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Yasemin Yavuz
Department of Restorative Dentistry, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey, yyavuz-21@hotmail.com

Sedef Kotanli
Department of Maxillo Facial Radiology, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey, sedefakyol@harran.edu.tr

Mehmet Sinan Doğan
Department of Pediatric Dentistry, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey, dtlider@hotmail.com

Kemal Doğan
Science and Technology Application and Research Center, Harran University, Sanlıurfa 63300, Turkey, kmldgn63@gmail.com

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Comparisons of Microleakage and Scanning Electron Microscope SEM Analyzes of The Use of Different Pulp Coverage Materials

Yasemin Yavuz[1*], Sedef Kotanli[2], Mehmet Sinan Doğan[3], Kemal Doğan[4]

[1]Department of Restorative Dentistry, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey
[2]Department of Maxillo Facial Radiology, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey
[3]Department of Pediatric Dentistry, Faculty of Dentistry, Harran University, Sanlıurfa 63300, Turkey
[4]Science and Technology Application and Research Center, Harran University, Sanlıurfa 63300, Turkey

Abstract

Background: The aim of this study was to compare three different pulp coverage materials with calcium silicate content considering microleakage in the cavity floor and evaluate the gaps with a stereomicroscope and scanning electron microscope SEM.

Methods: A total of 40 human molar teeth were used in this study, and class V (4 mm mesio-distal × 3 mm gingivo-occlusal × 3 mm depth) cavities were prepared. The samples were divided into four groups (N = 10), including NeoPutty(Nusmile), Biodentin (Septodont), and TheraCal PT (Bisco). All groups were restored using Single Bond Universal adhesive and Filtek Z250 (3M ESPE). The 0.5% basic fuchsin dye leakage was examined at 40× magnification under a stereomicroscope. SEM analysis revealed that the magnification was fixed at 1.00 KX on all the images. The gaps between the pulp coverage material and the cavity floor dentin were measured from four different points.

Results: The different microleakage scores were statistically significant ($p < 0.05$) when the pulp coverage materials were compared considering microleakage.

Conclusions: Within the scope of this study, the biocompatible pulp coating materials NeoPutty and Biodentin showed the least microleakage at the cavity floor and the smallest gaps on the dentin material combined surfaces. By contrast, TheraCal showed increased microleakage and large gaps.

Keywords: calcium silicate, gap, microleakage, pulp

INTRODUCTION

Restoration with an esthetic restorative material is the currently accepted form of treatment for dentin and enamel tissue loss due to decay. Composite restorative materials have become indispensable because of their superior esthetic properties and the absence of any laboratory process. However, the problems involving polymerization shrinkage of composite resins and microleakage development over time in large restorations have not yet been resolved. In deep restorations, the use of lining materials is necessary to prevent leakage, protect the pulp tissue, and stimulate remineralization. Calcium hydroxide has been used for many years as a pulp protective material below restorations.[1]

Calcium silicate materials have recently been used, which support the differentiation of cells produced by mineralized tissue and the formation of calcified tissue. By inducing type 1 collagen synthesis and the differentiation of pioneer cells to mineral expressing cells, these materials can initiate the process of mineralized tissue formation, such as cementogenesis, dentinogenesis, and osteogenesis.[2] The main components of these materials are tricalcium silicate and dicalcium silicate, which are both classified as hydraulic cements because of the formation of calcium silicate hydrate and calcium hydroxide by entering into a reaction with water.[3] When in contact with biological tissue fluids, the formation of hydroxyapatite-like crystals provides adhesion and impermeability.[4] These hydraulic materials are used clinically for pulp coverage, pulpotomy, apexogenesis, apexification, perforation repair, and root tip filling due to their bioactivities.[5] Calcium silicates have become a focus of interest for researchers because they support regeneration and repair without causing excessive inflammation, toxic reactions, or allergic reactions in the live biological system tissues.[6]

Vital pulp treatments aim to cover the pulp with an impermeable biomaterial, facilitate the formation of hard tissue, and complete the procedure by supporting the

