

Effect of green propolis addition to physical-mechanical properties of glass ionomer cements

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ABSTRACT

Objective: This study investigated the mechanical properties of glass ionomer cements (GICs) combined with propolis as a natural antimicrobial substance. **Material and Methods:** Typified green propolis, as an ethanolic extract (EEP) or in the lyophilized form (powder), was incorporated to specimens of Ketac Fil Plus, ChemFlex and Ketac Molar Easymix GICs. For each test, 8 specimens of each material were prepared. For water sorption and solubility tests, specimens were subjected to dehydration, hydration and re-dehydration cycles until a constant mass was obtained for each step. Measurements were recorded using a digital balance of 10^{-4} g precision. For the diametral tensile strength test, specimens were tested in a universal test machine at 0.5 mm/min crosshead speed after 24 h storage in deionized water. Data were evaluated by one-way ANOVA and Tukey's tests ($p < 0.05$). **Results:** The addition of propolis to GIC clearly increased water sorption compared to pure material. Solubility was material-dependent and was not clearly evident. For the diametral tensile strength test, association with propolis altered negatively only Chemflex. **Conclusion:** It may be concluded that incorporation of propolis to GICs alters some properties in a material-dependent condition.

Key words: Glass ionomer cements. Propolis. Solubility. Tensile strength.

INTRODUCTION

Currently, new approaches, techniques and materials have focused on maximum prevention and minimally invasive procedures in Dentistry. According to this philosophy, Atraumatic Restorative Treatment (ART) consists on the removal of carious tissue with hand instruments and restoration with an adhesive material^{8,28}. Glass ionomer cement (GIC) is the material of choice as it presents several benefits, mainly adhesion to dental substrates, fluoride release, recharge ability and reverse potential to reduce acidic environment³. GIC has also satisfactory biocompatibility and antimicrobial potential, which make this material attractive⁵.

Due to this antimicrobial property the association

of known antimicrobial substances, such as propolis, chlorhexidine and antibiotics to GICs has been extensively investigated^{7,12,23,30}. In specially challenging clinical situations as in ART, it can be of great interest.

Propolis is a natural resinous substance produced by honey bees^{9,20}. Bees extract it from plants exudates, which they process by using an enzyme found in their salivary glandules. The defense of plants against microorganisms explains the antimicrobial effect of propolis⁹. Propolis can vary extensively according to the origin and composition^{14,15,25}. It may be typified, in a process in which all components are chemically analyzed. Through this process, bioactive components are identified from original samples to be well applied¹⁴.

Diverse biological properties have been proven namely antimicrobial, antiinflammatory, anesthetic and healing actions^{6,15,19}. There are different forms of propolis, such as ethanolic and lyophilized. In scientific papers, the ethanolic extract of propolis (EEP) is the most commonly used, where ethanol works as a solvent or vehicle. Often, EEP is directly employed, resulting in effective antimicrobial potential²².

Despite these benefits, there are only few reports about the addition of propolis to GIC^{7,19,30}. These investigations have focused on antimicrobial effects, but physical-mechanical properties have been overlooked^{12,22}. Additionally, propolis application in Dentistry is mostly related to the use of EEP. There is a lack of reports on the use of the lyophilized form, which has been shown to have antibacterial, antiviral, analgesic and regeneration properties²⁴.

This study aimed at investigating the effect of 2 different forms of typified green propolis, EEP and lyophilized (L), associated with one conventional GIC and two high-density GICs, regarding mechanical properties. The null hypothesis was that there is no difference in material performance (water sorption, solubility, and diametral tensile strength test), with or without propolis.

MATERIAL AND METHODS

Propolis was obtained from typified pure extract of green propolis by high-efficiency liquid chromatography as presented in Figure 1¹⁴. The tested GICs (all shade A3) are presented in Figure 2. Each material solvated in ethanol was qualified as its abbreviation followed by E, being KPE, CE and KME, while in lyophilized form they were followed by L, being, KPL, CL and KML.

Water sorption and solubility

Eight specimens of each GIC were prepared in 3 different conditions: pure, associated to EEP or to lyophilized form, totalizing 72 samples. This test was conducted according to ISO 4049:1988¹¹. Powder and liquid were measured using a digital balance of 10⁻⁴ g precision (Tel Marke; Bel Quimis, São Paulo, SP, Brazil). After manual handling, the mixture was inject into previously isolated steel stainless moulds (15 mm x 0.5 mm) using Centrix syringe (Centrix, Centrix Dental, Shelton, CT, USA). A cellophane sheet was used to cover the inserted material. Digital pressure was exerted using a microscope glass slide for 20 s. After 15 min, specimens were removed from mould and lateral excess was removed using a #15 scalpel blade (Bard-Parker, Franklin Lakes, NJ, USA). For EEP groups (E) preparation, it was added to GIC-liquid using a pipette (Gilson, Roissy Ch de Gaulle Cedex, France) to obtain a final concentration of 1% of propolis into GIC³. To prepare specimens

using lyophilized propolis (L), it was added to GIC powder reaching a final concentration of 2%.

