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Antibacterial Activity of Different Pulp Capping Materials After Completed Setting Reaction

Sertleşme Reaksiyonu Tamamlanan Farklı Kuafaj Materyallerinin Antibakteriyel Etkinliği

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ABSTRACT

Introduction: The aim of this study is to investigate the antibacterial activity of different pulp-capping materials after completed setting reactions against Streptococcus mutans, Lactobacillus acidophilus and Enterococcus faecalis.

Methods: The antibacterial activity of four materials (Dycal-Dentsply, TheraCal LC-Bisco, ProRoot MTA-Dentsply and Biodentine-Septodont) was tested by agar-diffusion method. The standardized disc-shaped specimens were prepared in plastic-molds in accordance with the manufacturers' instructions (n=6). The specimens were placed in the wells prepared in agar-medium (Tyriptic Soy Agar-Merck) after completed setting reaction. Antibacterial activity was evaluated by measuring the inhibition zones around the materials after 24, 48 and 72 hours of incubation.

Results: Among the materials, limited inhibition zone (16±1 mm) against Streptococcus mutans was detected only in Dycal group at 24, 48 and 72 hours. The limited diffusion was observed against Enterococcus faecalis in Biodentine group, but this did not result with inhibition zone.

Discussion and conclusion: In the limitations of this in vitro study; the tested pulp-capping materials did not represent antibacterial activity after the completed setting reaction. A limited zone of inhibition against Streptococcus mutans was observed only in Dycal group. Further studies are necessary to clarify the antibacterial-action mechanism of pulp-capping materials and to develop innovative materials with high antibacterial activity.

Keywords: *Antibacterial, pulp capping, dental material*

ÖZ

Giriş ve Amaç: Bu çalışmanın amacı; sertleşme reaksiyonu tamamlanan farklı kuafaj materyallerinin Streptococcus mutans, Lactobacillus acidophilus ve Enterococcus faecalis'e karşı antibakteriyel etkinliğini incelemektir.

Yöntem ve Gereçler: Çalışmada dört farklı kuafaj materyalinin (Dycal-Dentsply, TheraCal LC-Bisco, ProRoot MTA-Dentsply ve Biodentine-Septodont) sertleşme reaksiyonu sonrası antibakteriyel etkinliği agar-difüzyon yöntemiyle test edildi. Materyallerden üretici önerileri doğrultusunda, plastik kalıplarda standart disk şeklinde örnekler hazırlandı (n=6). Agarlı besiyerinde (Tyriptic Soy Agar-Merck) hazırlanan kuyucuklara örnekler yerleştirildi. 24, 48 ve 72 saatlik inkübasyon sonrası materyal çevresinde oluşan inhibisyon zonları ölçülerek antibakteriyel aktivite değerlendirildi.

Bulgular: Test edilen kuafaj materyallerinden sadece Dycal grubunda 24, 48 ve 72. saatte Streptococcus mutans'a karşı sınırlı bir inhibisyon zonu (16±1 mm) oluşumu saptandı. Diğer gruplarda ise inhibisyon zonu oluşumu gözlenmedi (0 mm). Biodentine grubunda Enterococcus faecalis'e karşı sınırlı bir difüzyon izlenmesine rağmen, inhibisyon zonu oluşumu ile sonuçlanmadığı belirlendi.

Tartışma ve sonuç: Bu in vitro çalışmanın sınırları dahilinde; test edilen kuafaj materyallerinin sertleşme reaksiyonu sonrası antibakteriyel aktivite göstermedikleri saptanmıştır. Sadece Dycal grubunda Streptococcus mutans'a karşı sınırlı bir inhibisyon zonu oluşumu gözlenmiştir. Kuafaj materyallerinin antibakteriyel etki mekanizmasının anlaşılması ve ideal kuafaj materyali arayışı doğrultusunda antibakteriyel etkinliği yüksek kuafaj materyali geliştirilmesi için ileri araştırmalar gereklidir.