***Corresponding author:**
Yasemin Yavuz
Department of Restorative Dentistry, Faculty of Dentistry,
Harran University, Sanlıurfa, Turkey
E-mail: yyavuz-21@hotmail.com

remaining weak dentin tissue. Mineral trioxide aggregant, which was the original version first introduced for this purpose, had disadvantages, such as difficulties in manipulation in temporary pulp coverage, a long hardening period, and long application time to upper restorations. Therefore, new bioactive materials were developed. These materials include Biodentin (Septodont, St. Maur-des-Fossés, France), TheraCal PT (Bisco Inc., Schaumburg, IL, USA), and NeoPutty (NuSmile, Houston, TX, USA), which contain calcium silicate and have some advantages in application.[4–6]

Biodentin provides stimulation of growth factors that activate dentinogenesis and the differentiation of odontoblasts. Increasing the expression of TGF-B1 growth factor from pulp cells causes angiogenesis, the accumulation of progenitor cells, cell differentiation, and mineralization.[7–10] This growth factor has good adhesion due to the micromechanical adhesion to dentin tissue.[10,11] Reports also indicate its capability to form a mineral infiltration zone on the dentin surface. This layer (mineral infiltration zone) that forms within the intertubular structure of dentin is rich in carbonate ions.[12]

Biodentin, which has bioactive properties, has been reported to promote hard tissue regeneration without any signs of moderate or severe pulp inflammation response. Thus, Biodentin can meet restorative requirements because the entire procedure can be completed in a single session; moreover, it has simple clinical use and has superior mechanical, impermeability, and therapeutic properties.[10,13]

TheraCal LC was introduced as a material combining the desired properties of calcium silicate and the increasingly advantageous use of resin, which is hardened with a light source in vital pulp treatments. Since the introduction of TheraCal LC onto the market, it has been extensively studied in vitro and in vivo and different results have been obtained. Therefore, recommendations indicate that the use of TheraCal LC is limited to indirect pulp coverage in vital pulp treatments. TheraCal PT has been presented on the market as a new calcium silicate-based material, which is double cured and modified with resin. It can be used for pulpotomies and direct and indirect pulp coverage according to the manufacturer.[2,7,14]

NeoPutty has been introduced as packaged ready for use, which does not require any mixing procedure. As described by the manufacturer, NeoPutty has been designed for in vivo application in the presence of moisture coming from surrounding tissues.[4] This material is recommended for placement over the pulp at a minimum thickness of 1.5 mm and for completion of the restoration. However, the hardening time of this material remains unknown.[3,5]

Considering impermeability, a material defined as biocompatible should have good bonding with the pulp dentin complex and the upper final restoration. Microleakage in the interface between dentin and the pulp coverage material is responsible for postoperative sensitivity and the formation of secondary decay.[13,14]

Microleakage is crucial considering restoration survival. One of the main factors affecting the clinical life of the restoration is microleakage occurring in the dental hard tissue and restoration interface. The development of secondary decay in the restoration can lead to treatment failure.[15,16]

In vitro studies have often used the staining penetration method to determine and evaluate microleakage between dental hard tissues and filling materials. The method is extremely simple, repeatable, and does not contain reactive chemicals.[17,18]

Studies that have investigated NeoPutty and TheraCal PT considering microleakage and micro gaps were unavailable in the literature.

The new materials of NeoPutty, TheraCal PT, and Biodentin were compared in the current study. The aim of the study was to compare the interaction of dentin and pulp coverage material in the cavity floor considering microleakage and evaluate SEM scanning of the dental material interface. For the null hypothesis to be accepted, the results of three pulp coverage materials containing calcium silicate were examined and analyzed considering microleakage.

METHODS

Preparation of the Restorations

This in vitro study was conducted in the Restorative Dentistry Department of Harran University Dental Faculty. The study material comprised 40 human molar teeth with no decay, which had been extracted for periodontal or orthodontic reasons. The teeth were examined individually to ensure the absence of decay, fracture, or cracks in the crown section and no previous restoration. Soft tissue remnants on the teeth were removed with a scaler, and all the surfaces were cleaned and polished.

The teeth were stored in distilled water at room temperature until the study. Standard Class V cavities were opened on the buccal surface of each tooth using a cylindrical diamond burr (Plus, BR31B, P.R.C) under air and water cooling. The cavities were prepared with dimensions of 4 mm (mesio-distal) × 3 mm (gingivo-occlusal) × 3 mm (depth). During the cavity preparations, care was taken such that no deviation in the cavity dimensions was observed by using a periodontal probe with a millimetric tip. The teeth with complete cavity preparation were then randomly separated into four groups of 10, and the cavity liners were applied in accordance with the producer's offer.

