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Effects of the reinforced cellulose nanocrystals on glass-ionomer cements

Rafael Menezes-Silva[a,*], *Bruna Medeiros Bertol de Oliveira*[b], *Paulo Henrique Martins Fernandes*[a], *Lívia Yukari Shimohara*[a], *Fabiano Vargas Pereira*[c], *Ana Flávia Sanches Borges*[a], *Marília Afonso Rabelo Buzalaf*[d], *Renata Corrêa Pascotto*[b], *Sharanbir K. Sidhu*[e], *Maria Fidela de Lima Navarro*[a]

[a] *Department of Dental Materials, Endodontics and Operative Dentistry, Bauru School of Dentistry-FOB-USP, Al. Octávio Pinheiro Brisolla, 9-75, 17012-901, Bauru, SP, Brazil*

[b] *Department of Dentistry, State University of Maringa, Av. Mandacaru, 1550, 87080-000, Maringá, PR, Brazil*

[c] *Department of Chemistry, Federal University of Minas Gerais, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil*

[d] *Department of Biological Sciences, Bauru School of Dentistry-FOB-USP, Al. Octávio Pinheiro Brisolla, 9-75, 17012-901, Bauru, SP, Brazil*

[e] *Institute of Dentistry, Barts & The London School of Medicine and Dentistry, Queen Mary University of London, Turner Street, London, E1 2AD, UK*

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ABSTRACT

Objective. Glass-ionomer cements (GICs) modified with cellulose nanocrystals (CNs) were characterized and evaluated for compressive strength (CS), diametral tensile strength (DTS) and fluoride release (F^-).

Methods. Commercially available GICs (Maxxion, Vidrion R, Vitro Molar, Ketac Molar Easy Mix and Fuji Gold Label 9) were reinforced with CNs (0.2% by weight). The microstructure of CNs and of CN-modified GICs were evaluated by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) while chemical characterization was by Fourier transform infrared spectroscopy (FTIR). Ten specimens each of the unmodified (control) and CN-modified materials (test materials) were prepared for CS and DTS testing. For the fluoride release evaluation, separate specimens ($n = 10$) of each test and control material were made. The results obtained were submitted to the t-test ($p < 0.05$).

Results. The CN reinforcement significantly improved the mechanical properties and significantly increased the F^- release of all GICs ($p < 0.05$). The GICs with CNs showed a fibrillar aggregate of nanoparticles interspersed in the matrix. The compounds with CNs showed a higher amount of C compared to the controls due to the organic nature of the CNs. It was not possible to identify by FTIR any chemical bond difference in the compounds formed when nanofibers were inserted in the GICs.

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* *Corresponding author at*: Alameda Dr. Otávio Pinheiro Brisola, 9-75, Vila Nova Cidade Universitária, Bauru, São Paulo, 17012-901, Brazil.
E-mail address: rafa18ms@hotmail.com (R. Menezes-Silva).

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Significance. Modification of GICs with CNs appears to produce promising restorative materials.

1. Introduction

Since the 1950s, when the deleterious effects of mercury on the population that fed on contaminated fish from Minamata Bay in Japan were observed, a global movement has been observed to control and reduce its use in a variety of products, processes, and industries [1]. These actions culminated, in October 2013, with the signing of the Treaty of Minamata by 128 countries, with the aim of reducing the atmospheric emissions of the metal through environmental practices and the best techniques available for new ventures [1].

Dental amalgam is the only dental product that contains mercury. Although there is no evidence that it causes a deleterious effect on the human body in the quantity that is used in restorations [2], the restorative technique using dental amalgam, however, causes significant removal of healthy tooth structure and for this reason several international organizations have proposed the use of materials that allow procedures that preserve the maximum amount of tooth tissue based on Minimally Invasive Dentistry (MID) [3]. Thus, all countries that signed the Treaty of Minamata agreed on the gradual reduction of amalgam until banned from clinical use and on the need to improve alternative materials for amalgam as well as the invention of new materials [1].

Currently the two main dental materials available in restorative dentistry as alternatives to amalgam restorations are composite resins and glass-ionomer cements (GICs) [4].

GICs are materials with unique properties such as fluoride release, biocompatibility and the ability to adhere to dental structures. In general, compared with composite resins, the first restorative GICs had insufficient mechanical properties such as low compressive and tensile strengths as well as wear resistance [5].

In order to overcome the poor mechanical properties of the GICs, various modifications have been introduced into the cement powder and liquid, such as incorporation of bioautonomous apatite, zirconia, zinc, strontium oxide, fibers, stainless steel, silica, and titanium dioxide among others [6–14]. However, such modifications may be more expensive and may not be able to increase any mechanical property evaluated by 100%, and often compromising the manipulation of the GICs.