Anahtar Kelimeler: *Antibakteriyel, pulpa kuafajı, dental materyal*

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INTRODUCTION

Pulp capping is a treatment option aims to preserve pulp vitality via covering the exposed pulp tissue by a biocompatible material. Various materials have been used for this procedure until today. The ideal pulp capping material should have several expecting properties. These properties can be summarized as the stimulation of reparative dentin formation, maintaining pulp vitality, fluoride release to prevent secondary caries, bactericidal or bacteriostatic effect, adhesion to dentin and restorative material, resistant to the forces during restoration placement and usage, sterility, radiopacity and permanent seal.[1]

Pulp capping materials used in the treatment of deep caries lesions should have antibacterial properties. The pulp vitality can be preserved in deep caries lesions when the penetration of residual bacteria or their products to the root canal system and induction of the pulp inflammation are prevented. Deep caries lesions consist many types of bacterial strain. *Streptococcus mutans*, which is responsible for caries formation and *Lactobacillus acidophilus*, which is responsible for dentin caries are the main caries related microorganisms in the oral cavity.[2] *Enterococcus faecalis*, which is the resistant microorganism in root canals continues to exist in deep dentin caries.[3,4] For this reason, the usage of an effective pulp capping material against the responsible bacteria is important for the success of the treatment.

Different materials have been used as pulp capping agents and calcium hydroxide is one of the most popular agent considering the antimicrobial activity with its high pH value. The bactericidal action of calcium hydroxide depends on the release of hydroxyl ions in an aqueous environment.[5] However, it has some disadvantages such as high solubility, dissolution of the material after application and tunnel defects in the reparative dentin which create negative impact on the permanent sealing against bacterial infection.[6] Dycal (Dentsply, USA) is a radiopaque, self-setting calcium hydroxide-based pulp capping material used for direct and indirect pulp capping. The alkalinity of Dycal stimulates secondary dentin formation when it comes in direct contact with the pulp tissue.[7]

The calcium silicate and resin modified calcium silicate cements have been widely used as pulp capping materials in recent years. More studies are needed to confirm the clinical significance of resin-modified calcium silicates and to support their useage in vital pulp therapy other than indirect pulp capping.[6] ProRoot MTA (Dentsply, USA) and Biodentine (Septodont, France) are the calcium silicate based pulp capping materials widely used in the clinical practice recently. The calcium hydroxide formed during the setting reaction of ProRoot MTA produces precipitates composed of hydroxyapatite inducing mineralization. It has excellent biocompatibility and sealing ability when used for direct pulp capping.[8-10] Biodentine is a permanent and biocompatible dentin material that can be applied in a single session under the composite restoration or can be applied to the entire cavity during the observation period before the final restoration.[11] TheraCal LC (Bisco, USA) is designed as direct and indirect pulp capping material, facilitating immediate placement of the final restoration.[6] It is supplied ready-to-use in a syringe and has setting reaction by photopolymerization in a hydrophobic environment.[12]

The aim of this *in vitro* study is to investigate the antibacterial activity of different pulp capping materials consisting calcium hydroxide and calcium silicate after completed setting reactions against *Streptococcus mutans* and *Lactobacillus acidophilus*, which are the main caries related microorganisms and *Enterococcus faecalis*, playing role in resistant apical infections. The tested null hypothesis declares that there is no difference between antibacterial activities of different pulp capping materials after setting reaction against the oral microorganisms.

METHODS

Four different pulp capping materials consisting calcium hydroxide (Dycal-Dentsply, USA), resin modified calcium silicate (TheraCal LC-Bisco, USA) and calcium silicate (ProRoot MTA-Dentsply, USA and Biodentine-Septodont, France) were used in this *in vitro* study. The content and preparation details of the materials are listed in Table 1. The disc-shaped specimens were prepared in the standard plastic molds (8 mm diameter, 2 mm thickness) according to manufacturers' instructions *(n=6)*. After the setting reaction specific to each material, all specimens were stored at +4°C for 21 days. The antibacterial activity of each material was examined by culturing in separate petri dishes for each microorganism. Absence of antibacterial activity was predicted as 0 (zero) inhibition zone formation. In order to prevent possible material diffusion and interaction on the agar surface, an additional material that would constitute the negative and positive control group was not included in the experimental design.

