roughness (Sa, µm) and developed interfacial area ratio (Sdr, %)
Slika 2. Stupčasti prikaz srednjih vrijednosti ± standardnih devijacija hrapavosti površine (Sa, µm) i omjera razvijene spojne površine (Sdr, %)
Figure 3 Tridimensional representations of the surface topography of representative samples elaborated through optical profilometry
Slika 3. Trodimenzionalni prikazi površinske topografije reprezentativnih uzoraka, obrađeni optičkom profilometrijom







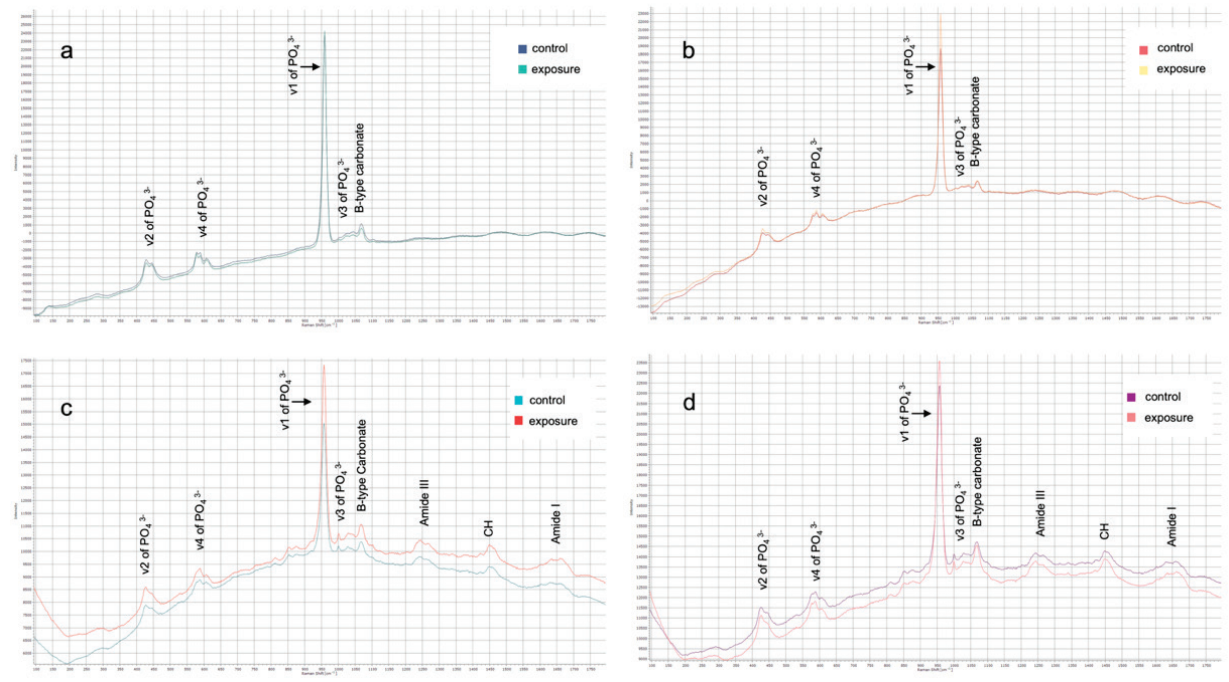


Figure 4 Mean (MPa) and standard deviation of shear bond strength (SBS)
Slika 4. Srednja vrijednost (MPa) i standardna devijacija smične čvrstoće veze (SBS)
Figure 5 Distribution of the modes of failure as observed with stereomicroscopy
Slika 5. Raspodjela načina loma opažena stereomikroskopijom
Figure 6 Images of representative samples for each type of failure mode, acquired through stereomicroscopy at 20x magnification, and through SEM-FEG at 150 xs and 2400x magnification. Notably, the procedures of sample preparation, which lead to dehydration and the observation under the high vacuum conditions of the SEM-FEG might induce additional cracks and fractures in the samples
Slika 6. Reprezentativni uzorci za svaki tip načina loma dobiveni stereomikroskopijom pri povećanju od 20 puta te SEM-FEG-om pri povećanjima od 150 i 2400 puta; važno je napomenuti da postupci pripreme uzoraka, koji su rezultirali dehidracijom, te promatranje u uvjetima visokoga vakuuma SEM-FEG-a mogu inducirati dodatne pukotine i frakture u uzorcima
Figure 7 Representative Raman spectra of samples from groups: (a) Enamel x FUJI x ozonized water; (b) Enamel x ACTIVA x ozonized gel; (c) Dentin x FUJI x ozonized water; (d) Dentin x ACTIVA x ozonized gel
Slika 7. Reprezentativni Ramanovi spektri uzoraka iz skupina: (a) caklina × FUJI × ozonirana voda; (b) caklina × ACTIVA × ozonirani gel; (c) dentin × FUJI × ozonirana voda; (d) dentin × ACTIVA × ozonirani gel

The samples were then gently removed from the resin stubs and placed in single containers with 4 mL HBSS and placed in an incubator at 37 °C for 7 days to allow the materials to completely set. The samples were then embedded in acrylic resin (Technovit 4071, Kulzer GmbH, Hanau, Germany), without involving the build-up, and allowed to cure for 24 hours. All procedures were performed by the same operator. A schematic of the experimental protocol is displayed in Figure 1.

Bond strength testing

Shear bond strength (SBS) was determined in a universal testing machine (zwickLine, ZwickRoell, Ulm, Germany) on 6 samples for each group. The shear force was applied at the restoration/tooth interface with a cylindric-shaped piston, parallel to the dental surface. A cross-head speed of 1 mm/min was chosen. Shear bond strength was determined as the peak load (N) at which the build-up detachment occurred, and the value in MPa was derived as N/mm^2, by previously measuring the actual area for each sample at the restoration/tooth interface by means of a caliper. Finally, the mean values and standard deviations were calculated for each group. The data were recorded in a Microsoft Excel spreadsheet.

