

Eugenol-Based Temporary Luting Cement Possesses Antioxidative Properties

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SUMMARY

Introduction Antioxidants protect against reactive oxygen species and expose beneficial anti-inflammatory activity when in contact with biological tissues. Dental materials that are used as temporary luting on fixed dental restorations are often in contact with injured gingival tissue, hence they should contain anti-inflammatory characteristics that are essential after prosthetic procedures preceding cementation of final restoration.

Objective The aim of this study was to investigate the antioxidant effect through the oxidation inhibition (OI) of mixed dental cement for temporary luting or their liquid component.

Methods Eight study groups were prepared each by ten samples: 1) *ex tempore* preparation of zinc-oxide eugenol paste (Kariofil Z Galenika, Serbia), 2) Viko Temp paste (Galenika, Serbia), 3) Temp Bond NE paste (Kerr, Germany), 4) ScutaBond (ESPE, Germany), 5) Cp-CAP paste (Germany, Lege Artis) and oil component of 6) Kariofil Z, 7) Viko Temp and 8) Cp-CAP. The samples were subjected to spectrophotometer to measure OI 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS) using Randox kit, United Kingdom. The control samples were pure ascorbic acid (1% w/v).

Results High values of OI exposed materials (groups 1, 5, 6, 7, 8) with content of eugenol (or its derivatives) in the range of 100–88.8% were statistically more significant than the values of non-eugenol substances (groups 2, 3, 4) with the range of 8.2–43.5%.

Conclusion Eugenol containing temporary fixation materials show significant antioxidative properties and therefore they may be used in those clinical situations where surrounding gingival tissue is injured during restorative procedure.

Keywords: antioxidant; periodontal disease; temporary luting cement; cement for temporary fixing; eugenol; free radicals

INTRODUCTION

Free radicals (FRs) are very reactive compounds with strong electrophilic properties due to unpaired electrons in the outer atomic/molecular orbit. Such powerful oxidizing agent creates inflammatory reaction damaging tissues and cell integrity [1]. FRs make oxidative damage on the biomolecules of all origins, such as constitutive and functional proteins, DNA, RNA or lipids. However, being in low (physiological) concentrations they have been found to produce positive physiological effects [2]. Evolutionary, extensive antioxidant systems are developed in living organisms to fight against harmful effect of FRs through different mechanism. Some of them include antioxidative enzymes such as superoxide dismutase, glutathione - S transferase and glutathione peroxidase [3].

Within inflamed, infected or somehow injured pulp and periodontal tissues inflammatory mediators are released during the oral tissues defense against pathogenic microorganisms and their metabolic and/or degradation products. Concomitantly, polymorphonuclears (PMN) respond to pathogens causing their damage but inducing host tissue lesions. During this defense, especially in cases of FRs hyperproduction, oxidative modification of some of host molecules and oral tissues damage can

occur where saliva has an important role with its antioxidants whether of enzyme or non-enzyme origin [4, 5, 6]. Strong correlation was found between inflammatory periodontal disease and FRs [7].

Up-to-date investigations have revealed anti-oxidative functions of lipoic acid and chelating of metal ions. Lipoic acid, soluble in fatty and water-based tissue, is often titled universal antioxidant due to its powerful blocking of oxidation process even more effective than well approved antioxidants such as vitamins C, E etc. Its potential beneficial role in dentistry might be in chelating sometimes harmful metal ions such as iron, zinc, copper, cadmium and mercury [3]. On the other side, many dental items contain nickel that has been used in dentistry for more than eighty years in both restorative work (fillings, crowns, bridges, partial dentures) and orthodontic appliances (wires, bands, brackets, implants etc.). The amount of nickel used in dental items can reach up to over 50%. Adverse effect of nickel allergy has been noted in the study of enhanced sensitization capacity of nickel at higher oxidation state [8].

