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Prevention of Bacterial Infiltration in Class 1 Temporary Restorations Using Zinc Oxide/Calcium Sulphate Filling Materials: An In Vitro Study

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Abstract: This study evaluated sealing properties of zinc oxide/calcium sulphate filling material (Plastor©) to prevent bacterial infiltration in simulated Class 1 cavities with and without the presence of a spacer apically the restoration. Twenty-eight experimental Eppendorf tubes containing Tryptic Soy Agar were prepared and divided into seven groups: group A (Plastor©), group B (Plastor© + cotton pellet), group C (Plastor© + cotton pellet soaked of m-cresyl acetate), group D (Plastor© + cotton pellet soaked of eugenol), group E (Plastor© + PTFE pellet), group F (positive control), and group G (negative control). All prepared vials were individually immersed in tubes containing 5 mL of a suspension of *Streptococcus mutans* (10^5 CFU/mL) previously prepared and then incubated at 37 °C for 5 and 10 days. Subsequently, agar was collected and tested for *S.mutans* using real-time PCR. Aliquots of each agar samples were separately seeded on TSA for *S. mutans* isolation. The real-time PCR tests were negative for *S. mutans* on all the vials at both T5 and T10, except for positive control resulted positive at both T5 and T10. The isolation tests showed bacterial growth of *S. mutans* only with the agar samples collected from the vials of positive control, confirming real-time PCR tests. This in vitro study evidenced complete sealing ability of zinc oxide/calcium sulphate in temporary restorations of Class 1 cavities without mechanical loading at 10 days and complete immersion in a bacterial suspension. Moreover, sealing ability is not influenced by presence of spacers.

Keywords: temporary filling materials; zinc oxide/calcium sulphate materials; bacterial infiltration; *Streptococcus mutans*

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1. Introduction

Temporary filling materials are used in many procedures and techniques in dentistry. In endodontics, they can be used in multiple-visit approach and/or after endodontic treatment until the final restoration is placed. The main goals of root canal treatments (RCTs) are undoubtedly the prevention or the resolution of periapical lesions and the retention of the function of the treated teeth as much as possible. The most significant factors leading to RCT failure are fundamentally related to the etiopathogenesis of endodontic diseases: the bacteria. In fact, endodontic pathologies are caused by endodontic microorganisms and their by-products, thus, its successful treatment strictly depends on the efficiency of bacteria eradication and recontamination prevention. Therefore, the basic goal of RCTs is to eliminate microorganisms from the root canal system and to remove any biological substrate which could aid microbial growth, such as pulp tissue remnants, through adequate chemo-mechanical disinfection of the endodontic system. Moreover, the quality of chemo-mechanical debridement is not the sole factor able to affect the long-term clinical

outcome. As a matter of fact, a tridimensional root canal filling is required to preclude bacterial recolonization of the endodontic system. Quality of both the root canal obturation and the coronal restoration influence the success rates of RCTs, confirming that satisfactory root fillings and proper coronal sealing are associated with an optimum root canal treatment outcome.

It is important to underline that after endodontic treatment, at least an immediate dentinal conditioning and sealing with a preliminary build-up using adhesive techniques is recommended, to reduce risks of dentinal contamination and to improve bonding strength of the final restoration [1]. Temporary filling materials can be used also in vital pulp therapies (pulp capping, apexogenesis), apexification or regenerative procedures until these treatments are completed [2–4]. Vital pulp therapies consists in the placement of biocompatible materials on the exposed pulp tissue to preserve its health, stimulate repair by mineralized tissue formation [4] and allow continued root development along the entire root length (apexogenesis) [3]. If the pulp is irreversibly inflamed or necrotic, root-end closure procedures are required when the apex has not fully formed. In fact, pulpal necrosis in permanent teeth that have not completed their root development leads to teeth with a very short root, roots with very thin walls and an inadequate crown-root ratio, which overshadows their survival prognosis [2,3]. The traditional apexification technique used calcium hydroxide ($Ca(OH)_2$) as intracanal dressing, a strong base with a high pH (approximately 12), that was originally used in endodontics as a direct pulp-capping agent. $Ca(OH)_2$ is formed by a powder that when in contact with an aqueous fluid dissociates into calcium and hydroxyl ions. This reaction induces a hard-tissue deposition and high antimicrobial activity. that the study was made on deciduous teeth, instead of immature permanent teeth. More recently, another type of apexification named as "apical MTA plug" was described using calcium silicate materials, formed by a biocompatible powder with fine hydrophilic particles that hardens in the presence of humidity.

