TJHSL
Turkish Journal of Health Science and Life

Investigation of antibacterial and some physical properties of dental luting cements with natural plant extracts

Tolga CANBOL[a], Serhat Süha TÜRKASLAN[a*], Emel SESLİ ÇETİN[c], Banu Esencan TÜRKASLAN[d]
Şule Sultan UĞUR[e], Merve KÖLE[f]

[a,b] *Suleyman Demirel University, Faculty of Dentistry, Department of Prosthetic Dentistry, Isparta, Turkey*
[c,f] *Suleyman Demirel University, Faculty of Medicine, Department of Medical Microbiology, Isparta, Turkey*
[d] *Suleyman Demirel University, Faculty of Engineering, Department of Chemical Engineering, Isparta, Turkey*
[e] *Suleyman Demirel University, Faculty of Engineering, Department of Textile Engineering, Isparta, Turkey*

ARTICLE INFO

RESEARCH ARTICLE

Article history:
Received: 21 February 2022
Accepted: 16 March 2022
Available : 23 March 2022

[a]https://orcid.org/0000-0002-9211-0295
[b]https://orcid.org/0000-0002-8933-7149
[c]https://orcid.org/0000-0001-5231-3824
[d]https://orcid.org/0000-0003-3948-6207
[e]https://orcid.org/0000-0002-6485-7172
[f]https://orcid.org/0000-0002-7516-7599

*Correspondence: Süha TÜRKASLAN
Süleyman Demirel University, East Campus, Faculty of Dentistry, 32200 Isparta, Turkey
e-mail: suhaturkaslan@yahoo.com

Turkish Journal of Health Science and Life
2022, Vol.5, No.1, 45-52.

ABSTRACT

Aim: The aim of this study is to improve the antibacterial properties and investigate the physical properties of modified dental luting cements (polycarboxylate cement, glass ionomer cement, dual-cure cement, composite resin cement) with oleuropein addition.
Materials and Methods: The dental luting cements were modified by adding Oleuropein 1% by weight. Unmodified luting cements were referred as the control group. In order to determine the antibacterial effect of each group Modified Direct Contact Test method and E. faecalis was used. The microhardness values of all samples was measured with Vickers microhardness tester. All samples were evaluated by Scanning Electron Microscopy (SEM).
Results: Fewer bacterial colonies were detected in the media of the experimental group compared to the control group. While the Vickers microhardness values of polymer-containing cements decreased with the addition of oleuropein, no statistical difference was found in polycarboxylate and glass ionomer cement.
Conclusion: According to the results of this study, the addition of oleuropein improved the antibacterial properties of the luting cements but physical properties of resin based cements are negatively affected. The results obtained may shed light on the studies to be carried out on the development of luting cements.
Key Words: Antibacterial, luting cements, oleuropein

INTRODUCTION

The primary purpose of luting cements is to hold the restoration in place and to provide a seal between the restoration and the tooth (1,2). Cementation is one of the last steps in the sequence of clinical procedures for indirect restorations on the prepared abutment tooth and when done with required care, future complications can be prevented (3,4). Unfortunately, some dentists do not pay the same attention to the cementation process as they do to other applications of restorative dental treatments. This may result in faulty occlusion and marginal adaptation mismatches and may require laborious disassembly and reconstruction of the prosthesis (5). Thus, dentin hypersensitivity, secondary dental caries, plaque accumulation and retention, and periodontal inflammation occur over time (6,7). In addition, one of the most important reasons for the failure of fixed partial dentures is secondary dental caries (8). More than 50% of dental restorations fail within ten years due to secondary caries, which has become the main cause of failure (9). Therefore, it is important to improve the antibacterial properties of luting cements used in the cementation of indirect restorations. Until today, many materials have been added to cements and studies have been made to improve their antibacterial properties. The common goal of all studies is to improve the antibacterial properties of luting cements and to ensure long-term sustainment of restorative materials in function, to protect the tooth and surrounding tissues. (10,11). The ideal luting cement to be used in dentistry should be biocompatible, prevent secondary caries and plaque formation, and besides have favorable physical properties like high strength and hardness (12). A cement that fully meets all these features has not yet been invented. The search for the ideal cement has been one of the interesting topics of dentistry in the past and also today.