Gingival tissue becomes injured frequently by different mechanical and chemical means especially during fixed restoration preparation. This tissue then comes into contact with the material for temporary fixation as well as temporary

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fixed restoration that are supposed to possess anti-inflammatory properties and for some time expresses antioxidant activity. There is a risk from the effect of harmful agents to the pulp-dentine complex of the prepared teeth and surrounding periodontal tissues during the observation period when the patient wears a temporary or permanent fixed restoration. Low solubility of adhesives/luting cement for temporary fixation, almost always present, may adversely affect the tooth pulp tissue via open dentinal tubules and pulp-periodontal communication and the surrounding gingiva. In this case the insoluble portion of applied adhesive temporary cement that is exposed to oral fluids should have a strong antioxidant and anti-inflammatory effect to the pulp and adjacent periodontal tissues. Specifically, any agent that reduces or catches FRs agents aids in the healing process is therefore of great importance to be detected. In addition, considering the detection of FRs, spectrophotometry exposed as itself accurately expressed method among the others.

FRs are also created during pathological tissues conditions. Besides, some cytostatics, waste in the air, hyperoxia /bar chamber, pesticides, some herbicides, cigarette smoke, alcohol, anesthetics in dental use and generally aromatic hydrocarbons might damage cells via FRs reaction [2]. Furthermore, residual monomers TEGDMA and HEMA always present in composite resins are responsible for GSH i.e. cysteine wasting due to originate of oxygen ionic groups which oxidize them. Spare GSH is then being wasted and is unable for quick intracellular synthesis [9]. The GSH lessening begins 15-30 min upon TEGDMA exposition to monomers and almost completes within 4 to 6 hours. Thus a low level of GSH depot causes inhibition on gingival and pulp cells. GSH oxidation may be the result of direct reaction between glutathione and monomers or their reactive metabolic intermediaries [10]. Photosensitive additives of composite resin materials, such as camphor-chinon and benzophenon, upon blue-light exposition directly cause increase in the concentration of intra- and extra-cellular reactive compounds that consume spare amount of reduced GSH [9].

Considering the foregoing, the goal of this investigation was to examine antioxidant power of different adhesives for temporary luting cements. The null hypothesis was that there would be no significant difference between eugenol and eugenol-free materials for temporary cementation at the level of $\alpha=0.05$.

OBJECTIVE

The aim of this study was to investigate the antioxidant effect through the oxidation inhibition (OI) of mixed dental cement for temporary luting or their liquid component.

METHODS

The approximate composition of temporary adhesive luting cement is described in Table 1 [11, 12].

Study group involves the next materials: 1) Kariofil Z; 2) Viko Temp, 3) Temp Bond NE, 4) Scuta Bond NE, 5) Cp-CAP; the liquid components of those preparations also underwent the experiment due to their meaningful antioxidative potential forming the groups 6) clove oil (Kariofil), 7) oil component of Viko Temp, and 8) Cp-CAP oil.

The control group involves 1% w/v ascorbic acid (vitamin C) samples of well-known full antioxidant effect of 100%.

Specimens preparation

The materials for the study were prepared ex tempore according to the manufacturer recommendation: 1) fully saturated powder by oil exposing glossy mixture upon spatulation, 2) quickly spatulated equal part of each components, 3) the same as 2, 4) the same as 2, 5) powder and liquid were measured helped by scoop and nozzle. A micropipette is used for accurate measuring of liquid component in groups 6, 7 and 8. Ten specimens were chosen for each experimental and three for control group. All specimens were operated under the same laboratory (environmental) conditions.

