Original Article

Antimicrobial properties, mechanics, and fluoride release of ionomeric cements modified by red propolis

Gêisa Aiane de Morais Sampaio[a]; Rogério Lacerda-Santos[b]; Yuri Wanderley Cavalcanti[c]; Gustavo Henrique Apolinário Vieira[a]; Cassiano Francisco Weege Nonaka[d]; Pollianna Muniz Alves[d]

ABSTRACT

Objectives: To evaluate the antimicrobial activity, mechanical properties, and fluoride release capacity of glass ionomer cement (GIC) used for cementing orthodontic bands and modified by ethanolic extract of red propolis (EERP) in different concentrations.

Materials and Methods: Two orthodontic GICs containing EERP at 10%, 25%, and 50%, were used. The following assays were carried out: cell viability tests against *Streptococcus mutans* and *Candida albicans*, diametral tensile strength, compressive strength, shear bond strength, microhardness, and fluoride release capacity. The statistical analyses of the antimicrobial tests, fluoride release, diametral tensile strength, compressive strength, and microhardness were performed using two-way analysis of variance and Tukey test ($P < .05$). Shear bond strength data were analyzed using one-way analysis of variance followed by Tukey test ($P < .05$).

Results: At the concentrations of 25% and 50%, EERP was shown to be a promising antimicrobial agent incorporated into GICs against *C albicans* ($P < .001$) and *S mutans* ($P < .001$). The fluoride release capacity of the GICs was not affected, and the EERP concentration of 25% was the one that least affected the mechanical properties of the cements ($P > .05$).

Conclusions: The GICs containing EERP at 25% showed a significant increase in their antimicrobial activity against *S mutans* and *C albicans*, while mechanical properties and fluoride release remained without significant changes. (*Angle Orthod.* 2021;91:522–527.)

KEY WORDS: Glass ionomer cement; Propolis; Antimicrobial

INTRODUCTION

Orthodontic bands play an important role in conventional orthodontic therapy. Cementation of bands on posterior teeth is necessary to improve anchorage and retention of fixed appliances.[1] However, the presence of these devices may cause biofilm accumulation in the region and demineralization of enamel adjacent to the orthodontic bands.[2]

In recent decades, glass ionomer cements (GICs) have been widely used for cementing orthodontic bands due to such clinical properties as biocompatibility and prevention of microleakage and demineralization.[1,3,4] With the advent of GICs for band cementation, caries and periodontal disease rates in orthodontic patients decreased considerably due to the lower amount of microleakage and their fluoride release capacity.[5] However, the composition of these cements can be modified by adding antimicrobial agents to improve their antimicrobial properties.[4,6–8]

Due to its antibacterial activity against microorganisms of the oral cavity,[9,10] researchers have suggested incorporating ethanolic extract of propolis to the GIC in order to increase its potential.[7,8,11–14] The use of this material in cementing orthodontic bands may contribute to a decrease in the bacteria in the region.[6,14]

Propolis is a resinous composition produced by honeybees from plant exudates found in the stem, leaves, and flowers.[15] Interest in its therapeutic use has

[a] Student, Graduate Program in Dentistry, State University of Paraíba, Campina Grande, Paraíba, Brazil.

[b] Professor, Department of Dentistry, Federal University of Juiz de Fora, Governador Valadares, Minas Gerais, Brazil.

[c] Professor, Graduate Program in Dentistry, Federal University of Paraíba, João Pessoa, Paraíba, Brazil.

[d] Professor, Graduate Program in Dentistry, State University of Paraíba, Campina Grande, Paraíba, Brazil.