Recently, it is thought that the reason for the antioxidant, anti-inflammatory, anti-cancer, hypoglycemic, hepatic, cardiac and neuroprotective effects of extra virgin olive oil is the polyphenols in its content (13). The three main phenols found in olive oil is hydroxytyrosol, tyrosol, and oleuropein. The most abundant phenolic compound in the leaves of the olive plant is oleuropein. Oleuropein concentration is lower in extra virgin olive oil and refined oil due to hydrolysis to tyrosol and hydroxytyrosol during processing (14). Bourquelot and Vintilesco discovered oleuropein in 1908 and stated that it is a heterosidic ester of elenoic acid and dihydroxyphenylethanol. Oleuropein is a bitter-tasting glycoside found in the bark, leaves and fruits of the olive tree (15,16). As a result of studies, it has been seen that oleuropein has many effects such as antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic, antiviral (17). The biological effects of oleuropein are a matter of curiosity about their potential for use in dentistry, and they were included in the content of luting cements in our study.

The aim of this study is to improve the antibacterial properties of luting cements by adding oleuropein and to investigate their physical properties.

2. MATERIALS AND METHODS

In this study, the antibacterial and physical properties of the products obtained by modifying luting cements utilizing oleuropein were investigated. In this study, polycarboxylate cement (Adhesor Carbofine, Pentron, Czech Republic) and glass ionomer cement (Aqua Cem, Dentsplay, Germany) which polymerized by ionic reaction and dual-cure resin (RelyX U200, 3M ESPE, Germany) and light-cured composite resin (Choice 2, Bisco, U.S.A.) luting cements were evaluated. Oleuropein, ≥ 98% herbal extract was obtained from the company that supplies natural and chemical products (Sigma-Aldrich, Germany).

2.1. Modification of Dental Luting Cements

The luting cements were modified using 1% weight oleuropein for each group. Oleuropein were added to the powder portion of conventional cements and to the total mass of resin-based cements in percent concentration. A precision scale (Weightlab Instruments WSA-224, Turkey) was used for weight measurements. Reganding conventional luting cements the powder portion and liquid was weighed separately and 1% weight oleuropein was added for the whole mass. After mixing them in a glass beaker using magnetic stirrer, the powder is mixed with the liquid and it was ready for further tests. Regarding composite cements the material was kept in light proof black plastic container and 1% weight oleuropein was added through the lid. Light curing unit is used in order to cure the material before further tests.

2.2. Testing Antibacterial Properties of Modified Luting Cements

Enterococcus faecalis ATCC 29212 strain was used as the test bacteria, to investigate the antibacterial properties of the modified cements. Each luting cement was evaluated separately. In addition to 1 control group consisting of unmodified cements and experimental group consisting of modified cements, positive and negative control groups were determined. At the end of the test, 20 wells of 10 different concentrations belonging to each of the experimental and control groups and 10 wells of the positive control group were cultivated.

The luting cements and instruments used in the experiment were sterile and kept in the experimental conditions for at least one hour before the tests.

2.2.1. Direct Contact Test

The Direct Contact test technique, modified by Eldeniz et al., was used to investigate the antibacterial properties of modified luting cements. Then, cultivating were made on solid media and measurements were made at the 6th, 12th and 24th hours and the number of colonies per milliliter was determined (18,19).

A total of 4 sterile 96-well flat bottom microtiter plates were used for each cement group. Oleuropein modified and unmodified luting cements were applied to the bottom of 2 wells for each group. After the working period of the cement samples, 10 μl of bacterial suspension prepared in 0.5 McFarland turbidity was added with the help of a micropipette on the cement samples applied to the wells in the microtiter plates and the positive control group wells. The microtiter plates were kept in an oven at 35±2°C for 1 hour to allow the bacterial suspension to evaporate and to allow direct contact of the test material with the bacteria. At the 1st hour, 200 μl of Brain Heart Infusion (BHI) Broth was added to the wells containing the cement samples, which were in direct contact with the bacteria, with the help of a micropipette. Then, 90 μl of BHI was added to 10 wells for each group and to a total of 50 wells in a microtiter plate for serial dilution. Then, 10 μl BHI was taken from the wells containing the cement samples using a micropipette and transferred to the well containing 90 μl BHI. Furthermore, 10 μl BHI was taken from this well and transferred to the other well, thus making 10-fold serial dilutions from 101 to 1010. BHI of 10 μl volume was taken from the well containing each dilution and cultivated into BHI agar solid media divided into 4 quadrants by drip method. This process was repeated at the 1st, 6th and 24th hours of incubation. Bacterial colonies were counted after 24 hours of incubation on the cultured media. The number of bacteria in countable dilutions was multiplied by the dilution coefficient to find the number of colony forming units per milliliter (CFU/ml).