For many years, there have been attempts to incorporate fibers as reinforcing agents in these materials to increase their strength [15,16].

Development in nanotechnology research has attracted the use of cellulose due to the fact that it comes from renewable and abundant sources, besides being non-toxic and possessing low thermal density and expansion [15]. The nanocrystals present in cellulose, in addition to carbon nanotubes, are considered excellent options for reinforcement, favoring improvements of the material's mechanical properties. Cellulose nanocrystals (CNs) have excellent dispersion in the matrix besides maintaining the integrity and purity of the material. In addition to these characteristics, CNs have a much lower cost when compared to carbon nanotubes [15].

The addition of CNs to GIC surpassed previous studies using macrofibers and microfibers [17,18]. CN-modified GIC resulted in greatly improved resistance to abrasion, and increased compression, diametral tensile strength and modulus of elasticity, using a lower concentration of cellulose in the material composition [19], besides being biocompatible [20].

Previous modifications of GICs [17–20] were made using a medium viscosity cement. There is a lack of studies with high viscosity GICs, in which the addition of nanoparticles could increase the mechanical properties of GICs to the levels of amalgam and composite resins. In addition, it is important to note that fluoride ions (F^-) released gradually by a GIC material confers an anticariogenic property to the restorative material [21], preventing new carious lesions [22]. Therefore, the objective of this study was to modify several commercial brands of medium and high viscosity restorative glass-ionomer cement with CNs, and to evaluate the effect of this on the compressive strength, diametral tensile strength and fluoride release of the resulting compounds.

2. Materials and methods

2.1. *Obtaining the cellulose nanocrystals*

The CNs were obtained through an acid hydrolysis process using sulfuric acid (64 wt%). Eucalyptus kraft pulp (20 g) was added to 160 mL of sulfuric acid solution under strong stirring at 50 °C. After 30 min of reaction, the dispersion was washed three times with distilled water using centrifugation steps. Finally, the material was dialyzed against deionized water. The material was then ultrasonicated over 2 min to obtain a dispersed aqueous suspension (concentration ~1% m/v).

2.2. *Specimens preparation*

Specimens of three commercial brands of medium viscosity restorative GICs [Maxxion (Ma) — DFL, Rio de Janeiro, Brazil; Vidrion R (V) — SSWhite, Rio de Janeiro, Brazil and Vitro Molar (VM) — DFL, Rio de Janeiro, Brazil] as well as two brands of high viscosity restorative GICs [Ketac Molar Easy Mix (KM) — 3M ESPE, St. Paul, USA and Fuji Gold Label 9 (F) — GC, Tokyo, Japan] were used.

For each material, there were test and control groups of ten specimens each. In the test groups (GICs–CN), the materials were modified by adding 0.2% of CN in relation to the total mass of the cement. To calculate the total mass of each species of each commercial brand, the ratio of powder:liquid by weight was taken into account. The CNs were added to the liquid of GICs, sonicated for 2 min to ensure complete homogenization and then incorporated to the GIC powder, respecting the powder:liquid ratio by weight and manually manipulated in accordance with the manufacturer's recommendations.

Handling of materials was carried out at controlled temperature (23° ± 1 °C) and relative humidity (50 ± 5%). The powder:liquid (P/L) ratio used was measured by weight and manipulation of materials was done according to the individual manufacturer's instructions. The unmodified GIC materials served as the controls for each commercial material.

2.3. *Sample calculation*

To determine the sample size (n), the comparison of means formula was used. The level of significance (Zα) and statistical power (Zβ) were considered 95% and 80%, respectively. The difference (D) to be detected among groups was stipulated at 16. The value referring to the standard deviation (sd = 11.49) was obtained after a pilot study. To this value, 20% was added to predict eventual losses. Thus, the minimum to be investigated became 9.69.

2.4. *Compressive strength (CS) and diametral tensile strength (DTS) evaluations*

For the mechanical tests, specimens (n = 10) of each group were prepared using a steel mold (6 mm in height and 4 mm in diameter) for CS tests and a separate steel mold (6 mm in height and 3 mm in diameter) for DTS tests, according to the relevant standards (ISO 9917-1: 2007 and ABNT NBR 7222:2011, 2011) [23,24]. In each instance, after mixing the cement was inserted into the mold with a syringe and covered by a polyester strip at the top and bottom surfaces. The specimen was then compressed between two steel plates and a screw clamp.

The whole assembly was stored at 37 °C and a relative humidity of at least 90%, for 1 h. The cement specimens were then carefully removed from the molds and stored for a further 23 h in distilled water at 37 °C before testing.

