UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
DEPARTAMENTO DE ODONTOLOGIA

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ARACAJU
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Trabalho de conclusão de curso apresentado à comissão de estágio de Graduação em Odontologia da Universidade Federal de Sergipe, sob a orientação do professor Dr. André Luis Faria e Silva.

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Does using a calcium hydroxide liner is necessary in restorations of deep cavities? Controlled randomized clinical trial

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Abstract

Objectives: This clinical trial evaluated the effectiveness of provisional restoration with resin-modified glass ionomer (RMGI) during stepwise caries removal when compared to using a calcium hydroxide liner.

Material and Methods: Caries lesions involving the inner third of dentin in posterior teeth were included in the study. Dentin thickness was radiographically measured, followed by analysis of clinical aspects (color, consistency and humidity) of dentin and bacteria counting. After partial caries removal, the cavities were restored with RMGI using (control) or not (experimental) a liner with calcium hydroxide. The patients returned after 60 days, while the symptom of irreversible pulpit/ necrosis (primary outcome) were assessed. The provisional restorations were removed after the delay period and the clinical aspects, dentin thickness and presence of bacteria were assessed. The relative risk to irreversible pulpit/necrosis was calculated. The effect of treatment and time of assessment on secondary outcomes was also statistically analyzed.

Results: The treatment did not affect the risk to irreversible pulpit/ necrosis, while restorations with this outcome presented thinner dentin on pulpal wall. In general; darker, harder, drier and less contaminated dentin was observed after the provisional restorations, regardless the treatment; while control resulted in darker dentin. No difference on dentin thickness was observed between the treatments.

Conclusions: RMGI seems be suitable to provisionally restore cavities during stepwise caries removal.

Clinical relevance: Applying calcium hydroxide liner seems does not provide any additional beneficial effect when used under restorations of deep carious lesions.

Keywords: Clinical trial, Dental caries, Glass ionomer cement.

1. Introduction

Despite recent developments on caries prevention and early diagnostic, untreated caries is still the most prevalent disease worldwide with a global prevalence of 35% for all ages combined [1]. The absence of early intervention results in the progression of early caries lesions to deep cavities involving the inner third of dentin, an occurrence that remains a significant challenge for clinicians [2-4]. The carious dentin removal prior to restorative procedure is commonly associated to significant risk to pulp damages, including pulpal exposure and irreversible pulpitis [5,6]. Considering the limited access of population to endodontic treatment in several countries, pulp damage can be followed by tooth extraction compromising the quality of life of patients and consisting in a significant public health problem [5,7,8].

Stepwise caries removal has been proposed to reduce the risk of pulp exposure when the carious tissue excavation is performed in two steps [9-11]. During the first procedure, the carious dentin is partially removed and the cavity is provisionally sealed until the second intervention. This approach allows the remineralization of the lesion and to induce development of tertiary dentin, thereby reducing the risk of pulpal exposure and postoperative complications after the second excavation step [12,13]. After 45 days to 6 months, complete excavation is performed followed by definite restoration of cavity. Among the provisional restorative procedures, the use of calcium hydroxide liner over the remaining carious dentin tissue is usually recommended due its ability to induce dentin remineralization [13, 14].

However, studies have demonstrated that simply sealing the cavity properly may be enough to allow the reorganization of carious dentin in a short period and the remineralization in longer periods [15.16]. Therefore, simpler provisional restorations with reduced number of clinical steps can result in similar outcomes to those observed for restorations using calcium hydroxide liners. Further to technique simplicity, the restorative material must present adequate biocompatibility to the pulp tissue, considering its insertion in deep cavities, sufficient mechanical properties to support occlusal loads, and proper margin seal. Despite inducing mild pulp damage when used in deep cavities, resin-modified glass-ionomers (RMGI) present acceptable biocompatibility [17,18], adequate mechanical properties for provisional restorations in occlusal cavities, antibacterial properties, fluoride release and ability to bond to tooth tissue [19-21].

