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ORIGINAL ARTICLE

Resin-Modified Glass Ionomer Nanocomposite: Subcutaneous Connective Tissue Response and Polymerization Shrinkage Resistance

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ABSTRACT

Objective: To evaluate the *in vitro* polymerization shrinkage resistance and *in vivo* tissue response of the resin-modified glass ionomer cement (RMGIC) Ketac N100 after implantation in the subcutaneous connective tissue of isogenic mice. **Material and Methods:** A total of 90 isogenic BALB/c mice were randomly distributed into nine groups, with each group receiving an implant of one of the following materials: ChemFil, Ketac N100, Compoglass, and Filtek Z350, as well as empty tubes serving as controls. The *in vitro* polymerization shrinkage was evaluated using Ultralux with an irradiance of 480mW/cm², while *in vivo* tissue response was assessed through histological examination of tissue samples at 7, 21, and 63 days post-implantation. Statistical analysis was performed using ANOVA with an F-test, adopting a significance level of 5% and using Tukey's test for multiple comparisons. **Results:** Compoglass and Filtek Z350 exhibited similar levels of polymerization shrinkage, with no significant differences between them. Ketac N100 demonstrated polymerization shrinkage stress comparable to Filtek Z350 resin, indicating its performance is closer to composite resin than conventional glass ionomer. The histological analysis of the *in vivo* tissue response revealed that Ketac N100 had a favorable biocompatibility profile, similar to ChemFil and Filtek Z350, with no significant adverse tissue reactions. **Conclusion:** Ketac N100 exhibited a favorable tissue response and intermediate polymerization shrinkage, closer to composite resins than conventional glass ionomers. Both *in vitro* and *in vivo* analyses demonstrated the material's potential for clinical use.

Keywords: Dental Materials; Glass Ionomer Cements; Polymerization.

Introduction

Glass ionomer cement (GIC) is considered one of the most versatile materials due to its excellent properties, such as biocompatibility, reduced dentin sensitivity, chemical adhesion to dental tissues, and a coefficient of thermal expansion similar to dentin. Based on their chemical composition, GICs can be classified into conventional, high-viscosity metal-reinforced, and resin-modified categories [1,2]. This wide range and the potential for new formulations have allowed for the expansion of their clinical applications [3]. In this context, the evolution of molecular engineering and the development of molecular nanotechnology have enabled the creation and application of materials and structures with nanoscale dimensions ranging from 0.1 to 100 nanometers. Moreover, incorporating nanoparticles into dental materials has enhanced their mechanical, chemical, and aesthetic properties [4].

Simultaneously, resin-modified GICs have made aesthetic improvements possible, such as increased translucency, and have prevented susceptibility to syneresis and imbibition processes [5]. In this regard, a resin-modified GIC with nanoparticles was introduced to the market (Ketac Nano Light-Curing or Ketac N100 – 3M ESPE, Saint-Paul, MN, USA), which contains approximately 69% filler content, with two-thirds of this filler being composed of nanoparticles [6]. Studies on resin-modified glass ionomer cement nanoparticulate (NRMGI) have predominantly assessed the mechanical and chemical properties of this material, such as fluoride release [7], surface roughness, biodegradation, bond strength, microhardness, and marginal microleakage [8]. However, evaluating the tissue compatibility of GICs remains crucial [9-13], as it can influence their suitability for restorations in deep cavities close to the pulp and gingival tissue. From a biological perspective, assessing polymerization shrinkage is also important because it is associated with the formation of gaps between the material and the dental surface, which can lead to microleakage and potentially trigger pulp reactions [9].

The null hypothesis for this study is that there is no significant difference in the inflammatory response and polymerization shrinkage between Ketac N100 and other materials evaluated (ChemFil, Compoglass, and Filtek Z350). Therefore, this study aimed to assess the pattern of the inflammatory response caused by NRMGI (Ketac N100) when in contact with the subcutaneous connective tissue of isogenic mice and its polymerization shrinkage.

