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ORIGINAL ARTICLE

# Infiltration by *Streptococcus mutans* in provisional crowns fixed by two types of cementing agents – pilot double-blind randomized clinical trial

# Infiltração por *Streptococcus mutans* em coroas dentárias provisórias fixadas por dois tipos de agentes cimentantes – estudo clínico randomizado duplo-cego piloto

Ana Luiza Aquino Rego[1], Vanessa Anielly Cruz Moura[1], Janaína Câncio de Holanda Prado[2], Francisca Jamila Ricarte Alexandrino[3], Romulo Rocha Regis[4], Karina Matthes de Freitas Pontes[4]

1. Dentistry and Nursing College, Federal University of Ceará (UFC), Fortaleza, Brazil. 2. Master's Degree in Dental Clinic by the Postgraduate Program in Dentistry Pharmacy, Dentistry and Nursing College, Federal University of Ceará (UFC), Fortaleza, Brazil. 3. Professor, Paulo Picanço School of Dentistry, Fortaleza, Brazil. 4. Professor, Department of Restorative Dentistry, Postgraduate Program in Dentistry, Pharmacy, Dentistry and Nursing College, Federal University of Ceará (UFC), Fortaleza, Brazil.

## Abstract

**Objective:** When provisional acrylic crowns are used for a long time, they become more susceptible to marginal leakage by cariogenic bacteria. The objectives of this pilot clinical study were to compare cement based on zinc oxide-eugenol and calcium hydroxide by contamination with *Streptococcus mutans*, and calculate the sample size for the continuation of this study. **Methods:** Individuals receiving provisional crowns and following inclusion/exclusion criteria, were randomly distributed into 2 groups: zinc oxide-eugenol (n=8); calcium hydroxide (n=9). The temporary crowns were made by a blind researcher and cemented by another. Patients were also blinded by the cement used inside their crowns. After 2 months, a cement sample from the crowns' peripheral inner face was collected, placed in a tube containing 1 mL of sterile saline, serially diluted, plated on Mitis Salivarius Bacitracin agar, and incubated for 48 hours. Colony-forming units (CFU/mL) were counted. A statistical power analysis was performed to calculate sample size (1-β=80%) and the Mann Whitney test to compare both cements (α=0.05). **Results**: Both cements were contaminated with *S. mutans*, with an average of 166.6 x 102 CFU/mL for calcium hydroxide and 435.3 x 102 CFU/mL for zinc oxide-eugenol, with no significant difference (p=0.311). The sample size calculated for this study was 36 per group. **Conclusion**: This pilot study suggests that there is important contamination inside provisional crowns used for two months, independent of the cement. The continuation of this study is needed, with a bigger sample size, to enable a comparison between the cements.

**Keywords:** Interim dental prosthesis; Fixed prosthesis. *Streptococcus mutans*; Calcium hydroxide; Zinc oxide-eugenol cement.

## Resumo

**Objetivo**: Quando coroas dentais provisórias são utilizadas por um longo período, elas se tornam susceptíveis à infiltração marginal por bactérias cariogênicas. O objetivo deste estudo clínico piloto foi comparar os cimentos a base de óxido de zinco e eugenol e hidróxido de cálcio pela contaminação com *Streptococcus mutans* e calcular o tamanho amostral para continuação deste estudo. **Métodos:** Indivíduos recebendo coroas provisórias e seguindo critérios de inclusão/exclusão, foram distribuídos aleatoriamente em dois grupos: óxido de zinco e eugenol (n=8); hidróxido de cálcio (n=9). As coroas provisórias foram feitas por um pesquisador cego e cimentadas por outro. Os pacientes também foram cegos quanto ao cimento utilizado dentro de suas coroas. Depois de 2 meses, amostras de cimentos foram coletadas da face interna periférica das coroas, colocadas em um tubo contendo 1 mL de solução salina estéril, diluídas de forma seriada, plaqueadas em ágar Mitis Salivarius Bacitracina e incubadas por 48 horas. Unidades formadoras de colônias (UFC/mL) foram contadas. Um teste de poder estatístico foi realizado para calcular o tamanho amostral (1-β=80%) e o teste de Mann Whitney para comparar os dois cimentos (α=0.05). **Resultados**: Os dois cimentos foram contaminados com *S. mutans*, com uma média de 166.6 x 102 UFC/mL para o hidróxido de cálcio e 435.3 x 102 UFC/mL para o óxido de zinco e eugenol, com nenhuma diferença significativa (p=0.311). O cálculo amostral para este estudo foi 36 indivíduos por grupo. **Conclusão**: Este estudo piloto sugere que existe importante contaminação dentro de coroas provisórias utilizadas por 2 meses, independente do cimento. A continuação deste estudo é necessária, com maior tamanho amostral, para possibilitar a comparação entre os cimentos.