Apexification presents the limitations of an interrupted root development, leaving the tooth with a fragile root structure and a poor crown-to-root ratio leading to higher risk of root fracture [2].

An alternative endodontic procedure for the treatment of necrotic immature teeth that may promote further root development by attempting to regenerate the pulp-dentin complex is regenerative endodontic procedure (REP). This conservative two-step procedure consists of the use of a combination of antimicrobials to reduce infection, no canal walls instrumentation, and induced apical bleeding to form a blood clot tightly sealed into the root canal in order to promote healing. It can be performed in cases of pulp necrosis secondary to trauma, decay, or dental anomalies. As for endodontic treatment, the success rate of these techniques is influenced by efficiency of bacteria eradication and recontamination prevention [2].

The principal objective of temporary restorations is to provide a tight seal of the access cavity, to prevent reinfection of root canal system/vital pulp from microorganisms present in saliva [5] and to guarantee protection of other materials located in cavity access/chamber floor or root canal system [6–9]. Previous studies demonstrated that bacteria present in natural human saliva will penetrate in <30 days through an entire root canal system obturated by a lateral or vertical condensation techniques [10]. Another advantage of temporary filling materials is the reduction in time to re-open cavity access in case of worsening of pulpal/periapical signs/symptoms or failure of vital pulp therapy with subsequent need of endodontic treatment [6,11]. The most commonly used materials for short-term temporizations contains zinc oxide eugenol and zinc oxide/calcium sulphate [1]. These compounds possess antimicrobial properties and good sealing properties, determined by its hygroscopic nature and high setting expansion [12,13]. Moreover, are simple to apply in cavity access with bulk techniques and easy to remove. Previous studies, evaluating marginal leakage of different temporary restorations, concluded that zinc oxide/calcium sulphate materials had better marginal seal in Class 1 and Class 2 cavity access preparations [6].

However, these materials lack mechanical properties and need to be minimum 3.5 mm in thickness [14] to prevent total leakage. Moreover, no mechanical loading would be preferable to maintain their stability in tooth cavity [15]. In addition to temporary filling material, a "spacer" or "barrier material" placed apically to the provisional restoration can be used in most clinical situations [16]. In endodontics, spacers can prevent unwanted materials (also the same temporary material) entering and blocking the canal space in multiple visit approach endodontic treatments [17]. In addition, spacers reduce the time required to access the root canal system and reduce the risk of chamber floor damage during removal of the temporary material [18]. Spacers can also be soaked with medicaments in specific emergency procedures [19]. When pulpectomy is not achievable, an emergency procedure for temporary relief of symptomatic acute pulpitis can be pulpotomy eventually associated to soaked pellets used as chamber floor medicaments [20–22].

One of these medicaments is eugenol, that can rapidly inhibit bacteria and kill viruses, with an analgesic effect to relieve the pain of patients [23]. Another chamber floor medicament is m-cresyl acetate that allows the removal of bacterial biofilm and is less irritating for periapical tissues than other compounds such as p-chlorophenols [24].

The probability of eliminating pain is related to the extent of the inflamed/necrotic tissue removal. However, if there is no sufficient time to for a complete removal of pulpal tissue (pulpectomy), pulpotomy will give a better pain-relieving effect compared with incomplete canal instrumentation [20].

Spacers soaked with water can be used to provide a suitable humidity source in therapies (vital pulp therapies, apexogenesis, apexification, and perforation repair) using calcium-silicate cements, that needs humidity for their setting time and bioactive properties. Calcium silicate-based cements, also called hydraulic cements, have gained popularity due to their excellent biological and physiochemical properties. The chemical basis of the setting mechanism of these sealers consists of a hydration reaction in which water is absorbed from dentin tubules, and a calcium silicate hydrate gel and calcium hydroxide are produced. Moreover, the latter is able to react with the phosphate ions to precipitate and form hydroxyapatite (HA). Calcium silicate cements have been recently introduced in endodontics for their enhanced properties compared to conventional sealers: high biocompatibility (nontoxicity); high chemical stability within the biological environment; shrinkage absence; expansion after setting; the absence of an inflammatory response in the case of extrusion into the periapical space; HA formation; high pH (strongly antibacterial); hydrophilicity; excellent sealing ability; and ease to use. Calcium silicate cements can be classified based on their chemical composition or their clinical application. According to the clinical context, hydraulic cements can be divided into intracoronal, intraradicular, and extraradicular materials whilst, considering the chemical constitution, they can be classified as hydraulic aluminate cement or hydraulic calcium silicate-based cement. Moreover, the latter can be divided into two different categories according to the cement components: Portland cement and calcium silicate-based cement [25,26]. The most commonly used spacers include cotton pellets and polytetrafluoroethylene (PTFE) tapes [16,18]. Cotton pellets have been widely used as an endodontic spacer and can be soaked with medicaments. Although, some authors described impairment in stability and displacement of the overlying restorative material during masticatory loading. Furthermore, the cotton fibers may interpose to the cavity walls-restoration interface, affecting the marginal seal of the provisional restoration and acting as a sponge by drawing fluids from the oral cavity to the internal cavity walls and root canal system [7]. PTFE tape is a versatile material that has been increasingly used for various purposes in dentistry and has been considered a suitable alternative spacer material with the potential to overcome the disadvantages of using cotton pellets. being a non-fibrous polymer, it reduces absorption and provides better support the provisional restoration [17,27]; although, PTFE cannot be soaked with medicaments such as cotton pellets. The use of PTFE tape as a spacer is associated with less tooth's contamination compared with cotton pellets, though the evidence available is limited and heterogeneous [16].