The antibacterial activity of the materials against oral microorganisms were investigated by agar diffusion test (Figure 1). The medium with agar (5 mm thickness, 20 mL) was poured into the sterile petri dishes with a diameter of 9 cm and allowed to freeze. Then, the overnight active liquid cultures of microorganisms ($5.8x10^6$ cfu/mL) were spread on the surface of the medium. After one hour at 37ºC, standard wells were created in the medium with the blunt tip of the Pasteur pipette, and the specimens were placed in the standard wells. *Streptococcus mutans* (DSM20523), *Lactobacillus acidophilus* (DSM 20079) and *Enterococcus faecalis* (ATCC 29212) strains were used in the study. The

sample size was calculated at a 95% confidence interval and a significance level of 0.05 according to the previous studies [13,14] The total sample size was calculated as 24 (n=6), considering a 95% power at a significance level of 0.05. Two technical replicates were performed in each group for the measurement of inhibition zones. Only one type of microorganism and one type of pulp capping material were tested in each petri dish *(n=6)*. After 24, 48 and 72 hours of incubation, the diameter of the inhibition zone around the each well was randomly measured from two points with the help of a digital caliper (Mitutoya Absolute Digimatic Caliper, Mitutoya Corp, Japan). The mean value (mm) was obtained for each sample.

Table 1: The contents and preparation details of the pulp capping materials.

Pulp capping material	Content	Preparation
Dycal (Dentsply)	Base: disalicylate ester of 1,3-butylene glycol, calcium phosphate, calcium tungstate, zinc oxide, iron oxide. Catalyst: calcium hydroxide, ethyl toluene sulfonamide, zinc stearate, titanium dioxide, zinc oxide, iron oxide.	Dispense equal volumes of base and catalyst pastes on the parchment paper pad provided. Replace container caps. Using a Dycal Liner applicator, stir immediately to mix thoroughly until a uniform color is achieved. Do not over-spatulate. Complete mixing within 10 seconds.
TheraCal LC (Bisco)	CaO, Sr glass, fumed silica, barium sulfate, barium zirconate, Portland cement type III, Bis-GMA and PEGDMA.	Layer is not to exceed 1 mm in depth. Cover all the exposed areas and extend TheraCal LC at least 1 mm onto sound dentin surrounding the exposure. Light cure between layers. Light cure each increment for 20 seconds.
ProRoot MTA (Dentsply)	Portland cement, bismuth(III) oxide, gypsum.	5 gram pouches and corresponding water ampoules to mix just the right amount for single-patient use.
Biodentine (Septodont)	Tricalcium silicate, dicalcium silicate, zirconium oxide, calcium carbonate, calcium oxide, iron oxide.	Pour 5 drops for each capsule. After that they mix at a speed of 4000 – 4200 rotations/min for 30 seconds.

(The content information and preparation details are in line with the manufacturer's declaration)