**Palavras-chave:** Próteses dentárias provisórias; Prótese fixa; *Streptococcus mutans*; Hidróxido de cálcio; Cimento de óxido de zinco e eugenol.

## INTRODUCTION

For a fixed prosthetic treatment to be biologically acceptable, it requires that the teeth, after proper preparation, are protected and stabilized by a provisional crown up to the permanent prosthesis installation, acting as a predictable determinant of form, occlusion, and aesthetics[1,2,3,4].

Besides having diagnostic importance, providing necessary information to the dentist about selective wear and tear of dental preparation, provisional crowns avoid damages with negative impact in the final restoration, such as pain, premature contact, and dental movement, thus becoming a necessary

**Correspondence:** Karina Matthes de Freitas Pontes. Department of Restorative Dentistry, Federal University of Ceará, Fortaleza, Brazil. E-mail: karinapontes@ufc.br
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and primordial stage for the clinical success of fixed prosthesis treatment[5,6,7,8,9,10].

Regardless of the method of preparation or fit accuracy, prosthetic crowns must be sealed with a luting agent, which usually has weak retentive properties to allow future planned removal of the restoration[11,12]. Although programmed to be used for a transitional period, there are situations in which long-term use may occur[7]. When this happens, they become more susceptible to cement solubility, marginal infiltration, bacterial infiltration, and caries[1]. Thus, the luting agents must provide good retention, mechanical and antimicrobial properties for the success of provisional crowns over a longer period[1].

The disintegration of cements through their decomposition or dissolution in oral fluids and the decrease of shear bond strength between them and dentin or between cement and restoration are reported as possible causes of marginal leakage[13]. Also, the occlusal forces to which the restoration is subjected after installation, as well as the temperature changes inside the mouth, can favor the microleakage[14].

In this way, the marginal adaptation accuracy of a provisional crown must be respected, between 50 µm to 120 µm[15,16] and an appropriate provisional cement must be chosen.[17]. Studies report that, among the various factors responsible for the quality of the seal and marginal retention, the characteristics of the cements, which allow intimate contact between the restorations and the surfaces of the prepared teeth, deserve a special focus[18].

Due to a large number of available cements, proper selection can be a difficult task and is usually based on the experience and preference of the dental surgeon, not considering a detailed knowledge of the materials[19]. However, none of the cementing agents available today fulfill all the requirements to be considered ideal for any clinical situation[20]. Therefore, the choice of the luting agent should be based, if possible, on marginal sealing ability and antibacterial action[21].

Zinc oxide-eugenol-based cements have the good sealing capacity, as well as resistance, and satisfactory antibacterial activity[21] Regarding the properties of calcium hydroxide-based cement, it presents low resistance and high solubility in many cases, besides they are not able to provide an effective long-term sealing when there is bacterial infiltration[22]. Provisional cements with antibacterial properties are powerful antimicrobial agents which can maintain control of the biofilm in the marginal space, preventing initial colonization of cariogenic bacteria[23].

Currently, some in vitro studies emphasize the comparison of marginal infiltration in provisional crowns made with different types of resins and different techniques, the resistance of temporary eugenol-containing and eugenol-free provisional cements, in addition to studies about the activity of cements modified with zinc-oxide nanoparticles against *Streptococcus mutans*[23,24,25]. However, no clinical studies were found about the microleakage of provisional crowns. The lack of clinical trials about this subject prevents decision-making based on scientific evidence.