Aim of this in vitro study was to evaluate the inhibiting effect of zinc oxide/calcium sulphate filling material, to bacterial infiltration in temporary restorations, with and without different types of spacers placed apically to the restoration.

2. Materials and Methods

2.1. Bacteriological Investigations

For this experiment, ATCC-700610 *Streptococcus mutans* (LGC, Milan, Italy) was used. For the bacterial suspension preparation, a sample of *S. mutans* was previously inoculated on plates of Triptic Soy Agar (TSA) (Liofilchem, Teramo, Italy), supplemented with 5% defibrinated sheep blood, and incubated for 24 h at 37 °C under aerobic conditions. After incubation period, N = 3 bacterial colonies were collected and Gram stained. To confirm the bacterial strain as *S. mutans*, the bacterial colonies were tested with real-time PCR. Subsequently, one colony was inoculated in tryptic soy broth (TSB) (Liofilchem, Teramo, Italy) and incubated at 37 °C for 24 h under aerobic conditions. Two mL of the 24 h-cultural medium were inoculated in 100 mL of TSB and incubated at 37 °C for 24 h under aerobic conditions. Therefore, the bacterial suspension was subjected to an additional characterization using real-time PCR to confirm the presence of *S. mutans* and was titrated for the bacterial suspension preparation which was used in the present study. The bacterial suspension's titration was carried out using plates of TSA inoculated with serial 10-fold dilutions starting from 10^{-1} to 10^{-10}. Two plates were used for each dilution. The results were recorded after 48 h of incubation at 37 °C for 24 h under aerobic conditions. The final bacterial suspension for the assays in the present study was calibrated at 10^5 colonies forming unity/mL (CFU/mL).

For the isolation of *S. mutans* from the samples, tryptic soy agar (TSA) supplemented with 5% defibrinated sheep blood was used.

2.2. Biomolecular Analysis

The DNA from the samples was isolated using the Power Soil Kit (Qiagen, Milan Italy), following the protocol indicated by the supplier, and the *S. mutans* nucleic acid was detected by real-time PCR using the *S. mutans*-specific primers and probe following the protocol described by Yoshida and co-authors [28], with minor modifications. Briefly, the primer set included the following: Smut 3368-Forward (5′-GCCTACAGCTCAGAGATGCTATTCT-3′) and Smut 3481-Reverse (5′-GCCATACACCACTCATGAATTGA-3′) with the fluorescent probe Smut3423-T (5′-FAM-TGGAAATGACGGTCGCCGTTATGAA-TAMRA-3′), which are specific for the specie-specific gene gtfB. In particular, for each real-time PCR test, 20 µL of a mixture containing 1 µL of extracted DNA, 1 µL TaqMan Universal PCR Master Mix (Applied Biosystems), 500 nM (each) sense and antisense primer, and 2500 nM TaqMan probe was placed in each well of a 96-well plate. Amplification and detection were performed using the C100 Touch Thermal Cycler (Biorad, Segrate, Italy) with the following cycle protocol: 95 °C for 10 min, and 45 cycles of 95 °C for 15 s, and 58 °C for 1 min. The DNA of ATCC-700610 *S. mutans* strain as provided by LGC, Italy, was used as positive control, whilst sterile DNA-ase/RNA-ase free water (Qiagen, Milan, Italy) was used as the negative control.