Protocol

Temporary luting cements were prepared at an ambient temperature of 21-23°C and relative humidity of 50-55%. Kariofil Z preparation was prepared by spatulation of components until glossy image of paste appeared. Other materials were prepared according to the manufacturer instructions for clinical usage. Liquid components were pasted directly out of tube/bottle (groups 6, 7 and 8). They

Table 1. List of investigated specimens of tested temporary cements (manufacturers and basic composition)

Material	Manufacturer	Cement type	Composition
Kariofil Z mix	Galenika, Belgrade, Serbia	Eugenol based	ZnO, eugenol oils
Viko Temp mix	Galenika, Belgrade, Serbia	Eugenol free based	ZnO, mineral oils, eugenol oils in traces
Temp BondNE mix	Kerr GmbH, Rastatt, Germany	Eugenol free based	Methacrylic resins, traces of eugenol oils
Scuta Bond mix	3M ESPE, MN, USA	Eugenol free based	Methacrylic resins, mineral oils
Cp-CAP mix	Lege Artis, Dettenhausen, Germany	Eugenol based	Cp-CAP oil, Canada balsam, Zr ₂ O, ZnO, Ca(OH) ₂ , Zn-acetate
Kariofil Z oil	Galenika, Belgrade, Serbia	Eugenol based	Eugenol oils
Viko Temp oil	Galenika, Belgrade, Serbia	Eugenol based	Mineral oils, traces of eugenol oils
Cp-CAP oil	Lege Artis, Dettenhausen, Germany	Eugenol based	Eugenol oils, Peruan balsam (oleoresins, cinnamic acid, volatile oils, benzyl derivatives) 8-hydroxyquinoline

were spatulated by a metal spatula angled at 45° while homogenous color was achieved. The same amount (weight) of each material (in eight experimental and three control groups) was dissolved in extraction medium of 0.9% NaCl and organic solvent. Weight concentration of the sample was $C\%=0.0025$ g/100 g. Antioxidativity was calculated by spectrophotometer using a set of reagents (Randox kit, UK) by oxidation inhibition of 2,2'-azino-di-3-ethyl-benzthiazoline-6-sulphonic acid (ABTS) as chromogene [13]. ABTS was rendered to incubation by peroxidase and water to create ketone radicals ABTS⁺. This was done to create a relatively stable bluish-green color of visible wavelength of 600 nm using the Perkin-Elmer spectrophotometer device (SPD). Antioxidants of the sample reacted in the way to suppress the creation of the aforementioned color. That grade of suppression is proportional to the concentration of the antioxidative agent in the sample expressing percentage numbers.

Statistical analysis

The power of antioxidativity on investigated materials was assessed statistically using the one-way analysis of variance (ANOVA). Tukey's post-hoc test was used to compute multiple pair-wise comparisons of data so as to determine significant differences between groups at the level of $p<0.05$.

RESULTS

The samples of the group 8 Oil (Cp-CAP) displayed insignificant statistical difference (high content of eugenol) in comparison to the groups 1, 5, 6, 7 and the control group but significant compared to the groups 2, 3 and 4 (Table 2).

Samples of Kariofil Z (mixture) – group 1, Jelly-oil – group 7 and Cp-CAP – group 5 exposed the similar relation of significance and with the same reason to the experimental and control group as two previously cited groups (6 and 8) (Table 2).

Values comparison of the Temp BondNE (group 3) vs. Viko Temp (group 2) revealed insignificant statistical difference but significant to the group 4 – Scuta Bond. Each of those groups exposed antioxidative potential significantly lower than the groups 6, 8, 1, 7 and 5 (Table 2).

One way anova analysis of investigated cements is presented in Table 3.

Table 2. Mean values of oxidation inhibition of investigated specimens

Material	Antioxidativity (mean±SD)
Kariofil Z mix	100±0
Viko Temp mix	31.4±0.68
Temp BondNE mix	43.5±2.53
Scuta Bond mix	8.2±0.61
Cp-CAP mix	100±0
Kariofil Z oil (Eugenol)	97.7±1.69
Viko Temp oil	96.6±0.55
Cp-CAP oil	88.8±0.66

Table 3. One-way variance analysis of investigated groups

Source	Type III sum of squares	df	Mean square	Significance
Corrected Model	96333.743	7	13761.963	0.000
Intercept	401138.650	1	401138.650	0.000
Cement	96333.743	7	13761.963	0.000
Total	497570.490	80		