Thus, this study aimed to evaluate the clinical effectiveness of using only RMGI as a provisional restorative material after the first excavation of stepwise caries removal, in comparison to a standard procedure using a calcium hydroxide liner [followed by RGMI restoration]. The hypothesis of this study was that restoring the cavity with RMGI only would result in similar clinical outcomes than using calcium hydroxide liner.

2. Material and Methods

This clinical trial was approved (CAAE # 27090414.0.0000.5546) by the scientific review committee and by the committee for the protection of human subjects of the local University. The study protocol was registered at clinicaltrials.gov under No. NCT02494193 and followed the CONSORT statements.

2.1 Study design.

This study was a randomized, single-blind, controlled trial with a parallel group and an allocation rate of 1:1. After partial caries dentin removal, deep carious cavities in the occlusal surface of posterior teeth were randomly allocated to be provisionally restored with RMGI using a calcium hydroxide liner (control) or not (experimental). The study was conducted at the dental clinic of the School of Dentistry at the Federal University of Sergipe from June 2014 to September 2015.

2.2 Inclusion and exclusion criteria

The participants were recruited by means of advertisements placed on the buildings of the University. Patients from both genders, aged between 15 and 30 y.o., presenting permanent premolars or molars with deep carious lesions involving the inner third of dentin tissue were included. The following exclusion criteria were used: presence of periapical or periodontal lesions, necessity of extensive indirect restorations, any diagnostic of pulpal alteration (cold test with refrigerant spray), root exposition or non-carious cervical lesion and history of hypersensitivity, and pulpal exposure during the caries removal.

2.3 *Sample Size Calculation*

The sample size calculation was based on data of the primary outcome ‘maintenance of pulp vitality’. The calculation was performed for binary outcome equivalence trial, considering a power test of 80%, a significance level of 5%, an equivalence limit between the control and experimental treatments of

20%, and the success rate of 90% for both treatments based on a previous study [22]. The sample size calculation showed that 78 patients (39 per group) were required, while the sample size was fixed in 102 patients considering 30% drop-out during the follow-up.

2.4 Randomization

A randomized list was computer-generated by a person not involved in the study and the treatment allocated for each carious cavity included in the study was inserted into sealed opaque envelopes numbered from 1 to 102. The cavities to be restored were numbered according to the sequence of enrollment. The operator responsible by interventions only opened the envelope at the time of the intervention.

2.5 Baseline Measurements

Prior to interventions, bitewing radiographs were taken to measure the thickness of remaining dentin bellow the caries lesion. Bitewing film holders were used to standardize the position of radiographs taken during all study. Self-cured acrylic resin was placed on the film holder and impressions of occlusal surfaces of antagonist teeth were taken, thus allowing us to relocate the device in the same position in the measurement performed after the intervention. Radiographic films of phosphorus plate were used, while the digitalized images were opened in the software ImageJ to measurement of remaining dentin thickness. Five straight lines were draw linking the cavity floor to pulpal chamber and the average length of these lines were calculated to estimate the remaining dentin thickness (Figure 1).

Prior to interventions, a single calibrator evaluator assessed the color, consistency and humidity of dentin tissue located on the cavity floor. The clinical aspect of the dentin was scored according to the following criteria: dentin consistency: 1 = very soft (tissue easily removed by dentin excavator, releasing fragments during removal procedure), 2 = soft (the probe is easily inserted into tissue), 3 = leathery (dentine spoon removes carious tissue when forced), and 4 = hard (similar to normal dentine; dentine color: 1 = yellow, 2 = fawn, 3 = light-brown, 4 = dark brown, and 5 = dark; and dentin humidity: absent or present.

2.6 Intervention

After local anesthesia and rubber dam isolation, the lesion was accessed with a diamond bur. The carious dentin tissue of lateral walls was removed using a sterile round steel bur compatible to the cavity size

at low speed. The softer tissue on the cavity floor was carefully removed with a dentin excavator until a slightly moist and reasonably soft dentin remains. In the cavities allocated to control treatment, the remaining dentin was covered with calcium hydroxide paste (power of $CaOH_2$ and distillated water) followed by hard-setting calcium hydroxide cement (Dycal; DY, Dentsply, Milford, DE, USA). The cavities were provisionally restored with RMGI Riva Light-cure (SDI, Baywaster, Vitoria, Australia). For the cavities allocated to experimental treatment, the RMGI was inserted directly over the remaining dentin.

