

Antimicrobial activity of a temporary sealant used in endodontic treatment: An *in vitro* study

Helena Baruffaldi Domingos¹, Lucio Souza Gonçalves¹, Milton de Uzeda¹

Correspondence: Dr. Milton de Uzeda
Email: miltonuzeda@terra.com.br

¹Department of Endodontics, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil

ABSTRACT

Objective: The present study is aimed to evaluate the antimicrobial action of Coltisol® in direct contact with human saliva. **Materials and Methods:** Twelve different individuals were selected. Saliva samples were evaluated at four different time periods: Baseline 1 (T1-initial control), T2 (2 h), T4 (24 h after contact with a standardized sample of a coronary sealer) and baseline 2 (T3-final control). Seeded plates were incubated at 37°C in a bacterial incubator for a period of 48–72 h. After the incubation period, the colony forming units were counted, and the results compared. **Results:** Differences were statistically significant. There was an inhibition of bacterial growth after the first 2 h of contact and an increase in the number of bacteria after 24 h of direct contact between the material and the saliva. Coltisol® presented bacterial growth inhibition in direct contact with saliva. This inhibitory effect tended to decrease over time, as shown by the two periods when the material was in contact with different samples of saliva. **Conclusions:** The antimicrobial activity of the material is an important feature; however, other physical and chemical properties of the coronary temporary sealer should be considered.

Key words: Antimicrobial, dental materials, saliva

INTRODUCTION

Saliva has important characteristics that can prevent microbial colonization in the oral cavity. However, some bacteria are resistant to the immune system and remain in the oral environment.^[1] Faulty apical sealings have been identified as the main cause of failures in endodontic treatment, and various studies have emphasized the importance of an adequate coronal sealing between sessions for successful endodontic therapy.^[2-4]

Microorganisms play a critical role in pulpal and periradicular diseases, whereas the aim of endodontic treatment is to maintain a healthy balance in the oral cavity. In order to avoid recontamination during endodontic treatment, a suitable sealing of the root canal and crown of the tooth is fundamental to prevent any contamination with the oral microbiota.^[2,3,5-7] Root canals are commonly filled with a temporary dressing between endodontic therapy sessions. This procedure aims to eliminate and prevent the proliferation of bacteria in the root canals. This dressing also works as a physical and chemical barrier against infection or re-infection of the root canal system by microorganisms present in

saliva in case of microleakage, fracture or loss of the temporary seal (of the tooth structure).^[8-10]

At the end of the endodontic treatment, a final sealing of the coronal portion of the restoration is necessary, since filled root canals in direct contact with saliva can be easily contaminated by solubilization of the sealer and the permeability of the filling.^[3,4,8,9] The bacterial penetration between the tooth and the restoration can be caused by microorganisms that colonize the tooth crown and invade the interface via saliva. The sealing material, however, can in turn provide antimicrobial activity, allowing the reduction or elimination of microorganisms remaining in the

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cavity or that penetrated through microleakages in the coronary sealer.^[9-12] The choice of Coltoso[®] for this study was based on results obtained by the study developed *in vitro* by Grillo, *et al.*,^[13] in which the antimicrobial activity of six coronary temporary sealing materials with different compositions was evaluated by the agar diffusion method inoculated with human saliva. In this research, all materials tested showed some antimicrobial activity against salivary microorganisms, and Coltoso[®] gave the best results.

It is worth noting the importance of *in vitro* testing of antimicrobial activity of these sealing materials in direct contact with human saliva considering their continuous action over a period of time. This procedure simulates what takes place in the oral cavity of the patient during endodontic treatment. Therefore, this study is aimed to evaluate the antimicrobial action of Coltoso[®] in direct contact with human saliva from 12 different individuals over four different time periods.

MATERIALS AND METHODS

The material tested in this study was Coltoso[®] (Vigodent, Rio de Janeiro, Rio de Janeiro, Brazil) based on the results revealed in the research conducted by Grillo, *et al.*^[13] The product consists of zinc oxide, hydrated zinc sulfate, calcium sulfate hydrous, diatomaceous earth, dibutyl phthalate copolymer and polyvinyl chloride.^[7]

This study consisted of a direct contact test in the culture broth, held in microdilution plates with 24 wells (Tissue Culture Test Plates 24[®] TPP, Switzerland). Each well contained: 2.0 mL of trypticase soy broth (Becton, Dickinson and Company, NJ, USA) and a portion of Coltoso[®], prepared by making a tablet in a metal mold (1 mm deep and 5 mm internal diameter). Inoculums consisted of 0.1 mL of stimulated human saliva, collected from 12 volunteers in sterile universal bottles, which were used and tested separately at different times, namely: Baseline 1 (T1-initial control), T2 (2 h), T4 (24 h after contact with standardized sample of coronary sealer) and baseline 2 (T3-final control) in duplicate. Sterile controls of Coltoso[®] tablet were also carried out in duplicate for each experiment.