One-way variance analysis revealed statistically high significance of differences between mean values of investigated groups ($p<0.01$): 1 vs. 2, 3 and 4; 2 vs. 4, 5, 6, 7 and 8; 3 vs. 4, 5, 6, 7 and 8, and 4 vs. 5, 6, 7 and 8. Analysis of variance showed that there was no significant difference between the groups 1 vs. 5, 6, 7 and 8; 2 vs. 3; 3 vs. 8; 5 vs. 6, 7 and 8; 6 vs. 7 and 8; 7 vs. 8.

Table 4. Comparison of oxidation inhibition values on eugenol and eugenol-free temporary fixing cements

Material type	Antioxidativity (%)
Eugenol based	96±1
Eugenol-free based	28±1

Student t-test revealed high statistical significant difference between eugenol (n=50) and eugenol-free (n=30) groups (samples): $p<0.001$, $t=1.64$.

The values of inhibition on ABTS chromogene for all eugenol materials in (groups 1, 5, 6, 7, 8) were noted as significantly different to the non-eugenol groups (2, 3, 4) thus the null hypothesis is to be rejected ($p<0.01$) (Table 4).

DISCUSSION

Essentially, this study has shown that eugenol based temporary luting materials present superior antioxidative properties when compared to their non-eugenol analogues. The highest values of antioxidativity that was expressed as the highest inhibition of ABTS chromogene (100.0%) noted in Eugenol oil – group 6 (Kariofil Z) might be explained by predominant presence of eugenol derivate with ortho OH group [14, 15, 16].

According to the manufacturer specification, 1.0 gr of Viko Temp mixture contains approximately 0.5 g ZnO and 0.165 g oil of clove that reacts as antioxidative substance. Although eugenol presence is of approximately 25% by weight, this material exposed low inhibition level (31.4%) that means pro-oxidant reaction due to the mineral oil components, additives and corrective substances of this preparation. Inhibition level was statistically significant in comparison to the samples of higher eugenol content and controls.

The second generation of Temp Bond is non-eugenol oil material Temp Bond NE. It was invented in order to enable acrylic resins polymerization in restorative materials such as temporary crowns and bridges as well as canal acrylic posts. This material exposed mean of 43.5% ABTS inhibition. Absence of eugenol (phenolic) components (somewhat in trace) that possesses high antioxidant power in this adhesive lowers the antioxidativity, which was confirmed by several studies [14-17]. This inhibition value is significantly lower than the samples in 1, 5, 6, 7, 8 and the control group. Scuta Bond as well as Temp Bond NE did not possess ZOE hence displayed a low level of oxidation inhibition of 7.2% in our study.

High value of oxidation inhibition in Cp-CAP mixture samples might be explained by Peruvian balsam

and its constituents. Actually, oleoresins content carotenoids, and phenolic groups (cinnamic acid), which are approved antioxidant agents. Unfortunately, the presence of $\text{Ca}(\text{OH})_2$ incorporated into the Cp-CAP mix stimulates the dentinogenesis but decreases the antioxidativity [18]. Slightly lower values of oxidation inhibition in group 5 (Cp-CAP mix) that was statistically insignificant in regard to its liquid part (group 8) might be of the presence of powder component [18]. This assumption has not yet been certified according to electron literature data review up-to-now. The intermediates of liquid and powder chemical reactions might cause such antioxidativity level of Cp-CAP mix.

VikoTemp oil component that contains eugenol oil in trace exposed moderate level of antioxidativity. Regarding this moderate low values of antioxidativity (group 7) in comparison to the eugenol-rich groups one can only speculate that polyvinyl components of group 7 samples favors the pro-oxidant process.