2.7 Microbiological analysis

Samples of dentin tissue removed were immersed in 5 mL of 0.9 % saline solution and homogenized in a mechanical agitator. Dilutions were performed until 10^{-6}, and 50 µl of this dilutions were cultivated in petri plate containing Rogosa or mitis salivarius agar to assess growing of *Lactobacillus ssp*. and *Streptococcus mutans*, respectively. All samples were incubated in 5% CO2 at 37℃ for 72 h. After incubation, the total number of colony-forming units per milliliter (CFU/ml) was counted from a representative area of each agar plate yielding 50–300 colonies using a stereoscopic microscope.

2.8 Evaluation

The patients were scheduled to return 60 days after the first intervention and those presenting any pain symptom indicating pulpal involvement (necrosis or irreversible pulpitis) before the re-evaluation were submitted to endodontic intervention and were excluded from the study. At the second session, a bitewing radiography was taken to evaluate any alteration on the distance between the cavity floor and the pulpal chamber. Following, the provisional restorations were carefully removed under anesthesia and rubber dam isolation. The clinical aspect of the dentin on the cavity floor was assessed and scores attributed to color and consistency, while the dentin was classified as dry or moist. The remaining carious dentin was removed with an excavator and samples of this tissue was evaluated regarding the presence of *Lactobacillus ssp*. and *Streptococcus mutans*. After the complete removal of carious dentin, the cavities were restored with composite resin.

2. 9 Statistical analysis

The absolute risks of each treatment and relative risk for pulp necrosis/ irreversible pulpitis was calculated with a confidence interval of 95%. The Fisher's exact test was used to assess possible statistical

difference between the relative risks. In order to assess possible differences between the cavities that presented negative or positive primary outcomes; clinical, radiographic and microbiological data on baseline were analyzed. Scores of consistency and color were submitted to Mann-Whitney test, while data from dentin thickness and CFUs were analyzed by T-test. Proportions between absence and presence of humidity were analyzed by Fisher's Exact test.

The following analyses were performed to evaluate the effect of 'treatment' and 'time of evaluation' on secondary outcomes. Data from consistency and color were submitted to Wilcoxon's test, comparing the time of evaluation, and Mann-Whitney test to assess differences between the treatments. Difference between the proportions of moist/ dried dentins were analyzed by Mcnemar's test, to assess the effect of the time of evaluation, and Fisher's Exact test to verify the effect of treatment.

Data from microbiologic analysis were transformed in Log_{10}, while zero count were previously converted to 1 to allow the transformation. Following, data for each bacterium evaluated were individually submitted to 2-way repeated-measure ANOVA. Data of dentin thickness was also submitted to 2-way repeated-measure ANOVA. The significance level was set at $\alpha = 0.05$ for all analyses.

3. Results

The results presented are relative to 75 cavities included in the study from a calculated sample size of 102. Twenty-one restored cavities (10 of control and 11 of experimental) did not return for the second evaluation, representing a drop-out of 28%. Fifty-four cavities (27 per treatment) were analyzed regarding the primary outcome, while 40 (20 per treatment) were analyzed for the other outcomes. The flow chart of study is illustrated at Figure 2.

The mean age (years) of the participants in this study was similar between the treatments (control = 23.6 ± 4.1 and experimental = 22.8 ± 4.7 years; p = 0.541; T-test). Regarding the gender, 43.2% of patients were male without significant difference between the patients allocated for each treatment (37.0 and 51.9 % of men for control and experimental allocations, respectively; p = 0.412; Fisher's Exact test).

The results regarding risk of pulp necrosis/ irreversible pulpitis is presented in Table 1, while both treatments resulted in the same absolute risk (26%). Restored cavities that presented pulp necrosis/ irreversible pulpitis differed from those with positive primary outcome only for the dentin thickness, while the

former showed shorter distance between the cavity floor and the pulp chamber (Table 2). No significant difference regarding the age was observed between the patients presenting negative (23.6 ± 4.1 year) and positive outcomes (23.1 ± 4.6 year; T-test, $p = 0.706$).