Group 1: The cavity material system used was NeoPutty, Single Bond Universal (3M ESPE, St. Paul, MN, USA), and Filtek Z250 (3M ESPE, St Paul, MN, USA).
Group 2: The cavity material system used was Biodentin, Single Bond Universal, and Filtek Z250.
Group 3: The cavity material system used was TheraCal PT, Single Bond Universal, and Filtek Z250.
Group 4: Control group with no cavity material applied.

The composite restorations were realized by using the same procedures in the control and experimental groups and the application of the different materials.

The finishing and polishing procedures were then performed on all the samples with aluminum covered disks (Sof-Lex, 3M ESPE, St. Paul, MN, USA). Following 24 h in an incubator at 37 °C (Nüve Incubator EN 500, Ankara, Turkey), the samples underwent 1500 thermal cycles (30 s waiting time) in thermal baths at 5 ± 2 °C and 55 ± 2 °C. The thermal cycles were conducted in the Dental Faculty Research Laboratory of Erciyes University.

Leaving a 1 mm border at the edges of the restoration, the entire tooth surface was then coated with two layers of nail varnish. When the nail varnish was dried, the teeth were placed in 0.5% basic fuchsin for 24 h and then removed and washed under running water. The teeth were separated into two equal parts horizontally in the bucco-lingual direction to pass through the center of the restoration. The stain leakage formed at the edges of the restoration was examined on each tooth by the same researcher at 40× magnification under a stereomicroscope (Olympus SZ60, Tokyo, Japan) (Figure 1). The stain leakage formed on the cavity walls at the interface of the tooth and the restoration was scored as follows:[19]
Score 0: no stain leakage;
Score 1: stain penetration to one-third of the depth of the cavity;
Score 2: stain penetration to two-thirds of the depth of the cavity;
Score 3: stain penetration as far as the cavity floor;
Score 4: stain penetration present in the cavity floor.

Four samples were randomly selected from each group for SEM analysis. The smear layer was removed by applying 37% orthophosphoric acid to the tooth surfaces for 5 s. The SEM (Zeiss EVO 50) images were then obtained at the Science and Technology Research and Application Center of Harran University. The dental samples were coated with gold at approximately 5 nm thickness with an electron microscopy system gold covering device before the images were obtained to ensure their conductivity. The images were taken with a secondary electron detector at EHT10.00 kV and a work distance of 11.0 mm. The magnification ratio was fixed at 1.00 KX on all the images. The gaps between the pulp coverage material and the cavity floor dentin were measured from four different points on the images (Figure 2).

This research was approved by the Harran University Clinical Research Ethics Committee's decision numbered HRU/22.09.05.

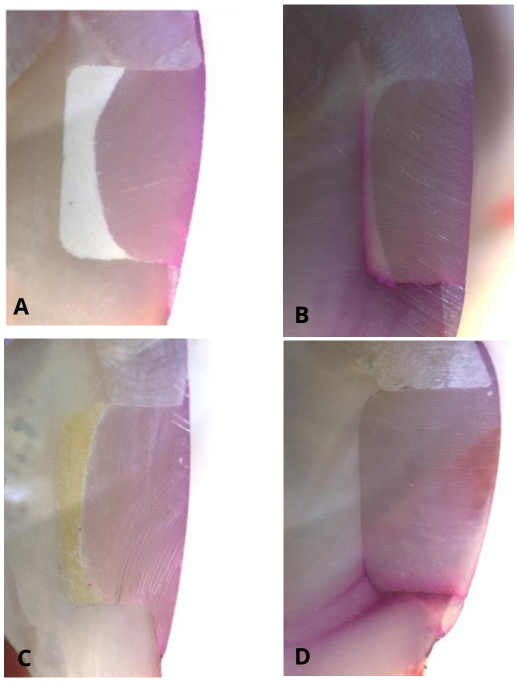

Figure 1. Stereomicroscope images at ×40 magnification (A) NeoPUTTY, (B) Biodentin, (C) TheraCal PT, (D) Control group.