2.3. Experimental Design

Twenty-eight experimental samples were prepared using sterile Eppendorf tubes 20 mm in length and filled with TSA for 10 mm (Figure 1A). The remaining space simulated mean depth of endodontic cavity access preparation from dental cusps to pulp chamber floor in molars, as reported in recent studies using Cone-beam computed tomography [29]. As temporary filling material was used Plastor© (Ghimas, Bologna, Italy), a premixed temporary filling material that contains mainly zinc oxide (40–50%), calcium sulphate (20–30%). As spacers were used sterile cotton pellets (Medirel, Agno, Switzerland) or PTFE pellets (TDV Dental, Pomerode, Brazil). Samples were divided in 7 groups (N = 4 vials/group): group A (Plastor©), group B (Plastor© + cotton pellet), group C

(Plastor© + cotton pellet soaked of m-cresyl acetate), group D (Plastor© + cotton pellet soaked of eugenol), group E (Plastor© + PTFE pellet), group F (positive control), and group G (negative control).

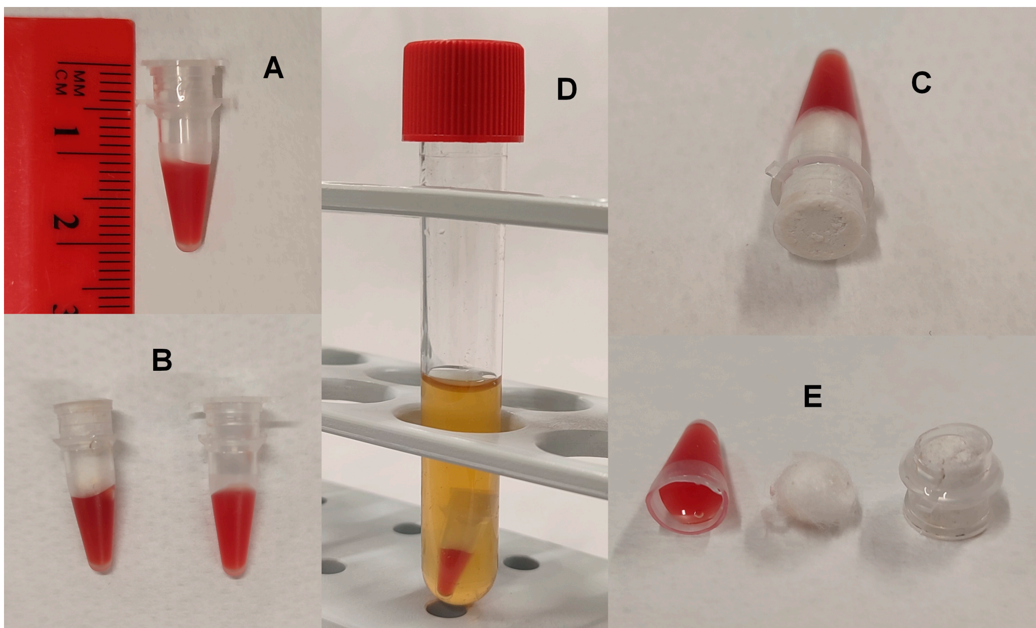

Figure 1. (**A**) Eppendorf tubes filled with Tryptic Soy Agar for 10 mm; (**B**) Eppendorf tubes filled with zinc oxide/calcium sulphate (**left**) and tubes filled with zinc oxide/calcium sulphate and spacer placed apically to restoration (**right**); (**D**) Eppendorf tube immersed in 5 mL of a suspension of *S. mutans* (10^5 CFU/mL) and then incubated at 37 °C for 5 and 10 days; (**C**) Zinc oxide/calcium sulphate filling material condensed and burnished; (**E**) Eppendorf tube cut to collect Agar.

Sterile spacers were gently immersed into the TCA, inside the Eppendorf tubes. In group C and D cotton pellets were soaked in 0.1 mL of m-cresyl acetate or eugenol immediately before the insertion. Plastor© was applied in a bulk technique in the access cavity, simulating a Class 1 cavity, with sterile instruments and subsequently condensed with a plugger to obtain a thickness at least of 5 mm (Figure 1B). The excess material was removed with a sterile cotton pellet lightly dampened with sterile saline to obtain a smooth surface. Subsequently, filling material was left in contact for 60 min with a sterile cotton pellet soaked with sterile saline solution to obtain its hardening (Figure 1C).

Preparation of the samples was aseptically performed by the same operator.

In the vials of group F (positive control) were filled with 20 µL of undiluted bacterial suspension of *S. mutans* before application of filling material, and in group G (negative control) were added 20 µL of sterile broth. All prepared vials were sterilized on the outer surface using a hypochlorite solution and were individually immersed in tubes containing 5 mL of a suspension of *S. mutans* (10^5 CFU/mL) previously prepared and then incubated at 37 °C (Figure 1D).