Each group was subjected to the CS test in an universal testing machine (Kratos Equipamentos, K5000, Cotia, Brazil) with a load cell of 200 kgf at a velocity of 0.75 mm min^{-1} and with its long axis in the vertical position, until the fracture occurred. For the DTS test, specimens of each group (n = 10) were subjected to the same load cell, but with a velocity of 0.5 mm min^{-1} and with the long axis in the horizontal position. The results obtained were converted into MegaPascals (MPa) by the accompanying software [23,24].

2.5. *Fluoride release*

For the assessment of fluoride release, specimens (n = 10) of each filling material were made using cylindrical teflon molds (11.0 mm in diameter and 3.0 mm thick).

For each specimen, the cement was mixed and inserted into the mold and covered with a polyester strip at the top and bottom surfaces and compressed between two glass plates. A nylon thread piece was introduced in the middle of the specimen, to allow it to be suspended within the storage media. After the initial curing reaction, the specimens were individually immersed in acrylic bottles, containing 20 mL of deionized water and stored at 37 °C for 14 days. The F^- release of each specimen was measured after 1, 6, 12, 24, 72, 168 and 336 hours by using an Orion fluoride-specific electrode (model 96-09, Thermo Fisher Scientific, Waltham, MA, USA) and a Procyon digital ion-analyzer (model SA-720, Orion Research Inc., Thermo Fisher Scientific). For this analysis, 1 mL of solution was mixed with an equal volume of TISAB II. All analyses were made in duplicate. The mean repeatability of the readings, based on duplicate samples, was 98%. The mV readings were then converted into $\mu\text{gF/mm}^2$ using the formula:

$$F^- \text{ amount released} * \text{total quantity of solution} / \text{specimen area.}$$

2.6. *Morphologic and chemical characterization*

2.6.1. *Transmission electron microscopy (TEM)*

TEM (Tecnai G2-12 – Spirit Biotwin FEI – 120 kV, Hillsboro, Oregon, USA) was used to characterize the microstructure of CNs. Drops of a diluted suspension of the CNs were deposited onto carbon Formvar-coated copper (300 mesh) TEM grids and negatively stained with 2% uranyl acetate to enhance the microscopic resolution.

2.6.2. *Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analysis*

Representative samples of each group were covered with a thin layer of gold-palladium. The prepared materials were observed by SEM, using an electron beam of 10–15 kV (CS-3500, Shimadzu, Kyoto, Japan). The qualitative elemental analysis of micro regions of the samples was performed using EDS (CS3200, Oxford, England).

2.6.3. *Fourier transform infrared spectroscopy (FTIR)*

Three samples were made for each control (unmodified) and test (modified) material. After mixing, the material was interposed between two polyester strips and compressed between two glass plates. After 1 h of initial curing, the GIC samples were ground in a mortar by an agate pestle and prepared to be measured using FTIR.

Spectra of the material samples were obtained with 128 scans in the interval between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1}, by means of FTIR, (Vertex70/70v spectrometer, Bruker, Billerica, Massachusetts, USA).

2.7. *Statistical analysis*

The results were analyzed using SPSS (Statistical Package for Social Sciences, IBM Inc., New York, NY, USA), version 20.0, for descriptive statistical analysis. After the normality (Shapiro–Wilk) and homogeneity of the tests of variance (Levene), the results of the mechanical properties and fluoride release were submitted to the t-test ($p < 0.05$).

Table 1 – Means, standard deviations and statistical significance of the results of the tests of compressive strength and diametral tensile strength for control and test groups of all materials tested.

GIC	Group	Compressive strength (MPa)		Diametral tensile strength (MPa)	
		Mean (DP)	p^*	Mean (DP)	p^*
Maxxion R	Ma	98.42 (3.67)	0.041	16.85 (8.12)	0.020
	Ma-CN	130.28 (25.30)		24.21 (2.26)	
Vidrion R	V	89.15 (6.38)	<0.001	30.16 (12.68)	<0.001
	V-CN	133.22 (26.42)		54.13 (19.22)	
Vitro molar	VM	97.58 (18.70)	<0.001	24.10 (9.87)	0.048
	VM-CN	134.46 (14.24)		30.09 (4.51)	
Ketac molar	KM	133.36 (23.78)	<0.001	22.99 (9.06)	<0.001
	KM-CN	198.42 (34.66)		48.08 (6.64)	
Fuji IX	F	157.37 (25.36)	0.003	32.47 (8.91)	0.038
	F-CN	208.26 (16.84)		48.25 (11.81)	

* T test for independent samples ($p < 0.05$).