After each contact time, 0.1 mL of inoculated broth was transferred to a Petri dish containing blood agar plates, prepared with tryptic soy agar (Becton, Dickinson and Company, NJ, USA) with the addition of 5% defibrinated sheep blood, and then evenly

spread across the dish surface with a Drigalski glass spatula. The seeded plates were incubated at 37°C in a bacterial incubator for a period of 48–72 h. After the incubation period, the count of colony forming units (CFUs) and comparison of the results were carried out.

The research project was previously submitted and approved by the Human Research Ethics Committee of Universidade Estácio de Sá (Rio de Janeiro, RJ, Brazil) and a term of consent was presented to all volunteers to sign.

Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Sciences software, version 19.0 (IBM, São Paulo, SP, Brazil). The Generalized Linear Model (GLM) for repeated measures was used to compare the mean values of the bacterial counts (CFU/mL) over the three time periods analyzed (baseline 1 [T1], 2 h [T2] and 24 h [T4]). Statistically significant differences in paired comparisons over time were also analyzed by the GLM method for repeated measures adjusted for multiple comparisons by the Bonferroni test. The Student's *t*-test for independent data was used to assess significant differences of the mean values of the bacterial count between T4 and T3 (after 24 h with and without Coltoso[®], respectively). The level of significance for all analysis was 5%.

RESULTS

Table 1 shows the number of CFUs.

Table 2 shows the mean values of bacterial count (CFU/mL) at baseline 1 (T1) after 2 h (T2) and

Table 1: Number of CFUs observed in cultures on blood agar

Samples	T1	T2	T3	T4
Volunteer 1	230×10 ⁴	60×10 ⁴	500×10 ⁴	500×10 ⁴
Volunteer 2	60×10 ⁴	60×10 ⁴	500×10 ⁴	500×10 ⁴
Volunteer 3	65×10 ⁴	100×10 ⁴	750×10 ⁴	1500×10 ⁴
Volunteer 4	40×10 ⁴	20×10 ⁴	800×10 ⁴	75×10 ⁴
Volunteer 5	25×10 ⁴	15×10 ⁴	100×10 ⁴	15×10 ⁴
Volunteer 6	150×10 ⁴	70×10 ⁴	1300×10 ⁴	80×10 ⁴
Volunteer 7	80×10 ⁴	40×10 ⁴	850×10 ⁴	50×10 ⁴
Volunteer 8	15×10 ⁴	23×10 ⁴	950×10 ⁴	750×10 ⁴
Volunteer 9	75×10 ⁴	50×10 ⁴	700×10 ⁴	750×10 ⁴
Volunteer 10	60×10 ⁴	45×10 ⁴	700×10 ⁴	400×10 ⁴
Volunteer 11	45×10 ⁴	15×10 ⁴	1000×10 ⁴	65×10 ⁴
Volunteer 12	40×10 ⁴	15×10 ⁴	1200×10 ⁴	10×10 ⁴

T1: Baseline 1, T2: 2 h, T3: Baseline 2, T4: 24 h, CFUs: Colony forming units

after 24 h (T4). Statistically significant differences were observed among the three-time periods ($P < 0.001$, GLM for repeated data).

When the time periods were compared two by two, statistically significant differences were observed only between time periods T2-T4 and T1-T4 ($P = 0.008$ and $P = 0.011$, GLM for repeated data adjusted for multiple comparisons) [Table 3].

The average bacteria count in T4 (after 24 h with Coltosol®) was also compared to the average observed after 24 h without the presence of Coltosol®, baseline 2 (T3) [Table 4]. The results showed a statistically significant difference ($P = 0.001$, *t*-test for independent data).