Harder dentin was observed after provisional restoration irrespective the procedure, while no difference was observed between the treatments (Table 3). Regarding color of dentin (Table 3), no difference was observed between the dentin of cavities allocated for each treatment. The score of color increased when the cavities were restored with both treatments but the control resulted in darkest dentin in the second evaluation. Irrespective of treatment, the provisional restoration was able to reduce the humidity of dentin without difference between the treatment at both evaluation times (Table 3).

The results for microbiological analysis are displayed in Table 4. Regarding *Lactobacillus ssp*., 2-way repeated-measure ANOVA showed that the 'treatment' did not affect the presence of bacteria ($p = 0.306$; interaction – $p = 0.076$), which was affected only by 'time of evaluation' ($p < 0.001$). It was observed a reduction on CFUs within the provisional restoration, irrespective the material used. Similarly, the 'treatment' ($p = 0.495$; interaction – $p = 0.055$) also did not affect the CFUs for *Streptococcus mutans*, whereas lowest values of CFU were observed for both groups after the provisional restoration removal ($p < 0.001$).

The results of radiographic evaluation are presented in Table 5. Two-way repeated-measure ANOVA showed that neither the 'treatment' ($p = 0.718$) nor the 'time of evaluation' ($p = 0.624$) affected the results; while the interaction between the factors also was not significant ($p = 0.305$).

4. Discussion

The caries removal in deep cavities requires careful excavation to avoid any pulp exposure followed by endodontic complications, while the stepwise or partial caries removal techniques have been indicated as conservative approaches to reduce this occurrence [10, 12]. Contrary to partial caries removal, the cavity is re-opened in the stepwise excavation when the remaining carious tissue can be removed with reduced risk of pulp exposure [10, 12]. Despite the absence of evidence about the necessity of reentry to increase the success of restorations [11,23], stepwise excavation was used in the present study to evaluate the effects of provisional restorative materials over the remaining dentin. We hypothesized that simplifying the provisional restoration by using only a single light-cured material would not affect the outcomes of stepwise excavation.

The results of the present trial demonstrated that both procedures evaluated were able to improve clinical aspects of remaining dentin and reduce the contamination of the tissue. Regarding the primary outcome, no difference on risk to pulp exposure was observed between the protocols leading us to accept the hypothesis of study.

The occurrence of irreversible pulpitis/ necrosis is strongly affected by any inflammation of pulp tissue and reduced thickness of remaining dentin. In fact, the restored cavities with diagnostic of irreversible pulpitis or necrosis are found closest to pulp tissue at baseline measurements. Regarding to pulp condition prior to intervention, only carious cavities in teeth presenting normal response to cold test were included. Cold test presents high positive (100%) and negative (90%) predictive values when used on diagnostic of pulp necrosis [24]. However, clinical tests of sensitivity are unable to precisely identify different degrees of pulp inflammation and the absence of painful symptoms cannot exclude the presence of any inflammatory involvement of the pulp [25]. Thus, imprecisions on measurement of prior pulp condition is a bias inherent to clinical trials involving restoration of deep carious lesions and any relation between the prior pulp condition and the primary outcome of the present study is speculative. Another factor associated to differences on pulp response is the age of patient, with older patients presenting reduced pulpal response and increased probability to irreversible pulpitis or necrosis [26]. Only patients aged between 15 and 30-year were included in this study seeking to reduce risk of bias related to age and no difference between restorations with negative and positive primary outcome was observed. Finally, the clinical and microbiological aspects were similar for cavities with different primary outcomes, not demonstrating any correlation between this factors and the risk to irreversible pulpitis or pulpal necrosis.