Material and Methods

Ethical Clearnce

The study design and parameters for evaluating tissue reaction were based on ISO 10993-6 [10]. The experiments were approved by the Committee on Animal Care and use of the School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil, under protocol number 2013.1.1403.58.8, following the guidelines and ethical regulations of the International Principles for Biomedical Research Involving Animals.

Subcutaneous Connective Tissue Study in Isogenic Mice

Ninety isogenic BALB/c mice, males aged 6-8 weeks and weighing between 15 and 20g, were randomly divided into nine groups, each containing 10 animals. Each animal received a sterilized polyethylene tube implant in the dorsal region, 10mm long and 1mm in diameter. After filling with different materials (experimental groups), empty tubes were also used as controls. The composition and characteristics of the materials used are detailed in Table 1.

For tube implantation, the animals were anesthetized with 10% ketamine (Agener National Chemical Union S/A, Embu-Guaçu, SP, Brazil) at a dose of 150mg/kg body weight and 2% xylazine (Dosaper, Calier Laboratories, SA, Barcelona, Spain) at a dose of 7.5mg/kg body weight. Subsequently, the dorsal region of the

animals was shaved and cleaned with 1% chlorhexidine (Farmoderm, Ribeirão Preto, SP, Brazil). A 1cm incision was made in the dorsal region, followed by tissue dissection and tube insertion into the connective tissue, with suturing using 4-0 silk thread (Vicryl; Johnson & Johnson: Ethicon Inc., New Brunswick, NJ, USA). The surgery was conducted under aseptic conditions, minimizing trauma in the implant area.

Table 1. Distribution of groups in different experimental periods.

Groups	Materials	N	Periods
I	Empty tube	10	7 Days
II	Empty tube	10	12 Days
III	Empty tube	10	63 Days
IV	ChemFil (Dentsply Sirona, Charlotte, NC, USA)	10	7 Days
V	ChemFil (Dentsply Sirona, Charlotte, NC, USA)	10	21 Days
VI	ChemFil (Dentsply Sirona, Charlotte, NC, USA)	10	63 Days
VII	Ketac N100 (3M ESPE, Saint-Paul, MN, USA)	10	7 Days
VIII	Ketac N100 (3M ESPE, Saint-Paul, MN, USA)	10	21 Days
IX	Ketac N100 (3M ESPE, Saint-Paul, MN, USA)	10	63 Days

During the experimental periods, the animals had free access to water and a standard diet. At the end of each experimental period, the animals were again anesthetized for the removal of the implant along with the surrounding tissues (skin and subcutaneous connective tissue) and then euthanized by anesthetic overdose. The removed tissues were fixed in buffered 10% formalin solution for 48 hours and underwent routine histotechnical processing. Serial sections of 4-5µm thickness were made parallel to the long axis of the tube and stained with hematoxylin and eosin. Both descriptive (qualitative) and quantitative (reactive granulomatous tissue) microscopic analyses of tissue reactions to the tested materials and empty tubes were performed at different experimental periods. Specimens were examined by an experienced pathologist using a binocular light microscope (Olympus Corp., Tokyo, Japan) at magnifications of 4x, 10x, 40x, and 100x.

Descriptive Microscopic Analysis

Each specimen underwent analyses and descriptions of morphological phenomena through optical microscopy at the tissue and cellular levels. Elements such as the presence of macrophages, multinucleated inflammatory giant cells, neutrophils, eosinophils, fibroblasts, and collagen fibers were considered. Efforts were made to identify particles of the tested material within the components of the reactive tissue.

Quantitative Microscopic Analysis

The total area of perimaterial granulomatous reactive tissue was measured in mm^2 at different time points using ImageJ 1.48 software (National Institute of Health, Bethesda, Maryland, USA). The results were correlated with the intensity of induced inflammation.