The used method of visible wavelength range by SPD applied in this study is enough confidential and simple for laboratory use for aforementioned reactant ABTS in comparison to others. In addition, many alternative methods exist for similar investigation on the antioxidativity potential such as electron-spin resonance [19, 20]. Chemiluminescence is based on the recording energy released during chemical reaction through the light emission [21]. For example, certain radical species give off radiation at specific wavelengths through combustion analysis. However, SPD displayed enough precise measuring and simplicity for evaluation on the suppression of oxidation. Besides those physicochemical methods the biological ones such as the model of protective antioxidant effect on red blood cells or PMNs are more sophisticated to apply due to complicated system for maintaining the vital cells. Although those methods are applicable for antioxidative studies of food they might be useful for similar research on the dental materials. Due to aforementioned lack of literature data about dental antioxidativity points out the necessity of research using that study model.

Although non-eugenol temporary cements for cast restorations exposing lower values of antioxidant potential in our investigation, their advantage in clinical use in cases where periodontal tissue is in good condition regarding their less microleakage in comparison to eugenol preparations [22, 23]. However, the results on systemic administration of vitamin C could be clinically beneficial in improving periodontitis-induced oxidative stress by down-regulating inflammatory gene expression [24].

Ureic acid, the most present antioxidant in saliva generates as the final product purine base degradation. It neutralizes FRs by their reduction where it converts into allantoin [25]. Some authors have noted that ureic acid presents around 70% out of all saliva antioxidative capacity during the ptyalism period [26].

A foreign body in oral tissue such as implant with consecutive periimplantitis provokes significantly lower values of ureic acid in human saliva as compared to the cases where inflammation is absent. This indicates at a necessary

precaution about cementing fix restoration in the vicinity of periimplantitis when there is the need to use a material of the high level of antioxidativity. Similar situation happens when material for temporary restoration exposes irritant features of some acrylic resins to the marginal gingiva. Excessive production of reactive oxygen species (ROS) in periimplant disease leads to the situation of excessive oxidative stress, which may be an important factor contributing to the destruction of peri-implant tissues [27].

High albumin concentration is found in passionate smokers as protective agent for FRs neutralization where combustion products and other fume toxins adhere to the periodontal tissues and prepared abutment that is often fully unprotected [28]. Studies from 2003 and 2007 have revealed that periodontal disease is associated with lower antioxidant capacity in whole saliva and revealed evidence of increased protein oxidation [29, 30].

That is to stress out the antioxidative role of temporary adhesives where for example preliminary study from 1998 favors those that contain a high percentage of ZOE [17]. It is important to emphasize to restrain oneself of alcoholic drinks and smoking as well as the significance of correct diet of natural approved antioxidants (vitamins A, C, E and selenium etc.) all the time during temporary fixation of fixed restorations. Furthermore, dental products that contain two-bond Zn ion might play a significant role in keeping the level of Zn-SOD as the most important intracellular antioxidative agent [15]. That is another evidence of ZOE mixture benefit for ample depot of Zn ions as well as eugenol antioxidant role. One should bear it in mind when mix ZOE preparation is to be applied (injured periodontal tissues, metal in oral cavity, smoker-patient etc.).

Although eugenol reacts with FRs, it simultaneously hinders methacrylate monomers polymerization. Thus, it is incompatible with resin restoratives but as unavoidable as ZOE luting is still in use due to their oxidant and anti-inflammatory effects for oral tissues frequently injured during lengthy dental operative procedures [23].

Japanese authors found that bis-eugenol acts as a potent inhibitor of nuclear factor-kB thus inhibiting lipopolysaccharide-stimulated expression of inflammatory cytokines at gene and protein level. This is the way eugenol exposes antioxidant and anti-inflammatory effect, which stem from the inhibition of prostaglandin synthesis and pyretic activity [31].