The baseline characteristics of cavities allocated to both interventions also were similar indicating that outcomes were mainly affected by properties of the materials themselves. In the present study, the reentry was performed with a short period (2 months) after the provisional restoration placement. Despite the short period, significant modifications on scores for all clinical criteria and on microbiological counting were observed. This period of provisional restoration was chosen in order to reduce the dropout rate of study, which was slightly lower than those expected, and allowed us to measure significant alterations on the outcomes. Regarding the primary outcome, both treatments presented similar risk (26%) to irreversible pulpitis or pulpal necrosis. A meta-analysis comparing RMGI and calcium hydroxide placed in deep cavities

demonstrated less inflammatory cell response with calcium hydroxide after 60 days, however; no relevant clinical significance also was observed [27]. Important observation of this review were the low level of evidence provided by included studies, which presented high risk of bias, and the necessity of further well-conducted clinical trials. The preliminary results of the present clinical trial, following the CONSORT statements, also indicated similar clinical outcomes when the deep cavities were dressed with calcium hydroxide or using only RMGI. In fact, it has been demonstrated that RMGI causes mild initial pulp alterations when used in deep cavities, while the inflammation reduces with time demonstrating acceptable biocompatibility of this material [17].

Modifications on color, consistency and humidity of dentin in the floor of cavities were observed after the provisional restoration removal irrespective the restorative protocol used. The outer carious dentin tissue (infected dentin) presents denatured collagen without potential for remineralization, whereas the organization of collagen structure increased toward to inner more mineralized dentin [28]. The outer infected dentin was removed during the first excavation procedure; and the provisional restorations were placed over more organized dentin. In-vitro, in-vivo and clinical studies have demonstrated indirect evidence that both RMGI and calcium hydroxide present ability to remineralize carious dentin [12, 15, 16, 18, 27, 29]. However; despite the evidence of ion exchange between these materials and dentin at a chemical level, evidence about interfibrillar or intrafibrillar remineralization of the carious dentin remains weak [30, 31]. In the present study, higher score of color for control treatment after the reentry was observed; which can suggest increased remineralization caused by calcium hydroxide due to higher amount of calcium ions released by this material. Even in the absence of true remineralization promoted by fluoride and calcium ions, the pH of calcium hydroxide and RMGI during the initial setting cause growth factors (TGF-βs) releasing from dentin matrix, inducing the odontoblast cells to produce tertiary dentin [32]. Thus, increased dentin thickness at the reentry session could be expected, but no difference was observed irrespective of treatment used. One possible explanation is that the presence of the significant thickness of radiopaque dentin remaining under the carious lesions on baseline (average higher 1.5 mm) impairs any effect of calcium hydroxide and glass ionomer on the pulp tissue.

Finally, the provisional restorations placement reduced the counting of *Lactobacillus ssp*. and *Streptococcus mutans* in the dentin. It has been demonstrated that proper sealing of the cavity alone is able to

reduce the microbiological content present in carious dentin due to limited the amount and complexity of nutrients available, irrespective the material used [33, 34]. Furthermore, the high pH of calcium hydroxide (around 9.5) causes damage to organelles and consequent cellular lyses [35], while the low pH of RMGI during the initial setting (about 4.0) affects the production of adenosine triphosphate and causes structural damage to the bacteria membrane due to presence of ions H^+ [36]. It is important to emphasize that the dentin contaminated despite the reduction observed on microbiota after the placement of provisional restorations. However, the presence of bacteria under restoration does not indicate the necessity of reentry to completely remove carious tissue. There was no evidence relating the presence of remaining bacteria under restoration and progression of carious lesions [37].

The preliminary results of the present clinical trial demonstrated that the placement of a calcium hydroxide liner under the provisional restoration during the stepwise excavation does not improve the outcomes when compared to using only RMGI. One limitation of the present clinical trial was the absence of follow-up of definitive restoration, whereas delayed pulp response can occur. Furthermore, the sample size of study will be completed yet and any difference on outcomes can be addressed with more patients evaluated.

5. Conclusion

Provisional restorations of deep carious lesions using only RMGI resulted in similar clinical, radiographic and bacterial outcomes than using a calcium hydroxide liner under the RMGI.

Compliance with Ethical Standards

Conflict of Interest: Author A declares that he has no conflict of interest.