Failure Mode

The debonded specimens were observed using a stereomicroscope (OPMI Pico; Carl Zeiss Meditec Inc., Jena, Germany) at 20x magnification, followed by a scanning electron microscopy (SEM-FEG Nova NanoSEM 450) examination to observe the morphology of the surface facing the dental interface and to determine the mode of failure. After being placed in an ultrasonic bath containing absolute alcohol for 5 minutes to remove possible surface debris, the specimens were mounted on aluminum stubs using a graphite-based conductive tape and sputter-coated with a 10 nm gold layer to improve conductivity. The modes of failure were visually classified as follows: (1) cohesive fracture within enamel/dentin if more than 75% of the surface of the detached specimen involves enamel/dentin; (2) mixed fracture, if between 25% and 75% of the surface of the detached specimen involves enamel/dentin; (3) adhesive fracture, if less than 25% of the surface of the detached specimen involves enamel/dentin; (4) cohesive fracture within the restorative material if the surface of the material is detached for more than 75%.

Micro-Raman Spectroscopy and SEM-EDX analysis of the interface

Four random samples for each group were previously selected to be kept in HBSS for 21 days, then sectioned perpendicularly to the bonded interface using a rotary microtome (Micromet Digital, Remet S.A.S., Bologna, Italia) at a speed of 3000 rpm under continuous water cooling, as near as possible to the center of the restorative material build-up.

The samples were cleaned by ultrasonication in absolute alcohol for 5 minutes to remove possible surface debris. Micro-Raman spectra were acquired on the surface of the transversal sections using a LabRAM spectrometer (Horiba - Jobin Yvon, Kyoto, Japan) to further describe the composition and the possible differences below the dental-restoration in-

Uzorci su zatim nježno skinuti sa smolnih nosača, postavljeni u pojedinačne posude s 4 mL HBSS-a te inkubirani na 37 °C tijekom 7 dana kako bi se materijali potpuno stvrdnuli. Nakon toga uloženi su u akrilatnu smolu (Technovit 4071, Kulzer GmbH, Hanau, Njemačka), bez uključivanja nadogradnje, te ostavljeni 24 sata da polimeriziraju. Sve postupke obavio je isti operator. Shematski prikaz eksperimentalnog protokola prikazan je na slici 1.

Ispitivanje čvrstoće veze

Smična čvrstoća veze (SBS) određena je na univerzalnom uređaju za testiranje mehaničkih svojstava (zwickLine, ZwickRoell, Ulm, Njemačka) na 6 uzoraka u svakoj skupini. Smična sila primijenjena je na sučelju restauracija/zub cilindričnim klipom, paralelno s dentalnom površinom. Odabrana je brzina poprečne glave od 1 mm/min. Smična čvrstoća veze određena je kao maksimalno opterećenje (N) pri kojemu se dogodilo odvajanje nadogradnje, a vrijednost u MPa izračunata je kao N/mm^2, pri čemu je prethodno izmjerena stvarna površina sučelja restauracija/zub za svaki uzorak s pomoću pomičnog mjerila. Na kraju su izračunate srednje vrijednosti i standardne devijacije za svaku skupinu. Podatci su zabilježeni u proračunskoj tablici Microsoftova Excela.

Način loma

Odvojeni uzorci promatrani su stereomikroskopom (OPMI Pico; Carl Zeiss Meditec Inc., Jena, Njemačka) pri povećanju od 20 puta, nakon čega je slijedilo ispitivanje skenirajućom elektronskom mikroskopijom (SEM-FEG Nova NanoSEM 450) zbog uvida u morfologiju površine okrenute prema zubnom sučelju i određivanja načina loma. Nakon 15-minutnog ultrazvučnog čišćenja u apsolutnom alkoholu radi uklanjanja mogućih površinskih ostataka, uzorci su postavljeni na aluminijske nosače s vodljivom trakom na bazi grafita te prevučeni slojem zlata debljine 10 nm radi poboljšanja vodljivosti. Načini loma vizualno su klasificirani kako slijedi: (1) kohezivni lom unutar cakline/dentina ako više od 75 % površine odvojenog uzorka uključuje caklinu/dentin; (2) miješani lom ako 25 % do 75 % površine odvojenog uzorka uključuje caklinu/dentin; (3) adhezivni lom ako manje od 25 % površine odvojenog uzorka uključuje caklinu/dentin; (4) kohezivni lom unutar restaurativnog materijala ako je površina materijala odvojena u više od 75 %.

Ramanova mikrospektroskopija i SEM-EDX analiza sučelja

Četiri nasumična uzorka iz svake skupine prethodno su odabrana za čuvanje u HBSS-u tijekom 21 dana, nakon čega su prerezana okomito rotacijskim mikrotomom (Micromet Digital, Remet S.A.S., Bologna, Italija) pri brzini od 3000 o/min uz kontinuirano vodeno hlađenje, što bliže središtu nadogradnje restaurativnog materijala.

Uzorci su očišćeni ultrazvučno u apsolutnom alkoholu 5 minuta radi uklanjanja mogućih površinskih ostataka. Ramanovi mikrospektri prikupljeni su na površini transverzalnih presjeka spektrometrom LabRAM (Horiba – Jobin Yvon, Kyoto, Japan) radi dodatnog opisa sastava i mogućih razlika ispod sučelja zub-restauracija. Kao izvor pobude ko-

terface. A solid-state Nd: YAG laser (wavelength: 532 nm) was used as the excitation source and was focused perpendicularly on the area near the interface and approximately 100 µm below the interface as a control, using a 100× objective.

The transversal sections of the samples were subsequently mounted on aluminum stubs using a graphite-based conductive tape and sputter-coated with a 10 nm gold layer to improve their electrical conductivity to be examined by scanning electron microscope (SEM-FEG Nova NanoSEM 450) equipped with an energy dispersive X-ray spectroscope (EDX - Quantax-200, Bruker Corp., Billerica, MA, USA). Backscattered electron scanning assay and EDX analysis of dentinal tubules and enamel prisms were performed near the interface and below the interface as a control, which allowed determining possible differences in the chemical composition in terms of the Ca, P and F contents of the target areas as an indication of bioactive ion release from the restorative materials.

