

Raimond NB van Duinen¹, Saroash Shahid², Robert Hill², Domagoj Glavina¹

In-vitro istraživanje temperaturnih promjena u pulpnoj komori nakon zagrijavanja pri stvrdnjavanju staklenoionomernog cementa

In-vitro Study on Temperature Changes in the Pulp Chamber Due to Thermo-Curing of Glass Ionomer Cements

¹ Zavod za dječju i preventivnu stomatologiju, Stomatološki fakultet Sveučilišta u Zagrebu, Hrvatska
Department of Paediatric and Preventive Dentistry, School of Dental Medicine University of Zagreb, Croatia

² Stomatološki i Medicinski fakultet Barts, London, UK
Barts and the London School of Medicine and Dentistry, London, UK

Sažetak

Staklenoionomerni cementi (SIC) primjenjuju se zbog mnogih svojstava poput izlučivanja fluora, kemijske adhezije na zubna tkiva, neznatne kontrakcije pri stvrdnjavanju, koeficijenta termalne ekspanzije vrlo sličnog zubu, niskog puzanja materijala i dobre stabilnosti boje. No zbog sporog stvrdnjavanja osjetljivi su na rani kontakt s vlagom. Primjena eksterne energije, poput ultrazvuka i zagrijavanja (thermo-curing), opisana je kao postupak za ubrzanje kemijske reakcije stvrdnjavanja i poboljšanja fizikalnih svojstava materijala. **Svrha:** Željelo se analizirati temperaturne promjene u pulpnoj komori nakon primjene zagrijavanja za ubrzanje stvrdnjavanja staklenoionomernog cementa. **Materijal i metode:** U istraživanju je korišten kapsulirani staklenoionomerni cement *Equia Forte* (GC). Temperaturne promjene u pulpnoj komori mjerene su termoelektričnim osjetnikom u kavitetima dubokima 2,6 i 4,7 mm s ispunima i bez ispuna. **Rezultati:** Uočena je porast temperature (ΔT) u pulpnoj komori od 3,7 °C. Promjena temperature (ΔT) u kavitetima dubine 2,6 i 4,7 mm bez ispuna iznosila je 4,2 °C i 2,6 °C. Nakon postavljanja ispuna porast temperature u pulpnoj komori bio je niži – od 1,9 °C do 2,4 °C. **Zaključak:** Može se zaključiti da je primjena postupka *thermo-cure* tijekom stvrdnjavanja SIC-a sigurna za pulpu i može se preporučiti u kliničkoj praksi.

Zaprimljen: 30. listopada 2016.
Prihvaćen: 6. prosinca 2016.

Adresa za dopisivanje
prof.dr.sc. Domagoj Glavina
Sveučilište u Zagrebu
Stomatološki fakultet
Zavod za dječju i preventivnu
stomatologiju
Gundulićeva 5, 10 000 Zagreb
tel. 4802 110
glavina@sfg.hr

Ključne riječi
priprema korijenskog kanala;
korijenski kanal; stakleno-ionomerni
cementi; temperatura

Uvod

Staklenoionomerni cementi (SIC) već se neko vrijeme mogu nabaviti na tržištu. Njihova klinička primjena u dentalnoj medicini preporučuje se zbog mnogih svojstava, poput izlučivanja fluora, kemijske adhezije na zubna tkiva, neznatne kontrakcije pri stvrdnjavanju, koeficijenta termalne ekspanzije vrlo sličnog zubu, niskog puzanja materijala i dobre stabilnosti boje. No zbog sporog stvrdnjavanja osjetljivi su na rani kontakt s vlagom (1, 2). U vezi s tim, smolasti materijali bili su bolji i korištenje eksterne radijacije (UV ili vidljivo svjetlo) za pokretanje njihove polimerizacije omogućavalo je kliničaru bolju kontrolu nad procesom stvrdnjavanja (*command set*). No dodatak smole štetno je djelovao na otpornost na abraziju, dimenzijsku stabilnost, stabilnost boje i, naravno, na biokompatibilnost. Istraživanja su pokazala da korištenje eksterne energije, poput ultrazvuka i radijacije, toplinom ubrza kemijske reakcije stvrdnjavanja staklenoionomernih cementa (3, 4). Također je dokazano da oboje poboljšava fizikalna svojstva (5, 6).

