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Ubrzavanje procesa izbjeljivanja zuba atmosferskim plazmenim mlazom

Atmospheric Pressure Plasma Jet as an Accelerator of Tooth Bleaching

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Sažetak

Svrha: Željela se ispitati učinkovitost atmosferskoga plazmenog mlaza (APM) kao izvora svjetlosti čiji učinak može brže razlagati vodikov peroksid u gelovima za izbjeljivanje i tako potaknuti brže i bolje izbjeljivanje. **Materijali i metode:** U zeleni čaj je osam sati bilo uronjeno 25 pastila hidroksilapatita. Nakon toga su osušene i podijeljene u pet skupina s po pet pastila. Uzorci su posebno tretirani gelovima za izbjeljivanje s 30-postotnim i 40-postotnim vodikovim peroksidom te u kombinaciji s atmosferskom plazmom. Tijekom procesa izbjeljivanja analizirana je optička emisijska spektroskopija i temperatura s pomoću pirometra. Boja pastila bila je određena RGB kolorimetrom. Za mjerenje pH vrijednosti korišteno je prije i poslije tretmana dodatnih 10 pastila kojima je pH izmjeren kontaktnim pH-metrom. **Rezultati:** Analizom promjene boja na pastilama prije i poslije tretmana, pokazano je da APM u kombinaciji s gelovima za izbjeljivanje poboljšava izbjeljivanje 32, odnosno 15 posto. Postupak izbjeljivanja s pomoću APM-a imao je bolji učinak u šest puta kraćem vremenu u odnosu prema tretmanu koji je predložio proizvođač. Optičkom emisijskom spektroskopijom dokazana je kemijska aktivnost plazme. Nakon tretmana APM-om, pH vrijednosti gela za izbjeljivanje pale su na 50 do 75 posto početnih vrijednosti, a temperatura na površini tretiranog uzorka porasla je s 8 na 10°C u odnosu prema početnim vrijednostima. **Zaključak:** Uporaba atmosferskoga plazmenog mlaza u kombinaciji s gelovima za izbjeljivanje daje bolje rezultate u kraćem vremenu i ne povećava temperaturu koja može oštetiti okolno tkivo.

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Ključne riječi
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Uvod

Tehnologija bazirana na plazmi sve je češće dio naše svakodnevnice. Električnim, temperaturnim, kemijskim i fizičkim svojstvima plazme koristimo se u raznim područjima suvremene industrije, uključujući mikročipiranje, informatičku, energetsku i avioindustriju te zaštitu okoliša. Nedavno je plazma potaknula veliko zanimanje i u biomedicini. Atmosferska plazma kemijski je aktivan, potpuno ili djelomično ionizirani plin, a nastaje djelovanjem energije na reaktivni plin, pri čemu počinje reorganizacija elektronske strukture u atomima i molekulama te se stvaraju pobuđene čestice i ioni. Plazme se mogu podijeliti na osnovi termodinamičke ravnoteže. Stanje plazme u kojoj postoji lokalna ravnoteža naziva se visokotemperaturnom ili vrućom plazmom jer temperature mogu dosegnuti i nekoliko desetaka tisuća kelvina (K), a plazma u kojoj nema jednakosti temperature različitih

Introduction

Plasma technology is becoming more and more part of our daily lives. Electrical, thermal, chemical and physical properties of plasma have been widely used in various fields including electronics, manufacturing, energy, aerospace and environmental industry. Recently, plasma has attracted increased attention in the biomedical field. Plasma is a partially ionized gas containing a large amount of highly reactive species including ions, electrons, free radicals and electronically excited neutrals. Plasmas can be classified according to thermodynamic equilibrium. Temperatures in the so called thermal plasma, which is in thermodynamic equilibrium, can reach as high as several 10,000 K. In non-thermal plasma, plasma which is not in thermodynamic equilibrium, temperatures can be as low as 300 K (1). Non-thermal plasmas can be divided according to pressure into the atmospheric presčestica naziva se niskotemperaturnom ili hladnom plazmom kod koje temperature mogu biti niske i do 300 K (1).

Niskotemperaturne plazme mogu se podijeliti prema tlaku na atmosferske i niskotlačne. Za aktivaciju niskotlačnih potrebne su vakuumska crpka i vakuumska komora, no one mogu biti poprilično skupe i komplicirane (2).

Različiti izvori plazme korišteni su za njihovo generiranje pri atmosferskom tlaku. Jedan od njih je atmosferski plazmeni mlaz (APM) koji se može podijeliti prema obliku elektrode na dielektrične bezelektrodne mlazove (DBM), izbojne mlazove s dielektričnom barijerom (IMDB), IMDB-u slične mlazove i plazmene mlazove s jednom elektrodom.

APM kao reaktivni plin, osim elektrona, pozitivno i negativno nabijenih iona te fotona u ultraljubičastom dijelu spektra, sadržava i aktivne radikale tzv. reaktivne kisikove radikale (RKR) O, OH, HO_2^-, O_2, HO_2, H_2O_2 i reaktivne dušikove radikale (RNR) NO,N_2,NO_2 koji mogu reagirati s biološkim materijalima na sobnim temperaturama. APM se može aplicirati na točno određeno mjesto u otvorenom prostoru bez bojazni od temperaturnog šoka, pa se može primjenjivati u području biomedicine. Zbog svojih kemijskih svojstava ima velik potencijal u inaktivaciji bakterija, sterilizaciji, koagulaciji krvnih žila, cijeljenju rana, tretmanu kancerogenih stanica i raznih infekcija (4 – 8). Plazmatska tehnologija također ima velike mogućnosti u području dentalne medicine (9).

Jedan od popularnijih estetskih zahvata u dentalnoj medicini jest izbjeljivanje zuba. Svaki dan sve više pacijenata traži mogućnost za estetsku korekciju osmijeha s pomoću neinvazivnih i sigurnih metoda. Izbjeljivanje zuba može se obavljati na dva načina – kao *izbjeljivanje kod kuće* kada se dva do tri tjedna pacijent koristi individualnim udlagama te niskom koncentracijom karbamid-peroksidnog gela za izbjeljivanje i *izbjeljivanje u ordinaciji* gdje liječnik dentalne medicine, gelovima za izbjeljivanje baziranima na vodikovu peroksidu i karbamid peroksid gelovima u koncentracijama od 15 do 45 i od 30 do 37 posto, postiže željeni učinak za 30 do 60 minuta po jednom posjetu (10 – 12). Mehanizam izbjeljivanja temelji se na aktivaciji gela koji se počinje razlagati na radikale koji napadaju i oksidiraju organske molekule u zubu i tako ga izbjeljuju (13). Za povećanje učinkovitosti gela primjenjuju se razni oblici svjetlosti: halogena, infracrvena svjetleća dioda (LED), laser i plazmatski luk (14,15). No uporaba izvora svjetlosti, kako bi se poboljšao učinak izbjeljivanja, i dalje je upitna. Neki autori ističu da izvori svjetlosti ne poboljšavaju izbjeljivanje (16), neki pak tvrde da poboljšavaju (17). No uspješnost postupka izbjeljivanja zuba temelji se na koncentraciji gela i trajanju postupka (18). Produženo vrijeme izbjeljivanja može rezultirati neželjenim posljedicama, kao što su promjene na površini cakline, demineralizacija i smanjenje tvrdoće (19, 20).