Statistical analysis

The Shapiro-Wilk test was used to assess the normal distribution of the data. The nonparametric

Kruskal-Wallis test followed by the Dunn's test with Bonferroni correction for post hoc pairwise multiple comparisons when appropriate (or their parametric counterparts for normally distributed variables) were used to investigate the differences in bond strength and EDX ion values between the different groups and subgroups. The EDX values between the two target areas of each sample were compared using the Wilcoxon signed-rank test or ANOVA. The modes of failure were recorded as percentages and were analyzed by the Fisher's exact test. Possible interactions between substrate, material, failure mode and bond strength were explored using the rank-transformed two-way ANOVA, followed by one-way post-hoc analyses when appropriate. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using Stata 16.1 software (StataCorp Lp, College Station, Texas, USA).

Results

Surface Roughness

No significant differences in surface roughness - Sa (p>0.4, ANOVA) were observed between the ozone-treated groups and the control, either in the enamel or the dentin.

With regards to Sdr (developed interfacial area ratio), a slightly greater value was observed in the dentin treated with gaseous ozone and ozonized water, compared to the untreated control group (p=0.03, ANOVA, Tukey-Kramer post-hoc test). No other significant differences were found among the other groups (Figure 2 and Figure 3).

Bond strength

During the build-up of restorations, 11 specimens failed due to immediate lack of retention and were therefore excluded from the study (6 for FUJI, 5 for Activa). The mean measured area at the interface between the material build-up and the dental substrate was 16.1 mm^2. The mean mea-

rišten je Nd:YAG laser u čvrstom stanju (valna duljina 532 nm) usmjeren okomito na područje uz sučelje i približno 100 µm ispod sučelja kao kontrola, uz korištenje objektiva s povećanjem od 100 puta.

Transverzalni presjeci uzoraka zatim su postavljeni na aluminijske nosače s vodljivom trakom na bazi grafita te prevučeni slojem zlata debljine 10 nm radi poboljšanja električne vodljivost, kako bi se pregledali pretražnim elektronskim mikroskopom (SEM-FEG Nova NanoSEM 450) opremljenim energijski disperzivnim rendgenskim spektroskopom (EDX - Quantax-200, Bruker Corp., Billerica, MA, SAD). Pretraga povratno raspršenim elektronima te EDX analiza dentinskih tubula i caklinskih prizmi obavljene su uz sučelje i ispod sučelja kao kontrola, čime su se mogle utvrditi moguće razlike u kemijskom sastavu kad je riječ o udjelu kalcija (Ca), fosfora (P) i fluora (F) u ciljanim područjima kao pokazateljima bioaktivnog oslobađanja iona iz restaurativnih materijala.

Statistička analiza

Shapiro-Wilkov test korišten je za procjenu normalnosti raspodjele podataka. Neparametrijski Kruskal-Wallisov test, praćen Dunnovim testom s Bonferronijevom korekcijom za *post hoc* višestruke usporedbe parova kada je primjenjivo (ili odgovarajući parametrijski ekvivalenti za varijable s normalnom raspodjelom), korišteni su za ispitivanje razlika u čvrstoći veze i EDX vrijednosti iona između različitih skupina i podskupina. EDX vrijednosti između dvaju ciljanih područja svakog uzorka uspoređene su Wilcoxonovim testom predznaka za uparene uzorke ili ANOVA-om. Načini loma zabilježeni su kao postotci i analizirani Fisherovim egzaktnim testom. Moguće interakcije između supstrata, materijala, načina loma i čvrstoće veze istražene su dvosmjernom ANOVA-om na rang-transfomiranim podatcima, uz naknadne jednofaktorske *post hoc* analize kada je primjenjivo. Vrijednost p < 0,05 smatrana je statistički značajnom. Statistička analiza obavljena je u programu Stata 16.1 (StataCorp LP, College Station, Texas, SAD).

Rezultati

Hrapavost površine

Nisu utvrđene statistički značajne razlike u hrapavosti površine (Sa) (p > 0,4, ANOVA) između skupina tretiranih ozonom i kontrolne skupine, ni na caklini ni na dentinu.

S obzirom na parametar Sdr (omjer razvijene spojne površine), u dentinu tretiranom plinovitim ozonom i ozoniranom vodom zabilježena je blago povišena vrijednost u usporedbi s netretiranom kontrolnom skupinom (p = 0,03, ANOVA, Tukey-Kramerov post hoc test). U ostalim skupinama nisu pronađene druge statistički značajne razlike (slika 2. i 3.).

Čvrstoća veze

Tijekom izrade nadogradnji restauracija nije uspjelo 11 uzoraka zbog neposrednog izostanka retencije te su isključeni iz istraživanja (6 za FUJI, 5 za ACTIVA-u). Srednja izmjerena površina na sučelju između nadogradnje materijala i dentalnog supstrata iznosila je 16,1 mm^2. Srednja izmjerena

sured SBS value was 3.59±3.23 MPa, with a minimum of 0.05 MPa and a maximum of 11.69 MPa. The mean values and standard deviations (SD) for each group are displayed in Figure 4.

No statistically significant difference in terms of shear bond strength - SBS (p>0.5, Kruskal-Wallis) could be found with the control group as a result of different ozone treatments within the same substrate/material, which suggests that the application of ozone has little to no effect on the bond strength.

These findings are confirmed by multivariate analyses, indicating that ozone treatment does not significantly influence the SBS (p=0.55), while both the restorative material and the substrate (p<0.0001 both), and their interaction (p=0.004) have a significant effect on SBS (rank-transformed two-way ANOVA, one-way ANOVA). With regards to the material-specific performance, ACTIVA yielded significantly higher mean SBS values both when used on enamel (+ 5.41 MPa, 95% CI [3.79; 7.03]) and dentin (+ 1.43 MPa, 95% CI [0.32; 2.54]) compared to FUJI (Table 2, Mann-Whitney test). As for the substrate-specific results, the overall SBS values were higher for the enamel compared to the dentin, with small and non-significant differences for FUJI (+ 0.42 MPa, 95% CI [-1.12; +1.95]; p=0.55, Mann-Whitney test), and a significant difference for ACTIVA (+ 4.39 MPa 95% CI [3.15; 5.64]; p<0.01, Mann-Whitney test).