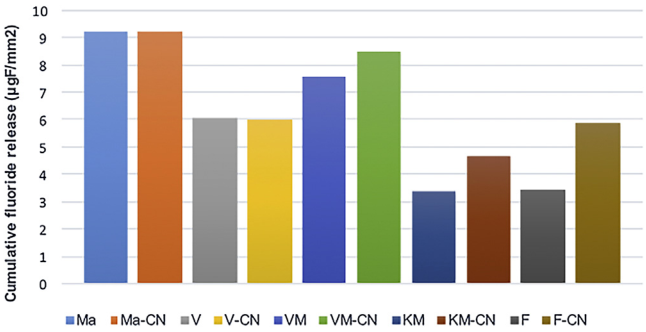

Fig. 1 – Cumulative fluoride release (μg/mm^2) of control and test material groups in 14 days.

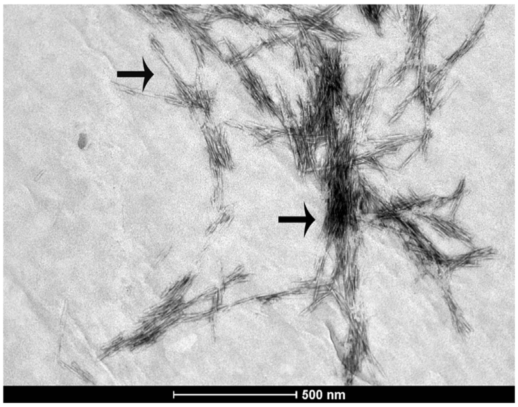

Fig. 2 – TEM images showing individual nanocrystals and also some laterally aggregated nanoparticles (arrowed).

3. Results

The mean values for the CS and the DTS with the standard deviations for the tested materials are summarized in Table 1. In general, the CN reinforcement significantly improved the mechanical properties tested for all conventional GICs ($p < 0.05$). The groups with CN reinforcement present an increased value for CS and DTS, respectively: 31.86% and 43.67% for Ma; 49.43% and 79.47% for V; 37.79% and 24.85% for VM; 48.78% and 109.13% for KM and 32.33% and 48.60% for F.

The means and standard deviations of fluoride release (μgF/mm^2) for all groups are summarized in Table 2. The addition of CN to Ma significantly increased the F^- release until the first 24 h and after 14 days. For the V material, the addition of CN significantly increased the F^- release after the first hour, 24 h and 14 days. For VM, the addition of CN significantly increased the F^- release after 6 h, 12 h and 3 days of evaluation. The addition of CN to KM increased the F^- release until the first 24 h. The addition of CN to F increased the F^- release in all the evaluation periods.

The total amount of F^- ions released for the 15 days is, in descending order: Ma-CN > Ma > VM-CN > VM > V > V-CN >F-CN > KM-CN > F > KM (Fig. 1).

Fig. 2 represents a typical TEM image of the CNs obtained from eucalyptus. The image shows individual nanocrystals and also some laterally aggregated nanoparticles. The aggregation is expected and occurs due to the strong hydrogen bonds' interaction during the drying and/or staining the sample for TEM observation. From several TEM images of CNs, we measured the mean values of length and diameter as being 145 ± 25 nm and 6 ± 2 nm, respectively.

The SEM images of the GIC–CN compounds can be seen in Fig. 3.

The very fine format of CNs facilitated its homogeneous dispersion in the GIC liquid and its more uniform insertion and distribution throughout the cement mass [25]. The SEM images of the GIC–CN compounds showed a fibrillar aggregate of nanoparticles interspersed in the matrix of the GIC, suggesting the formation of a single complex, which indicates interaction of the nanofibrils (formed by the union of the nanocrystals) with the cement matrix and the particles of powder that are not attacked. The long fibers observed seem to be formed from a lateral aggregation of the CN within the GIC matrix. These fibers were shown to be interconnected, in addition to adhering to the particles of GIC. The EDS analysis of the GICs showed the presence of Ca, F and C in the organic matrix of GICs. The GIC–CN compounds showed the same pattern with higher amounts of C due to the organic nature of the CNs (Fig. 3).

The FTIR spectra of the GICs and GIC–CN materials tested are displayed in Fig. 4. The absorption bands indicate the