Specimens were stored in desiccators at 37°C containing silica gel. The discs were weighed daily and the complete cycle was repeated until a constant mass (m_1) be obtained, i.e., until the mass loss of each specimen was not more than 0.1 mg *per* 24-h cycle. The thickness of each specimen was measured at 4 points using an electronic digital caliper (Mitutoyo Corporation, Tokyo, Japan). Thereafter, the specimens were stored in water at 37°C for 7 days (6 mL of water *per* specimen). The specimens were daily reweighed, after being carefully wiped with an absorbent paper. When constant weight was obtained, this value was recorded as m_2 . After this weighing, the specimens returned to the desiccators, the entire mass reconditioning cycle was repeated and the constant mass was recorded as m_3 .

The values for water sorption and solubility, in micrograms *per* cubic millimeter, were calculated using the following equations: $S=(M_2-M_3/V)$ and $SB=(M_1-M_3/V)$. Data were subjected to one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons ($P<0.05$).

Diametral Tensile Strength test

Eight specimens for each group were prepared with dimensions of 6 mm diameter x 3 mm high. After 1 h, the specimens were individually immersed in deionized water in plastic vials for 24 h. Diametral tensile strength (DTS) was performed using a universal test machine (Kratos Equipamentos Industriais Ltda, Cotia, SP, Brazil) at a crosshead speed 0.5 mm/min. It was calculated using the equation: $2P/\pi DT$, where: P= load applied; D= diameter of the cylinder, T= thickness of the cylinder, π =(constant) 3.14. DTS values [kgf/cm²] were converted into MPa as follows: $DTS[MPa]=DTS[Kgf/cm^2] \times 0.09807$. Data were subjected to ANOVA and Tukey's tests for individual comparisons at 0.05 level of significance.

RESULTS

KM showed the least water sorption and KFL the greatest water sorption, with mean values varying from 101.0 to 189.6 $\mu\text{g}/\text{mm}^3$ (Table 1). GICs associated with lyophilized propolis or EEP showed significantly more water sorption than each pure group ($P<0.05$). The lyophilized form promoted greater water sorption in comparison to groups with EEP. KF presented less water sorption than C, and was not statistically different from KM, both indicated for ART. Thus, the null hypothesis that there is no difference of water sorption among GICs with or without propolis was rejected.

CL presented the least water solubility whereas KFL presented the highest solubility, varying from

Number	Compounds	Sample BRP1 (mg/g)
1	6-Propenoic-2,2-dimethyl-8-prenyl-2H-1-benzopiran acid	12.61
2	3,5-Diprenyl-4-hydroxycinnamic acid (ARTEPILLIN C®)	29.50
3	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 1)	0.94
4	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 2)	2.52
5	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 3)	1.21
6	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 4)	0.98
7	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 5)	1.34
8	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 6)	1.37
9	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 7)	6.37
10	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 8)	3.55
11	3,5-Diprenyl-4-hydroxycinnamic acid* (derivative 9)	0.41
12	3-Prenyl-4-hydroxycinnamic acid	6.51
13	Caffeic acid	1.70
14	Caffeoylquinic acid 1**	2.58
15	Caffeoylquinic acid 1**	1.05
16	Caffeoylquinic acid 1**	10.16
17	Caffeoylquinic acid 1**	16.34
18	Caffeoylquinic acid 1**	0.83
19	Cinnamic acid*** (derivative 1)	9.33
20	Cinnamic acid*** (derivative 1)	2.35
21	Cinnamic acid*** (derivative 1)	65.05
22	p-Coumaric acid	14.56
23	Kaempferide	21.88
24	Kaempferol	2.51
25	Pinobanksin	33.21
	Total (mg/g of propolis <i>in natura</i>)	254.57
	Total (%) (m/m)	25.46

* Same UV spectrum of 3,5-Diprenyl-4-hydroxycinnamic acid, with different retention time. Expressed in 3,5-Diprenyl-4-hydroxycinnamic acid

** Same UV spectrum of Caffeic acid, with different retention time. Expressed in Caffeic acid

*** Same UV spectrum of Cinnamic acid, with different retention time. Expressed in Cinnamic acid

Source: Marcucci¹⁴ (2000).