Corresponding author: Geisa Aiane de Morais Sampaio, Universidade Estadual da Paraíba, Rua das Baraúnas, 351, Bairro Universitário, Campina Grande, Paraíba 58429-500, Brazil
(e-mail: geisasampaio8@gmail.com)

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been exacerbated by scientific evidence regarding the prevention of diseases due to its beneficial properties, including antimicrobial, anti-inflammatory, antioxidant, antiviral, and healing effects.[16–18]

Red propolis is found in the northeastern region of Brazil, especially in the coastal areas, and has been the target of numerous chemical and pharmacologic studies that have generated great interest in the world scientific community because of its good biological, antimicrobial and antifungal activity.[15] Therefore, red propolis is a promising antimicrobial agent to incorporate in orthodontic GIC.

Adding antimicrobial agents to GICs can, however, result in changes in their physical and biological properties. Thus, in this study we proposed to evaluate the antimicrobial activity, mechanical properties, and fluoride release capacity of GIC used to cement orthodontic bands and modified by ethanolic extract of red propolis (EERP) in different concentrations. The null hypothesis was that adding EERP to cement does not change its properties.

MATERIALS AND METHODS

Preparation of the EERP

The present study used EERP from the coasts of Paraíba (João Pessoa, Paraíba, Brazil), obtained from crude extract red propolis. For each 25 g of propolis, it was dissolved in 250 mL of 80% (vol/vol) ethanol solution. The extract was then filtered twice on filter paper to remove excess wax. Then, the EERP was prepared at concentrations of 10%, 25%, and 50% and placed in an amber glass bottle at room temperature.[8]

Preparation of Propolis Containing GIC

Two orthodontic GICs, Meron (VOCO, Cuxhaven, Cuxhaven, Germany) and Riva (SDI, Bayswater, Victoria, Australia), were used. For the control groups, the cements were handled according to the manufacturers' instructions. For the test groups, the 10%, 25%, and 50% concentrations of EERP were incorporated into the liquid of the cements during their manipulation in a ratio of one drop of the liquid (tartaric acid) to one drop of the EERP solution, using the same dosing nozzle, then spatulated with the cement powder to obtain a solid material.[5] The samples were thus distributed into eight groups: MC (control), M10, M25, M50, RC (control), R10, R25, and R50.

Analysis of Antimicrobial Activity

Cement specimens ($n = 3$ per microorganism) were prepared by inserting the materials into silicone molds (10 mm $\times$ 5 mm). Suspensions of *Streptococcus mutans* (ATCC 25175) and *Candida albicans* (ATCC 90028) were established at densities equivalent to 1×10^8 colony-forming units (CFUs)–*S mutans*/mL and 1×10^6 CFUs–*Candida*/mL, respectively, with a spectrophotometer.

The specimens were positioned horizontally in a 24-well plate, and, in each well, *S mutans* and *C albicans* biofilms were formed from the mixture of 0.2 mL of the inoculum of the microorganisms in 1.8 mL of supplemented BHI medium with 1% sucrose (Merck & Co, Kenilworth, NJ, USA) for *S. mutans* and RPMI 1640 medium supplemented with 100 mM glucose (Merck KGaA, Darmstadt, Hesse, Germany) for *C albicans*, and incubated at 37°C for 24 hours in aerobiosis.

To measure the cellular viability of the microorganisms adhered to the specimens, the biofilms were collected after 24 hours by transferring the specimens to polypropylene tubes containing 2 mL of saline solution and subjected to vigorous vortexing for 60 seconds to obtain suspensions of biofilms. The suspensions were diluted serially in concentrations ranging from 10^{-1} to 10^{-5}. These dilutions were seeded on BHI agar plates (Kasvi, São José do Pinhais, Paraná, Brazil) for *S mutans*, and on Sabouraud dextrose agar plates (Kasvi, São José do Pinhais, Paraná, Brazil) for *C albicans*, using the drop method (10 μL). The plates were then incubated at 37°C in aerobiose for 24 hours. Viable microorganisms were counted in the dilutions where there was growth between 6 and 60 colonies.