Statistical Analysis

The Shapiro-Wilk normality test was employed to determine if the distributions were normal. One-way ANOVA was used to compare differences between materials and the control, with pairwise comparisons conducted through Tukey's post-test, adopting a significance level of 5%. All analyses were performed using GraphPad Software, version 5.0a (GraphPad Software, LLC, Boston, MA, USA).

Polymerization Shrinkage Tensile Measurement Test

Glass rods with a diameter of 5mm and a height of 5cm were prepared, with one of the surfaces sandblasted with aluminum oxide to provide a rough surface for adhesion. The adhesive was applied to the

sandblasted surface for each material and polymerized according to the manufacturer's instructions (detailed in Table 2), and the compositions are included.

Table 2. Materials used, spatulation time, polymerization, and final setting time according to the manufacturer.

	Spatulation Time	Polymerization Time	Final Prey Time
ChemFil	40 Seconds	You don't need to	10 minutes
Vitremer	45 Seconds	40 Seconds	5 minutes
Ketac N100	20 Seconds	40 Seconds	5 minutes
Compoglass	You don't need to	40 Seconds	5 minutes
Z 350	You don't need to	40 Seconds	5 minutes

The rods were attached to the EMIC DL-2000 Universal Testing Machine for the tensile test. The distance between the machine bars was standardized at 2mm. The ratio of adhered to non-adhered faces, corresponding to the C Factor, was set at 2.5. An extensometer was attached to the rods, and the materials were placed on the rod after spatulation, following the manufacturer's instructions.

A total of 50 specimens were obtained, with 10 for each studied material. Four materials (Ketac N100, Filtek Z350, Compoglass F – Ivoclar Vivadent, Amherst, NY, USA; Vitremer – 3M ESPE, St Paul, USA) were selected for the polymerization shrinkage tensile measurement test. Vitremer and Compoglass F were included to provide comparative data on the performance of resin-modified glass ionomer cement against other material types. Filtek Z350 was included as a representative of composite resins, and Ketac N100 was the primary material of interest in this study. ChemFil (Dentsply Sirona, Charlotte, NC, USA) was the control material due to its established use as a conventional glass ionomer. Photopolymerization was conducted using Ultralux (Dabi Atlante, Ribeirão Preto, SP, Brazil), with an irradiance of 480mW/cm². The choice of irradiance was based on the manufacturer's recommendation and the common practice in evaluating similar materials. ChemFil (Dentsply Sirona, Charlotte, NC, USA) was polymerized following its specific setting time. Photopolymerization, when required, was conducted using two devices on opposite sides, perpendicular to the rods, at a distance of 1cm from the material. The irradiance was assessed by a previously calibrated radiometer (brand and model should be added here) at a distance of 1cm. The radiometer was calibrated according to the manufacturer's guidelines to ensure accuracy. After the completion of polymerization, the contraction force (in kgf) was recorded and considered as a contraction in tf. An additional waiting time of 5 minutes was observed, and the contraction force (in kgf) generated after this period (t 5min) was recorded.