Introduction

Glass ionomer cements (GIC) have been used for several years. Their application in clinical dentistry has been driven by several properties such as fluoride release, chemical adhesion to tooth, negligible setting shrinkage, and coefficient of thermal expansion close to tooth, low creep, and good colour stability. However, the cement is vulnerable to early exposure to moisture due to slow setting characteristics (1,2). Resin-based materials were better in this respect and the use of external radiation (ultra violet or visible light) to initiate their polymerization improved this and gave the clinician greater control over the setting process ("command set"). However, the addition of resins produced adverse effects on abrasion resistance, dimensional stability, colour stability and certainly biocompatibility. The uses of external energy such as ultrasound and radiant heat have been reported to provide acceleration of the setting chemistry of GICs. (3,4). Both are reported to enhance physical properties (5, 6).

Commercial GICs have recently become available which advocate the use of "thermo setting" via the use of radiant

Komercijalni staklenoionomerni cementi s uputama proizvođača za primjenu tehnike *termičkog stvrdnjavanja* (*thermo-curing*) korištenjem radijacijske topline iz prenosive LED lampe, postali su dostupni na tržištu. No postoji zabrinutost da takva izloženost toplini može potaknuti patološke promjene u pulpi zuba. Zato je svrha ovoga *in-vitro* istraživanja bila analizirati promjene temperature unutar pulpne komore zuba tijekom zagrijavanja kako bi se ubrzalo stvrdnjavanje SIC-a.

Materijal i postupci

U istraživanju je korišten kapsulirani komercijalni staklenoionomerni cement *Equia Forte* (GC Corporation, Japan). Za *thermo-curing* korišteno je LED svjetlo koje preporučuje proizvođač (D-Light Duo, GC Corporation, Japan), a isporučuje se zajedno s materijalom. Izlazna toplina lampe izmjerena je dodiranjem njezina vrha s termoelektričnim osjetnikom i registracijom maksimalne topline nakon 90 sekunda.

Nakon toga učinjen je na vestibularnoj površini nekarioznog zuba mali ulaz za termoelektrični osjetnik (svi zubi za istraživanje nabavljeni su iz banke tkiva Queen Mary University London- QMUL).

Zub je postavljen iznad vodene kupke u plastični pokrov (bez uranjanja) kako bi se osigurao vlažan okoliš na temperaturi od 37 °C (slike 1. i 2.). Radijacijska toplina dobivena uporabom LED lampe primijenjena je tijekom 90 sekunda na okluzalnu plohu zuba te su promjene temperature u pulpnoj komori zuba registrirane termoelektričnim osjetnikom. Nakon toga je kavitet dubok 2,6 mm (mjereno od vrha kvržice) prepariran stomatološkom turbinom i dijamantnim brusilom. Zub je vraćen u plastični pokrov te su registrirane promjene temperature tijekom aplikacije radijacijske topline 90 sekunda.

Za mjerenje promjena temperature tijekom termičkog stvrdnjavanja (*thermo-curing*) staklenoionomernog ispuna u zub je, u skladu s uputama proizvođača, postavljen ispun od materijala *Equia Forte* boje A3. Staklenoionomerni materijal potisnut je u kavitet tehnikom pritiska prstom (*finger press*).

heat from a portable LED lamp. However, there have been concerns that exposure to heat from these sources could lead to damage to tooth pulp. Therefore, the aim of this *in vitro* study was to analyze temperature changes in the pulp chamber when using radiant heat to accelerate the set of GICs

Materials and Methods

Encapsulated commercial GIC *Equia Forte* (GC Corporation, Japan) was used in this study. Thermo-curing was performed using the LED light (D-Light Duo, GC Corporation, Japan) recommended by the manufacturer.