Funding: The work was supported by the Department of Restorative Dentistry, Federal University of Sergipe, Brazil**.**

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figures captions.

Fig 1. Measurement of dentin thickness bellow of carious cavity.

Fig 2. Flow diagram of the clinical trial.

Table

Table 1. Analysis of risk (confidence interval of 95%) to pulp necrosis/ irreversible pulpits regarding each treatment.

Treatment	Absolute risk	Relative risk	p-value[1]
Control	0.26 (0.13/ 0.45)		
		1.00 (0.41/ 2.46)	1.000
Experimental	0.26 (0.13/ 0.45)		

1. Fisher's Exact test.

Table

Table 2. Results of comparison of the clinical, radiographic and microbiological conditions between the restorative procedures that resulted in negative and positive response to primary outcome.

Primary outcome	Clinical aspects			Dentin thickness** (in mm)	Microbiological analysis (CFU in Log_{10})**	
	Consistency* (scores 1 to 4)	Color* (scores 1 to 5)	Humidity (presence/ absence)		*Lactobacillus ssp.*	*Streptococcus mutans*
Positive	2.00 (1.00/ 2.00)	1.00 (1.00/ 2.00)	3/ 37	1.71 (0.74)	2.77 (0.61)	1.73 (0.24)
Negative	1.00 (1.00/ 2.00)	1.00 (1.00/ 1.00)	0/ 14	0.88 (0.71)	2.70 (0.95)	1.85 (0.15)
p-value	0.063[1]	0.514[1]	0.560[2]	< 0.001[3]	0.786[3]	0.144[3]

* Median (1^{st}/ 3^{rd} quartiles); ** Means (standard deviation)

1. Mann-Whitney test; 2. Fisher's Exact test; 3. T-test.

Table

Table 3. Results of clinical aspects of dentin remaining in cavity floor.

Treatment	Median (1st/ 3rd quartiles) for Consistency			Median (1st/ 3rd quartiles) for Color			Presence/ absence of humidity		
	Baseline	Reentry	p-value[1]	Baseline	Reentry	P-value[1]	Baseline	Reentry	P-value[3]
Control	2.00 (1.00/ 2.75)	4.00 (3.00/ 4.00)	< 0.001	1.00 (1.00/ 2.00)	4.00 (3.25/ 4.75)	< 0.001	18/ 2	1/ 19	< 0.001
Experimental	2.00 (1.00/ 2.00)	4.00 (3.00/ 4.00)	< 0.001	1.00 (1.00/ 2.00)	2.50 (2.00/ 4.00)	< 0.001	19/ 1	0/ 20	< 0.001
	0.557	0.759	p-value[2]	0.358	0.024	p-value[2]	1.000	1.000	p-value[4]

Scores from 1 to 4 for consistency, and 1 to 5 for color.

1. Wilcoxon's test; 2. Mann-Whitney test; 3. Mcnemar's test; 4. Fisher's Exact test.

Table

Table 4. Means (standard deviation) of CFU/ml (in Log_{10}).

Treatment	*Lactobacillus ssp.*		*Streptococcus mutans*	
	Baseline	Reentry	Baseline	Reentry
Control	2.8 (0.5)	0.8 (0.5)	1.7 (0.1)	1.1 (0.3)
Experimental	2.8 (0.7)	0.6 (0.2)	1.8 (0.3)	1.0 (0.3)
Pooled average	2.8 (0.6)^{A}	0.7 (0.4)^{B}	1.7 (0.2)^{A}	1.0 (0.3)^{B}

For pooled averages, distinct letters indicate statistical difference ($p < 0.05$).