Failure mode analysis

The distribution of the modes of failure is displayed in Figure 5. Overall, the most frequent mode of failure was adhesive fracture (57.65%), followed by mixed failure (24.71%).

Regardless of the combination of substrate and restorative material used, no statistically significant differences were found in the distribution of modes of failure among the ozone treatment subgroups (p>0.6, Fisher's exact test), suggesting that the ozone treatment does not remarkably affect the mode of failure.

On the other hand, the distribution of modes of failure was significantly correlated with the substrate and the restorative material applied (p<0.001 for both, Fisher's exact test). Specifically, adhesive failure was significantly more frequent for dentin compared to enamel (p<0.05, adjusted Pearson residuals), while cohesive failure within the tooth structure was more frequent in the enamel (p<0.001, adjusted Pearson residuals). As for the material-specific distribution, adhesive failure was more frequent for FUJI (88.10%, p<0.001, adjusted Pearson residuals), while mixed or cohesive fracture within the tooth structure prevailed for ACTIVA (p<0.01, adjusted Pearson residuals). When considering the specific substrate, the distribution of modes of failure was significantly different between enamel and dentin for ACTIVA (p<0.001, Fisher's exact test, adjusted Pearson residuals), with an increased rate of cohesive fracture within the enamel and an increased rate of adhesive or mixed fractures for the dentin. Conversely, FUJI showed a non-statistically significant different distribution between enamel and dentin (p=0.5, Fisher's exact test).

A correlation between the SBS values and the mode of failure was found (p=0.0001; Kruskal-Wallis test). Specifi-

vrijednost smične čvrstoće veze (SBS) iznosila je 3,59 ± 3,23 MPa, uz minimalnu vrijednost od 0,05 MPa i maksimalnu vrijednost od 11,69 MPa. Srednje vrijednosti i standardne devijacije (SD) za svaku skupinu prikazane su na slici 4.

Nije utvrđena statistički značajna razlika u smičnoj čvrstoći veze (SBS) (p > 0,5, Kruskal-Wallis) u usporedbi s kontrolnom skupinom kao posljedica različitih tretmana ozonom unutar istog supstrata/materijala, što upućuje na to da primjena ozona ima malen ili nikakav učinak na čvrstoću veze.

Ti su nalazi potvrđeni multivarijatnim analizama koje pokazuju da tretman ozonom ne utječe značajno na SBS (p = 0,55), a restaurativni materijal i supstrat (p < 0,0001 za oba), kao i njihova interakcija (p = 0,004), značajno utječu na SBS (dvosmjerna ANOVA na rang-transformiranim podatcima, jednosmjerna ANOVA). Kad je riječ o materijalno specifičnoj učinkovitosti, ACTIVA je postigla statistički značajno više srednje vrijednosti SBS-a i na caklini [+ 5,41 MPa, 95 % CI (3,79; 7,03)] i na dentinu [+ 1,43 MPa, 95 % CI (0,32; 2,54)] u usporedbi s FUJI-jem (tablica 2., Mann-Whitneyjev test).

Kad je riječ o rezultatima specifičnima za supstrat, ukupne vrijednosti SBS-a bile su više na caklini u odnosu na dentin, uz male i neznačajne razlike za FUJI [+ 0,42 MPa, 9 5% CI (-1,12; +1,95)]; p = 0,55, Mann-Whitneyjev test) te značajnu razliku za ACTIVA-u [+ 4,39 MPa, 95 % CI (3,15; 5,64)]; p < 0,01, Mann-Whitneyjev test).

Analiza načina loma

Raspodjela načina loma prikazana je na slici 5. Ukupno je najčešći način loma bio adhezivni (57,65 %), nakon čega slijedi miješani (24,71 %).

Neovisno o kombinaciji supstrata i restaurativnog materijala, nisu pronađene statistički značajne razlike u raspodjeli načina loma među podskupinama tretiranima ozonom (p > 0,6, Fisherov egzaktni test), što upućuje na to da tretman ozonom ne utječe zamjetno na način loma.

Suprotno tomu, raspodjela načina loma bila je značajno povezana sa supstratom i primijenjenim restaurativnim materijalom (p < 0,001 za oba, Fisherov egzaktni test). Konkretno, adhezivni lom bio je značajno češći na dentinu u odnosu na caklinu (p < 0,05, prilagođeni Pearsonovi reziduali), a kohezivni lom unutar zubne strukture bio je češći na caklini (p < 0,001, prilagođeni Pearsonovi reziduali). Kad je riječ o raspodjeli specifičnoj za materijal, adhezivni lom bio je češći za FUJI (88,10 %, p < 0,001, prilagođeni Pearsonovi reziduali), a kod ACTIVA-e prevladavali su miješani lom ili kohezivni lom unutar zubne strukture (p < 0,01, prilagođeni Pearsonovi reziduali).

Pri razmatranju specifičnog supstrata, raspodjela načina loma bila je statistički značajno različita između cakline i dentina za ACTIVA-u (p < 0,001, Fisherov egzaktni test; prilagođeni Pearsonovi reziduali), uz povećanu učestalost kohezivnog loma unutar cakline te povećanu učestalost adhezivnih ili miješanih lomova na dentinu. Nasuprot tomu, FUJI je pokazao statistički neznačajno različitu raspodjelu između cakline i dentina (p = 0,5, Fisherov egzaktni test).

Utvrđena je povezanost između vrijednosti SBS-a i načina loma (p = 0,0001; Kruskal-Wallis test). SBS je pokazao

cally, the SBS values showed a decreasing pattern, starting from cohesive failure within the tooth, followed by cohesive failure within the material, mixed failure, and finally adhesive failure, with the SBS values associated with adhesive failure being significantly lower than those observed for the other failure modes ($p<0.01$, Dunn's post-hoc test). Representative Scanning Electron Microscope with Field-Emission Gun (SEM-FEG) and stereomicroscope images for each failure mode are displayed in Figure 6.