Analysis of Mechanical Properties

For diametral tensile strength (DTS) and compressive strength (CS) tensile tests, the specimens ($n = 10$) were prepared by inserting the material into cylindrical silicone molds (6 mm $\times$ 3 mm DTS and 4 mm $\times$ 8 mm CS). After inserting the material, a polyester strip was placed on the upper surface, and a glass plate was manually pressed to obtain a regular surface of the specimen. After 5 minutes, the specimens were stored at 37°C in 100% moisture for 24 hours, and their dimensions were measured with a digital caliper (Mitutoyo, Kawasaki, Kanagawa, Japan).

The assays were performed in a universal testing machine (Instron Corporation, Norwood, MA, USA) with a loading cell of 5 kg and a speed of 0.5 mm/min. The load was applied along the diameter of the specimen, and the maximum force before rupture was recorded. Subsequent equations were then applied to each specimen to obtain DTS and CS tests results, where $DTS = 2F/\pi dt$ and $SC = 4F/\pi d2$, where F is the bursting load, d is the diameter, and t is the height of the specimen.

For Vickers microhardness tests, the specimens ($n = 5$) were prepared by inserting the material into

cylindrical silicone molds (6 mm × 3 mm) following the same procedures for manipulation, insertion, and cure previously described; then, the specimen surfaces were polished. Vickers microhardness measurements were performed using an HMV microdurometer (Shimadzu Corporation, Quioto, Quioto, Japan) with 200 g load over 15 seconds. In each specimen, three equidistant notches were performed, obtaining 15 measurements per group.

For the shear bond strength tests, 80 bovine incisors were kept in 0.1% thymol solution until the moment of the experiment. The teeth were segmented using a diamond disc (KG Sorensen, Cotia, São Paulo, Brazil) in a straight handpiece at low rotation around the cervical third of the roots and in the incisal third of the crown. Each tooth was then positioned horizontally in cylindrical arrays of polyvinyl chloride tubes (20 mm × 10 mm) and fixed with acrylic resin (VIPI, Pirassununga, São Paulo, Brazil). The buccal surfaces were polished with a rubber cup (KG Sorensen, Cotia, São Paulo, Brazil) and pumice (S.S. White, Juiz de Fora, Minas Gerais, Brazil) at low speed for 10 seconds, washed, and dried for 10 seconds.

Metal matrices for orthodontic bands (Morelli, Sorocaba, São Paulo, Brazil) were cut (5 mm × 10 mm) and metal brackets (Morelli, Sorocaba, São Paulo, Brazil) were welded to them. The GICs were manipulated, and each matrix was cemented to the center of the buccal surface of the tooth. After 5 minutes, the specimens (n = 10) were stored at 37°C in 100% moisture for 24 hours. The tests were run in a universal testing machine (Instron Corporation, Norwood, MA, USA) using a die with a 5 kg loading chisel at a speed of 1mm/min. The results were obtained in N and divided by the base area of the bracket, providing results in MPa.

After the tests, the buccal surface of each test specimen was evaluated in a stereoscopic magnifying glass (Carl Zeiss, Göttingen, Niedersachsen, Germany) with 8× magnification to quantify the Adhesive Remnant Index (ARI): 0 = no cement adhered to the enamel; 1 = less than half of the cement adhered to the enamel; 2 = more than half of the cement adhered to the enamel; 3 = all of the cement adhered to the enamel.

Fluoride Release Analysis

The specimens (n = 3) were made using silicone molds (10 mm × 5 mm) and stored at 37°C and at 100% moisture for 30 minutes. After this period, each specimen was weighed with a precision analytical balance (Shimadzu Corporation, Quioto, Quioto, Japan) and placed in 2 mL of deionized water by the Milli-Q purification system and maintained in an oven at 37°C. Fluoride release was measured after 24 hours using a selective ion electrode connected to an ion analyzer (Thermo Scientific, Waltham, MA, USA) previously calibrated with standards of 0.2 to 5.0 ppm F in Total Ionic Strength Adjustment Buffer (TISAB II) at 50%. The readings were made in millivolts (mV) and transformed into μg/mL (ppm F) by linear regression of the calibration curve.