Svrha istraživanja bila je ispitati učinak APM-a na postupak izbjeljivanja i može li se APM-om bolje i brže postići efekt izbjeljivanja, a da se ne poveća temperatura koja bi mogla oštetiti potporni aparat zuba ili zubnu pulpu. Za ovo istraživanje korištene su pastile pripremljene od praha hidroksilapatita (zbog uniformnosti). Radne hipoteze bile su: (H1) APM ne poboljšava efekt izbjeljivanja; (H2) nema pro-

sure plasmas (APP) and low pressure plasmas. For generating low pressure plasmas, vacuum chamber and vacuum pumps are required which can be very expensive and complicated (2). Various types of plasma sources have been developed to generate plasmas at atmospheric pressure. One of them is the non-thermal APP jet, which can be further subdivided according to their electrode design: dielectric-free electrode (DFE) jets, dielectric barrier discharge (DBD) jets, DBD-like jets and single electrode (SE) plasma jet (3).

The APP jet contains all reactive species as mentioned above including active radicals such as: Reactive Oxygen Species (ROS) O, OH^-, HO_2^-, O_2, HO_2, H_2O_2 and Reactive Nitrogen Species (RNS) NO, N_2, NO_2. Both ROS and RNS have the potential to react with biological materials while their temperature remains near room temperature. Because the APP jet can be applied to desired sites in open space without damaging the surrounding tissues, it has recently attracted much interest in biomedicine due to its potential applications in bacteria inactivation, tissue sterilization, blood coagulation, wound healing, suppressing the melanoma cancer cell and treatment of corneal infections (4-8). Also, in dentistry the plasma technology has shown great potential (9).

One of the most popular esthetic procedures in dentistry is tooth bleaching and every day more and more patients are seeking to improve their smiles with an effective and safe method. Tooth bleaching can be generally divided into two types: "in home bleaching" in which the patient uses a tray containing a low concentration of carbamide peroxide (CP) gel for two or three weeks and "in office bleaching" in which dental practitioners use a higher concentration of hydrogen peroxide (HP) gels 15-45% and higher concentrations 30-37% of CP gels, which is directly applied to the teeth for a total period of 30-60 minutes (10-12). The mechanism of bleaching is based on the HP or CP gel's ability to penetrate tooth structure and produce free radicals that oxidize organic stains within the tooth (13). For better results in office, tooth bleaching products available on the market can be combined with a supplementary light source (i.e. laser, LED, OLED, halogen lamp or plasma arc) (14, 15). However, information on the effectiveness of these light sources in the literature is conflicting. Some authors claim that use of the light sources increases the efficiency of tooth bleaching (16) while others reported it had no clinically significant effect on tooth bleaching (17). The success of tooth bleaching depends on the concentration of the gel and total treatment duration (18). The time needed for tooth bleaching is of great importance, longer time produces better bleaching results. However, increased application time may cause enamel surface alterations, such as loss of mineral content and microhardness (19, 20).

The aim of this study was to evaluate if APP jet as activator can accelerate the degradation of hydrogen peroxide and provide more effective results in a shorter period of time without a significant temperature increase which may cause damage of the tooth and surrounding tissue. For this purpose pastilles were used because they provide more uniformly colored test sample than teeth. Research hypotheses: (H1) The mjene u pH vrijednostima gelova aktiviranih APM-om tijekom procesa izbjeljivanja; (H3) aktivacija APM-a povećava temperaturu na površini tretiranog uzorka.

Materijali i metode

Eksperimentalni postav

Eksperimentalni postupak za tretiranje gela na pastilama grafički je prikazan na slici 1.

APM korišten za ovo istraživanje sastojao se od bakrene elektrode (žica promjera 100 μm) uložene u staklenu cjevčicu (vanjski promjer 1,5 mm i dužina 5 cm) i spojene na izvor napona od 25 kHz i 2,5 kV (21). Kao radni plin korišten je helij (čistoća 4,6) pri protoku od 2 l/min. Uzorci (pastile) su se nalazili 13 mm od vrha elektrode.

Tijekom tretmana mjerena je temperatura na površini tretiranog uzorka i optička emisijska spektroskopija (OES). Za spektroskopiju korišten je optički spektrometar Avantes AvaSpec 3648 (Avantes Inc., Apeldoorn, Nizozemska) nominalne rezolucije 0,8 nm u rasponu od 200 do 1100 nm s optičkim vlaknom na čijem se kraju nalazila leća postavljena okomito u odnosu na APM (slika 1.). Integracijsko vrijeme između mjerenja bilo je prilagođeno na 250 ms. Minijaturni infracrveni senzor (MI, Cole-Parmer, Vernon Hills, Illinois, SAD), pirometar s omjerom udaljenosti 10 : 1 od objekta, bio je postavljen 10 cm od uzorka pod kutom od 45º. Zbog nepoznavanja emisivnosti gela, za njezino određivanje korišten je K-tip termočlanka. Uzorci (pastila i pastila s gelom za izbjeljivanje) zagrijavani su u pećnici i temperatura se istodobno mjerila pirometrom i termočlankom. Nakon mjerenja pirometar je kalibriran.

Uzorci i eksperimentalni postupak

Od praha hidroksilapatita (Hydroxylapatite for analysis, ACROS Organics Co., Fair Lawn, NJ, SAD) bilo je pripremljeno 35 pastila promjera 12,5 mm i debljine 2,5 mm. Svaka je sadržavala 400 mg hidroksilapatita izmjerenog na vagi (Mettler PM200, Greifensee, Švicarska) i zatim prešanog pod tlakom od 20 bara (Universal GP1, BanjaLuka, BiH). Kako bi pastile dobile na čvrstoći, sušene su dva sata na temperaturi od 150 °C u suhom sterilizatoru (Instrumentarija, ST-01/02, Zagreb, Hrvatska) (22). Otopina za bojenje bila je pripremljena od 2 g zelenoga čaja (Fanning's, Cedevita d.o.o., Zagreb, Hrvatska) uronjenog pet minuta u 100 mL vruće vode. Nakon što se otopina ohladila na sobnu temperaturu, u nju su osam sati bile uronjene pastile kako bi dobile boju (18).