DISCUSSION

The use of coronary temporary sealing materials between sessions or at the end of endodontic therapy is one of the factors determining the success or failure of treatment. These materials are intended to temporarily seal the tooth, preventing the

entry of fluids, microorganisms, and other debris in the root canal system and avoid the loss of the medication.^[12,14-17] The coronary temporary sealing materials must present adhesiveness, low solubility, high mechanical strength, dimensional stability with a coefficient similar to the tooth tissue, antimicrobial activity, esthetically acceptable thermal expansion and allow easy placement and removal in the oral cavity. However, the incorrect preparation of the access cavity, misplacement and poor adaptation of the material in the cavity walls and the absence of a dental wear temporary crown sealer can cause microleakages.^[8,13,15,17,18] The antimicrobial activity is perhaps the most important property of a temporary crown sealer material. Whenever there is a flaw in the mechanical properties of the material or a lack of professional skill, the biological properties of the material can prevent or at least minimize contamination or recontamination of the root canal.^[9,11,12] Therefore, a direct contact with saliva test reveals this characteristic of a temporary crown sealer more reliably, as in the case of Coltosol® here.^[9,17]

Several authors have evaluated the antimicrobial activity of temporary coronary sealers using agar diffusion tests by measuring inhibition zones. However, we emphasize the importance of evaluating the antimicrobial activity of the sealer in direct contact with human saliva *in vitro*, in order to test the continuous action of the material during a given period of time, trying to simulate the clinical reality.^[8,13,17] This study is aimed to evaluate the antimicrobial action of Coltosol® in direct contact with human saliva from different individuals over different periods of time. Comparing the mean count of CFUs between an initial control (T1-baseline 1) and two-stroke direct contact with the saliva of the material, T2 (2 h), T4 (24 h) statistically significant differences were found. There was an inhibition of bacterial growth after the first 2 h of contact and an increased number of bacteria after 24 h of direct contact of the material with the saliva.

When treated individually, the mean values of the initial and final controls showed a statistically significant increase, as expected. When comparing the mean between the two times of direct contact, the difference was also statistically significant, with a bacterial growth increase, indicating a reduction of the inhibitory action of the material over microbial colonization along time.

The analysis of the values between the ultimate control (T3-baseline 2) and T4 (24 h) suggested

Table 2: Mean values of bacterial count (CFU/ml) in 3 time periods

Times	Mean	SD	P
T1	73.8	60.0	<0.001
T2	42.8	26.8	
T4	391.3	449.0	

P value refers to the GLM for repeated measures comparing the three times. T1: Baseline, T2: 2 h, T4: 24 h, SD: Standard deviation, GLM: Generalized linear model, CFU: Colony forming unit

Table 3: Comparison of mean values of bacterial count (CFU/ml) between time periods

Times		95% CI		P
		Lower	Upper	
T1	T2	-1.45	63.4	0.630
T2	T4	-604.6	-48.8	0.008
T1	T4	-586.2	-166.4	0.011

P value refers to the GLM for repeated measures, adjusted for multiple comparisons by Bonferroni test. T1: Baseline, T2: 2 h, T4: 24 h, CI: Confidence interval, GLM: Generalized linear model, CFU: Colony forming unit

Table 4: Comparison of mean values of bacterial count (CFU/ml) between T3 and T4

Times		95% CI		P
		Lower	Upper	
T3	T4	165.2	610.7	0.001

P value refers to student *t*-test for independent data. T3: 779.2 CFU/ml (values of bacterial count after 24 h without Coltosol®), T4: 391.3 UFC/ml (values of bacterial count after 24 h with Coltosol®), CI: Confidence interval, CFU: Colony forming unit

there was some bacterial inhibition. However, this comparison is not a clinical reality because in the oral cavity there is a constant renewal of saliva, which does not occur in this *in vitro* study.

Another aspect that must be considered is the limitations of the study, especially the a low number of samples tested, due to the use of direct contact *in vitro* and quantitative culture test. However, this methodology seems to be more reliable than the antimicrobial activity in agar diffusion test results, since it favors greater contact of dental materials with microorganisms, as well as allowing their wider dissemination in the culture broth. It should be pointed out that the use of Coltosol[®] as a temporary crown sealant aims to decrease the microbiota present in the tooth coronal portion during treatment or in a waiting period for a final restoration.

According to the results, it was concluded that Coltosol[®] inhibits microbial growth when it is in direct contact with saliva. However, this inhibitory effect tends to decrease over time, as shown by the two-time periods when the material was in contact with different saliva samples.

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Conflicts of interest

There are no conflicts of interest.

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