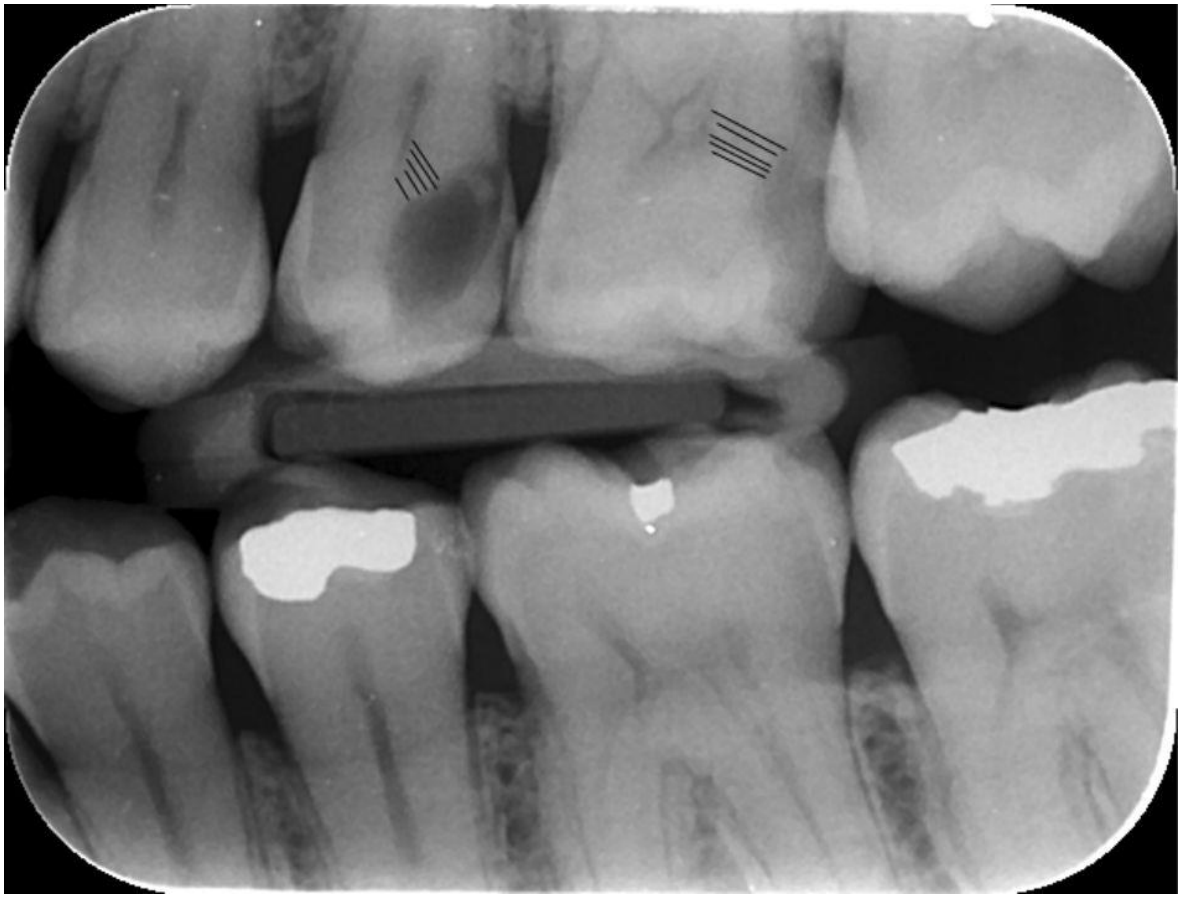
Table

Table 5. Results of radiographic analysis measuring the distance (mm) between the cavity floor and the pulp chamber.