Two Eppendorf tubes were collected for each group, positive and negative control after 5 (T5) and 10 (T10) days of incubation. After disinfection of the external surface using 5.25% sodium hypochlorite, the vials were opened aseptically and their content was used for the detection and in vitro isolation of *S. mutans* by real time PCR and inoculation on TSA, respectively.

3. Results

3.1. Bacteriological Investigation

The preliminary assay for *S. mutans* cultivation allowed to obtain a bacterial suspension with a titer of 10^8 CFU/mL. The suspension was diluted with TSB (Liofilchem, Teramo,

Italy) to obtain a bacterial concentration of 10^5 CFU/mL used in the experimental study. The isolation test of *S. mutans* from agar samples collected from cutted Eppendorf tubes showed bacterial growth only with the Agar samples collected from the tubes of Group F (positive control). The bacterial colonies isolated in TSA supplemented with 5% sheep blood resulted Gram-positive and were confirmed as *S. mutans* using specific real-time PCR.

3.2. Molecular Analysis

The real-time PCR tests carried out on agar samples collected at T5 and T10 were negative for *S. mutans* on all the vials of groups from A to E and the ones of group G (negative control). The group F samples (positive control) were positive in RT-PCR with a Ct value of 22.50 and 19.00 at T5 and T10, respectively (Table 1).

Table 1. Detection of *S. mutans* by isolation (I) and real-time PCR (RT) in agar samples from Eppendorfs vials after 5 days (T5) and 10 days (T10) of incubation.

Groups	T5		T10	
	I	RT	I	RT
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	-	-	-	-
E	-	-	-	-
F	+	+ (22.50) *	+	+ (19.00) *
G	-	-	-	-

Legend: +: positive sample; -: negative sample. * In brackets the Ct values of RT.

4. Discussion

None of the groups except group F (positive control) presented bacterial infiltration during the time of our experiment. The use of Eppendorf tubes as experimental model of cavity access was considered to exclude possible communications present in natural teeth (exposed dentinal tubules, lateral canals, enamel/cementum cracks) that could determine false positive bacterial infiltrations. The complete immersion of Eppendorf tubes in a bacterial suspension of *S. mutans* was a condition not naturally observable in in vivo studies, considered to test the sealing ability of filling material and temporary restorations in simulated extreme experimental conditions. Agar culture medium was inserted in Eppendorf tubes to have a solid base to support spacer and filling materials and to facilitate the extraction of cells and genetic material for PCR and microbiological tests. Positive control demonstrated the accuracy of microbiological analysis and negative control excluded external contaminations during collection procedures of samples. Moreover, absence of *S. mutans* genome was confirmed through RT-PCR, in order to further support isolation results. In other words, we have planned to carry out also the real-time PCR assay which, as is well known, is able to detect the nucleic acids of pathogens. Therefore, even the presence of dead pathogens eventually passed through the restoration may be detected. Accordingly, the lack of isolation of *S. mutans* from the Agar samples of Eppendorf tubes was confirmed as the absence of microorganisms that passed through the filling material.

Streptococcus mutans is generally considered to be a major pathogen of human caries and is also involved in periodontal diseases. Moreover, *S. mutans* can migrate from periodontal pocket into endodontic system without clinically coronal leakages such as caries, traumatic tooth fractures, or old restorations. *S. mutans* strains are classified into serotypes c, e, f, and k on the basis of the biochemical composition of serotype-specific polysaccharides. From the literature, it is known that approximately 70–80% of strains found in the human oral cavity are classified as serotype c, followed by the serotype e (~20%), and the serotypes f and k (less than 5% each). The virulence of *S. mutans* as a dental pathogen mostly resides in its ability to adhere and form biofilms on tooth surfaces, produce large quantities of organic acids, and tolerate low pH and oxidative stress. The oral host tissue attachment

could be due to a group of glycotransferases, which act as a cell surface protein antigen, recognized to have a collagen-binding activity. The most described collagen-binding proteins (CBPs), described only in the *S. mutans* strains, are the Cnm and Cbm proteins. These act as adhesins, i.e., they have the ability to adhere to type I collagen, thus promoting the biofilm formation, the development, and the duration of the periodontal pathologies. In addition, *S. mutans* strains can became invasive, and the cnm gene is required for *S. mutans* adherence to endothelial and epithelial cells and intracellular invasion, also being an important virulence factor for systemic disease secondary to bacteremia such as endocarditis. It is well-known that *S. mutans* cnm-positive strains have the ability to invade the endothelial cells with a subsequent periodontal vascular damage, and the possibility to migrate in bloodstreams with potential life-threatening pathologies such as infective endocarditis or disseminating infections, especially in patients with pre-existing cardiovascular conditions or compromised immune system.