Statistical Analysis

Statistical analyses of the data were performed using the Statistical Package for the Social Sciences (version 20, SPSS, Inc, Chicago, IL, USA) based on 95% significance levels ($P < .05$) for statistical significance determination. Data distribution analysis was performed using Kolmogorov-Smirnov tests. For analysis of the antimicrobial effect, given a non-normal distribution, the data underwent log transformation ($\log_{10}$); the normal distributions being confirmed by the same statistical tests.

In the tests of antimicrobial analysis, fluoride release, DTS, CS, and microhardness, the comparative analysis between groups was performed using two-way analysis of variance (ANOVA) and Tukey multiple comparisons tests for post hoc ($P < .05$). For the results of the tests of shear bond strength, ANOVA to a fixed factor (one-way ANOVA) was used, followed by the Tukey test ($P < .05$). Kruskal-Wallis nonparametric tests were used for the ARI results, followed by Dunn multiple comparison tests.

RESULTS

In the cell viability tests (*S mutans* and *C albicans*), no statistically significant differences were observed between Meron and Riva materials ($P > .05$). For the different concentrations of propolis, there was a decrease in the cell viability of *S mutans*, with the groups of concentrations 25% and 50% differing significantly from each other ($P < .05$). A decrease in the cell viability of *C albicans* was also observed, with the groups 25% and 50% differing significantly from the control group ($P < .05$) and with no statistical difference between the 10%, 25%, and 50% groups (Table 1).

Results of the DTS tests showed significant differences between the materials, with Meron cement more resistant ($P < .05$). The cements with 10% and 50% concentrations of propolis exhibited a significant decrease in DTS resistance compared with the control ($P < .05$) (Table 2).

For CS results, no statistically significant differences were observed between the Meron and Riva materials ($P > .05$). For the different concentrations of propolis, there was no statistically significant difference from the

Table 1. Comparison Among Groups for Cellular Viability Tests With *Streptococcus mutans* (log_{10} CFU/mL) and *Candida albicans* (log10 CFU/mL)

	S mutans				*C albicans*			
	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**
Control	8.27 (0.26)	8.19 (0.25)	8.23 (0.24)^{x}	-	7.32 (0.42)	7.08 (0.60)	7.20 (0.49)^{x}	-
RP10%	8.17 (0.23)	7.96 (0.22)	8.06 (0.24)^{x}	-	6.90 (0.42)	6.52 (0.06)	6.73 (0.35)^{x,y}	-
RP25%	7.38 (0.13)	7.42 (0.08)	7.40 (0.10)^{y}	-	5.38 (0.55)	6.32 (0.17)	5.85 (0.63)^{y}	-
RP50%	7.23 (0.14)	6.89 (0.17)	7.06 (0.23)^{z}	-	5.47 (1.09)	6.29 (0.45)	6.04 (0.81)^{y}	-
Total	7.76 (0.51)	7.61 (0.55)	-	.470	6.33 (0.48)	6.51 (0.55)	-	.434
*P**	-	-	.001	-	-	-	.004	-

[a] RP indicates red propolis; CFU indicates colonies forming units.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

control; however, the concentration of 50% exhibited a significant decrease in CS compared with the concentration of 25% ($P < .05$) (Table 3).

In the Vickers microhardness analysis, no statistically significant differences were observed between Meron and Riva materials ($P > .05$), as well as among the different concentrations of propolis and controls ($P > .05$) (Table 4). For the results of shear bond strength, significant differences were observed between the materials, with Riva cement appearing to have greater resistance ($P < .05$). For the different concentrations of propolis, there was no significant difference among groups ($P > .05$) (Table 5). Regarding ARI results, no significant statistical differences were observed ($P > .05$) (Table 6).

For the fluoride ion release analysis, no statistically significant differences were observed between the Meron and Riva materials ($P > .05$), as well as among the different concentrations of propolis and the controls ($P > .05$) (Table 7).