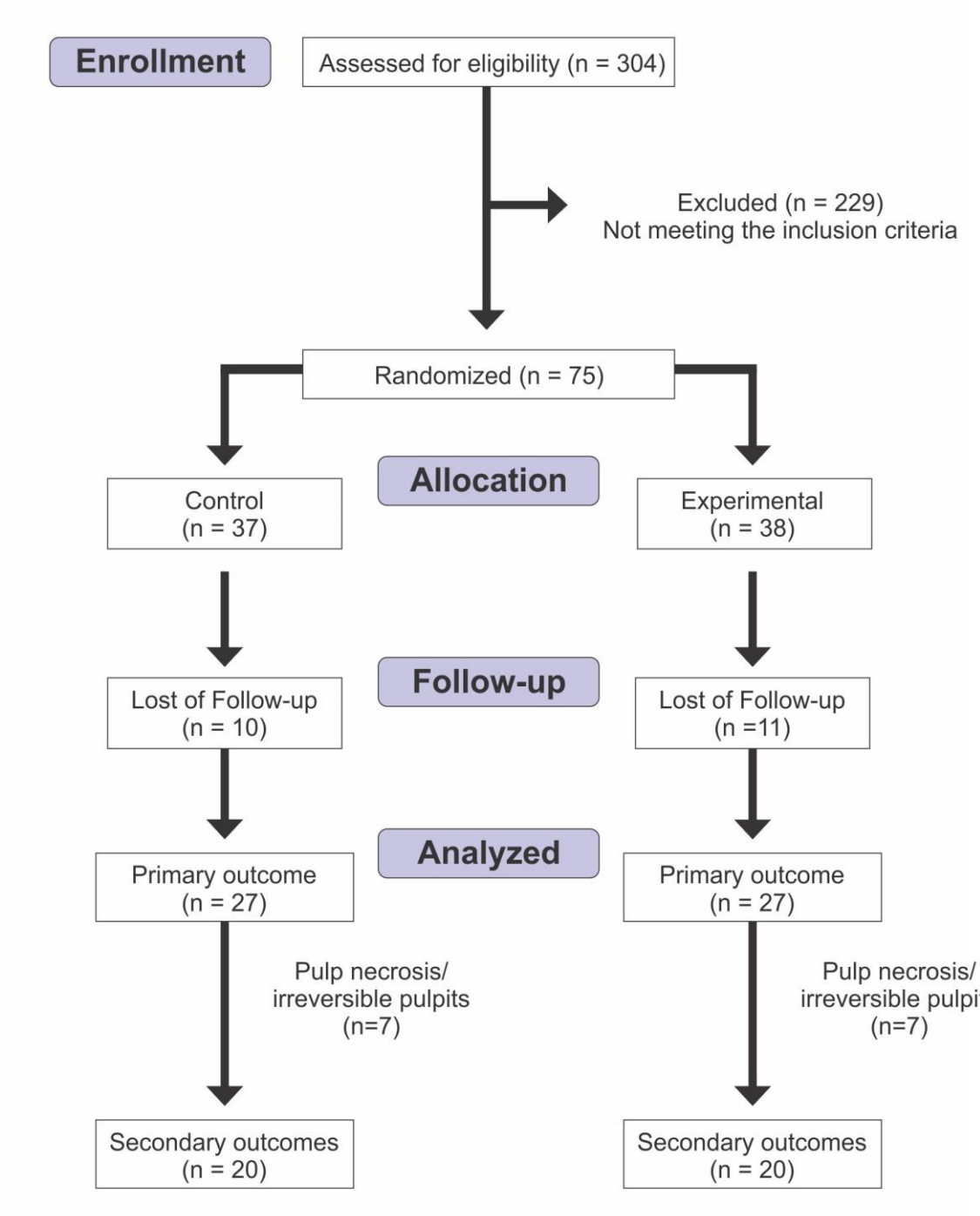
Treatment	Moment of evaluation	
	Baseline	Final
Control	1.70 (0.71)	1.75 (0.67)
Experimental	1.71 (0.79)	1.59 (0.55)

2-way repeated-measured ANOVA: 'treatment' – p = 0.718; 'moment of evaluation' – p = 0.624; interaction – p = 0.305.

Figure



Figure


Enrollment
Assessed for eligibility (n = 304)
Excluded (n = 229)
Not meeting the inclusion criteria
Randomized (n = 75)
Allocation
Control
(n = 37)
Experimental
(n = 38)
Follow-up
Lost of Follow-up
(n = 10)
Lost of Follow-up
(n =11)
Analyzed
Primary outcome
(n = 27)
Primary outcome
(n = 27)
Pulp necrosis/
irreversible pulpits
(n=7)
Pulp necrosis/
irreversible pulpits
(n=7)
Secondary outcomes
(n = 20)
Secondary outcomes
(n = 20)