The distribution frequency of the strains carrying the cnm gene among oral isolates has been estimated to be approximately 10–20% and the detection of *S. mutans* in the oral sites has been subject of interest, not only due to its primary role in caries onset but also due to its association with extra-oral infections. In particular, the serotype k was the most recent serotype, and it was recognized to possess the most prominent being as the defect of the glucose side chain in serotype-specific rhamnose–glucose polymers, which is related to the highest incidence of this serotype in the cardiovascular specimens.

Moreover, know-how and technologies for bacteriological investigations and biomolecular analysis of this bacterial species were already available. For these reasons, *S. mutans* was chosen for this study as it represents an important oral pathogen for its virulence factors and ability to adhere on to solid surfaces, forming and sustaining a polysaccharide-encased biofilm.

This experimental model presents some limits as only one bacterial species has been evaluated, denying any possible interaction in the oral microbial community. This could generate different microbial dynamics in terms of the capacity of bacteria to infiltrate fillings.

The duration of the experiment of 10 days was considered as a reasonable time compared to clinical timing of appointments in a multi-visit approach endodontic treatment, in emergency pulpotomy procedures or in vital pulp therapies using calcium-silicate materials to attend setting time of bioactive material [2,7,18]. Calcium silicate materials offered an improvement in terms of timing, with only one appointment needed to perform it and another one after 72 h to control setting of the material and fill the rest of the canal, respect to apexification with $Ca(OH)_2$ that required changing of intracanal dressing every 2–3 months until the operator feels a barrier when probing the apex with an endodontic file [3]. Risk of infection of remaining parts of the pulp between the emergency pulpotomy and complete pulpectomy or incomplete canal instrumentation in endodontic treatment can be avoided with a temporary restoration that guarantee coronal sealing.

Presence of spacers apically to restoration do not interfere with sealing abilities of temporary filling material and no differences were observed using cotton pellets or PTFE pellets.

Because no mechanical loading was applied on restorations, this could be a limitation of the study and could explain the different observations of other authors on bacterial contamination between cotton pellets and PTFE used as spacers in temporary restorations. A correct mechanical loading influence stability and subsequent marginal adaption of restorative materials, and results of in vitro experiments could differ from in vivo observations [16,17]. Control of mechanical loading on temporary restoration should be evaluated especially in posterior teeth (molars and premolars), while in anterior teeth (canines and incisors) the cavity access was generally not influenced by occlusion and mandibular movements [30,31].

The use of chamber floor medicaments does not influence sealing ability or bacterial infiltration of temporary filling material and their use in some procedures should be recommended only for its antibacterial and/or analgesic properties. Chamber floor

medicaments can secondarily improve benefits of pulpotomy with analgesic effects and/or antibacterial properties with a minor contribution respect pulpotomy procedure [32]. It is well known that endodontic infections are mixed infections with complex floral interactions and medicaments effective against a single microorganism may not be effective against a mixed infection, resulting in a poor antibacterial effectiveness [21].

5. Conclusions

The experiment evidenced complete sealing ability of temporary filling material containing zinc oxide/calcium sulphate at 10 days of complete immersion in a bacterial suspension, simulating an extreme clinical condition. Use of spacers apically the restoration do not interfere with sealing properties of temporary filling material nor use of spacers soaked with eugenol or m-cresyl acetate used as chamber floor medicaments.

Even though mechanical loading was not a study-parameter, the experimental design sufficiently reproduces temporary restorations in teeth without occlusal contact points or occlusal reduction. Zinc oxide/calcium sulphate filling materials showed complete marginal seal with a mean thickness of 5 mm, which can be easily processed for endodontic cavity accesses [29]. Other advantage of these materials is their application in the cavity with bulk techniques, simpler and faster than application modes of other filling materials.

This in vitro study demonstrated that zinc oxide/calcium sulphate filling materials had complete marginal sealing ability at least of 10 days in Class 1 cavities without mechanical loading. Moreover, its sealing ability is not influenced by the presence of spacers.