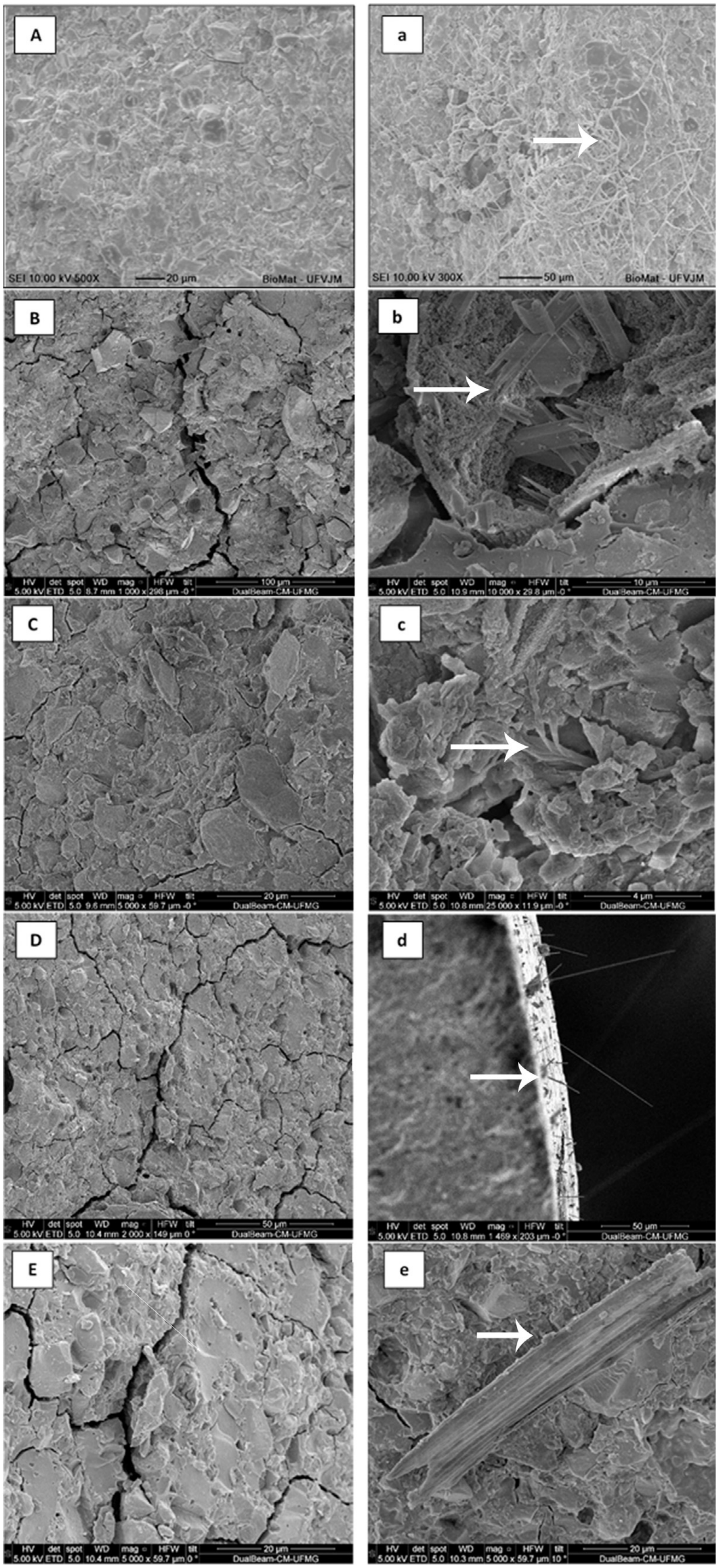

Fig. 3 – SEM images of the GICs with CNs, showing a fibrillary aggregate of nanoparticles (arrowed) interspersed in the matrix: Ma (A) (500×); Ma-CN (a) (300×); V (B) (1000×); V-CN (b) (10,000×); VM (C) (5000×); VM-CN (c) (25,000×); KM (D) (20,000×); KM-CN (d) (1469×); F (E) (5000×); F-CN (e) (5000×).

presence of OH and SI–OH (3455 cm^{-1}), OH (3267 cm^{-1}), COO^{-} (1720 cm^{-1}), C=O (1637 cm^{-1}), aluminum polyacrylate (1447 cm^{-1}), calcium polyacrylate (1409 cm^{-1}) and Si–OH (1190 cm^{-1}). The spectra demonstrated that it was not possible to identify any chemical bond difference during the setting reaction of the GIC.

4. Discussion

The aim of the present study was to develop a CN-reinforced GIC that could be used to replace amalgam in dental restorations. The results of this study showed that CN incorporation improved the compressive and diametral tensile strength of all materials tested. It is important said that the proportion of CN added to GICs caused agglutination to occur easily in these cements, and provided a working time similar to that of the commercial GICs, without changing their final setting time clinically observed, thus respecting the characteristics of clinical relevance of this restorative material. Regarding the cost, 1 kg of CN costs US $500. Due to the small amount of CN that is used for reinforcement, it would increase the cost of each kit of GIC by approximately US 3 cents.