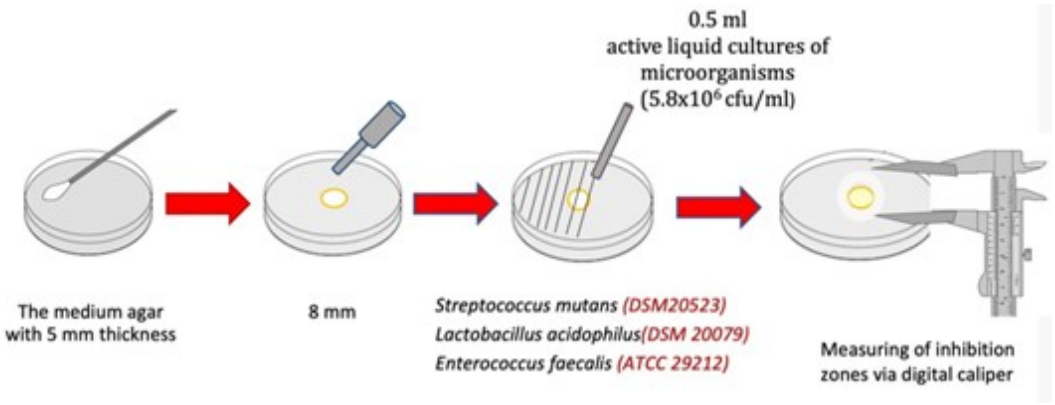

Figure 1: The stages and details of agar diffusion test applied to determine the antibacterial activity

Statistical Analysis

In statistical analyses, GraphPad Prism Software-La Jolla was used. One Way ANOVA and post Hoc Tukey test were used for antibacterial activity. The statisitcal significant difference was considered as $p<0.05$.

RESULTS

The mean inhibition zones obtained for each material after 24, 48 and 72 hours are presented in Table 2. After 24 hours of incubation, a limited zone of inhibition (16±1 mm) against *Streptococcus mutans* was detected only in the Dycal group ($p<0.05$) and the inhibition zone formation was not observed in other groups (Figure 2). At the end of 48 hours, while the inhibition zone formed in the Dycal group against *Streptococcus mutans* remained the same, no inhibition zone formation was observed in the other groups (Figure 3). It was determined that there was no difference at the end of 72 hours (Figure 4). Although a limited diffusion was observed against *Enterococcus faecalis* in the Biodentine group, it did not result in the formation of an inhibition zone (Figure 5).

Table 2: The mean inhibition zones (mm) after 24, 48 and 72 hours of incubation

	Streptococcus mutans			*Lactobacillus acidophilus*			*Enterococcus faecalis*		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Dycal	16±1	16±1	16±1	0	0	0	0	0	0
TheraCal LC	0	0	0	0	0	0	0	0	0
ProRoot MTA	0	0	0	0	0	0	0	0	0
Biodentine	0	0	0	0	0	0	0	0	0