DISCUSSION

The EERP led to a decrease in cell viability of *S mutans* in the groups in which propolis was added. The cements with concentrations higher than 25% exhibited better antimicrobial activity, differing statistically from the control group. Other studies have also demonstrated an increase in antimicrobial activity of GIC against *S mutans* with addition of ethanolic extract of yellow propolis at concentrations of 25% and 50% by means of minimum inhibitory concentration measurement tests[8] and an agar diffusion test.[14] An antimicrobial action against *S mutans* was also demonstrated by the addition of lyophilized ethanolic extract of yellow propolis to the GIC powder at concentrations of 0.75% and 1.25% by agar diffusion and bacterial adhesion tests.[13]

A decrease in the cellular viability of *C albicans* was also observed in the groups of cements with 25% and 50% concentrations of EERP, differing significantly from the control group. This was probably due to the antimicrobial action that EERP exerts on *Candida* spp.[15,19] This was in agreement with a study by Freires et al.[20] that showed similar results with a propolis species from Northeast Brazil. Also, Haghdoost et al.[21] found significantly decreased *Candida* formation using ethanolic extract of yellow propolis, with direct concentration dependency, using a germ tube formation assay.

Regarding the evaluation of mechanical properties, it was observed that the 25% EERP showed no significant alteration of the DTS and CS, unlike the other concentrations. Troca et al.[7] also observed a significant decrease in the resistance to DTS of restorative GICs containing ethanolic extract of green propolis, in addition to an increase in water absorption

Table 2. Comparison Among Groups for Diametral Tensile Strength (MPa)[a]

	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**
Control	27.93 (6.09)	16.83 (6.33)	22.67 (8.29)^{y}	-
RP10%	17.19 (6.49)	14.00 (8.70)	15.59 (7.65)^{z}	-
RP25%	27.28 (5.51)	12.81 (6.22)	20.05 (9.37)^{yz}	-
RP50%	23.68 (6.73)	10.27 (5.53)	16.98 (9.12)^{z}	-
Total	24.02 (7.37)^{y}	13.39 (6.95)^{z}	-	.001
*P**	-	-	.008	-

[a] RP indicates red propolis.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

Table 3. Comparison Among Groups for Compression Strength (MPa)[a]

	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**
Control	27.83 (5.71)	20.97 (4.12)	24.58 (6.02)^{xy}	-
RP10%	25.34 (7.68)	22.37 (8.77)	23.93 (8.12)^{xy}	-
RP25%	28.93 (6.61)	26.06 (5.97)	27.49 (6.30)^{x}	-
RP50%	18.65 (7.66)	20.96 (5.79)	19.80 (6.69)^{y}	-
Total	25.35 (7.74)	22.68 (6.47)	-	.095
*P**	-	-	.008	-

[a] RP indicates red propolis.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

Table 4. Comparison Among Groups for Vickers Microhardness (HV)[a]

	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**
Control	74.86 (16.49)	84.62 (8.43)	79.74 (13.38)	-
RP10%	97.58 (17.85)	87.60 (9.02)	92.59 (14.33)	-
RP25%	88.62 (13.98)	89.94 (8.02)	89.28 (10.77)	-
RP50%	74.66 (12.90)	85.54 (10.94)	80.10 (12.65)	-
Total	83.93 (17.31)	86.93 (8.67)	-	.461
*P**	-	-	.067	-

[a] RP indicates red propolis.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

and solubility of the cement. Another study also verified the decrease in CS and increase in the solubility of cements containing ethanolic extract of green propolis.[22] This can be attributed to propolis interference with the reaction of glass particles and polyacrylic acid, thus increasing the number of unreacted particles in the structure.[7,22]

For Vickers microhardness, no significant difference among the groups were found, which showed that the addition of EERP in the concentrations of 10%, 25%, and 50%, did not affect the microhardness of the cements. Altunsoy et al.[12] showed that adding ethanolic extract of propolis to a conventional GIC at the same concentrations increased the Vickers microhardness of the material and did not affect its microleakage.