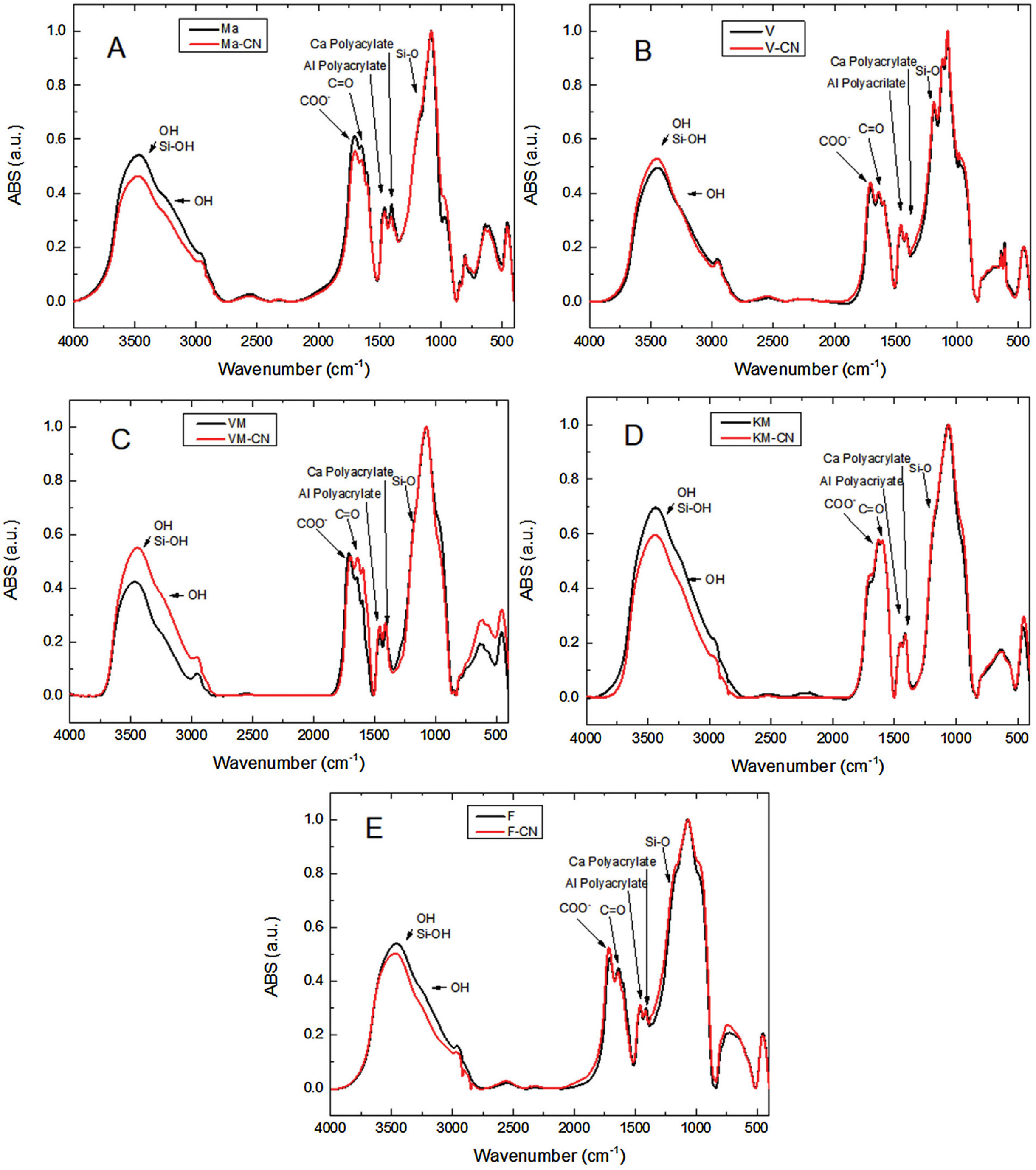

Fig. 4 – FTIR spectra of GICs and GIC–CN with the functional groups identified. (A) Ma and Ma-CN spectra. (B) V and V-CN spectra. (C) VM and VM-CN spectra. (D) KM and KM-CN spectra. (E) F and F-CN spectra.

Table 2 – Means and standard deviations for fluoride release (μgF/mm^2) of control and test material groups.