2.3. Vickers Microhardness Test

Vickers microhardness measurements were made using Vickers microhardness tester (HWMMT-X3, Matsuzawa, Japan). For the test, 5 samples per group were prepared. The cements mixed in accordance with the manufacturer's recommendations and were placed in a special stainless-steel mold with 4 mm diameter and 6 mm length. They were removed from the mold after setting and the Vickers hardness values of the cements, were measured under 100 g load for 15 seconds. Measurements of each sample were accomplished in three different points, one at the midpoint and two at the sides of mid area, and the average of three values were calculated.

2.4. Characterization

Morphological characterization of modified and unmodified cements was performed using scanning electron microscope (SEM) (FEI Quanta FEG 250) with a pressure value of 70 Pascal in low vacuum mode. The materials were examined at x500 magnifications.

2.5. Statistical Method

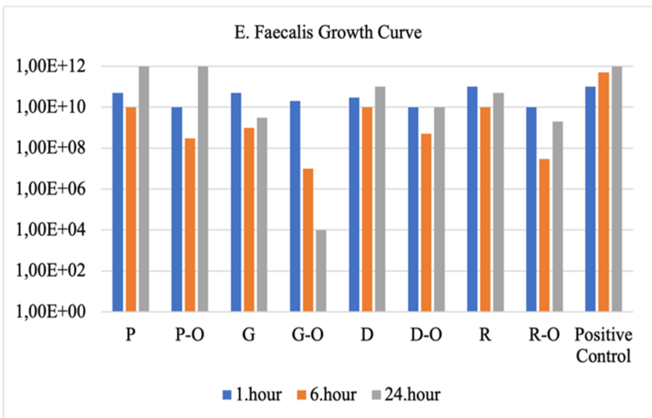
The data obtained in the research were analyzed using the SPSS 25.0 (Statistical Package for Social Sciences) program. While evaluating the data, descriptive statistical methods (number, percentage, mean, standard deviation, minimum, median and maximum) were used.

3. RESULT AND DISCUSSION

3.1. Antibacterial Properties

The obtained values were analyzed in 4 different groups and each cement was evaluated by comparing the modified and unmodified forms (Figure 1).

Figure 1. Time-Related Growth Curve of E. Faecalis Bacteria in Test Samples



In Group 1, each polycarboxylate cement (P) and oleuropein modified polycarboxylate cement (PO) test samples showed a decrease in bacterial count between 1-6 hours and the values increased between 6-24 hours. PO showed less bacterial counts than P by measurements conducted per hour.

In Group 2, the glass ionomer cement (G) and oleuropein modified glass ionomer cement (GO) test samples showed a decrease in the number of bacteria between 1-6 hours and the values increased between 6-24 hours. GO showed a decrease in bacterial count between 1-6 and 6-24 hours. GO showed less bacterial counts than G by measurements conducted per hour.

In Group 3, the dual cure cement (D) and oleuropein modified dual cure cement (DO) test samples showed a decrease in bacterial count between 1-6 hours and increased values between 6-24 hours. Measurements revealed that DO showed less bacterial counts than D.

In group 4, the composite resin cement (R) showed increased bacterial count between 1-6 and 6-24 hours. On the other hand, oleuropein modified composite resin cement (RO) showed a decrease in the number of bacteria between 1-6 hours and an increase between 6-24 hours. RO showed less bacterial counts than R per hour of measured.

The results show that oleuropein added cements showed a significant antibacterial effect among all groups.