Many phenol compounds of plant origins are frequently present in dental luting preparations as confirmed in experimental samples. Those substances i.e. phenol agents of synthetic origin possess significant antioxidative effect inhibiting lipoperoxidation. Some herbal phenol substances could trigger oxidation damage to non-lipid bio-molecules in DNA reducing Fe^{3+} to Fe^{2+} and reducing superoxide to the level of H_2O_2 [15]. Phenols show a double action reacting as antioxidants at lower but as pro-oxidants at higher doses under certain conditions. The development and progression of oral disease might be modified not only by these natural and synthetic phenol substances but by saliva antioxidants and its metal ions as well as dental materials that contain antioxidant agents [16]. Hence one

should stress out the significance of accurate measuring of constituents especially when phenol compound is present in liquid part and should be fully saturated to Zn-oxide and other ingredients of powder component.

Further study should be designed to evaluate the influence of every single component of dual luting systems for temporary fixation regarding their antioxidativity if it would be possible to detect and separate them in accurate proportion.

CONCLUSION

This study proved that eugenol containing materials present high values of oxidation inhibition (88.8-100%) in contrast to non-eugenol substances with (8.2-43.5%). There was no statistically significant difference considering inhibition

oxidation among the eugenol-containing groups. Arriving to the conclusion, due to unknown composition of investigated materials it is only to be speculated that those semi-hard or powdered substances possess prooxidant power because their liquid-oil component were proved to expose antioxidant effect in the range of approximately 96-100% oxidation inhibition. Further studies are necessary to evaluate the influence of every single component of dual luting systems for temporary fixation in terms of their antioxidativity.

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Привремени цементи на бази еугенола имају антиоксидативно дејство

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КРАТАК САДРЖАЈ

Увод Антиоксиданси штите жива ткива од реактивних кисеоникових слободних радикала, чиме испољавају пожељно антиинфламаторно дејство у контакту са живим ткивом. Стоматолошки материјали који служе за привремено цементирање протетичких рестаурација често су у контакту с ледираним ткивом гингиве, па стога треба да поседују пожељне антиинфламаторне особине, врло битне након протетичких процедура које претходе цементирању фиксних рестаурација.

Циљ рада Циљ испитивања је био да се помоћу забележених вредности инхибиције оксидације (ИО) утврди антиоксидативни ефекат неколико припремљених препарата за привремено цементирање и њихових течних компонената.

Методе рада По десет узорака је припремљено у осам експерименталних група: 1) *ex tempore* замешана цинк-оксидна (ZnO) еугенол паста (*Kariofil Z Galenika*, Србија); 2) паста *Viko Temp* (*Galenika*, Србија); 3) паста *Temp Bond NE* (*Kerr*, Немачка); 4) паста *ScutaBond* (*ESPE*, Немачка); и 5) паста *Sp-CAP* (*Lege Artis*, Немачка); њихове течне компоненте: 6) уље *Kariofil Z*; 7)

уље *Viko Temp*; и 8) уље *Sp-CAP*. Узорци су подвргнути спектрофотометру ради мерења вредности ИО на *ABTS* применом сета реагенса (*Randox kit*, Велика Британија). Контролну групу су чинили узорци чисте аскорбинске киселине (1% w/v).

Резултати Високе вредности ИО испољили су узорци у групама еугенолних материјала 1, 5, 6, 7 и 8 у распону од 88,8% до 100%, што је било статистички значајно у односу на ниже вредности код нееугенолних материјала (распон 8,2–43,5%) у групама 2, 3 и 4.

Закључак Није забележена статистичка значајна разлика у вредностима ИО међусобним поређењем еугенолних група ($p > 0,05$). Поређењем вредности ИО између нееугенолних група, само је код групе 4 утврђена статистички значајна разлика према групама 2 и 3 ($p < 0,01$). Поређењем узорака 2 и 3 забележена је статистички безначајна разлика за вредности ИО ($p > 0,05$).

Кључне речи: антиоксиданс; пародонтално обољење; цемент за привремено цементирање; цемент за привремено фиксирање рестаурације; еугенол; слободни радикали

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