Micro-Raman Spectroscopy and SEM-EDX analysis of the interface

The analysis of micro-Raman spectra yielded no remarkable differences among groups, either with regard to the restorative material tested or to the control or ozone treatment applied. The typical spectrum of hydroxyapatite (HAP – $[Ca_{10}(PO_4)_6(OH)_2]$) could be observed in all the samples, both at the interface and below the interface, in all groups (phosphate ν1shift at 960 cm^{-1}, and carbonate ν1 shift at 1070 cm^{-1}). In the dentin substrate groups, a slight trace of increased organic components, signaled by weak peaks at the locations corresponding to the Raman shift of methylidyne (-CH, 1450 cm^{-1}) and amides (amide III at 1246/1270 cm^{-1}, amide I at 1667 cm^{-1}) was observed in some samples, which is compatible with the physiological composition of dentin (38). Representative spectra are displayed in Figure 7.

The results of semi-quantitative energy dispersive X-ray (EDX) chemical analyses are reported in Table 2, showing the overall mean composition of the enamel and the dentin at the restoration-tooth interface and in the sub-interfacial area considered as control. No significant differences ($p>0.05$, ANOVA) in ion composition were observed at the restoration-tooth interface among different ozone-treated groups, regardless of the substrate or the material used, suggesting that ozone treatment does not affect a possible ion release of the materials at the interface (Table 3). No significant differences were found regarding the Ca/P ratios ($p>0.5$, Wilcoxon's test for paired data). Conversely, F ions were significantly higher ($p<0.01$, T-test for paired data) at the interface than in the sub-interfacial area considered as control, regardless of the substrate and the material, thus indicating a possible F release by both materials at the interface, with no significant differences between them ($p>0.05$, ANOVA).

Discussion

The effect of ozone application on the adhesion of resin-based restorative materials remains controversial due to ozone's strong oxidizing potential. Oxidizing agents such as bleaching compounds can alter surface morphology and inhibit polymerization by reacting with monomer radicals, ultimately compromising adhesion to dental substrates (39–41).

In the present study, ozone application did not adversely affect the bond strength or failure modes of the two resin-based materials tested on enamel and dentin, thus confirming the first null hypothesis. This finding may be attributed to ozone's intrinsic instability: once applied, ozone rapidly reacts with organic compounds and decomposes, leaving mini-

opadajući uzorak, počevši od kohezivnoga loma unutar zuba, zatim kohezivnoga loma unutar materijala, miješanoga loma i naposljetku adhezivnoga loma, pri čemu su vrijednosti SBS-a povezane s adhezivnim lomom bile statistički značajno niže od onih zabilježenih za ostale načine loma ($p < 0,01$, Dunnov post hoc test). Reprezentativne SEM-FEG i stereomikroskopske slike za svaki način loma prikazane su na slici 6.

Ramanova mikrospektroskopija i SEM-EDX analiza sučelja

Analiza Ramanovih mikrospektara nije pokazala zamjetne razlike među skupinama, ni s obzirom na ispitani restaurativni materijal, ni s obzirom na kontrolu ili primijenjeni tretman ozonom. Tipični spektar hidroksiapatita [HAP – $(Ca_{10}(PO_4)_6(OH)_2)$] zabilježen je u svim uzorcima, i na sučelju i ispod sučelja, u svim skupinama (fosfatni ν1 pomak na 960 cm^{-1} te karbonatni ν1 pomak na 1070 cm^{-1}). U skupinama s dentinom kao supstratom u nekim je uzorcima uočen blagi trag povećanih organskih komponenti, naznačen slabim pikovima na položajima koji odgovaraju Ramanovu pomaku metilidina (–CH, 1450 cm–1) i amidnih skupina (amid III na 1246/1270 cm–1, amid I na 1667 cm–1), što je u skladu s fiziološkim sastavom dentina (38). Reprezentativni spektri prikazani su na slici 7.

Rezultati polukvantitativnih kemijskih analiza energijski disperzivnim rendgenskim spektrometrom (EDX) nalaze se u tablici 2. te prikazuju ukupni srednji sastav cakline i dentina na sučelju restauracija - zub i u subinterfacijalnom području koje je razmatrano kao kontrola. Nisu utvrđene statistički značajne razlike ($p > 0,5$, ANOVA) u ionskom sastavu na sučelju restauracija-zub među različitim skupinama tretiranima ozonom, neovisno o supstratu ili korištenom materijalu, što upućuje na to da tretman ozonom ne utječe na moguće oslobađanje iona materijala na sučelju (tablica 3.). Nisu utvrđene značajne razlike u omjerima Ca/P ($p > 0,5$, Wilcoxonov test za uparene podatke). Nasuprot tomu, F ioni bili su značajno viši ($p < 0,01$, t-test za uparene podatke) na sučelju nego u subinterfacijalnom području koje je razmatrano kao kontrola, neovisno o supstratu i materijalu, što upućuje na moguće oslobađanje fluorida iz oba materijala na sučelju, bez značajnih razlika između njih ($p > 0,05$, ANOVA).

Rasprava

Učinak primjene ozona na adheziju restaurativnih materijala na bazi smole ostaje sporan zbog snažnog oksidacijskog potencijala ozona. Oksidansi, poput sredstava za izbjeljivanje, mogu promijeniti površinsku morfologiju i inhibirati polimerizaciju reakcijom s monomernim radikalima, čime se u konačnici narušava adhezija na zubne supstrate (39 – 41).