U ovom istraživanju koristili smo se dvama različitim gelovima za izbjeljivanje: DASH (Discus Dental, LLC, Los

optical effect of bleaching is not affected by APP jet activation (H2) There is no change in pH values of bleaching gels activated by APP jet during bleaching (H3) Additional APP jet activation can lead to surface temperature increase.

Materials and Methods

Experimental setup

Experimental setup for treating gel-pastille samples is presented in Figure 1. Atmospheric pressure plasma (APP) jet used in this investigation was a single electrode jet also known as plasma needle. Copper electrode (wire 100 microns in diameter) inserted in a glass tube (outer diameter 1.5 mm, length 5 cm) was connected to a 25 kHz and 2.5 kV power supply (21). Normally, around 1 W of power is transferred to the sample. Helium (4.6 purity), at flow rate 2 l/min, was used as a working gas. Samples (pastille, bleaching gel) were placed on a non-conductive holder approximately 13 mm from the tip of the electrode to the gel surface.

During the treatment, surface temperature of the sample and the optical emission spectroscopy (OES) of the APP jet were measured. Fiber optic spectrometer Avantes AvaSpec 3648 (Avantes Inc., Apeldoorn, The Netherlands) with a 0.8 nm spectral resolution in the range from 200 to 1100 nm was used. Collimating lens at the end of the optical fiber was placed at the beginning of the jet, perpendicular to the jet axis as seen in Figure 1. The integration time was set to 250 ms. Miniature Infrared Sensor (MI, Cole-Parmer, Vernon Hills, Illinois, USA) pyrometer with 10:1 distance-to-target ratio was mounted approximately 10 cm from the sample at an angle of about 45°. A pyrometer was used because it provides non-contact measurement and is not affected by plasma as thermocouples. Because the emissivity of the used samples is not known, a calibration using K-type thermocouple was performed. Samples (pastille and pastille with bleaching gel applied) were heated in an oven and then the temperature was measured simultaneously with pyrometer and thermocouple while the samples were cooling down. From these measurements, pyrometer calibration curves for our samples were determined.

Samples and experimental procedure

35 pastilles were made out of 400 mg hydroxylapatite powder (Hydroxylapatite for analysis, ACROS Organics Co., Fair Lawn, NJ, USA). Pastilles 12.5 mm in diameter and 2.5 mm thick were compressed under the pressure of 20 bar (Universal GP1, Banja Luka, Bosnia and Herzegovina) out of hydroxylapatite powder weighted with scale (Mettler PM200, Greifensee, Switzerland). In order to obtain strength, the pastilles were then dried for 2 hours at the temperature of 150°C in a dry sterilizer (Instrumentaria, ST-01/02, Zagreb, Croatia) (22). Solution was made from 2 g of green tea (Fanning's, Cedevita d.o.o., Zagreb, Croatia) boiled in 100mL of distilled water for 5 minutes and after that the tea was cooled to room temperature. The pastilles were then immersed into the tea for 8 hours in order to gain color (18).

In this study, we used two different bleaching gels: DASH (Discus Dental, LLC, Los Angeles, California, USA), Angeles, California, SAD) koji sadržava 30-postotni vodikov peroksid i BOOST (Ultradent Products, Inc., South Jordan, Utah, SAD) koji sadržava 40-postotni vodikov peroksid. Za potrebe mjerenja uzorci su bili podijeljeni u dvije skupine (n = 10), ovisno o korištenom gelu (DASH ili BOOST). Svaka skupina zatim je podijeljena u dvije podskupine: G1 kontrolna podskupina bila je tretirana samo gelom za izbjeljivanje bez APM-a, a G2 podskupina bila je tretirana gelom za izbjeljivanje u kombinaciji s APM-om. Dodatna skupina od pet pastila bila je tretirana samo APM-om bez gela za izbjeljivanje. Za potrebe mjerenja pH vrijednosti izdvojene su dvije skupine po pet pastila i svaka je tretirana gelom za izbjeljivanje u kombinaciji s APM-om. Prva skupina pastila tretirana je postupkom izbjeljivanja prema preporuci proizvođača.

Gel se nanosio aplikatorom na površinu pastile debljine 1,5 mm i ostavio 15 do 20 minuta (DASH/BOOST), a zatim se uklonio plastičnom špatulom. Odmah nakon toga mjerila se vrijednost crveno zeleno plavo (*red green blue,* RGB) indeksa na pastili. Postupak se ponavljao tri puta. U drugoj skupini na površinu pastila bio je nanesen gel debljine 1,5 mm i tretirana je APM-om devet minuta. Nakon toga se plazmom uklonio gel s površine pastila i izmjerio RGB indeks. U trećoj skupini pastile su tretirane samo APM-om bez gela.

Prosudba učinkovitosti izbjeljivanja, pH mjerenje i statistička analiza

Za mjerenje boje zuba korišteni su uređaji poput spektrofotometra i kolorimetra (23, 24).

Odmah nakon tretmana, RGB vrijednosti bile su izmjerene kolorimetrom (PCE-RGB 2, PCE Instruments, Southampton, Ujedinjeno Kraljevstvo). Za svaku pastilu izmjerene su pet puta prije tretmana i pet puta nakon tretmana, kako bi se smanjila statistička pogreška. Zato što osim izbjeljivanja nije bilo drugih parametara koji bi mogli utjecati na ishod izbjeljivanja, korišten je RGB model boja. RGB model boja temelji se na miješanju crvene, zelene i plave s različitim intenzitetima oblikujući pritom konačnu boju spektra. Boja u RGB modelu prostora stoga je prikazana kao RGB triplet. Svaka od tri boje ima svoje numeričke vrijednosti od nule do maksimuma (u našem primjeru 1023). Crna boja označava vrijednosti prikazane kao 0,0,0, a bijela je maksimalna vrijednost pojedine boje (1023, 1023, 1023) ili tzv. bijela točka. Zato se Euklidova udaljenost između izmjerenih RGB vrijednosti i bijele točke može definirati kao bijela linija u prostoru RGB modela boja. Dakle, može se reći da je razlika u boji ΔRGB definirana kao razlika udaljenosti između bijele linije pastila prije tretmana i bijele linije istih dužina nakon tretmana. Aritmetička srednja vrijednost za ΔRGB i njihove standardne devijacije izračunata je nakon pet uzastopnih mjerenja obavljenih na pet pastila tretiranih u jednakim uvjetima.