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References

1. Križnar, I.; Seme, K.; Fidler, A. Bacterial Microleakage of Temporary Filling Materials Used for Endodontic Access Cavity Sealing. *J. Dent. Sci.* **2016**, *11*, 394–400. [CrossRef] [PubMed]
2. Staffoli, S.; Plotino, G.; Nunez Torrijos, B.; Grande, N.; Bossù, M.; Gambarini, G.; Polimeni, A. Regenerative Endodontic Procedures Using Contemporary Endodontic Materials. *Materials* **2019**, *12*, 908. [CrossRef] [PubMed]
3. Murray, P.E. Review of Guidance for the Selection of Regenerative Endodontics, Apexogenesis, Apexification, Pulpotomy, and Other Endodontic Treatments for Immature Permanent Teeth. *Int. Endod. J.* **2022**, 1–12. [CrossRef] [PubMed]
4. Buonavoglia, A.; Lauritano, D.; Perrone, D.; Ardito, F.; Troiano, G.; Dioguardi, M.; Candotto, V.; Silvestre, F.-J.; lo Muzio, L. Evaluation of Chemical-Physical Properties and Cytocompatibility of TheraCal LC. *J. Biol. Regul. Homeost. Agents* **2017**, *31*, 1–9.
5. Buonavoglia, A.; Latronico, F.; Greco, M.; D'Abramo, M.; Marinaro, M.; Mangini, F.; Corrente, M. Methicillin-Resistant Staphylococci Carriage in the Oral Cavity: A Study Conducted in Bari (Italy). *Oral Dis.* **2010**, *16*, 465–468. [CrossRef] [PubMed]
6. LAI, Y.; PAI, L.; CHEN, C. Marginal Leakage of Different Temporary Restorations in Standardized Complex Endodontic Access Preparations. *J. Endod.* **2007**, *33*, 875–878. [CrossRef] [PubMed]
7. Naoum, H.J.; Chandler, N.P. Temporization for Endodontics. *Int. Endod. J.* **2002**, *35*, 964–978. [CrossRef]
8. Troiano, G.; Perrone, D.; Dioguardi, M.; Buonavoglia, A.; Ardito, F.; lo Muzio, L. In Vitro Evaluation of the Cytotoxic Activity of Three Epoxy Resin-Based Endodontic Sealers. *Dent. Mater. J.* **2018**, *37*, 374–378. [CrossRef]
9. Abdellatif, D.; Amato, A.; Calapaj, M.; Pisano, M.; Iandolo, A. A Novel Modified Obturation Technique Using Biosealers: An Ex Vivo Study. *J. Conserv. Dent.* **2021**, *24*, 369. [CrossRef]
10. Khayat, A.; Lee, S.-J.; Torabinejad, M. Human Saliva Penetration of Coronally Unsealed Obturated Root Canals. *J. Endod.* **1993**, *19*, 458–461. [CrossRef]