U ovom istraživanju primjena ozona nije nepovoljno utjecala na čvrstoću veze ni na načine loma dvaju ispitivanih materijala na bazi smole na caklini i dentinu, čime je potvrđena prva nulta hipoteza. Taj nalaz može se pripisati intrinzičnoj nestabilnosti ozona: nakon primjene ozon brzo reagira s organskim spojevima i razgrađuje se ostavljajući minimalnu količinu rezidualnog oksidansa na zubnoj površi-

mal residual oxidant on the dental surface (42). The short exposure time used in this study, compared with that of other oxidizing agents, may have further reduced any potential inhibitory effects on polymerization (43). Similar results were reported by other authors (5, 21, 28, 29, 44), although Rodrigues et al. observed reduced dentin bond strength when ozone was applied prior to etching (45). These discrepancies could be due to differences in sample preparation, adhesive systems, or material characteristics. In the present study, ozone was applied before etching/surface conditioning, similarly to other authors, and found no relevant differences among groups (5, 29). The dual-curing, self-adhesive nature of one of the materials tested may also explain its lower sensitivity to oxygen inhibition relative to conventional light-curing composites (46). Additionally, beyond its dual-curing behavior, a relatively low viscosity and hybrid resin-based composition of ACTIVA may promote better surface wettability and adaptation to oxidized substrates, which could further reduce its sensitivity to oxygen-related inhibition effects (47). Furthermore, the ozone concentrations used here (40 µg/mL) were within a high but clinically relevant range, whereas other studies employed higher and less realistic values (7, 45, 48). Methodological differences may also account for inconsistent findings across studies. The micro-tensile bond strength test, though widely used for its versatility and efficient specimen use, is highly sensitive to procedural variability (45, 49–53). Given the brittleness and limited thickness of enamel, shear bond strength testing is generally considered more reliable for enamel substrates (54, 55). Hence, to allow consistent testing on both enamel and dentin substrates, shear bond strength testing was selected for the present study.

With regard to failure mode, ozone treatment did not produce appreciable effects compared to the control group, regardless of the formulation and the substrate. Adhesive failure was the most common fracture mode, followed by mixed type failure, in line with the relevant literature (5, 32, 45, 56). Lower SBS values were typically associated with adhesive failures, suggesting limited micromechanical integration between the tooth surface and the restorative material. Nonetheless, the clinical relevance of failure mode distribution remains controversial (30). Overall, despite the relatively low SBS values observed, SEM-FEG analysis confirmed the presence of well-defined and interpretable failure patterns at the tooth–restoration interface, supporting the validity of the bond strength measurements and excluding major interfacial defects or specimen preparation artifacts (Figure 6).

The second null hypothesis was rejected, as ACTIVA demonstrated significantly higher bond strength than the RMGIC FUJI, particularly on enamel, whereas FUJI showed lower but more uniform results across substrates. These differences likely reflect inherent material properties and are in agreement with some previous findings (57), although contrasting reports exist (58, 59). These discrepancies could be related to variations in etching, bonding, or storage protocols. In the present study, ACTIVA was applied using an etch-only protocol without a separate bonding agent, in accordance with one of the manufacturer's recommended clinni (42). Kratko izlaganje primijenjeno u ovom istraživanju, u usporedbi s drugim oksidansima, moglo je dodatno smanjiti potencijalne inhibicijske učinke na polimerizaciju (43). Slične rezultate naveli su i drugi autori (5, 21, 28, 29, 44), iako su Rodrigues i suradnici zabilježili smanjenu čvrstoću veze na dentinu kada je ozon primijenjen prije jetkanja (45). Ta odstupanja mogu biti posljedica razlika u pripremi uzoraka, adhezivnim sustavima ili svojstvima materijala. U ovom istraživanju ozon je primijenjen prije jetkanja/kondicioniranja površine, slično kao što su učinili neki drugi autori, i nisu pronađene relevantne razlike među skupinama (5, 29). Dvostruko stvrdnjavajuća, samoadhezivna priroda jednoga od ispitivanih materijala također može objasniti njegovu manju osjetljivost na inhibiciju kisikom u odnosu na konvencionalne svjetlosno polimerizirajuće kompozite (46). Dodatno, uz dvostruko otvrdnjavanje, razmjerno niska viskoznost i hibridni sastav ACTIVA-e na bazi smole mogu pridonijeti boljoj sposobnosti vlaženja površine i adaptaciji na oksidirane supstrate, što bi dodatno moglo smanjiti osjetljivost na inhibicijske učinke povezane s kisikom (47). Nadalje, u ovom su istraživanju primijenjene koncentracije ozona (40 µg/mL) bile visoke, ali klinički relevantne, a u nekim drugim istraživanjima koristile su se veće i manje realne vrijednosti (7, 45, 48). Metodološke razlike također mogu objasniti nedosljedne nalaze među istraživanjima. Mikrovlačni test čvrstoće veze, iako široko primjenjivan zbog svestranosti i učinkovite uporabe uzoraka, vrlo je osjetljiv na proceduralnu varijabilnost (45, 49 – 53). S obzirom na krhkost i ograničenu debljinu cakline, smični test čvrstoće veze općenito se smatra pouzdanijim za caklinske supstrate (54, 55). Stoga je, zbog dosljednog testiranja vezivanja na caklini i dentinu, u ovom istraživanju odabran smični test čvrstoće veze.

Što se tiče načina loma, tretman ozonom nije pokazao zamjetne učinke u odnosu na kontrolu, neovisno o formulaciji i supstratu. Adhezivni lom bio je najčešći, a slijedio ga je miješani lom, u skladu s relevantnom literaturom (5, 32, 45, 56). Niže vrijednosti SBS-a tipično su bile povezane s adhezivnim lomovima, što upućuje na ograničenu mikromehaničku integraciju između zubne površine i restaurativnog materijala. Ipak, klinička relevantnost raspodjele načina loma ostaje kontroverzna (30). Općenito, unatoč razmjerno niskim vrijednostima SBS-a, SEM-FEG analiza potvrdila je prisutnost jasno definiranih i interpretativnih načina loma na sučelju zub - restauracija, što podupire valjanost mjerenja čvrstoće veze i isključuje veće defekte sučelja ili artefakte pripreme uzoraka (slika 6.).

Druga nulta hipoteza odbačena je jer je ACTIVA pokazala značajno veću čvrstoću veze od RMGIC FUJI-ja, osobito na caklini, dok je FUJI pokazao niže, ali ujednačenije rezultate između supstrata. Te razlike vjerojatno odražavaju intrinzična svojstva materijala i u skladu su s nekim prethodnim nalazima (57), iako postoje i suprotni izvještaji (58, 59). Ta odstupanja mogu biti povezana s varijacijama u protokolima jetkanja, aplikacije adheziva ili pohrane. U ovom istraživanju ACTIVA je aplicirana uz protokol *etch-only* bez zasebnoga adheziva, u skladu s jednom od kliničkih opcija koje preporučuje proizvođač za direktnu aplikaciju. Iako je u kliničkoj praksi u nekim indikacijama česta primjena dodatnog

ical options for direct placement. While the use of an additional bonding agent is common in clinical practice for some indications, we adopted here a simplified, manufacturer-supported protocol to allow a more direct and controlled evaluation of the intrinsic self-adhesive properties of both materials and their interaction with ozone-treated dental tissues.