PH vrijednosti izmjerene su pH metrom ExStik EC 500 s kontaktnom elektrodom (Flir Commercial Systems, Inc., Nashua, New Hampshire, SAD), a bio je kalibriran puferskim otopinama pH 4, 7 i 10 (Hanna instruments, Ann Arbor, Michigan, SAD). Kalibracija se ponavljala poslije svakog mjerenja. Početne pH vrijednosti gelova za izbjeljivanje

which is a 30% HP gel and BOOST (Ultradent Products, Inc., South Jordan, Utah, USA), which is a 40% HP gel. For measuring the bleaching effect of each gel, specimens were divided into two groups (n=10) depending on used bleaching gel (DASH or BOOST). Each group was then divided into two subgroups: (G1) control - bleaching gel without APP jet activation and (G2) bleaching gel + APP jet activation. One separate group (n=5) was formed for treatment with only APP jet without bleaching gel. As for the pH measurement, two groups were formed (n=5); each for different gel in combination with APP jet.

Whitening procedure, using only bleaching gels, was performed according to the manufacturer's instructions. 1.5 mm thick layer was applied on the pastille, left for 15/20 minutes (DASH/BOOST) and then removed using a plastic spatula. Immediately after the gel was removed, RGB of the pastille was measured. Three such cycles were performed on the same pastille. In the second procedure, first a 1.5 mm layer of bleaching gel was applied on the pastille and then treated with APP jet for 9 minutes. After the treatment, the gel was removed and RGB of the pastille was measured. In the third whitening procedure, the pastilles were treated for 9 minutes only with APP jet. RGB was measured immediately after that.

Bleaching evaluation, pH measurements and statistical analysis

Instruments such as spectrophotometers and colorimeters are used to measure the colors of different materials as well as tooth color (23, 24). Immediately after the treatment, RGB (red, green, blue) values of pastilles were measured with colorimeter (PCE-RGB 2, PCE Instruments, Southampton, United Kingdom). RGB values of each pastille were measured 5 consecutive times before the treatment and 5 consecutive times after the treatment in order to minimize the measurement error. Because only the differences between bleaching efficiency of different procedures were evaluated, RGB color space could be used. The RGB color model is based on mixing red, green and blue with different intensities thus forming the final color. In RGB space the color is therefore presented as an RGB triplet. Each of the three colors can vary from zero to maximum value (in our case 1023), where black is represented as point (0, 0, 0) and white is at a point (1023, 1023, 1023). Euclidian distance between measured RGB and white point can be defined as white distance. The color change DRGB can therefore be defined as a difference between the white distance of pastille before the treatment and the white distance of the same pastille after the treatment. Arithmetic mean value of DRGB and its standard deviation were evaluated over 5 consecutive measurements of 5 different pastilles treated under the same conditions.

The pH was measured using ExStik EC 500 pH meter with contact pH electrode (Flir Commercial Systems, Inc., Nashua, New Hampshire, USA). The pH meter was standardized by Hanna instruments buffer solutions of pH 4, 7 and 10 (Hanna instruments, Ann Arbor, Michigan, USA) and was re-calibrated after each measurement. Initial pH value of both bleaching gels was measured on a glass plate prior

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izmjerene su na staklenoj pločici prije tretmana. Za mjerenje pH vrijednosti nakon tretmana APM-om korišteno je po pet pastila za svaku vrstu gela – ukupno njih 10. Gel za izbjeljivanje nanosio se na površinu pastile i, nakon tretmana APM-om, pH elektroda je bila u kontaktu s gelom 10 minuta pri sobnoj temperaturi od 21°C i vlažnosti zraka od 56 posto. pH vrijednosti mjerene su svaku minutu te je ukupno za svaki gel zabilježeno 10 vrijednosti. Između svake serije mjerenja pH elektroda je bila isprana mlazom vode iz slavine kako bi se uklonile naslage. Nakon toga isprana je deioniziranom vodom i osušena sterilnom gazom (Lola Ribar d.d., Zagreb, Hrvatska). Nakon kalibriranja isti je postupak bio primijenjen za sljedeću pastilu.

Za statističku analizu korišten je Origin Pro 8,5 (OriginLab Corporation, North Hampton, MA, SAD).

Rezultati

Optička emisijska spektroskopija tijekom tretmana APM-a na pastilama i APM-a u kombinaciji s BOOST-om na pastilama, prikazana je na slici 2. Najizraženija linija spektra pripada atomu helija i molekuli dušika. Osim tih dviju linija uočeni su OH, N_2^+, O i H ioni. Intenzitet linija spektra izraženiji je u kombinaciji APM+BOOST, u odnosu na samo APM. Slične linije spektra vidljive su i kod drugog gela za izbjeljivanje – DASH-a. Na slici 3. su rezultati promjene boje ΔRGB-om nakon tretmana. Promjena boje ΔRGB-om za BOOST pokazuje bolje rezultate u odnosu na DASH. Najbolji rezultati postignuti su kombinacijom gelova za izbjeljivanje DASH/BOOST s APM-om, s time da su nešto bolji rezultati dobiveni kombinacijom APM+BOOST u odnosu prema kombinaciji APM+DASH. APM u kombinaciji s BOOST-om izbjeljivao je 20 posto bolje u odnosu prema tretmanu samo s BOOST-om u šest puta kraćem vremenu, a APM u kombinaciji s DASH-om pokazao je čak 32 posto bolje izbjeljivanje od primjene samo gela.

Za gelove DASH i BOOST izmjerene su pH vrijednosti prije tretmana i nakon tretiranja APM-om (tablica 1.). Nakon tretmana APM-om, pH vrijednosti za DASH bile su gotovo dva puta niže u odnosu na početne vrijednosti, a za BOOST je pH vrijednost pala na 75 posto početne vrijednosti. Temperaturne vrijednosti izmjerene na površini pastila, odnosno gela, pokazale su da tretman APM-om povećava temperaturu. Kod pastila tretiranih APM-om temperatura doseže 30 °C. Pri tretiranju pastila kombinacijom APM+DASH, temperatura doseže maksimalnih 32 °C, u odnosu na APM+BOOST od 30,5 °C.

to the treatment. In order to measure pH value of bleaching gels after the APP jet treatment, 5 pastilles were used for each type in total of 10 pastilles. The bleaching gel was placed on pastille and after the APP jet treatment the pH electrode was placed in contact with the gel for a period of 10 minutes at room temperature 21°C and humidity 56%. Each minute the pH value was recorded for total of 10 measurements for each sample. The electrode was washed between samples measurements under a stream of water to remove gel debris. The electrode was then rinsed using deionized water and dried with sterile gaze (Lola Ribar d.d., Zagreb, Croatia). After re-calibration, the procedure was repeated for the next pastille.

Data analysis and graphical presentation of the results was performed with Origin Pro 8.5 (OriginLab Corporation, North Hampton, MA, USA).