11. Iandolo, A.; Amato, A.; Martina, S.; Abdel, L.D.; Pantaleo, G. Management of Severe Curvatures in Root Canal Treatment with the New Generation of Rotating Files Using a Safe and Predictable Protocol. *Open Dent. J.* **2020**, *14*, 421–425. [CrossRef]
12. Schwartz, R.S.; Fransman, R. Adhesive Dentistry and Endodontics: Materials, Clinical Strategies, and Procedures for Restoration of Access Cavities: A Review. *Tex Dent. J.* **2011**, *128*, 547–570. [CrossRef] [PubMed]
13. Çiftçi, A.; Vardarlı, D.A.; Sönmez, I.Ş. Coronal Microleakage of Four Endodontic Temporary Restorative Materials: An in Vitro Study. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2009**, *108*, e67–e70. [CrossRef] [PubMed]
14. Webber, R.T.; del Rio, C.E.; Brady, J.M.; Segall, R.O. Sealing Quality of a Temporary Filling Material. *Oral Surg. Oral Med. Oral Pathol.* **1978**, *46*, 123–130. [CrossRef]
15. Weston, C.H.; Barfield, R.D.; Ruby, J.D.; Litaker, M.S.; McNeal, S.F.; Eleazer, P.D. Comparison of Preparation Design and Material Thickness on Microbial Leakage through Cavit Using a Tooth Model System. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2008**, *105*, 530–535. [CrossRef] [PubMed]
16. Mathew, A. Microbial Contamination Comparison between Cotton Pellet and Polytetrafluoroethylene Tape Endodontic Spacers: A Systematic Review. *Eur. Endod. J.* **2021**, *6*, 143–150. [CrossRef] [PubMed]
17. Sattar, M.M.; Patel, M.; Alani, A. Clinical Applications of Polytetrafluoroethylene (PTFE) Tape in Restorative Dentistry. *Br. Dent. J.* **2017**, *222*, 151–158. [CrossRef]
18. Vail, M.M.; Steffel, C.L. Preference of Temporary Restorations and Spacers: A Survey of Diplomates of the American Board of Endodontists. *J. Endod.* **2006**, *32*, 513–515. [CrossRef]
19. Boutsiouki, C.; Frankenberger, R.; Krämer, N. Clinical and Radiographic Success of (Partial) Pulpotomy and Pulpectomy in Primary Teeth: A Systematic Review. *Eur. J. Paediatr. Dent.* **2021**, *22*, 273–285. [CrossRef]
20. Hasselgren, G.; Reit, C. Emergency Pulpotomy: Pain Relieving Effect with and without the Use of Sedative Dressings. *J. Endod.* **1989**, *15*, 254–256. [CrossRef]
21. Ohara, P.; Torabinejad, M.; Kettering, J.D. Antibacterial Effects of Various Endodontic Medicaments on Selected Anaerobic Bacteria. *J. Endod.* **1993**, *19*, 498–500. [CrossRef] [PubMed]
22. Buonavoglia, A.; Leone, P.; Solimando, A.G.; Fasano, R.; Malerba, E.; Prete, M.; Corrente, M.; Prati, C.; Vacca, A.; Racanelli, V. Antibiotics or No Antibiotics, That Is the Question: An Update on Efficient and Effective Use of Antibiotics in Dental Practice. *Antibiotics* **2021**, *10*, 550. [CrossRef] [PubMed]
23. Song, H.; Lei, Y.; Xing, Z.; Liu, M. Minocycline plus Zinc Oxide Eugenol Cement Might Be A Promising Alternative for Acute Pulpitis. *Evid.-Based Complement. Altern. Med.* **2022**, *2022*, 4653602. [CrossRef] [PubMed]
24. Schilder, H.; Amsterdam, M. Inflammatory Potential of Root Canal Medicaments. *Oral Surg. Oral Med. Oral Pathol.* **1959**, *12*, 211–221. [CrossRef] [PubMed]
25. Gandolfi, M.G.; Iacono, F.; Agee, K.; Siboni, F.; Tay, F.; Pashley, D.H.; Prati, C. Setting Time and Expansion in Different Soaking Media of Experimental Accelerated Calcium-Silicate Cements and ProRoot MTA. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2009**, *108*, e39–e45. [CrossRef]
26. Zanza, A.; Reda, R.; Vannettelli, E.; Donfrancesco, O.; Relucenti, M.; Bhandi, S.; Patil, S.; Mehta, D.; Krithikadatta, J.; Testarelli, L. The Influence of Thermomechanical Compaction on the Marginal Adaptation of 4 Different Hydraulic Sealers: A Comparative Ex Vivo Study. *J. Compos. Sci.* **2023**, *7*, 10. [CrossRef]
27. Ossiboff, R.J.; Sheh, A.; Shotton, J.; Pesavento, P.A.; Parker, J.S.L. Feline Caliciviruses (FCVs) Isolated from Cats with Virulent Systemic Disease Possess in Vitro Phenotypes Distinct from Those of Other FCV Isolates. *J. Gen. Virol.* **2007**, *88*, 506–517. [CrossRef]
28. Yoshida, A.; Suzuki, N.; Nakano, Y.; Kawada, M.; Oho, T.; Koga, T. Development of a 5′ Nuclease-Based Real-Time PCR Assay for Quantitative Detection of Cariogenic Dental Pathogens *Streptococcus mutans* and *Streptococcus sobrinus*. *J. Clin. Microbiol.* **2003**, *41*, 4438–4441. [CrossRef]
29. Azim, A.A.; Azim, K.A.; Deutsch, A.S.; Huang, G.T.-J. Acquisition of Anatomic Parameters Concerning Molar Pulp Chamber Landmarks Using Cone-Beam Computed Tomography. *J. Endod.* **2014**, *40*, 1298–1302. [CrossRef]
30. Katona, T.R.; Eckert, G.J. The Mechanics of Dental Occlusion and Disclusion. *Clin. Biomech.* **2017**, *50*, 84–91. [CrossRef]
31. Kurokawa, M.; Kanzaki, H.; Tokiwa, H.; Handa, H.; Nakaoka, K.; Hamada, Y.; Kato, H.; Nakamura, Y. The Main Occluding Area in Normal Occlusion and Mandibular Prognathism. *Angle Orthod.* **2016**, *86*, 87–93. [CrossRef] [PubMed]
32. Gavriil, D.; Kakka, A.; Myers, P.; O'Connor, C.J. Pre-Endodontic Restoration of Structurally Compromised Teeth: Current Concepts. *Br. Dent. J.* **2021**, *231*, 343–349. [CrossRef] [PubMed]

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