Both materials exhibited some degree of technique sensitivity, as evidenced by early detachment or failure during build-up (57, 60), which suggests the importance of maintaining optimal humidity and temperature to ensure effective chemical interaction and adhesion. Relatively large standard deviations observed are consistent with the inherent variability of shear bond strength testing, which is highly sensitive to microscopic differences in substrate morphology, surface preparation, and unavoidable operator-dependent factors during manual specimen preparation and testing. This further suggests the need for continued research to improve this kind of experimental settings.

Energy-dispersive X-ray analysis (EDX) revealed no significant differences in the elemental composition of the interfacial area among groups, indicating that ozone treatment did not alter ion exchange or material-tooth interactions. Trace elements such as Na, N, Mg, and Si likely originated from natural substitution processes or polishing residues (61–63). While no notable differences were found in the Ca/P ratios, higher fluoride levels at the interface, independent of substrate or ozone exposure, suggest fluoride release from both materials, consistent with their bioactive potential (64,65). However, the study of this property requires further dedicated studies. Similarly, no remarkable ozone-induced modifications were observed in the micro-Raman spectra (66).

Surface roughness (Sa) and developed interfacial area ratio (Sdr) were not significantly affected by ozone treatment although minimal oxidation-induced surface changes, particularly in dentin, cannot be excluded. The slight increase in Sdr observed after gaseous ozone and ozonated water treatment may reflect a mild surface "micro-cleaning" or oxidative modification, leading to a small increase in the developed surface area. However, since this change was limited in magnitude, it did not translate into improved bond strength, indicating that these subtle surface modifications were insufficient to enhance either micromechanical retention or interfacial bonding effectiveness. Overall, these variations are unlikely to be clinically relevant, which aligns with reports indicating that ozone preserves enamel microhardness and morphology, producing only negligible changes in surface texture (67,68).

To the best of our knowledge, this is one of few studies to comprehensively investigate the effect of ozone on the adhesion and interfacial properties of bioactive restorative materials, assessing both enamel and dentin substrates and comparing multiple ozone formulations under standardized conditions. The use of human enamel samples further enhances the clinical relevance of the findings

(5). However, this study has a number of limitations. The preliminary nature of this study and a relatively small sample size, selected for feasibility reasons, limit the statistical pow-

adheziva, ovdje je primijenjen pojednostavljeni protokol koji je podupirao proizvođač kako bi se omogućila izravnija i kontroliranija procjena intrinzičnih samoadhezivnih svojstava obaju materijala i njihove interakcije s dentalnim tkivima tretiranim ozonom.

Oba materijala pokazala su određeni stupanj osjetljivosti na tehniku, što je vidljivo u ranom odvajanju ili neuspjehu tijekom izrade nadogradnje (57, 60), a to upućuje na važnost održavanja optimalne vlažnosti i temperature radi učinkovite kemijske interakcije i adhezije. Razmjerno velike standardne devijacije u skladu su s intrinzičnom varijabilnošću smičnog testa čvrstoće veze koji je vrlo osjetljiv na mikroskopske razlike u morfologiji supstrata, pripremi površine te neizbježne čimbenike ovisne o operatoru tijekom ručne pripreme i testiranja uzoraka. To dodatno upućuje na potrebu daljnjih istraživanja radi unaprjeđenja takvih eksperimentalnih postavki.

EDX analiza nije pokazala značajne razlike u elementarnom sastavu interfacijalnog područja među skupinama, što pokazuje da tretman ozonom nije promijenio ionsku razmjenu ni interakcije materijal-zub. Elementi u tragovima poput natrija (Na), dušika (N), magnezija (Mg) i silicija (Si) vjerojatno potječu iz prirodnih supstitucijskih procesa ili ostataka poliranja (61 – 63). Iako nisu utvrđene značajne razlike u omjerima Ca/P, više razine fluorida na sučelju, neovisno o supstratu ili izloženosti ozonu, upućuju na oslobađanje toga elementa iz obaju materijala, što je u skladu s njihovim bioaktivnim potencijalom (64, 65). Međutim, proučavanje ovog svojstva zahtijeva daljnja, ciljano dizajnirana istraživanja. Slično tomu, u Ramanovim mikrospektrima nisu uočene zamjetne ozonom inducirane promjene (66).

Hrapavost površine (Sa) i omjer razvijene spojne površine (Sdr) nisu bili značajno promijenjeni tretmanom ozonom, iako se minimalne oksidacijom inducirane promjene površine, osobito u dentinu, ne mogu isključiti. Blagi porast parametra Sdr nakon tretmana plinovitim ozonom i ozoniranom vodom može odražavati blago *mikročišćenje* površine ili oksidacijsku modifikaciju, što malo povećava razvijene površine. Međutim, budući da je ta promjena bila ograničenog opsega i nije se pretvorila u povećanje čvrstoće veze, to pokazuje da su takve suptilne površinske modifikacije bile nedostatne za poboljšanje mikromehaničke retencije ili učinkovitosti interfacijalnog vezivanja. Općenito, vjerojatno nije riječ o klinički relevantnim varijacijama, a nalazi su u skladu s izvješćima prema kojima ozon ne utječe na mikrotvrdoću i morfologiju cakline, uz tek zanemarive promjene u površinskoj teksturi (67, 68).