Results

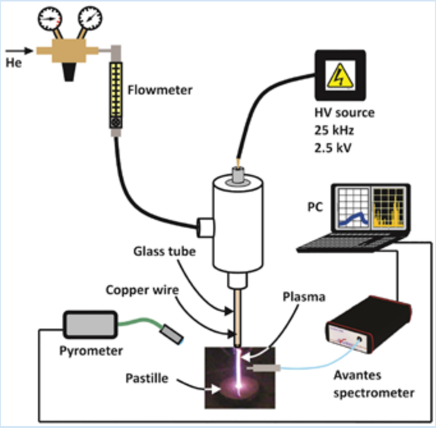
Typical optical emission spectra for APP helium jet without a sample and for jet during the treatment of BOOST gel on pastilles are presented in Figure 2. The strongest emission lines in the presented OES spectra belong to helium atom and nitrogen molecule. Apart from these two sets of lines, OH, N_2^+, O and H emission lines are also visible. It can be observed that intensities of the emission lines of the APP+BOOST spectrum are slightly higher than the ones of the free APP spectrum. Similar OES results can be observed for APP jet during the DASH gel treatment. In Figure 3, the results of color change DRGB immediately after the treatments are shown. The DRGB color change for BOOST gel showed better results than for DASH. The best results were obtained when APP jet was in conjunction with bleaching gels. Slightly better results were obtained when APP jet was in conjunction with BOOST than with DASH.

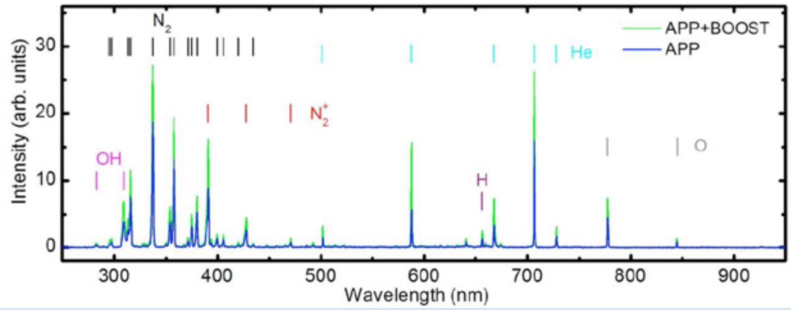
The pH values of DASH and BOOST gel were measured before the treatment and after the treatment with APP (Table 1). After the APP treatment, the pH value of DASH gel was approximately 2 times lower than before the treatment, while in the case of BOOST gel, the pH value dropped to about 75% of its initial value. The pH was also measured each minute for another 10 minutes after the treatment with APP jet. These values did not change dramatically and no trend was observed.

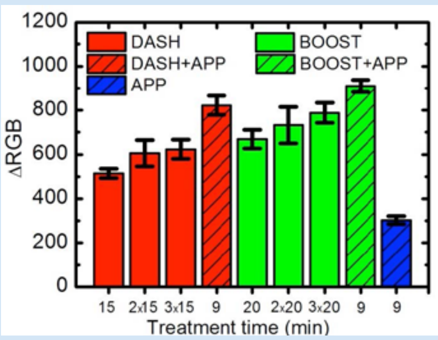
The non-contact temperature measurements of the pastille or gel surface show that the treatment with APP rises the surface temperature of the samples. In the case of the pastille to about 30°C, the surface temperature of DASH and BOOST gel rises to a slightly higher temperature of about 32°C and 30.5°C, respectively (Figure 4).

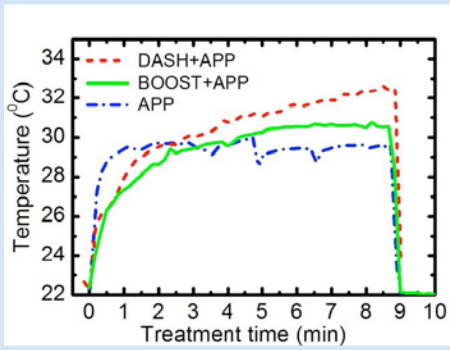
Tablica 1. pH vrijednosti gelova DASH i BOOST prije 9-minutnog tretmana APM-om i nakon njega
Table 1 pH measurements of the DASH and BOOST gel before and after the 9 minute treatment with APP.

	pH mean value	pH standard deviation
DASH prije tretmana • DASH before the treatment	4.64	0.08
DASH nakon tretmana • DASH after the treatment	2.05	0.15
BOOST prije tretmana • BOOST before the treatment	7.25	0.01
BOOST nakon tretmana • BOOST after the treatment	5.34	0.08









Slika 1. Eksperimentalni postav
Figure 1 Experimental setup.
Slika 2. Optička emisijska spektroskopija za atmosferski plazmeni mlaz i za atmosferski plazmeni mlaz u kombinaciji s BOOST-om (40-postotni vodikov peroksid)
Figure 2 Optical emission spectra for free atmospheric plasma jet and for plasma jet during the treatment (at 1 min) of pastille with BOOST gel.
Slika 3. Aritmetička srednja vrijednost promjene boje (DRGB) za pastile tretirane samo gelom za izbjeljivanje (DASH, BOOST), APM-om u kombinaciji s gelovima za izbjeljivanje i samo APM-om
Figure 3 Arithmetic mean value of color change (DRGB) for pastilles treated only with bleaching gels (DASH, BOOST), with APP in conjunction with bleaching gels and only with APP.
Slika 4. Temperaturni porast na površini uzoraka tijekom tretmana samo APM-om i u kombinaciji s gelovima DASH i BOOST
Figure 4 Time evolution of the sample surface temperature during the treatment with only APP and with APP in conjunction with DASH or BOOST gel.

Rasprava

Optička emisijska spektroskopija pokazuje porast intenziteta za APM u kombinaciji s gelovima za izbjeljivanje u odnosu na samo APM (slika 2.). Gelovi korišteni u ovoj studiji sadržavali su vodikov peroksid u različitim koncentracijama. Najveća promjena uočena je za OH liniju spektra (308 nm). Iako je radni plin helij, zbog difuzije okolnoga zraka u plazmu, raste količina dušika u samom spektru (25). Kada okolni zrak promijeni sastav zbog kemijski aktivne uloge plazme, neke molekule ispare iz gela pa se spektar mijenja. To je razlog za promjenu OH linije spektra vidljivog 10 mm iznad tretiranoga uzorka gela i kada je optičko vlakno postavljeno okomito na gel. Osim toga, kada bi OES bio mjeren uz plazmeni mlaz ili bi kut između mlaza i optičkoga vlakna bio drukčiji, promijenili bi se omjeri između spektralnih linija. Zato bi, ako bi se leća OES-a usmjerila niže prema uzorku, porastao intenzitet OH linije u odnosu na liniju dušika. Kako bismo procijenili učinak izbjeljivanja u različitim postupcima, RGB promjena boje na pastilama izmjerena je prije i poslije tretmana. Pri izbjeljivanju samo gelovima vodikova peroksida (ΔRGB) rezultati su prikazani nakon prvoga, dru-