The heat output of the lamp was measured by touching the tip of the lamp with the thermocouple and recording the maximum heat after 90 seconds. Thereafter, a small entrance was made in the facial surface of non-carious molars procured from Queen Mary University London (QMUL) tissue bank.

The tooth was placed above a water bath (without immersing it into water) in a plastic tent to ensure humid environment at 37°C (Figures 1,2). Radiant heat was applied for 90 seconds to the occlusal surface using the LED lamp and temperature changes in the pulp chamber were recorded using the thermocouple. Following this, a 2.6 mm deep cavity (measured from the cusp tips) was cut into the same molar using a high speed handpiece and a diamond bur. The tooth was placed back in the plastic tent and temperature changes in the pulp chamber were recorded while using radiant heat for 90 seconds.

To measure the temperature changes while thermo-curing a GIC restoration, the tooth was then restored with *Equia Forte* A3 shade following the manufacturer's instructions. The GIC was packed into the cavity using the "finger press" technique. The GIC was thermo-cured for 90 seconds and the temperature changes in the pulp chamber were recorded. First, the temperature changes in the pulp chamber were re-



Slika 1 i 2. Postavljanje eksperimenta
Figures 1 and 2 Experiment set-up

Stakleni ionomer grijan je (*thermo-cure*) 90 sekunda i pritom su zabilježene promjene temperature u pulpnoj komori. Najprije su mjerene promjene temperature na razini od 2,6 mm, a nakon toga kavitet je produbljen do 4,7 mm (mjereno od vrha kvržice). Temperaturne promjene u pulpnoj komori s ispunom i bez ispuna izmjerene su kako je opisano (slike 1. i 2.). Postupak je ponovljen tri puta da bi se osigurali reproducirani rezultati.

Rezultati

Dobiveni rezultati pokazuju da je maksimalna temperatura, emitirana iz LED lampe korištene u istraživanju, iznosila 60 °C, a postignuta je za 60 sekunda. Nakon toga nije bilo daljnjeg rasta. Primjenom svjetla na koronarnu površinu zuba bez prepariranog kaviteta, povećanje temperature (ΔT) u pulpnoj komori iznosilo je 3,7 °C. Promjena temperature (ΔT) u kavitetima dubine 2,6 mm i 4,7 mm bez ispuna, bila je 4,2 °C i 2,6 °C. Ipak, nakon postavljanja ispuna promjena temperature (ΔT) bila je manja – od 1,9 °C do 2,4 °C (slike 3. – 5.).

Rasprava

Acido-bazna reakcija neutralizacije staklenoionomernih cemenata može se ubrzati korištenjem eksterne energije, kao što su ultrazvuk (2, 3, 4) i toplina (5, 6, 7). To je posebno korisno za prevladavanje osjetljivosti na vlagu koja štetno utje-

corded at the level of 2,6 mm. Subsequently, the cavity in the tooth was extended to 4.7 mm (measured from the cusp tip). Temperature changes in the pulp chamber with and without GIC filling were measured as described above (Figures 1 and 2).

This process was performed 3 times to ensure the reliability and reproducibility of research.