Prema našim saznanjima, ovo je jedno od rijetkih istraživanja koje je sveobuhvatno ispitalo učinak ozona na adheziju i interfacijalna svojstva bioaktivnih restaurativnih materijala, uz procjenu i cakline i dentina te usporedbu više formulacija ozona u standardiziranim uvjetima. Primjena humanih uzoraka cakline dodatno povećava kliničku relevantnost nalaza (5). Ipak, ovo istraživanje ima više ograničenja. Preliminarna priroda istraživanja i razmjerno mali uzorak, odabrani radi izvedivosti, ograničavaju statističku snagu nalaza (33). Nadalje, korišteni su nekariozni supstrati radi procjene osnovnih učinaka ozona kao završnog koraka dekontaminacije; u buduća istraživanja trebalo bi uključiti tkiva zahvaćena kari-

er of the findings (33). Moreover, non-carious substrates were used to evaluate baseline effects of ozone as a final decontamination step; future research should include carious tissues to reflect clinical conditions more closely. Finally, the influence of varying ozone concentrations and application times were not examined. Further investigations are required to provide insights into potential dose-dependent effects.

Conclusions

Within the limitations of this preliminary *in vitro* study, ozone treatment did not significantly influence the surface topography, the short-term adhesive performance, or interfacial ion-related findings of the tested restorative materials on enamel and dentin, regardless of formulation. However, these results are restricted to specific experimental conditions adopted, including a limited sample size, a short observation period, and the absence of artificial aging or long-term stability assessments. Moreover, only surface-related and interfacial properties were investigated, and no conclusions can be drawn regarding possible biological effects on the dental pulp or deeper tissues. The present findings suggest that ozone does not adversely affect the immediate bond strength under testing conditions and may be used as a final decontamination step prior to restoration, selecting the appropriate formulation according to practical considerations such as ease of application, clinical objectives, safety, and availability. Nevertheless, further studies with larger samples, longer-term aging protocols, and biological evaluations are required before any definitive clinical recommendations can be made.

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jesom kako bi se uvjeti što više približili kliničkoj stvarnosti. Konačno, utjecaj različitih koncentracija ozona i vremena primjene nije ispitivan te zahtijeva dodatna istraživanja zbog uvida u moguće učinke ovisne o dozi.

Zaključci

U okviru ograničenja ovoga preliminarnog istraživanja *in vitro*, tretman ozonom nije značajno utjecao na površinsku topografiju i kratkoročnu adhezivnu učinkovitost, ni na interfacijalne nalaze povezane s ionima za ispitivane restaurativne materijale na caklini i dentinu, neovisno o formulaciji. No ovi rezultati ograničeni su na specifične eksperimentalne uvjete, uključujući ograničenu veličinu uzorka, kratko razdoblje praćenja te izostanak umjetnog starenja ili procjene dugoročne stabilnosti. Nadalje, ispitivana su isključivo obilježja povezana s površinom i sučeljem te se ne može zaključci o mogućim biološkim učincima na zubnu pulpu ili dublja tkiva. Dobiveni nalazi upućuju na to da ozon u ispitivanim uvjetima ne narušava neposrednu čvrstoću veze te se može primijeniti kao završni korak dekontaminacije prije restauracije, uz odabir formulacije prema praktičnim razmatranjima poput jednostavnosti primjene, kliničkih ciljeva, sigurnosti i dostupnosti. Ipak, prije donošenja bilo kakvih konačnih kliničkih preporuka potrebna su daljnja istraživanja s većim uzorcima, protokolima dugotrajnijeg starenja i biološkim procjenama.

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Izjava o informiranom pristanku: Informirani pristanak pribavljen je od svih ispitanika uključenih u istraživanje. U skladu sa smjernicama i propisima lokalnoga Etičkog povjerenstva, dobrovoljci su bili informirani o uporabi njihovih ekstrahiranih zuba u istraživanju te je dobivena njihova usmena suglasnost.

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Doprinos autora: **F. V.** – konceptualizacija; **F. V., A. N., G. B., P. S.** i **L. G.** – metodologija; **T. F.** i **L. G.** – validacija; **F. V.** i **T. F** – formalna analiza.; **F. V., A. N., G. B., P. S.** – istraživanje; **F. V.** i **T. F** – kuriranje podataka; **F. V.** – priprema i pisanje izvornog nacrta; **A. N., G. B., P. S., T. F., U. C.** i **L. G.** – pisanje teksta, pregled i uređivanje; **U. C.** i **L. G** – nadzor. Svi autori pročitali su tekst i složili se s objavljenom verzijom.

Sažetak

Cilj: U ovom preliminarnom istraživanju *in vitro* ispituje se kako različite formulacije ozona utječu na adheziju, površinsku topografiju i oslobađanje iona na sučelju dvaju pedijatrijskih bioaktivnih restaurativnih materijala. **Materijali i metode:** Standardizirane površine cakline i dentina (n = 96) dobivene iz ekstrahiranih humanih zuba randomizirane su u 8 skupina koje se razlikuju prema protokolu obrade (bez obrade, ozon u plinu, ozonirana voda, ozonirani gel) i prema restaurativnom materijalu (Fuji II LC, ACTIVA Bioactive Restorative). Adhezivna učinkovitost procijenjena je nakon 7 dana pohrane (HBSS, 37 °C) ispitivanjem smične čvrstoće veze (SBS) i mikroskopskom analizom načina loma. Hrapavost površine (Sa) mjerena je optičkom profilometrijom; elementarni sastav na sučelju analiziran je Ramanovom spektroskopijom te skenirajućom elektronskom mikroskopijom s energijski disperzivnim rendgenskim detektorima (SEM-EDX). **Rezultati:** Obrada ozonom nije značajno utjecala na SBS [srednja vrijednost 3,59 ± 3,23 MPa (min 0,05 MPa; max 11,69 MPa)] načine loma ni na hrapavost površine ($p > 0,05$ za sve domene). Kemijske analize nisu pokazale zamjetne razlike, uz moguću iznimku minimalnog oslobađanja fluorida. **Zaključci:** Neovisno o mediju primjene, tretmani ozonom nisu pokazali značajan učinak na kratkoročno adhezivno svojstvo ni za jedan restaurativni materijal, ni na kvalitetu vezivanja s caklinom i dentinom. Dodatno, nisu utjecali na površinsku topografiju, ni na potencijalnu bioaktivnost na sučelju ispitivanih materijala. No ti preliminarni rezultati ograničeni su na specifične eksperimentalne uvjete pa potrebna daljnja istraživanja prije donošenja bilo kakvih konačnih kliničkih preporuka.

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