Group	1 h	p^*	6 h	p^*	12 h	p^*	24 h	p^*	3 days	p^*	7 days	p^*	14 days	p^*
Ma	1.72 (0.18)	<0.001	2.19 (0.19)	<0.001	1.56 (0.16)	<0.001	1.42 (0.56)	0.031	1.04 (0.32)	0.054	0.81 (0.23)	0.467	0.49 (0.05)	0.011
Ma-CN	1.91 (0.41)		2.31 (0.23)		1.51 (0.17)		1.21 (0.38)		1.07 (0.26)		0.72 (0.08)		0.52 (0.10)	
V	0.27 (0.11)	0.006	0.79 (0.19)	0.871	0.81 (0.06)	0.703	0.69 (0.10)	0.004	1.22 (0.19)	0.655	1.22 (0.11)	0.790	1.08 (0.05)	0.009
V-CN	0.14 (0.05)		0.80 (0.08)		0.82 (0.08)		0.82 (0.08)		1.19 (0.07)		1.23 (0.09)		1.01 (0.05)	
VM	1.05 (0.45)	0.483	0.95 (0.14)	0.003	0.91 (0.20)	0.013	1.00 (0.18)	0.632	1.34 (0.15)	0.066	1.25 (0.11)	0.664	1.07 (0.13)	0.748
VM-CN	1.20 (0.42)		1.23 (0.20)		1.25 (0.34)		1.04 (0.20)		1.47 (0.14)		1.22(0.11)		1.09 (0.08)	
KM	0.28 (0.18)	0.038	0.30 (0.06)	<0.001	0.32 (0.07)	<0.001	0.32 (0.09)	0.021	0.60 (0.14)	0.069	0.76 (0.14)	0.652	0.81 (0.06)	0.943
KM-CN	0.57 (0.37)		0.76 (0.17)		0.52 (0.07)		0.46 (0.14)		0.76 (0.22)		0.79 (0.16)		0.80 (0.15)	
F	0.80 (0.15)	<0.001	0.31 (0.06)	<0.001	0.34 (0.08)	<0.001	0.48 (0.13)	0.047	0.49 (0.11)	0.014	0.50 (0.11)	0.013	0.49 (0.07)	0.003
F-CN	0.89 (0.16)		0.97 (0.47)		0.91 (0.32)		0.78 (0.43)		0.79 (0.33)		0.80 (0.33)		0.72 (0.19)	

* T test for independent samples ($p < 0.05$).

Mechanical resistance is a fundamental property for the indication of a restorative material for areas directly exposed to masticatory loads [26]. When the medium viscosity GICs (V, Ma and VM) were reinforced with CN, they acquired the resistance of high viscosity GICs. Silva et al. [19] previously reported that the addition of a small amount of CN in a conventional GIC (Vidrion R) led to significant improvements in all of the mechanical properties evaluated. The compressive strength for CN-modified Vidrion R, V-CN, increased by 17% compared with the control group and diametral tensile strength increased by 71%. In the present study, the CS for the same material was increased to 49% and DTS up to 80%. The improvement of mechanical properties can be due to the ability of CN to bind to the hydroxyl groups of the glass particles and to the carboxylic groups of the polyacrylic acid by means of hydrogen bonding; Petri et al. [27] showed that the flexural strength of a commercial GIC was considerably improved by the addition of a tiny amount of chitosan. They observed that the network formed by chitosan and polyacrylic acid around the inorganic particles must have reduced the interfacial tension between the components of the GIC, thus improving the mechanical performance of the cement.

The differences between the increases in the strengths of the different groups tested depends on the size of the particles of the cements. The anionic nature of the CNs contributes to the strong electrostatic interaction between the positive charges of the GIC and the negative charges of the CN, contributing to its dispersion and reinforcement of the cements [28].

In addition to systematic reviews of the literature concluding that there are no differences between resin and amalgam restorations as a function of the Minamata Treaty, new clinical trials between these conventional materials are not necessary. However, new studies between composite resin, as control, and GIC are required [29–31].

A more recent study, which clinically compared conventional restorations with the high viscosity GIC Equia Fil (GC Corporation, Tokyo, Japan) and composite resin, showed that both materials showed good clinical performance for posterior tooth restorations during the evaluation of 6 years for both class I and class II, and none of the materials was superior to the other [32].

Mickenautsch analyzed the state of the art of direct restorations in posterior permanent teeth using high viscosity GICs and concluded that when used in Atraumatic Restorative Treatment (ART) restorations they presented a clinical performance similar to amalgam restorations [33]. The following year, Kielbassa et al. suggested that Equia Fil could indeed be a good alternative for dental amalgam in countries where health services do not cover composite resins or in situations where people have allergies to polymers [34]. According to the manufacturer, the newly developed Equia Forte (evolution of Equia Fil), has a compressive strength equal to 219 MPa [35], which is still considered very close to the values of the high viscosity GICs reinforced by CN.

High viscosity GICs have demonstrated effectiveness in single surface restorations made using ART in permanent and deciduous teeth [36]. If the CNs were incorporated in medium viscosity GICs they could also be indicated for single surface restorations made using ART. High viscosity GICs enhanced with CNs improved the strength of the cements even further and therefore their indication for ART could be extended to class II restorations.