Discussion

OES spectra of the APP jet during the treatment of bleaching gels on a pastille shows the intensive increase of emission lines compared to APP jet without the sample (Figure 2). However, the ratio between intensities of APP jet without the sample and with the sample is not the same for all spectral features. While gels used in this study consist of hydrogen peroxide, one of the biggest relative increases can be observed for OH emission lines (308 nm). Even though the working gas is helium, because of the diffusion of the ambient air into the plasma, there is a lot of nitrogen in the OES spectra (25). When the surrounding air changes its composition, because of the chemically active role of plasma on the gel, some molecules evaporate from the sample and the OES spectra of the light emitted by the plasma also changes. This is why the change in OH emission lines is also observed about 10 mm above the treated gels, while as previously mentioned, the OES spectra were measured at the beginning of the jet and the optical fiber was positioned perpendicular to the jet. Moreover, if OES would be measured lower along the jet or if the angle between jet and the gog i trećeg ciklusa mjerenja. Kao što je već spomenuto, jedan ciklus traje 15 minuta za DASH i 20 minuta za BOOST. Izbjeljivanje na pastilama nakon trećeg ciklusa, za DASH nije bilo znatno bolje u odnosu na dva ciklusa od po 15 minuta. No dva ciklusa od po 15 minuta za 20 posto su bila bolja u odnosu na samo jedan ciklus. Gel DASH u kombinaciji s APM-om u trajanju od devet minuta postigao je 32 posto bolje rezultate od tretmana samo DASH-om (3 x 15 min). Pri uporabi gela BOOST, izbjeljivanje je bilo za 8 posto bolje u odnosu na svaki sljedeći ciklus. No BOOST u kombinaciji s APM-om u trajanju od devet minuta, pokazao je 20 posto bolje rezultate od tretmana samo BOOST-om (3 x 20 min). Treba istaknuti da kod uporabe BOOST-a, nakon produljenog djelovanja s APM-om, nastaje kompletna deformacija gela, što nije imalo utjecaja na efekt izbjeljivanja. Izbjeljivanje se također može postići samo uporabom APM-a, ali su rezultati otprilike tri puta lošiji u odnosu na kombinaciju APM-a i gela. Slični rezultati objavljeni su u studiji Choe i suradnika (26) – oni su se koristili APM-om s različitim reaktivnim plinovima. U studiji Nama i suradnika (27) korištena su tri različita izvora (APM, plazmatski luk i diodni laser) za aktivaciju karbamid-peroksidnog gela i APM je pokazao najbolje rezultate.

Prema podatcima proizvođača, gelovi za izbjeljivanje imaju razmjerno neutralni pH kako bi se smanjila eventualna oštećenja tvrdih zubnih tkiva zbog kiseloga, odnosno lužnatoga medija. No za većinu gelova korištenih pri izbjeljivanju zuba pronađeno je da su kiseli, a osobito oni primjenjivani u ordinacijama, sadržavaju veću koncentraciju vodikova peroksida, odnosno karbamid peroksida (28). Tvrda zubna tkiva u jako kiselom mediju ili jako bazičnom mediju mogu se oštetiti zbog produženog djelovanja ili čestoga korištenja. Zubne paste za izbjeljivanje koje se rabe svaki dan, trebale bi imati neutralni pH, no istraživanje je pokazalo da neke imaju vrlo nizak pH – 3,67 ± 0,06 (29). Kiseli ili neutralni gelovi za izbjeljivanje imaju isti učinak izbjeljivanja *in situ* i *in vitro* (30). U ovoj studiji bila je izmjerena pH vrijednost za DASH od 4,64 ± 0,08 a za BOOST je pH bio blizu neutralnoga – 7,25 ± 0,01. Izmjerena pH vrijednost za DASH bila je ispod dopuštene granične vrijednosti za caklinu u iznosu od pH = 4,5-5,5 i stoga može demineralizirati tvrda zubna tkiva. Prema literaturi, niska pH vrijednost pripisuje se niskim koncentracijama kalcija i fosfatnih iona te visokim koncentracijama natrijevih i kloridnih iona u gelovima za izbjeljivanje (31). Nakon tretmana APM-om u kombinaciji s gelovima za izbjeljivanje, pale su pH vrijednosti za obje vrste gela (tablica 1.). To se povezuje s kemijskom aktivnošću plazme. Aktivni radikali koji se nalaze u plazmi – RKR i RDR mogu reagirati s gelovima za izbjeljivanje i tako smanjiti pH vrijednosti gela, no to ne mora nužno utjecati na promjenu u kemijskoj strukturi cakline. Daljnja istraživanja potrebna su kako bi se utvrdila kemijska interakcija koja može sniziti pH vrijednosti gelova za izbjeljivanje nakon tretmana APM-om. Dulje izlaganje zuba i oralnoga tkiva niskim koncentracijama pH može rezultirati neželjenim posljedicama, kao što su demineralizacija (32) i resorpcija korijena (33). Stoga, smanjenjem vremena interakcije s pomo-

fiber was different, the ratios between spectral features would change (25). Therefore, if the collimating lens of the OES spectrometer was pointed down toward the sample, the intensity of the OH emission lines compared to e.g. nitrogen emission lines would probably increase.

In order to evaluate the bleaching effect of different procedures, RGB color change of the pastilles before and after the treatment was determined. In case of treatment with only bleaching gels, DRGB results are presented after the first, second and third cycle. As mentioned before, one cycle is 15 and 20 minute long treatment with DASH and BOOST, respectively. The whitening of the pastilles after the third DASH treatment is not considerably better than with only two consecutive 15 minute treatments. However, two 15 minutes DASH treatments are almost 20% better than only one. When using APP in combination with DASH gel for 9 minutes there were 32% better results in comparison with only DASH gel (3 x 15 minutes). In the case of BOOST gel, whitening is approximately 8% better with each additional cycle. When BOOST is treated for 9 minutes with APP jet the DRGB is about 15% higher than with three 20 minute BOOST treatment cycles. Here it should be mentioned that after prolonged plasma treatment, the bleaching gel deformed and changed viscosity. However, this did not reduce the bleaching effect. The bleaching effect can also be achieved with APP treatment only, but it is almost 3 times lower than plasma in conjunction with bleaching gels. Similar whitening effects were also reported in the study by Choi et al. (26) where they used APP with different gases. In the study by Nam et al. (27), three different sources (APP, plasma arc lamp and diode laser) for activation of carbamide peroxide were used, and APP obtained the best bleaching results.