3.2. Vickers Microhardness Measurements

The mechanical properties of luting cements were also evaluated by Vickers microhardness test (Table 1). The Vickers microhardness values were decreased in all samples with the addition of oleuropein. In Groups D and R, Vickers microhardness values were decreased when compared with the non-added samples and the results were statistically significant. On the other hand no statistically significant difference was observed in the Vickers microhardness values of

Table 1. Descriptive Statistics of the Variables of the Vickers Test Used in the Study

		N	MİNİMUM	MEDIAN	MAXİMUM	AVG	SD	F	P	B
GROUP 1	P	15	19	26.8	33	26.23	4.18	4.227	0.021*	
	PO	15	17.7	22.7	27.6	22.02	2.99			
GROUP 2	C	15	63.5	74.1	88.7	75.67	7.68	13.316	0.000*	
	CO	15	65.8	71.4	82.5	72.63	4.95			
GROUP 3	D	15	35.9	53.7	58.6	51.83	6.41	15.074	0.000*	2<1
	DO	15	24.7	43.8	50.4	42.77	6.27			
GROUP 4	R	15	56.8	63.5	67	62.52	3.41	20.458	0.000*	2<1
	RO	15	43.3	60.8	65.8	57.58	6.3			

F: Anova test statistic, *p<0.05, 1: X, 2: XO the oleuropein-modified samples in Groups G and P when compared with the non-added samples.

3.3. SEM Analysis

In order to compare the surface properties of the unmodified and modified versions of randomly selected cement samples, the samples were examined by SEM at x500 magnifications (Figure 2, 3).

Figure 2. SEM images of unmodified samples of luting cements: polycarboxylate (a), glass ionomer (b), dual cure (c) and composite resin (d) at x500 magnification.

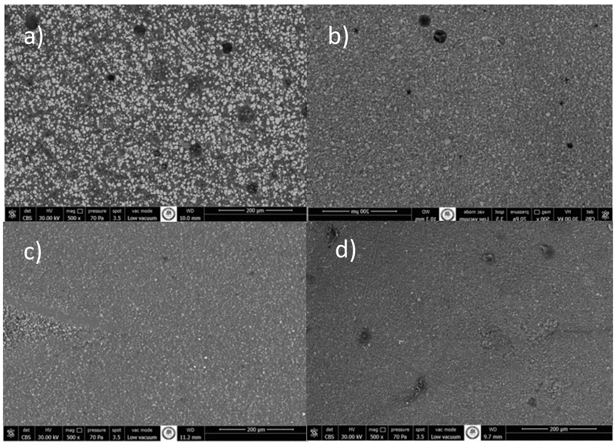
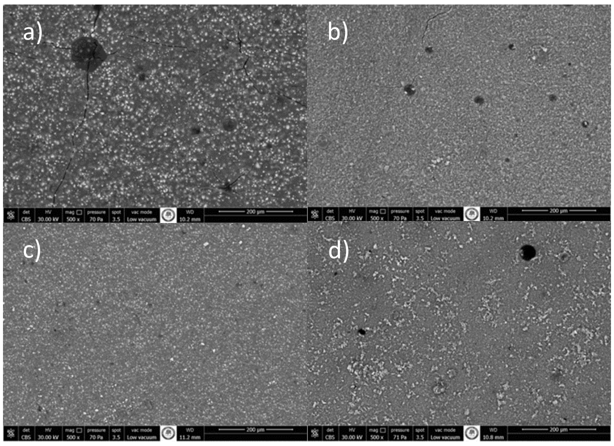


Figure 3. SEM images of modified samples of luting cements: polycarboxylate (a), glass ionomer (b), dual cure (c) and composite resin (d) at x500 magnification.



SEM images show that all groups except R-O have homogeneous surface structure in their modified and unmodified samples. A surface structure difference is observed between R and R-O. Regarding the glass ionomer cement, unlike G, cracks associated with the pores are observed in the surface structure of the G-O group.

According to SEM images, P and P-O samples had the largest pore structure in terms of size and number. Among these samples, pore-related cracks were observed in the P-O sample, which has the highest number of pores and volume. The high volume and porous structure number of polycarboxylate cement in SEM images obtained in this study are similar to the studies in the literature (20).

Over time, studies have been carried out with addition of many materials to dental cements to improve their antibacterial properties in order to prevent caries formation and protect the health of periapical and periodontal tissues. Some of these studies have shown that the addition of chlorhexidine salts reduces the mechanical properties of glass ionomer cement, such as compressive strength and bond strength to dentin. (21–23). In another study, high doses of chlorhexidine were reported to present some undesirable biological responses, including inhibition of protein synthesis and mitochondrial activity, and were considered toxic upon contact with pulp cells (24).