For this reason, we intended to conduct a clinical trial comparing two cements under provisional crowns in active use by patients, to obtain real results, with the variations which occur within the oral cavity, such as temperature, ph, chewing forces, eating habits, parafunctional habits, presence of saliva, hygiene level, etc. These variations are not present on in vitro assays. So, the objective of this preliminary clinical study was to compare calcium hydroxide-based and zinc oxide-eugenol cements used for cementation of provisional prosthetic crowns, as to their contamination by marginal infiltration of *Streptococcus mutans* after two months in function, testing the hypothesis that there would be no difference between the cements. Once this is a pilot study, the other purpose was the sample size calculation.

## MATERIALS AND METHODS

### Ethical aspects

This study was conducted following Helsinque Declaration standards and had its project approved by the Committee of Ethics in Research of the Federal University of Ceará (COMEPE), with a document number 224/12, COMEPE protocol number 114/12.

### Trial design

This is a pilot double-blind randomized clinical trial with two parallel groups, conducted in Brazil.

### Participants

Patients over 18 years old, of both genders, who attended the Federal University of Ceará, seeking dental prosthesis, were recruited for this research.

Sixty-four patients were screened, of which 42 met the inclusion criteria. The inclusion criteria were: patients should have an anterior or posterior dental element with an indication of a total single crown; the dental element in question should present satisfactory endodontic treatment; the teeth should allow a cervical preparation term respecting periodontal biological space, being restricted to 0.5 mm subgingival; patient must have periodontal health, with a good level of oral hygiene and low bacterial plaque index. Patients framed in the inclusion criteria and who agreed to the treatment, read and signed the Informed Consent Form.

Exclusion criteria were: patients who reported allergy or sensitivity to acrylic resin components; dental elements with an indication for endodontic re-treatment or extraction; patients with a high index of bacterial plaque or dental calculus; patients with periodontal infections, presenting bleeding and/or pouches with a depth of probing greater than 3 mm; smoking

patients or those who refused to wait up to two months for the preparation of a definitive prosthetic crown.

The withdrawal criteria were: patients who did not appear within two months after cementing the provisional crown for the collection of cement and microbiological analysis; patients whose provisional crowns were released before the date set for collection; patients who presented periodontal problems involving the treated tooth within the waiting period.

### Interventions

Each selected patient underwent a periapical radiographic examination of the dental element that required the provisional crown, applying the bisecting technique. In cases where the radiographed tooth presented adequate endodontic treatment, the coronary dental remnant was prepared with diamond tips nos. 1014, 3216, 2200, 4138 (KG Sorensen), making a cervical notch across its perimeter at a subgingival level (0.5 mm). Parts of a tooth with unsupported enamel or dentin with a thickness of less than 1 mm have been removed by wear. The main root canal had the filling materials removed in 2/3 of its length, using a heated Rhein tip (Golgran). Then a new radiograph was taken to check the filling removal. Then, drills were used (Dentsply, Maillefer), with a diameter corresponding to the conduit, to regularize its walls and to widen its diameter.

After the dental preparation was completed, the provisional crown was prepared. All these crowns were prepared by two previously calibrated operators who did not know which cement would be used in each patient.

An orthodontic wire number 0.5 (Morelli) was adapted to the prepared root canal, relubricated with self-curing acrylic resin (Dencor, Classic) to be subsequently attached to the provisional crown, thus ensuring intra-radicular retention of the restoration.

For posterior teeth, the functional sculpture technique was employed, using a poly (methyl-methacrylate) - PMMA – auto polymerizing acrylic resin (Dencor, Classic) in the previously selected color. This technique consisted of the manipulation of a portion of acrylic resin, following the powder/liquid proportions recommended by the manufacturer, using a spatula 7 (Golgran) in a glass jar with a lid. Upon reaching the plastic phase of its polymerization, the resin was taken and accommodated to the preparation. The operator requested that the patient occluded on the resin and made an opening, closing, and excursive movements to pre-sculpt the provisional crown. After the polymerization was finished, the surplus of resin has been removed with tungsten carbide drill ref. 1251 (Edenta) in low rotation and the occlusal sculpture was finalized with a ref. 1574 drill (Edenta), also in low rotation. The occlusal contacts were verified for removal of any premature contact, also the interproximal contacts. Marginal adaptation, retention, and stability were checked, and, where necessary, it was made a cervical reline. Finally, the polishing was performed using silicone rubbers impregnated with aluminum oxide in three different granulations, refs. 0679PM, 0669HP, 0659HP (Exa-Technique, Edenta).