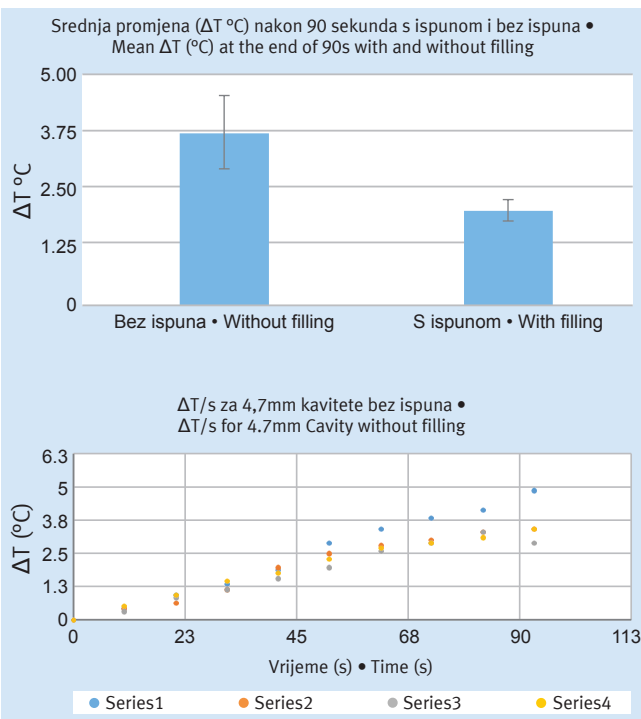
Results

The results showed that the maximum temperature from the LED thermocuring light used in this study was 60°C. The light reached its maximum temperature after 60 seconds. After that, there was no further rise in temperature.

On applying the light to the tooth from the coronal surface without any cavity preparation, the temperature rise (ΔT) in the pulp chamber was 3,7°C. ΔT for the 2.6mm and 4.7mm deep cavity without placing any restoration was 4,2°C and 2,6°C respectively. However, after the restoration has been placed, the ΔT range in pulp chamber was lower ranging from 1.9°C to 2.4°C (Figures 3-5).

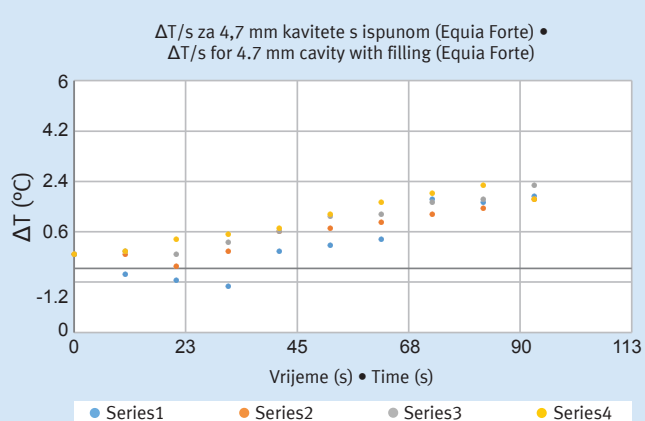
Discussion

The acid base neutralization reaction of Glass Ionomer Cements can be accelerated through the use of external energy such as ultrasound (2,3,4) and heat (5,6,7). This is particularly useful in overcoming the moisture sensitivity which



Slika 4. Promjena temperature u kavitetima bez ispuna dubine 4,7 mm
Figure 4 Temperature changes in the 4.7mm deep cavity without filling

Slika 3. Promjena temperature u kavitetima s ispunom i bez ispuna nakon 90 sekunda na dubini od 4,7 mm
Figure 3 Temperature changes in the cavities with and without filling after 90s on the depth of 4.7mm



Slika 5. Promjena temperature u kavitetima s ispunom dubine 4,7 mm
Figure 5 Temperature changes in the 4.7mm deep cavity with filling

če na svojstva SIC-a (8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). Iako ultrazvuk ubrzava stvrdnjavanje, njegova je klinička primjena komplicirana zbog čega je za tu tehniku manji komercijalni interes. S druge strane, toplina se može postići LED lampom, kao što to preporučuje *GC Corp.* za svoj sustav *Equia Forte*.

Kao što je to i kod svjetlosno aktiviranih sustava za izbjeljivanje (21), *toplinsko stvrdnjavanje* staklenoionomernih cementa može rezultirati intrapulpnim povećanjem temperature. No ovo istraživanje pokazuje da zagrijavanje do 90 sekunda uređajem GC D-light Duo povećava temperaturu u pulpnoj komori od 2 do 3 °C, iako temperatura na površini može biti i 50,2 °C. To se događa zato što su staklenoionomerni cementi toplinski izolatori, kako su istaknuli Gavic i suradnici, pa je i niska toplinska provodljivost u staklenoionomernim ispunima debljine 4 mm i 2 mm (22). No njihovo istraživanje nije bilo obavljeno na zubu. Rezultati su također pokazali manji porast temperature u ispunima sa SIC-om postavljenima u kavitet u usporedbi s kavitetima bez ispuna. Tijekom reakcije stvara se voda, što može hladiti zub. Pad temperature također može biti objašnjen nižom početnom temperaturom materijala za ispun – nakon što je postavljen iznosila je 23 °C.