Figure 1- Amount of each identified component by high-efficiency liquid chromatography analysis of *in natura* propolis

-50.56 to 1.25 $\mu\text{g}/\text{mm}^3$ (Table 2). Except for KFL, all GICs showed negative values of solubility. For KF, there was no difference among the subgroups of this product. For KM groups, both associations of the GIC with propolis showed greater solubility compared to control group KM; however, KML did not differ significantly from KM and KME. CE and C demonstrated significantly greater solubility in water compared to CL. KF reached higher values of solubility in water when compared to C and KM, both indicated to ART. Data revealed distinct performances among tested materials and solubility was material-dependent. The null hypothesis that there is no difference in water solubility of GICs with

or without propolis is partially accepted.

KME showed the lowest DTS and C the highest DTS, varying from 7.74 to 18.77 MPa (Table 3). When GICs were not associated with any kind of propolis, DTS reached the highest values compared to GIC-propolis groups. C demonstrated the highest value with significant differences from all groups, except for KF with the second highest DTS means. KM was less resistant to KFL, which reached the third greatest resistance under DTS test. Only C was significantly higher to its propolis-associated counterparts. KF was not significantly different from C and KM. Propolis form seems not to influence differently each tested GIC. As the performance

Products/Abreviation	Manufacturer	Composition*	Classification	Lot/ Expiry	Weight Proportion P/L
Ketac Molar Easymix (KM)	3M ESPE, Dental Products, St. Paul, MN, USA	Power: Calcium aluminum lanthan fluorosilicate glass, copolymer, pigments Liquid: Acrylic acid, maleic acid copolymer, tartaric acid, benzoic acid	High-density GIC	Power: 12.5 g 223626 (04/2007) Liquid: 8.5 mL 212425 (06/2007)	2.9:1.0
ChemFlex© (C)	DENTSPLY, Rio de Janeiro, RJ, Brazil	Power: Estrontium fluoraluminium silicate glass Liquid: Poli acrylic acid, tartaric acid, pigments	High-density GIC	Power: 15 g 0503001430 (03/2008) Liquid: 6 mL 0412000907 (10/2007)	3.8:1.0
Ketac Fil Plus (KF)	3M ESPE, Dental Products, St. Paul, MN, USA	Power: Calcium aluminum lanthan fluorosilicate glass, strontium. Liquid: Water, acrylic an maleic acids copolymer, tartaric acid and benzoic acid	Conventional GIC	Power: 10 g 254776 (06/2009) Liquid: 8.3 mL 255496 (07/2009)	3.2:1.0

Figure 2- Materials tested

Table 1- Means (standard errors) of water sorption

Material	Water Sorption ($\mu\text{g}/\text{mm}^3$)	
	($\mu\text{g}/\text{mm}^3$)	
KFL	189.6 (9.75)	A
CL	186.6 (4.81)	A
CE	177.0 (4.60)	A
KML	153.8 (2.93)	B
KME	148.3 (3.09)	B
C	146.4 (2.78)	B C
KFE	125.0 (1.40)	C
KF	114.8 (6.50)	D
KM	101.0 (2.55)	D

Different letters indicate statistically significant differences among materials ($P < 0.05$). L= lyophilized; E= ethanolic extract of propolis

was material-dependent, the null hypothesis that there is no difference on the DTS of the GICs with or without propolis was partially accepted.

DISCUSSION

Dentistry has focused on different technologies to develop new materials and approaches to dental restorations^{2,10}. With this objective, association of

Table 2- Means (standard errors) of water solubility ($\mu\text{g}/\text{mm}^3$)

Material	Solubility ($\mu\text{g}/\text{mm}^3$)	
KFL	1.25 (9.61)	A
KF	-10.32 (1.15)	A B
KFE	-14.39 (2.24)	A B C
KME	-16.13 (3.03)	A B C D
CE	-19.74 (2.35)	B C D E
C	-24.31 (5.44)	B C D E F
KML	-30.76 (2.05)	C D E F
KM	-42.28 (1.15)	F G
CL	-50.56 (2.85)	G

Different letters indicate statistically significant differences among materials ($P < 0.05$). L= lyophilized; E= ethanolic extract of propolis

available materials with different substances has led to a promissory field, but further research has to be done to allow this actual application. Following this rationale, propolis as an easy available natural substance seems to be a great option for dental treatment. However, few studies still reported this^{19,30}. Due to their relevant antimicrobial features, conventional GIC is the material of choice to caries high-risk patients^{27,28}. Since the introduction of the ART approach, high-density materials with

Table 3- Means (standard errors) of diametral tensile strength (MPa)

Material	Diametral tensile strength (MPa)	
C	18.77 (2.34)	A
KF	17.33 (1.97)	A B
KFL	12.90 (0.85)	B
KM	12.46 (0.62)	B
CE	11.94 (0.82)	B
CL	10.76 (0.76)	B
KML	9.57 (0.76)	B C
KFE	8.10 (0.82)	B C
KME	7.74 (0.56)	D

Different letters indicate statistically significant differences among materials ($P < 0.05$). L= lyophilized; E= ethanolic extract of propolis

conventional setting have been introduced in order to clinically serve in more stressful areas as posterior teeth^{27,28}. Although these materials have a conventional setting, their high density could influence the capacity of propolis to affect these materials and so they were included in the present study.