Reinforced GIC could also be used in conventional treatment of patients, especially those with high dental caries, as these materials have adhesion to tooth surface and anticariogenic properties due to the fluoride release and continuous fluoride uptake [37]. The variation in the F^- release among the different types of GICs may be associated with the powder and liquid compositions, the time and method of agglutination [38], as well as the pH and storage conditions [39].

The concentration of nanocrystals used in these compounds may have provided an ideal interaction between the crystals themselves and the cement matrix during the chemical reaction with the formation of a reinforcing structure, whereas sulfuric acid treatment provided the surface of the fibers with large amounts of COO^- clusters.

The presence of some amount of amorphous phase in the diffractogram is due to the polymeric nature of the cellulose, which showed characteristics typical of the group called cellulose I of allomorphic structure, a basic constituent of wood pulp fibrils [40] and the silica gel formed by the attack of the polyacrylic acid to the surfaces of the loading particles, during the setting reaction of the cement. It has been observed that the great crystallinity of the GIC–CNs is related to the loading particles of the GICs, which are glass particles.

The SEM/EDS and FTIR of the compounds of this study showed the same characteristics of the GICs and CN as reported in the literature [19]. The use of CN in the GICs showed the formation of a network of long fibers with uniform width and random distribution inside the GIC matrix. The fiber network is formed by CN interconnected and adhering to the GIC particles. The formation of this structure may be explained by the significant nature of self-association of the CN, which is advantageous for the formation of supporting architectures for load percolation into the cement matrix; such architectures exhibit the formation of rigid CN networks in which the transfer of force is facilitated mainly by the hydrogen bond between the CN [41]. As a result of this arrangement, we observed a considerable reinforcement effect of the GIC.

The molecular structures of the nanocrystals, as well as all the compounds developed in the study, presented vibrational excitation resulting from the covalent bonds of the specific functional groups present in the materials [42]. It was not possible to identify by FTIR any chemical bond difference in the compounds formed when nanofibers were inserted in the GICs, as the results obtained were similar comparing the GIC and GIC–CN spectra for all materials of this study.

A recent article evaluating studies published on the mechanisms of fluoride action against dental caries during the last 65 years suggested a need to pay more attention to this topic since it can provide a scientific basis for the use of fluoride [43]. Fluoroaluminosilicate is the main component of GIC. As it is more soluble than barium and strontium, it is able to release more fluoride [44]. The high fluoride release in the first 24 h is due to the high erosion rate of newly agglutinated ionomeric cements [45]. The F^- release analysis is extremely important because, through the release of its ions, the ionomer is able to maintain a favorable remineralization site, because fluoride interferes with the metabolism of bacteria, binding to the enamel makes it more resistant to acids and decreases tooth demineralization [21,22].

The addition of CNs to GICs significantly increased the release of F^- at least in one of the periods tested in this study for all brands. The effect appears to be greater and sustained on the high viscosity GICs (KM and F). Studies that reinforced the GICs with chitosan, which is a similar biocomposite to CN, showed that the release of F^- from GIC is catalyzed [27]. Furthermore, de Amorim et al. [36] demonstrated that the fluoride release was significantly higher with nano chitosan particles in GIC compared to conventional GIC over 7 days. Considering these factors, the results of this study suggest that GIC–CN reinforcement will improve the anti-cariogenic and mechanical properties for high strength applications.

Nicholson et al. 1998 [46] reported that fluoride appears to enhance the compressive strength of GICs, being capable of achieving compressive strengths well above 200 MPa at 24 h, when on the other hand, fluoride-free cements reach compressive strengths above 100 MPa only. One reason for this is that fluoride seems to increase the reactivity of the glasses [47] and also can form strong hydrogen bridges with the aluminium carboxylate complexes in the GIC matrix [46,48]. Considering these factors, it is likely that the interaction of the CN particles with the components of the GIC powders in this study contributed to increased fluoride release and for the CN–GICs tested to achieve high values of compressive strengths. This result is important to improve the survival rate of glass-ionomer restorations especially in high stress-bearing areas.

Although GICs have improved significantly since they were launched in the market, they still present weakness in terms of mechanical strength [49,50]. Hence, the CN–GICs could be a way to improve the GICs as a whole, and as replacement materials for dental amalgam in the future.

In conclusion, the addition of a small amount of a nano particulate renewable material in the form of cellulose nanocrystals significantly increased the compressive and diametral tensile strengths of restorative GICs, as well as the release of fluoride. Modification of GICs with CNs seems to produce very promising restorative materials to be indicated as alternatives to dental amalgams. It is necessary for further studies to validate these findings and to verify the required conditions to lead us to an outstanding material for physical-chemical and biological performances.

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