For anterior teeth, a stock tooth was used (Vipi Dent), whose vestibular facet was adapted to the abutment tooth and the palatal face of the crown was carved with self-curing acrylic resin. The criteria for occlusal and proximal contacts, marginal adaptation, retention, stability, and finishing were also verified and corrected, using the same materials mentioned for the posterior teeth.

The operators who participated in this stage were unaware of which group each patient belonged to. The patients were also unaware of the type of cement that would be used to fix their provisional crown. A third operator, not involved in examining the patient and making the provisional crowns, carried out the cementation procedures, following the random distribution of the patients in the groups.

The patients who met the inclusion criteria were randomly distributed into two groups, according to the cement that would be used in fixing their provisional crowns. In one group it was used the calcium hydroxide cement (Liner, Vigodent), and in the other, the zinc oxide and eugenol cement was applied (Provy, Dentsply).

The cementation of the provisional crowns followed a protocol initiated by the cleaning of the provisional crown and the prepared tooth with a jet of distilled water, followed by the relative insulation of the prepared tooth and air-jet drying. Then it was made the manipulation of the selected dental cement, according to the manufacturer's instructions, using the spatula 22 (Duflex, SSWhite) and sterile glass plate. The manipulated cement was placed with a thin layer over the entire inner surface of the provisional crown, including its margins, then the provisional crown was inserted into the prepared tooth until complete adaptation, awaiting the cement fixation for the removal of the surplus with an exploratory probe.

Patients were advised of the care they should have while using the provisional crowns, communicating to researchers any problems that occurred during the three months of waiting, such as fractures or loosening.

After two months of cementation, patients were called to have their temporary crowns removed and for collection of cement sample, but of these, only 17 patients remained in the study, as 1 patient gave up, 2 patients missed the consultation and 22 patients dropped the provisional ones before two months. After the collection, the crowns were cemented again, and the patients were referred for completion of the prosthetic treatment.

The operator who cemented the provisional crowns was responsible for removing them. The removal consisted of the use of a spatula number 7 (Golgran) adapted to the vestibular cervical margin of the provisional crown, being careful not to

cause trauma to the marginal gingival tissue.

The removed crown was handled cautiously so that there was no contamination of its inner surface. The cement collection was made with a sterile dentin spoon number 5 (Duflex, SSWhite), making a single scrapping movement across the inner perimeter of the crown, parallel and 0.5mm distant from the cervical margin.

The collected cement was packed in a sterile tube, containing 1 mL of sodium chloride P.A. solution (Vetec). Next, the microbiological analysis was performed by a fourth operator, without knowledge of the cement used, in laminar flow. The suspensions containing the cements used were homogenized in vortex-type apparatus (AP 56). After homogenization, 10 microliters of each suspension were collected and plated in Mitis Salivarius Bacitracin agar culture (MicroMed) for growth assay of *Streptococcus mutans*.

The seeded plates were incubated at 37˚C, for 48 hours, in a heating chamber with 5% of $CO^2$. Finally, the colony-forming units (CFU/mL) were counted in each plate. Data were tabulated for statistical analysis.

### Outcomes

The outcome was the mean of CFU/mL of *S. mutans* from each collected cement.

### Sample size

The data were collected using the BioEstat 5.3 software (Mamirauá Sustainable Development Institute), in which it was concluded that the sample size for each group should be 36 patients, for an 80% power (1-β = 80%).

### Randomization

For the allocation of the participants, a computer-generated list of random numbers was used. Participants were randomly assigned following simple randomization procedures to 1 of 2 treatment groups.

### Blinding

Patients, clinical operators (who made the provisional crowns), microbiological operators, and statistical researchers were blinded. Only one researcher, who cemented the crowns, was not blinded.

### Statistical methods

Descriptive statistical analysis and the Mann Whitney test using GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla) were used for comparison of the groups (α = 0.05).