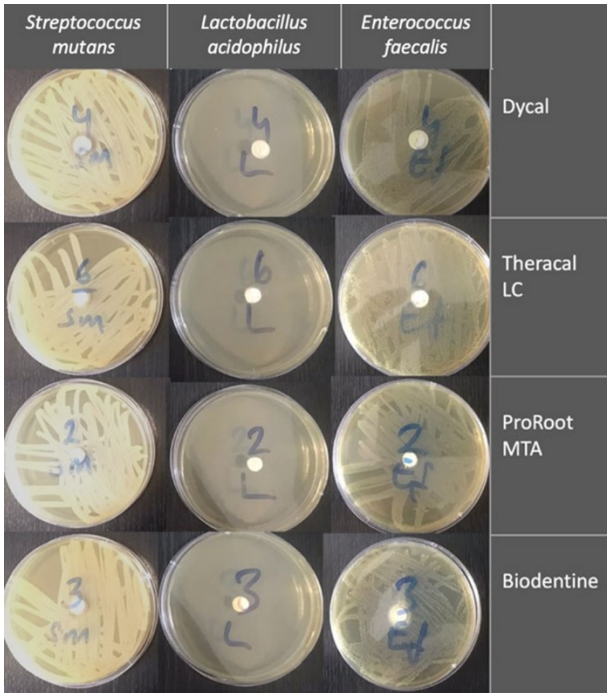

Figure 2: The representative images of the inhibition zones after 24 hours

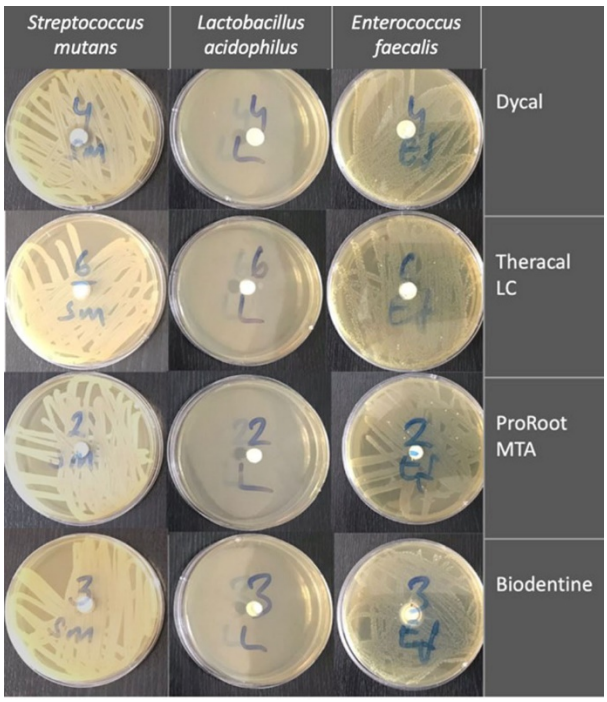

Figure 3: The representative images of the inhibition zones after 48 hours

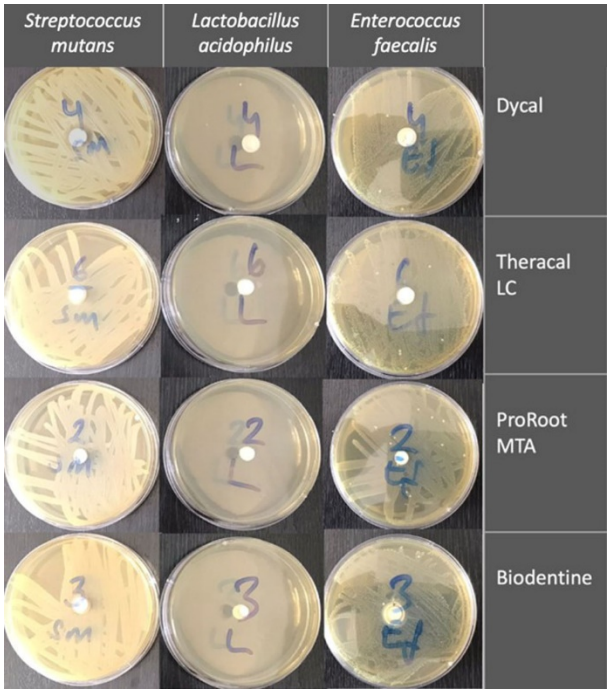

Figure 4: The representative images of the inhibition zones after 72 hours

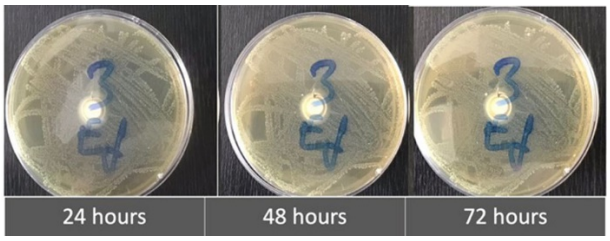

Figure 5: The representative images of the diffusion fields against *Enterococcus faecalis* in Biodentine group

DISCUSSION

In the present study, differences were verified regarding antibacterial activity of pulp-capping materials after setting reaction. Based on this findings, the null hypothesis was rejected. The agar diffusion test is known as an acceptable and easy method to test and determine dental materials antimicrobial activity at first step .[15-17] The mentioned method was used to investigate the antibacterial activity of different pulp capping materials consisting calcium hydroxide and calcium silicate after completed setting reactions against *Streptococcus*

mutans, *Lactobacillus acidophilus* and *Enterococcus faecalis* in this *in vitro* study. But the size of the inhibition zones formed in the agar well technique is not appropriate to determine the entire antibacterial activity, as the technique depends mostly on the diffusion capacity of the materials. In this study, the antibacterial activities of different pulp capping materials that have completed the setting reaction and become solid were investigated. Therefore, the potential for different diffusion degrees that may occur during the curing reaction of the mixed capping materials to affect the study findings was eliminated. This design of the study made it possible to apply the agar diffusion method. It may be useful to examine the antibacterial activity of the examined materials in further studies with different methods reflecting clinical conditions such as tooth-cavity model to support the study findings.