The results of the tests of shear bond strength and ARI did not show significant differences between the cements with addition of EERP and conventional cements. Similar results were found in studies that verified the shear strength of a GIC with a liquid containing 1% ethanolic extract of yellow propolis[23] and GIC with 10%, 25%, and 50% concentrations.[8,14] These findings suggest that the addition of ethanolic extract of propolis does not affect the shear bond strength of GIC.

Adding EERP to the GIC did not affect the fluoride release capacity of the cements. Other studies, however, found an increase in the fluoride release of a GIC with its liquid containing 1% ethanolic extract of yellow propolis[23] and a GIC with its powder containing 1.25% lyophilized ethanolic extract of yellow propolis.[13]

Table 5. Comparison Among Groups for Shear Bond Strength (MPa)[a]

	Meron, Mean (SD)	Riva, Mean (SD)	*P**
Control	0.160 (0.046)[Y]	0.218 (0.066)[Z]	.033
RP10%	0.149 (0.072)	0.180 (0.081)	.383
RP25%	0.118 (0.040)	0.169 (0.083)	.104
RP50%	0.130 (0.063)	0.174 (0.071)	.160
*P**	.373	.455	-

[a] RP indicates red propolis.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

Table 6. Adhesive Remnant Index Scores and Mean Values Exhibited by Groups

	Adhesive Remnant Index Scores[a]				
Groups	0	1	2	3	*P**
Meron control	0	1	4	5	2.4
Meron 10%	0	1	1	8	2.7
Meron 25%	1	1	2	6	2.3
Meron 50%	2	1	1	6	2.1
P value*	-	-	-	-	.575
Riva control	2	0	4	4	2.0
Riva 10%	2	1	2	5	2.0
Riva 25%	1	3	0	6	2.1
Riva 50%	5	0	2	3	1.3
P value*	-	-	-	-	.469

[a] 0, no remaining adhesive; 1, less than half the remaining adhesive; 2, more than half of the remaining adhesive; 3, all remaining adhesive.
* Test of Kruskal-Wallis and multiple comparison of Dunn ($P < .05$).

Regarding the two brands of cement tested, it was observed that the Meron cement showed a higher resistance to DTS. For the other tests, no significant differences were observed between the cements. This was probably due to the similarities in their compositions.

Finally, the results of the present study demonstrated that EERP, at concentrations of 25% and 50%, was shown to be a promising antimicrobial agent to add to orthodontic GICs. The fluoride ion release capacity was not affected, and 25% EERP had the least effect on the mechanical properties of the cements. However, further studies using other concentrations of EERP are recommended, as well as other mechanical and clinical trials. In addition, only the ethanolic extract was used in this study other forms of red propolis should be incorporated into the GIC and studied.

CONCLUSIONS

- In summary, adding EERP to GIC at 25% concentration increased the antimicrobial capacity of the

Table 7. Comparison Among Groups for Fluoride Release Test (μg/mL)

	Meron, Mean (SD)	Riva, Mean (SD)	Total, Mean (SD)	*P**
Control	4.53 (0.05)	4.68 (0.11)	4.60 (0.11)	-
RP10%	4.47 (0.03)	4.50 (0.01)	4.48 (0.02)	-
RP25%	4.54 (0.04)	4.60 (0.01)	4.57 (0.04)	-
RP50%	4.53 (0.05)	4.56 (0.04)	4.55 (0.04)	-
Total	4.52 (0.05)	4.58 (0.08)	-	>.05
*P**	-	-	>.05	-

[a] RP indicates red propolis.
* Two-way analysis of variance with Tukey multiple comparison set. Different letters indicate statistical difference between the lines ($P < .05$).

cement against *S mutans* and *C albicans* without affecting its mechanical properties and ability to release fluoride.

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