## RESULTS

Following the withdrawal criteria, 25 patients were lost during the study: 22 were due to the release of the provisional crown before two months, 12 of which had been cemented with calcium hydroxide and 10 with cement containing zinc oxide-eugenol; three patients did not come to a 2-month returning session. Seventeen patients remained in the study, so 17 teeth were restored: 8 had their provisional crowns cemented with zinc oxide-eugenol (5 anterior teeth and 3 premolars) and 9 had it cemented with calcium hydroxide (5 anterior teeth and 4 premolars).

Both cements showed contamination through marginal infiltration after two months of function. In the samples, calcium hydroxide cement had a minimum of 17 x 102 CFU/mL of *S. mutans* and a maximum of 1056 x 102 CFU/mL, having an arithmetic mean of 166.6 x 102 CFU/mL and a standard deviation of 337.6 x 102 CFU/mL, while zinc oxide and eugenol cement presented a minimum of 4 x 102 CFU/mL of *S. mutans* and a maximum of 1680 x 102 CFU/mL, having an arithmetic mean of 435.3 x 102 CFU/mL and a standard deviation of 592.1 x 102 CFU/mL, but statistically, this difference was not significant (p = 0.3117). Complete data and statistical analysis are presented in tables 1, 2, and 3.

**Table 1.** CFU/mL (x102) individual counting in the groups calcium hydroxide (HC) and zinc oxide-eugenol (OZE).

| Patient | Hc | Patient | Oze |
|---|---|---|---|
| 1 | 56. | 1 | 390. |
| 2 | 21. | 2 | 85. |
| 3 | 184. | 3 | 132. |
| 4 | 66. | 4 | 1680. |
| 5 | 17. | 5 | 4. |
| 6 | 56. | 6 | 221. |
| 7 | 22. | 7 | 960. |
| 8 | 21. | 8 | 10. |
| 9 | 1056. | | |

Table 2. Descriptive statistical analysis (x102 CFU/mL).

| | HC | OZE |
|---|---|---|
| Number of values | 9 | 8 |
| Minimum | 17.00 | 4.000 |
| 25% Percentile | 21.00 | 28.75 |
| Median | 56.00 | 176.5 |
| 75% Percentile | 125.0 | 817.5 |
| Maximum | 1056 | 1680 |
| Mean | 166.6 | 435.3 |

| | HC | OZE |
|---|---|---|
| Std. Deviation | 337.6 | 592.1 |
| Std. Error | 112.5 | 209.3 |
| Lower 95% CI of mean | -92.92 | -59.77 |
| Upper 95% CI of mean | 426.0 | 930.3 |
| D'Agostino & Pearson omnibus normality test | | |
| K2 | 24.09 | 6.989 |
| P value | < 0.0001 | 0.0304 |
| Passed normality test (alpha=0.05)? | No | No |
| P value summary | *** | * |
| Coefficient of variation | 202.67% | 136.04% |
| Sum | 1499 | 3482 |

**Legend:** HC= calcium hydroxide; OZE= zinc oxide-eugenol

**Table 3.** Statistical analysis, with Mann Whitney test (α=0.05).

| **P value** | **0.3117** |
|---|---|
| Exact or approximate P value? | Gaussian Approximation |
| P value summary | ns |
| Are medians signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| Sum of ranks in column A,B | 70 , 83 |
| Mann-Whitney U | 25.00 |

## DISCUSSION

This was a pioneering clinical trial, which verified the bacterial infiltration by *Streptococcus mutans* of cementing agents taken directly from the inside of provisional prosthetic crowns of patients. All previous similar studies were in vitro assays and all agree that clinical research is required to confirm laboratory findings.

In the samples, contamination was observed by marginal infiltration of *S. mutans* in both cements, confirming the null hypothesis that there would be no difference between them, which corroborates with the in vitro study of Bonecker et al., 2010[17], in which a comparison of coronary infiltration by dye exposure was made in provisional cemented prostheses with two different types of temporary luting agents: one based on calcium hydroxide and another one based on zinc oxide-eugenol. In the aforementioned study, no significant differences were found between the two cements, implying that both have the similar marginal sealing abilities.

However, as these cementing agents have different fixation mechanisms, different reactions to humidity, and variable dimensional stability, there is a tendency for them to produce different marginal sealing abilities[26]. However, according to what was observed in this study, although the two cements used had different sealing qualities, this difference was not significant to indicate the best one to be used in the longer term.