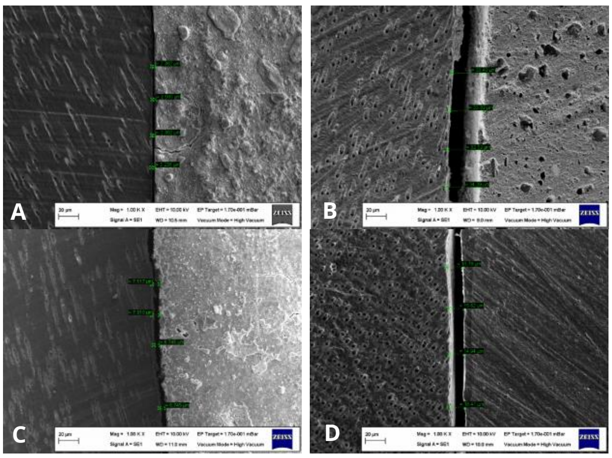

Figure 2. (A) SEM images of the cavity floor of the samples applied with NeoPUTTY (B) SEM images of the cavity floor of the samples applied with TheraCal PT (C) SEM images of the cavity floor of the samples applied with Biodentin (D) SEM images of the samples of the control group.

Statistical Analysis

Data were analyzed using SPSS Statistics version 23.0 software for Windows (IBM Corporation, Armonk, NY, USA). Descriptive statistics were stated as mean, median, and standard deviation values, numbers, and percentages. Normality of the data distribution was tested using the Shapiro–Wilk test. The data were not normally distributed; thus, the Kruskal–Wallis test was applied to determine significant differences between the groups.

RESULTS

The microleakage scores of the cavities for the different pulp coverage materials (Biodentin, NeoPutty, and TheraCal PT) and the control samples are shown in Table 1. In the comparison of the microleakage of the pulp coverage materials, cavities restored with TheraCal PT showed significantly higher microleakage than those restored with NeoPutty and Biodentin.

The micro gap width values between the dentin and pulp coverage material in the experimental groups and between the dentin and the restorative material in the control group are shown in Table 2. When comparisons were made between the groups of the interface micro gap width, the cavities restored with TheraCal PT showed a significantly larger gap width than those with NeoPutty and Biodentin ($p < 0.05$) (Table 2).

Table 1. Microleakage scores in cavities restored with TheraCal PT, Biodentin, and NeoPutty

Type of pulp coverage material	N	Microleakage Scores					Median	*p*
		0	1	2	3	4		
Biodentin	10	0	10	0	0	0	1	0.001*
NeoPutty	10	0	10	0	0	0	1	
TheraCal PT	10	0	0	2	4	4	3	
Control	10	0	0	6	1	3	2	

Sample size: 40 ($p < 0.05$)

Table 2. Comparisons of the micro gap width values between pulp coverage materials and dentin using SEM

Type of pulp coverage material	N	Mean	Std Deviation	Median	Std Error of Mean	*p*
NeoPutty	4	3.46200	0.727198	3.40000	0.363599	0.003*
TheraCal PT	4	33.4750	0.73668	33.5450	0.36834	
Biodentin	4	7.2543	0.60007	7.1810	0.30004	
Kontrol	4	15.9675	0.77672	16.1150	0.38836	

*Kruskal–Wallis Test. Statistically significant at $p < 0.05$

DISCUSSION

The increasingly predominant role of calcium silicate in restorative dentistry can be explained by the high biocompatibility, thereby promoting the formation of a high-quality dentin bridge, and the impermeability of the region covering the pulp.[1,20] The stain penetration method is the most widely used technique for obtaining information regarding impermeability to bacteria, fluids, chemical substances, molecules, and ions between the restoration and dental hard tissues. This method can provide information regarding new restorative materials.[11]

The aim of this in vitro study was to compare the levels of impermeability of different materials with calcium silicate content that are used for pulp coverage through the stain penetration method. The null hypothesis of the study was rejected. The study results showed a statistically significant difference in microleakage between the control group and the groups where NeoPutty, Biodentin, and TheraCal PT were used as vital pulp coverage materials. This difference can be explained by the polymerization shrinkage of the resin of the composite restoration in the TheraCal PT and control groups. Previous studies have reported impaired adaptation to cavity walls in resin-based materials due to polymerization shrinkage, thereby inducing microleakage occurrence.[21] In a study by Yıkılgan *et al*., as the system material in resin restorations, resin-modified calcium silicate cement and the use of fluid composite and resin-modified glass ionomer cement did not prevent microleakage; however, a lower rate of microleakage compared with the control group without any system material used was found.[21]