Različita su mišljenja o tome koliki porast temperature šteti pulpi. Zach i Cohen (23) istraživali su utjecaj povišenja temperature na pulpu rezus majmuna. Njihovo istraživanje pokazalo je da povećanje temperature od 5,6 °C (iznad tjelesne temperature, tj. 42,6 °C) nepovratno oštećuje pulpu 15 posto tih primata, a porast od 16,6 °C oštećuje pulpu njih 100 posto. Suprotno ovomu, u nekim istraživanjima (24, 25, 26) istaknuto je da, kad je riječ o ljudima, povećanje intrapulpane temperature od 8,9 °C do 14,7 °C ne potiče patološke promjene. Ovi rezultati mogu sugerirati da je humana pulpa otpornija na toplinsku ozljedu, štoviše, izmjereni pragovi u navedenim istraživanjima viši su negoli povećanje intrapulpane temperature u ovom istraživanju. Osim toga, izmjerena povećanja temperature usporediva su i u nekim slučajevima niža od povećanja intrapulpane temperature nakon primjene svjetlosno aktiviranih lampi za izbjeljivanje.

Zaključak

Na temelju dobivenih rezultata može se zaključiti da korištenje eksterne topline tijekom stvrdnjavanja SIC-a nema kao posljedicu štetno pregrijavanje pulpnog tkiva i patološki učinak. Zbog toga se primjena eksterne topline (*thermo-curing*) kao tehnike stvrdnjavanja na zahtjev (*Command set*) i tehnike za poboljšanje mehaničkih svojstava i adhezije SIC-a može preporučiti u kliničkoj praksi.

Sukob interesa

Nije bilo sukoba interesa.

adversely affects the properties of GIC (8,9,10,11,12,13,14,15,16,17,18,19,20). Although ultrasound accelerated the setting, its use was clinically difficult. Consequently, there has been less commercial interest in the technique. Heat on the other hand can be applied through LED lamps such as those marketed by GC corp with its Equia Forte system.

As with the use of light activated bleaching systems (21), thermo-setting GIC can lead to intra pulpal temperature rises. However, the current study showed that the application of heat for up to 90 seconds using GC D-light Duo leads to 2-3°C rise in temperature in the pulp chamber even though the surface temperature rose as high as 50.2°C. This happened because GICs are thermal insulators and as previously noted by Gavic et al. there is low thermal conductivity in GIC restorations with the thickness of 4mm and 2mm (22). However, their study was not performed in a tooth. The results also showed a lower rise of temperature with the GIC applied in the cavity compared with those that were not applied in the cavity. Water is formed during the reaction, which possibly cools the tooth. Also, the drop in temperature can be explained by a lower initial temperature of the filling material which is around 23°C when placed in the cavity.

An extensive debate has been ongoing as to adverse effects of temperature rises on the pulp. Some research was carried out to examine whether temperature rise can be lethal to the pulp. Zach and Cohen (23) investigated the effect of temperature rise on the pulp of rhesus monkeys. Their study reported that a temperature rise of 5.6°C (above body temperature, meaning above 42.6°C) led to irreversible damage in 15% monkeys. It was found that the temperature rise of 16.6°C led to pulp damage in 100% monkeys. In contrast, some studies (24,25,26) found that the rise in temperature in human pulp between 8.9°C and 14.7°C does not produce any pathology. Although this may suggest that human pulp is more resilient to heat damage, the thresholds reported by these studies are higher than the intrapulpal increase observed in the current study. Moreover, these temperature rises are comparable and in some cases lower than the intrapulpal temperature rises reported with the use of the light activated bleaching lamps (24).