On the other hand, according to the results of the study by Arora et al., 2016[24], it was observed that provisional crowns cemented with zinc oxide-eugenol cements presented a greater infiltration, and the same study concluded that a possible explanation for this is due to the fact that this cement has a high film thickness, which results in inadequate settling of the provisional crowns and, therefore, increases the chances of micro infiltration.

Due to the high thickness film formation of eugenol-based cements, Yu et al., 2014[27], recommended its limited use in clinical practice, as there may be larger amounts of water absorption, which results in the reduction of mechanical properties, resulting in micro infiltration.

However, Andrade et al., 2018[23], found that a temporary cement modified with zinc oxide nanoparticles exhibited antibacterial activity when in contact with *Streptococcus mutans*.

In this study, a period of two months was considered for the analysis of the cements, and it was possible to verify that this period provided an infiltration of *S. mutans*, serving as a warning to dental surgeons and patients for contraindication of prolonged use of temporary crowns. If the evaluation had been done weekly or fortnightly after cementation, perhaps we would have observed some antimicrobial action of these cements.

Nanda et al., 2015[28] have studied samples of saliva and plaque, confirming that *Streptococcus mutans* was determined as the main species of bacteria that cause dental caries, since the high count of this microorganism is related to the high occurrence of this disease. In our study, it was possible to observe that the count was quite high in both cements for a considerable part of the patients, which leads us to imagine that, if these temporary crowns remained longer, there could be an incidence of caries, thus damaging the remaining tooth.

An important feature in providing a suitable seal is the solubility of the cement – it can be considered an important cause of failure, thus contributing to the occurrence of caries, in addition to the recurrent loss of retention[18]. In 2009, Francisconi et al[22]. carried out a study in which the solubility of different calcium hydroxide cements was evaluated, and it was observed that there were significant differences among all of them, with some brands of calcium hydroxide-based cements being more soluble than others. In addition, they state that they are not capable of providing an effective long-term seal when bacterial infiltration is present. In this study, although a tendency to greater contamination by *S. mutans* in the zinc oxide-eugenol cement was observed, both cements were also responsible for the early release of the provisional crowns of the 22 withdrawn patients.

More concrete data are needed regarding the provisional cementation of prosthetic crowns, evaluating the retention force and the influence of the time factor on each cement, and whether this factor should be taken into account when choosing the type of temporary cement. In this study, after two months, it was possible to have a bacterial infiltration index in both cements, but it was not possible to determine which one had greater resistance to microleakage.

Another question is about how much the materials and techniques used to make the provisional dental prosthesis can influence the marginal microleakage. In this trial, direct techniques with PMMA autopolymerizing acrylic resin were used. Direct technique is important because it is done in a single session and has a low cost for patients and institutes. John, Muthukumar, and Vasantha Kumar (2015)[29] observed that PMMA provisional crowns made by direct technique presented less amount of microleakage than those fabricated by indirect technique. Naqash, Alfarsi, and Hussain (2019)[30] verified that provisional crowns made by PMMA with the direct technique have a vertical marginal gap of 103µm, which is clinically acceptable[15,16].

This study was performed in a standardized way and respecting the blinding of all the operators and patients involved. However, it presented some limitations that had repercussions on the sample size. The main limiting factor was the release of provisional crowns before the stipulated deadline. This is the reason why this was considered as a pilot or preliminary research. On the other hand, we did not find other clinical studies about this subject, which makes this work very important. It is suggested, therefore, the possibility of its continuation, in order to reach the ideal n. The calculation of sample size estimated 36 volunteers per group, in order to reach a statistical power of 80%.

More clinical trials should be carried out with the purpose to search for a material that best corresponds to the expectations of cementation. Combinations of temporary luting agents, with other resins and techniques of making an interim prosthesis, using shorter time analysis and bacteria involved in periodontal diseases, may be objectives of future studies.

## CONCLUSION

Within the limits of the present pilot clinical study and according to the obtained results, it can be concluded that, after two months in function, the provisional crowns had an important marginal infiltration by *Streptococcus mutans*, with both cements. The continuation of this study is necessary, with bigger sample size, in order to enable a reliable comparison between the cements.

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