In the evaluation of microleakage under a light microscope in the current study, Biodentin and NeoPutty showed similar results of successful impermeability compared with TheraCal PT. NeoPutty and Biodentin were successful considering impermeability, but they can also be compared considering application in vital pulp treatments. The short hardening time of Biodentin (minimum 12 mins) is accepted as an advantage among calcium silicate cements, whereas no waiting time has been reported by the manufacturer for NeoPutty. The recommended hardening of NeoPutty has not occurred in vital pulp treatment for completion of the restoration in a single session; however, this condition can be changed to the corresponding minimum pressure when completing the restoration. Therefore, fixation of the cavity with a fluid resin material is recommended. The results of Biodentin in the current study supported other studies related to microleakage and were consistent with the literature.[1,16,22,23]

Lovan *et al*. evaluated the effect of tooth wetness on Biodentin considering marginal microleakage and showed good marginal impermeability of Biodentin restorations in dry and wet hard tissues despite the wetness at the occlusal and cervical margins.[11] In a comparison of bacterial leakage in apical coverage, Refaeı *et al*. found Biodentin to be more successful than ProRoot mineral trioxide aggregate and calcium-enriched mixture.[24] Koubi *et al*. compared Biodentin and resin-modified glass ionomer cement restorations with the open sandwich technique using the in vitro glucose diffusion method and concluded the absence of a statistically significant difference.[13]

Choudary D. used the open sandwich technique of calcium silicate-based Biodentin and full filling material and also concluded the absence of a statistically significant difference between the two restoration techniques considering in vitro impermeability.[16] No study could be found in literature that has evaluated microleakage of the following: TheraCal PT, which is a new calcium silicate-based material, dual-cure hardened with resin content; and NeoPutty, which is packaged ready for use and does not require any mixing process. In the stain penetration method applied in this study, the highest score of 4 (microleakage present) was observed in the TheraCal PT group and the highest score in the Biodentin and NeoPutty groups was 1 (stain penetration as far as one-third of the depth of the cavity). This difference was assumed to be due to polymerization shrinkage of the resin content in TheraCal PT.

The investigation of microleakage using the stain penetration method was supported by the SEM analysis results of the dentin material interface. In the SEM examination of the interface between the different calcium silicate materials and the dentin, NeoPutty was observed to provide the best adaptation to dentin and formed a small gap (mean 3.46 μm) (Table 2, Figure 2). The other gap values were as follows: mean 7.25 μm for Biodentin, mean 15.96 μm in the control group, and the largest gap was found in TheraCal PT at 33.37 μm (Table 2, Figure 2). The vacuum process in SEM analysis studies may cause dry shrinking in the dental hard tissue and the material. The water-based chemistry of calcium silicates may also change the nature of dentin interface material adaptation in this process.[25] Therefore, techniques, such as confocal and atomic force microscopy, which do not change the structural properties of materials, can be used to investigate the interface properties.[26]

The interaction of pulp coverage materials with dentin is clinically important considering ion leakage from the material to dentin tubules and reaching the dental pulp. Gaps between the materials and the tooth structure can be attributed to re-colonization by bacteria, causing the formation of secondary decay.[26]

Calcium silicate cements interact with the underlying dentin by the movement along the interface of calcium ions, creating a mineral infiltration region.[26] However, no such movement has been observed and only the accumulation of calcium phosphate in the interface has been recorded in previous studies.[27]

In studies of the tooth–material interface using confocal microscopy, Hadis M. et al. reported the presence of phosphorus accumulation in the Biodentin–dentin interface but no elemental migration was observed for silesium, aluminum, or zinc.[26]

In the current study, the placement techniques and thicknesses of all three materials complied with the maufacturer's instructions. However, the type of dentin to which the cement was applied, the form in which the smear layer occurred, and the sample preparation methodology could have affected the results. Thus, further studies with large samples are necessary for this subject to be understood effectively.

CONCLUSIONS

Within the limitations of this study, the biocompatible pulp coverage materials, namely NeoPutty and Biodentin, showed minimal microleakage in the cavity floor and fewer gaps between the dentin and filling material. TheraCal, which is completed with a hardening light source, showed significantly increasing microleakage and large separation in the dentin material surfaces.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare related to this study.

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