Conclusion

Based on the obtained results it could be concluded that the use of external heat during the setting of GIC material does not lead to harmful overheating of the pulp tissue, hence it does not cause any pathological conditions. The application of external heat (*Thermo-curing*) as a “Command set” method and technique for improving mechanical properties and adhesion of GIC materials can be part of regular clinical practice.

Conflict of interest

None declared

Abstract

The application of the Glass Ionomer Cements in clinical dentistry is recommended due to properties such as fluoride release, chemical adhesion to tooth, negligible setting shrinkage, and coefficient of thermal expansion close to tooth, low creep, and good color stability. However, the cement is vulnerable to early exposure to moisture due to slow setting characteristics. The uses of external energy such as ultrasound and radiant heat (Thermo-curing) have been reported to provide acceleration of the setting chemistry and enhance physical properties. **Aim:** The aim of this in vitro study was to analyze temperature changes in the pulpal chamber when using radiant heat to accelerate the setting of GICs. **Materials and methods:** The encapsulated GIC Equia Forte was used for this study. The temperature changes in the pulp were measured using thermocouple in the cavities which were 2,6 and 4,7mm deep with and without filling. **Results:** The results showed that a temperature rise (ΔT) in the pulp chamber was 3,7°C. ΔT for the 2.6mm and 4.7mm deep cavity and without placing any restoration the temperature was 4,2°C and 2,6°C respectively. After the restoration has been placed, the ΔT range in the pulp chamber was lower ranging from 1.9°C to 2.4°C. **Conclusion:** It could be concluded that Thermo-curing of the GIC during the setting is safe for the pulp and can be recommended in clinical practice.

Received: October 30, 2016

Accepted: December 6, 2016

Address for correspondence

Professor Domagoj Glavina, PhD
University of Zagreb
School of Dental Medicine
Department of Pediatric and Preventive
Dentistry
Gundulićeva 5, HR-10 000 Zagreb
Tel: +385 1 4802 110
glavina@sfz.hr