In another study, silver nanoparticles were added to improve the antibacterial properties of resin cements. However, silver nanoparticles have limited use as biocidal materials in liquid systems due to their low colloidal stability observed in dentistry. It is difficult for silver to separate from the restorative material and reach bacteria in the liquid (25). It is possible to have an antibacterial effect at low concentrations with smaller particles. However, silver is difficult to disperse in other materials due to the high aggregation of NAg, which leads to a lower surface energy and therefore a lower antibacterial effect (26). Another disadvantage of the use of silver in dentistry is that it is expensive and it changes color due to the oxidation reaction (27). All these disadvantages have led researchers to seek new additive materials for antibacterial cements.

In this current study, a white colored material oleuropein was used to modify various luting cements, in order to improve antibacterial properties and the natural origin was promising. Vickers hardness test is conducted in order to determine if the mechanical properties were adversely affected. The results are encouraging regarding antibacterial potential and physical properties are challenging.

Casas-Sanchez et al. tested the antibacterial properties of oleuropein, and their results reveals that oleuropein interacted with phosphatidylglycerol (PG) monolayers at a higher rate and affected gram-positive bacteria (28). One of the important properties of oleuropein is its antioxidant structure and its ability to clean up free radicals (29). In different studies, it has been reported that hydroxytyrosol and oleuropein inhibit the inflammatory responses of mouse and human monocytes and macrophages by blocking COX2 expression (30).

Oleuropein, which is the most studied phenolic compound found in olive fruits and leaves, has been reported to have a highly effective antimicrobial effect against gram-positive and gram-negative bacteria and fungi such as candida albicans in in vitro studies (31,32).

Oleuropein has shown an immunomodulatory effect in the treatment of sepsis, it has been reported to show this effect by promoting phagocytosis or suppressing the biosynthesis of proinflammatory cytokines (33). In addition, oleuropein has been reported to inhibit the growth rate of microorganisms (34).

Phenolic compounds, such as oleuropein, show their antimicrobial effects by denaturing proteins and adversely affecting cell membrane permeability. These compounds damage cell membranes and break down cell peptidoglycans, cause to leakage of cytoplasmic components such as protein, inorganic phosphate, glutamate, and potassium (35). In this study 1% wt oleuropein presented sufficient antibacterial effect. The percent is kept to a minimum as 1% wt in order to keep the changes in physical properties of the luting cements constant. Studies show that oleuropein may be the subject of more studies in the field of dentistry.

In this study, Direct Contact Test was used to examine the contribution of the antibacterial properties of oleuropein to luting cements. In addition, Vickers microhardness test was used to examine the physical properties of oleuropein-modified luting cements compared to the control group. The surface properties of randomly selected cement samples, which were subjected to the Vickers microhardness test, were examined by SEM.

When SEM images evaluated, it can be concluded that the polycarboxylate cement samples had the largest pore structure in size and number. Among these samples, cracks associated with the pores were observed in the PO sample with the largest number and volume of pores. The high volume and number of porous structures of polycarboxylate cement in the SEM images obtained in this study are similar to other studies in the literature (36).

Cracks are seen in the PO sample show that the surface structure of the sample has deteriorated as a result of the Vickers test and explain its statistically significantly lower value than P.

Oleuropein is a well-known material with antibacterial effect in the light of studies in the literature, but no study accomplished evaluating its effect on luting cements. As a result, oleuropein -modified luting cement samples showed significant antibacterial properties, but Vickers microhardness values were decreased in all luting cement samples, but no statistically significant difference was found in the P and G groups.

4. CONCLUSION

Studies have shown that oleuropein has many effects such as antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic, and antiviral (17). In this study, the contribution of the antibacterial properties of oleuropein to luting cements was investigated. As a result, luting cement samples modified with oleuropein showed significant antibacterial properties, all samples showed a decrease in Vickers microhardness values. The results are promising for the use of oleuropein to improve the antibacterial properties of dental luting cements. However, further investigations must be conducted to improve the physical properties of modified luting cements as well.

Acknowledgements: This study was produced from Tolga Canbol's thesis conducted by Prof. Dr. Süha Türkaslan at Süleyman Demirel University, Faculty of Dentistry, Department of Prosthetic Dentistry.

Financial Support: This study is supported by the Scientific Research Projects Coordination Unit of Süleyman Demirel University with the project numbered TDH-2020-7496.

Conflicts of Interest: The authors declared that there is no conflict of interest.

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