The manufacturer suggested that the products have relatively neutral pH to minimize the potential damage which could be caused by highly acidic or highly basic solutions. However, most of the bleaching gels were found to be acidic, especially in-office bleaching products (28). The effects of acidic or basic solutions depend on the exposure time and how often the product is used. Whitening toothpastes, which are used every day, should also have a neutral pH; it is reported that some toothpastes had a highly acidic pH as low as 3.67±0.06 (29). Acidic and neutral in-office bleaching agents have the same whitening efficiency *in situ* and *in vitro* (30). In this study DASH gel had pH values of 4.64 ± 0.08 while BOOST gel was close to neutral with pH 7.25 ± 0.01. The acid pH measured for 30% HP (DASH) was below the critical level for enamel which is in between 4.5-5.5 and can cause hard tissue demineralization. According to the literature, this can be attributed to the low concentrations of calcium and phosphate ions and high concentrations of sodium and chloride ions in bleaching gels which can cause undersaturation with respect to hydroxyapatite (31). After the APP jet treatment in conjunction with bleaching gels, the pH values dropped for both types of gels (Table 1). This drop is related to chemical activity of APP jet. Active radicals such as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are present in plasma and can react with bleachću APM-a prezentiranog u ovoj studiji, može se smanjiti vrijeme izlaganja zuba niskom pH i ublažiti neželjene posljedice.

Prema dostupnoj literaturi uporaba izvora svjetlosti u kombinaciji s gelovima za izbjeljivanje povećava temperaturu u pulpnoj komori (34 – 36). U ovoj studiji temperatura generirana APM-om, mjerena na površini tretiranih uzoraka, nije prešla granicu od 33 ºC. Maksimalni temperaturni porast zabilježen je nakon upotrebe APM-a u kombinaciji s BOOST-om od 8 ºC, odnosno 10 ºC za DASH. U slučaju BOOST-a, nakon nekoliko minuta od početka tretmana APM-om, temperatura je od sobne dosegnula granicu malo iznad 30 ºC i ostala je konstantna tijekom tretmana, a kod DASH-a je nakon tri minute dosegnuta temperatura od 30 ºC i rasla je tijekom cijelog tretmana do konačnih 32 ºC. Razni izvori svjetlosti koji služe za ubrzavanje procesa izbjeljivanja povećavaju temperaturu na površini zuba i u pulpnoj komori (18). Zach i Cohen otkrili su na modelima majmuna da povećanje temperature u pulpnoj komori od 5,5 ºC rezultira ireverzibilnim oštećenjima u pulpi (37). No kako je dentalno tkivo slabo termalno vodljivo, površinsko zagrijavanje zuba ne mora značajno zagrijavati pulpu (38). U studiji Eldeniza i suradnika (39) diodni laser je, među svjetlima za izbjeljivanje, najviše povećao temperaturu (11,7 ºC), a infracrvena svjetleća dioda (LED) pokazala je najmanje povećanje temperature. U studiji Luka i njegovih kolega (16) opisana je aplikacija infracrvenog lasera, CO_2lasera, halogene svjetiljke i argonskog lasera u kombinaciji s 35-postotnim vodikovim peroksidom i 10-postotnim karbamid-peroksidnim gelom kod kojega se povećava temperatura u pulpnoj komori i na površini zuba. Klarić i suradnici (34) zaključili su da je izvor svjetlosti za izbjeljivanje ZOOM 2 potaknuo najveći porast temperature u pulpnoj komori i na površini zuba od 21,1 ºC, odnosno 22,8 ºC nakon femtosekundnog lasera za koji su izmjerene vrijednosti bile 15,7 ºC i 16,8 ºC. I učinak netaknute cirkulacije pulpe ili simulirani protok krvi može disperzirati određenu količinu topline prije oštećenja stanica pulpe (40,41). Naš *in vitro* model nije mogao ponoviti sve navedeno te je moguće da je povećanje temperature od 10º C na površini tretiranog uzorka zapravo mnogo niže i ispod praga koji bi oštetio pulpu.

Nedostatak ove studije jest to što nije mogla biti provedena *in vivo*. Pri korištenju izvora svjetlosti za ubrzavanje procesa izbjeljivanja temperatura na površini i unutar pulpne komore ovisi o vrsti svjetla i trajanju tretmana. Obično dulja uporaba svjetla povećava temperaturu jer se više svjetlosne energije pretvara u toplinsku (42). Klinički ishod je širenje tekućine unutar dentinskih tubula i pulpe, što potencijalno završava hiperemijom i postoperativnom preosjetljivošću (43). Vrijeme aplikacije za APM iznosi devet minuta, što je oko šest puta kraće u odnosu na vrijeme koje je predložio proizvođač gela za izbjeljivanje. Pri korištenju APM-a temperatura uzoraka ispod je tjelesne temperature, što znači da pacijent neće osjetiti bol u slučaju topline niti će susjedno tkivo biti oštećeno zbog temperaturnog šoka (27). Zato smatramo da je APM primjenjiv u terapijama koje zahtijevaju kemijsku aktivaciju bez povećanja temperature, kao što je izbjeljivanje zuba.

ing gel thus lowering pH value of the gel which can lead to potentially less harmful effect on the tooth surface without alterations of the enamel structure. Further research is needed to investigate the main chemical interaction which can lower pH value of bleaching gels. Subjecting the teeth and oral tissues to a low or high pH for an extended period of time may cause adverse side effects such as enamel demineralization (32) and root resorption (33). Therefore the reduction of bleaching time with APP jet treatment, as presented in this study, and consequently shortening the exposure of teeth to low pH can decrease the side effects described above.

According to the available literature, the use of the light activation in conjunction with bleaching gels generates higher intrapulpal temperatures (34-36). In the present study, during the bleaching procedure, the heat generated by APP jet was measured on the surface of the treated samples and in all cases it did not pass 33˚C. The maximum temperature increase was 8˚C and 10˚C above baseline for BOOST and DASH gel, respectively. In the case of BOOST gel, in a few minutes of treatment the temperature increased from room temperature to slightly above 30°C and remained constant throughout the treatment, while in the case of DASH gel the surface temperature increased to 30°C in about 3 minutes and continued to rise slowly to about 32°C in 9 minute treatment. The effects of various lights used to accelerate the bleaching process on surface and pulp chamber temperature increases have been investigated (18). Zach and Cohen found in a monkey model that a 5.5°C temperature rise was likely to cause irreversible pulpal damage (37). Since dental tissue has a low thermal conductivity, superficial heating of a tooth does not significantly heat the pulp (38). It was found that the increase in the pulp chamber temperature with most bleaching lights was above this critical threshold. In the study by Eldeniz et al. (39), the diode laser induced significantly higher temperature increases than any other curing unit (11.7°C) while the light emitting diode unit produced the lowest temperature change (6.0°C). In a study by Luk et al. (16) the application of infrared laser, CO2 laser, halogen lamp and argon laser in combination with 35% hydrogen peroxide gel and 10% carbamide peroxide gel caused significant temperature increase inside the pulp chamber and on the tooth surface. Klaric et al. (34) found that the ZOOM2 light source led to the largest increase in mean pulpal and tooth surface temperatures of 21.1 and 22.8 °C, followed by focused femtosecond laser which increased the pulpal and surface temperatures by up to 15.7 and 16.8 °C. Also, the effect of an intact pulpal circulation or simulated blood flow is able to dissipate some of the applied heat before pulpal cells are damaged (40, 41). Our *in vitro* model was not able to replicate this, so it is possible that the 10°C surface temperature rise is in reality lower and therefore below the threshold for possible pulp damage.