Key words

Root Canal Preparation; Dental Pulp
Cavity; Glass Ionomer Cements, Tem-
perature

References

- Fourie J, Smit CF. Cervical microleakage in Class II open-sandwich restorations: an in vitro study. *SADJ*. 2011 Aug;66(7):320-4.
- Azevedo ER, Coldebella CR, Zuanon AC. Effect of ultrasonic excitation on the microtensile bond strength of glass ionomer cements to dentin after different water storage times. *Ultrasound Med Biol*. 2011 Dec;37(12):2133-8. Epub 2011 Oct 27.
- Shahid S, Billington RW, Hill RG. The effect of ultrasound on the uptake of fluoride by glass ionomer cements. *J Mater Sci Mater Med*. 2011 Feb;22(2):247-51.
- Moshaverinia A, Ansari S, Moshaverinia M, Schrickler SR, Chee WW. Ultrasonically set novel NVC-containing glass-ionomer cements for applications in restorative dentistry. *J Mater Sci Mater Med*. 2011 Sep;22(9):2029-34.
- Baloch F, Mirza A, Baloch D. An in-vitro study to compare the microhardness of glass ionomer cement set conventionally versus set under ultrasonic waves. *Int J Health Sci (Qassim)*. 2010 Nov;4(2):149-55.
- Coldebella CR, Santos-Pinto L, Zuanon AC. Effect of ultrasonic excitation on the porosity of glass ionomer cement: a scanning electron microscope evaluation. *Microsc Res Tech*. 2011 Jan;74(1):54-7.
- Towler MR, Bushby AJ, Billington RW, Hill RG. A preliminary comparison of the mechanical properties of chemically cured and ultrasonically cured glass ionomer cements, using nano-indentation techniques. *Biomaterials*. 2001 Jun;22(11):1401-6.
- Kleverlaan CJ, van Duinen RN, Feilzer AJ. Mechanical properties of glass ionomer cements affected by curing methods. *Dent Mater*. 2004 Jan;20(1):45-50.
- Yan Z, Sidhu SK, Carrick TE, McCabe JF. Response to thermal stimuli of glass ionomer cements. *Dent Mater*. 2007 May;23(5):597-600.
- Fagundes TC, Barata TJ, Bresciani E, Cefaly DF, Carvalho CA, Navarro MF. Influence of ultrasonic setting on tensile bond strength of glass-ionomer cements to dentin. *J Adhes Dent*. 2006 Dec;8(6):401-7.
- Rafeek RN. The effects of heat treatment on selected properties of a conventional and a resin-modified glass ionomer cement. *J Mater Sci Mater Med*. 2008 May;19(5):1913-20.
- Skrinjaric K, Vranic DN, Glavina D, Skrinjaric I. Heat-treated glass ionomer cement fissure sealants: retention after 1 year follow-up. *Int J Paediatr Dent*. 2008 Sep;18(5):368-73.
- Talal A, Tanner KE, Billington R, Pearson GJ. Effect of ultrasound on the setting characteristics of glass ionomer cements studied by Fourier transform infrared spectroscopy. *J Mater Sci Mater Med*. 2009 Jan;20(1):405-11.
- Thanjal NK, Billington RW, Shahid S, Luo J, Hill RG, Pearson GJ. Kinetics of fluoride ion release from dental restorative glass ionomer cements: the influence of ultrasound, radiant heat and glass composition. *J Mater Sci Mater Med*. 2010 Feb;21(2):589-95.
- O'Brien T, Shoja-Assadi F, Lea SC, Burke FJ, Palin WM. Extrinsic energy sources affect hardness through depth during set of a glass-ionomer cement. *J Dent*. 2010 Jun;38(6):490-5.
- Guglielmi CA, Mohana A, Hesse D, Lenzi TL, Bonini GC, Raggio DP. Influence of ultrasound or halogen light on microleakage and hardness of enamel adjacent to glass ionomer cement. *Int J Paediatr Dent*. 2012 Mar;22(2):110-5. doi: 10.
- Gorseta K, Glavina D, Skrinjaric I. Influence of ultrasonic excitation and heat application on the microleakage of glass ionomer cements. *Aust Dent J*. 2012 Dec;57(4):453-7.
- Menne-Happ U, Ilie N. Effect of gloss and heat on the mechanical behaviour of a glass carbomer cement. *J Dent*. 2013 Mar;41(3):223-30.
- Gorseta K, Negovetic Vranic D, Glavina D and Skrinjaric I. Effects of polymerisation unit on the flexural strength of Glass Carbomer. *Int J Paediatr Dent* 2009; 19 (Supp.1): 75-75.
- Fabián Molina G, Cabral RJ, Mazzola I, Brain Lascano L, Frencken JE. Biaxial flexural strength of high-viscosity glass-ionomer cements heat-cured with an LED lamp during setting. *BioMed Research International* 2013; 2013: 1-6. doi: 10.1155/2013/838460.
- Sulieman M, Rees JS, Addy M. Surface and pulp chamber temperature rises during tooth bleaching using a diode laser: a study in vitro. *Br Dent J*. 2006 Jun 10;200(11):631-4.
- Gavic L, Gorseta K, Glavina D, Czarnecka B, Nicholson JW. Heat transfer properties and thermal cure of glass-ionomer dental cements. *J Mater Sci Mater Med*. 2015 Oct;26(10):249.
- Zach L, Cohen G. Pulp response to externally applied heat. *Oral Surg Oral Med Oral Pathol*. 1965 Apr;19:515-30.
- Carrasco TG, Carrasco-Guerisoli LD, Fröner IC. In vitro study of the pulp chamber temperature rise during light-activated bleaching. *J Appl Oral Sci*. 2008 Sep-Oct;16(5):355-9.
- Kupietzky A, van Duinen R. Report on the clinical technique of thermo-curing glass-ionomer sealant. *Quintessence Int*. 2015 Sep;46(8):699-705.
- Tolidis K, Dionysopoulos D, Gerasimou P, Sfeikos T. Effect of radiant heat and ultrasound on fluoride release and surface hardness of glass ionomer cements. *J Appl Biomater Funct Mater*. 2016 Nov 2;14(4):e463-e469.