The antibacterial activity is expected from the ideal pulp capping material to protect the pulp tissue against secondary infection caused by microleakage or residual bacteria.[18,19] This protection is possible with the antibacterial activity of the pulp capping material. The studies have focused on the antibacterial activity of the freshly mixed pulp capping materials.[20,21] However, one of the main points to be considered is the antibacterial activity after the completed setting reaction. It is also critical to maintain antibacterial activity in the processes of mineralization and hard tissue formation. It was stated that regeneration of the pulp-like tissue progressed further with the formation of dentin bridge-like mineralized tissue at 14 days.[22] In another study, it was indicated that a progressive increase in Dentin Matrix Protein-1 gene expression has reached the highest intensity on 21 day.[23] It is known that 21 day is critical for mineralization after pulp capping. Therefore, the antibacterial activity of pulp capping materials after setting reaction was evaluated subsequent to 21 days waiting period in this study.

It was stated that the most effective material against *Streptococcus* strains was Dycal, and it was reported that Biodentine did not form an inhibition zone against *Streptococcus mutans*.[19] In this study, Dycal was also effective against *Streptococcus mutans* and no antibacterial activity was observed with Biodentine. The antibacterial activity of Dycal is attributed to the release of free hydroxyl radicals and subsequent pH rise.[24] From this point of view, the antibacterial activity of Dycal may be related with the solubility of the material. Contrary to our findings, Dycal showed the lowest antibacterial activity in a study in which the antibacterial effects of the materials were evaluated at three different time period after mixing. The antibacterial activity of Biodentine remained at the same standard levels in a week although antibacterial activity of MTA getting decreased.[25] The main difference of the mentioned study from this study is the methodology including direct contact test to evaluate antibacterial activity.

Considering the antibacterial activity of MTA products, the efficiency was determined against *Streptococcus mutans*, but no activity was determined against *Enterococcus faecalis*.[20] A decrease in antibacterial activity was observed after setting reaction.[26] In this study, no antibacterial activity was observed in the MTA group after the completed setting reaction.

The turbid spots with no inhibition zone was determined against *Streptococcus mutans* and *Enterococcus faecalis* around the freshly mixed MTA products.[14] Similarly, in this study, the diffusion areas against *Enterococcus faecalis* were detected in the Biodentine group, but it did not result in an inhibition zone. The formation of these diffusion fileds may attributed to reactions against the bacteria or the solubility of the material. Further studies are necessary to explain this phenomen in detail.

Among the antibacterial activities of freshly mixed ProRoot MTA, Biodentine and glass ionomer cement, the widest inhibition zone developed against *Streptococcus mutans* and Biodentine had higher antibacterial activity compared to MTA.[21,27] The small inhibition zones (0.5–1.0 mm) against *Streptococcus mutans* and *Enterococcus faecalis* were determined 24 hours after the completed setting reaction for three different MTA products and TheraCal LC.[14] However, no inhibition zones were detected in ProRoot MTA and TheraCal LC groups in this study after the setting reaction and 21 days waiting period.

CONCLUSION

In the limitations of this *in vitro* study; the tested resin modified calcium silicate and calcium silicate containing pulp-capping materials did not represent antibacterial activity after the completed setting reaction. A limited zone of inhibition against *Streptococcus mutans* was observed only in Dycal group. Further studies are necessary to clarify the antibacterial action mechanism of pulp capping materials and to develop innovative materials with high antibacterial activity**.**

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