Another shortage in interpretation of the results in this study is that, apart from the surface experiment, this type of study could not yet be conducted *in vivo*. Where light is used to accelerate bleaching, the intrapulpal and surface temperatures also depend on the light application period and type of light used. Usually, a longer light irradiation pro-

Ova studija ima nekoliko ograničenja. Istraživanje je provedeno na pastilama hidroksilapatita koje se razlikuju od pravih zuba prema kemijskom sastavu, površini, boji i fizičkim obilježjima. Pastile su korištene radi veće objektivnosti kao eksperimentalna metoda u ispitivanju potencijalnog učinka APM-a na izbjeljivanje i služile su kao eksperimentalni i jedinstveni model jednakog sastava, veličine i boje. To je razlog zašto su prikladan izbor za usporedbu učinka pri različitim tretmanima izbjeljivanja. S druge strane, ljudski ili goveđi zubi različiti su po kemijskim, fizičkim i optičkim svojstvima te bi se teško ustanovio pravi učinak APM-a. Osim toga, pastile imaju ravnu površinu, što je važno tijekom mjerenja promjene boje RGB kolorimetrom. Temperaturne vrijednosti izmjerene u ovoj studiji ne mogu se izravno primijeniti na temperaturne promjene *in vivo*. Daljnje studije s različitim gelovima za izbjeljivanje u kombinaciji s APM-om ili drugim tipovima plazme na prirodnim zubima nužne su radi preciznog određivanja porasta temperature na površini uzorka.

Zaključak

Istraživanje je dokazalo da se APM može koristiti kao kemijski aktivator jer ubrzava degradaciju vodikova peroksida i tako ubrzava proces izbjeljivanja u kraćem vremenu od onoga navedenog u preporukama proizvođača. Mjerenje površinske temperature tijekom tretmana APM-om pokazuje da je ova vrsta tretmana učinkovitija od konvencionalnih izvora svjetlosti. Tretman APM-om u kombinaciji s gelovima za izbjeljivanje (30-postotni vodikov peroksid i 40-postotni vodikov peroksid) mogao bi jednog dana biti postupak izbora pri izbjeljivanju zuba.

Zahvala

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Sukob interesa

Ne postoji.

duces a higher temperature rise because more light energy is converted into heat (42). The application time for APP jet was 9 minutes which is considerably shorter than the time proposed by the manufacturer of bleaching gels used in the study. Clinical outcome of this heat generation is expansion of the liquids inside the dentinal tubules and the pulp which in combination with dehydration of the bleached hard dental tissues result in hyperemia and post-operative sensitivity (43). As the temperature of the sample surface with APP jet treatment is under the body temperature, which means that a patient could not feel a sense of pain from cold or heat and the tooth pulp or tooth surrounding tissue will not be thermally damaged (27). For this reason, APP jet is applicable to therapies that require chemical activation without temperature increase such as tooth bleaching.

This study has a few limitations. The research was conducted on pastilles of hydroxylapatite, which are different from human teeth in their chemical composition, surface color or physical characteristics. The pastilles were used as an experimental model to investigate the potential bleaching effect of APP jet. They served as experimental and uniform models and were equal in their composition, size and color. That is why the pastilles serve as a good and suitable choice for comparison of the effects of different bleaching treatments. On the other hand, human or bovine teeth are all different in their chemical, physical and optical characteristics and the true effect of the APP jet cannot be reliably determined. Pastilles also have a flat surface, which is important for color measurement when using the RGB colorimeter. Also, the temperature values measured in this study cannot be directly applied to temperature changes *in vivo*. Further studies with different concentrations of bleaching agent in conduction with APP jet or other types of plasma using human teeth are necessary to characterize the precise surface temperature rise.

Conclusion

In this study it was proved that APP jet can be used as a chemical activator which can accelerate the degradation of hydrogen peroxide. This provides more effective results of tooth bleaching in a shorter period of time than the procedures proposed by the manufacturers. The measurements of surface temperature during the APP treatment suggest that this kind of treatment has a greater capability than conventional light sources. APP jet treatment combined with bleaching gels (30 and 40% HP) could therefore become a procedure that could be safely used for in office tooth bleaching without a significant temperature increase.

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Conflict of interest

None declared.

Abstract
Objective: To study the effect of atmospheric pressure plasma (APP) jet as a potential accelerator of the degradation of hydrogen peroxide in bleaching gels which could lead to better and faster bleaching. **Material and Methods:** 25 pastilles of hydroxylapatite were colored in green tea for 8 hours and were randomly divided into five groups (n = 5). The bleaching process was performed with 30% and 40% hydrogen peroxide (HP) gel alone and in conjunction with helium APP jet. During the bleaching treatment, optical emission spectroscopy and non-contact surface temperature measurement using pyrometer were performed. Color of the pastilles was determined by a red–green–blue (RGB) colorimeter. PH values of bleaching gels were measured before and after the plasma treatment on additional 10 pastilles using a pH meter with contact pH electrode. **Results:** The color measurements of pastilles before and after the treatment showed that treatment with APP jet improved the bleaching effect by 32% and 15% in the case of 30 % and 40% HP gel. Better results were obtained approximately six times faster than with a procedure suggested by the bleaching gel manufacturer. Optical emission spectroscopy proved that plasma has a chemically active role on the gel. After the APP treatment, pH values of bleaching gels dropped to about 50–75% of their initial value while the surface temperature increased by 8–10˚C above baseline. **Conclusion:** The use of plasma jet provides more effective bleaching results in a shorter period of time without a significant temperature increase which may cause damage of the surrounding tissue.

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Key words
Tooth Bleaching; Gels; Hydrogen Peroxide; Atmospheric Pressure Plasma Jet; Spectrum Analysis; Durapatite

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