As GIC is a hydrophilic material, it is critical under highly moist conditions. Water plays an important role on the physical-mechanical properties of GICs as they are based on an acid-base reaction¹⁷. GICs restorations are susceptible to gain and loss of water, making superficial protection a relevant procedure⁴. In the present study, no protection was made to test the maximum potential of each material under water critical storage.

Analyses of water sorption revealed values from 101.0 to 189.6 $\mu\text{g}/\text{mm}^3$, which are in agreement with previously reported investigations^{4,5,17}. Cefaly, et al.⁵ (2003) evaluated water sorption of high-density viscosity GICs used in ART and found means of 137.66 $\mu\text{g}/\text{mm}^3$ (Fuji IX), 100.97 $\mu\text{g}/\text{mm}^3$ (Ketac Molar) and 120.34 $\mu\text{g}/\text{mm}^3$ for Ketac Fil Plus, which are similar to the values of water sorption of the present study.

In the present study, groups in which GIC was associated with lyophilized propolis showed greater sorption. Water excess is not clinically desired as it can be detrimental to the physical properties. However, when GICs were associated with EEP, the values were more similar to the control groups, making this option more indicated to be clinically applied. Additionally, it has been to consider that in this test, no superficial protection was performed and this care can reduce this limitation once in a clinical service.

Solubility in water is also a relevant property, as a soluble material can reach pulp tissue through

dentinal tubules. If a material presents beneficial characteristics, solubility can be positive; on the other hand, toxic products can compromise biological functions of pulp¹³.

The values of the present study ranged from -50.56 to 1.25 $\mu\text{g}/\text{mm}^3$. There are different methods to analyze the effect of solubility^{1,21}, which impairs the comparison with previous investigations. However, our findings are in accordance with those of Mortier, et al.¹⁷ (2004).

Except for KFL, all materials showed negative values, which can indicate that these materials suffered water sorption in a level that could have masked the actual solubility in water. It can be explained by the high hydrophilicity of GIC-based materials. Addition of propolis seems to optimize this property.

According to the results of this test, all groups presented solubility similar to that of the respective control groups, which is considered clinically acceptable by previous studies. In laboratory, it was observed evident solubility of both combinations using lyophilized and EEP forms. Water storage presented yellow coloration, which can indicate release of propolis. As propolis is a natural and biocompatible substance^{15,20}, its release is clinically interesting acting synergically to fluoride with anticariogenic properties.

As combination of GIC and propolis has demonstrated alterations, mechanical strength was important to be investigated. DTS is usually applied to test brittle materials as GIC^{4,18,23,29} and so was properly used in this investigation.

DTS values varied from 7.74 to 18.77 MPa, similar results of previous reports^{4,29}. Yap, Pek and Cheang²⁹ (2003) evaluated high-density GICs with values of 12.27 MPa (Fuji IX GP) and 10.55 MPa (Fuji IX GP Fast) after 24 h water storage. Except for Chemflex, all tested groups were not statistically different, which indicates that no significant alterations were verified. It suggests that this combination can be applied.

However, some difficulties observed in this study have to be mentioned. Addition of lyophilized propolis led to a hard manipulation. When EEP was added, the opposite was verified. It requires attention to material preparation to obtain the adequate consistency and to not compromise material's properties. Another limitation is attributed to color change of groups in which propolis was associated with GIC. As the material turned into yellow, it could be indicated as base or liner not to compromise restorative color match.

Based on the results of this investigation, the use of propolis combined with GICs is promising, in spite of some limitations. Other types of propolis, such as red one, are getting available presenting successful performance²⁶. It would be of particular

interesting as a resource against caries disease and prevention to secondary caries. However, more studies should be conducted to analyze the potential and risks before clinical use. Other technologies, such as the incorporation of hydroxyapatite and fluorapatite nanobioceramics to GIC are under investigation using ethanol based sol-gel technique and it also seems to be promising¹⁸. However, these strategies are mostly focused on mechanical and not antimicrobial features. All these new data highlight GIC as a dental material with excellent perspectives. Special attention should be directed to the limitation of GICs, as stated by Mjör¹⁶ (2007), who called the attention to studies investigating their anticariogenic potential, as one of the basic problems is that the release of anticariogenic agent may result in material degradation¹⁶. Additionally, controlled clinical trials have to be done to verify the correlation to *in vitro* strength and anticariogenic observations¹⁶.

The properties evaluated in this study are useful to consider the factors to be further investigated in order to enhance the applicability of combining GIC and propolis in a single product.

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