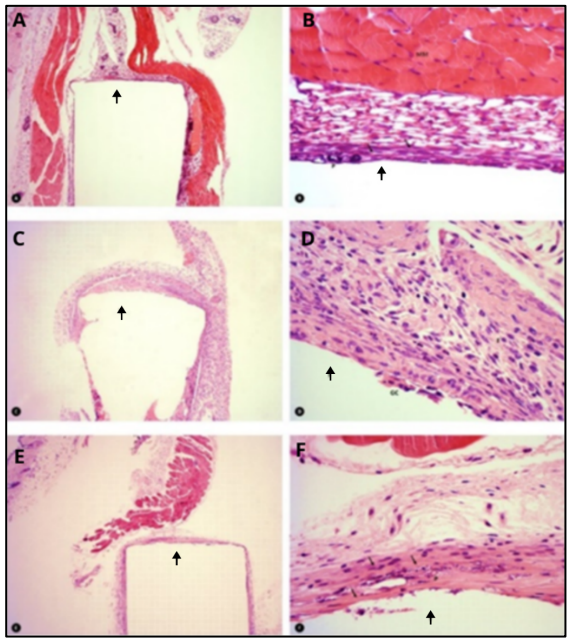
Statistical Analysis

The force obtained was divided by the rod's area to calculate the stress exerted during polymerization. These data were transformed into MPa and subjected to statistical analysis using E-Views 6.0 software. The data distribution in the groups at different times was similar to a normal distribution after the Shapiro-Wilk test. For materials with initial and final times, the paired t-test was applied to compare between times for each material. Next, one-way ANOVA with F-test was used for group comparisons at each time point, with a significance level of 5%. Tukey's post-test was applied, given the homogeneity of variances for all groups ($p>0.05$ in Levene's test).

Results

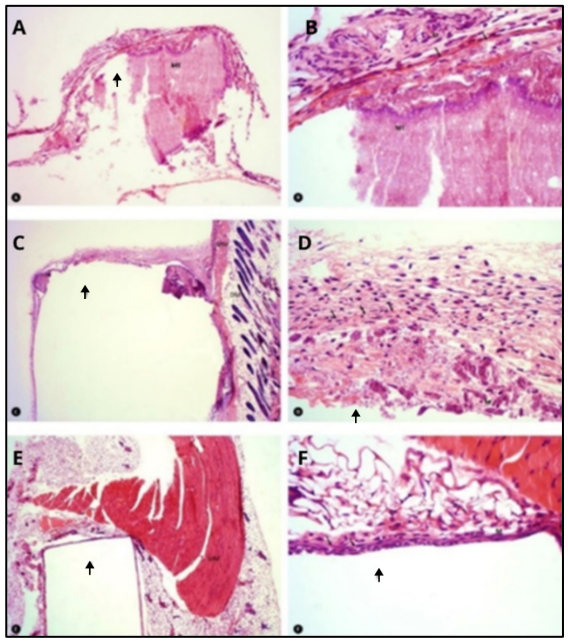
Subcutaneous Connective Tissue Study in Isogenic Mice

The descriptive microscopic analysis (qualitative) results are presented as captions for Figures 1 to 3.



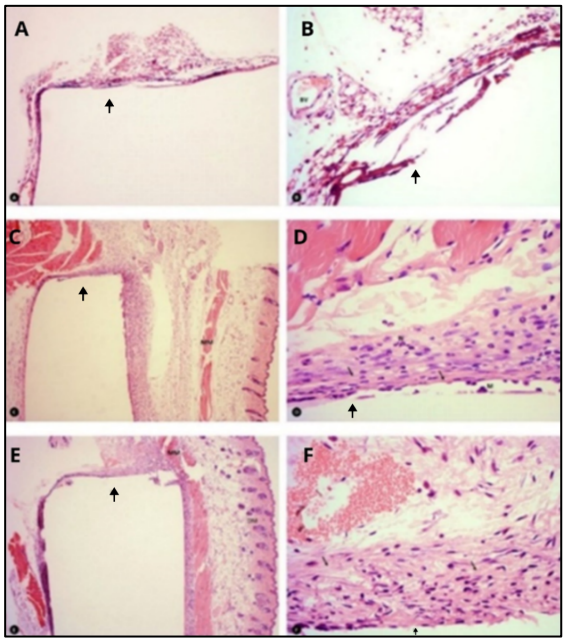
HE; A, C, and E = x4; B, D, and F = x40. T=Polyethylene tube; BV=Blood vessel; MM=Skeletal muscle; M=Macrophage; DM=Dermis.

Figure 1. Empty Tube: 7 days (A and B): Reactive tissue predominantly composed of young, ovoid, or fusiform fibroblasts with delicate and discreetly organized collagen bundles, newly formed blood vessels (BV), monocyte-shaped macrophages, and neutrophils.21 days (C and D): Tissue appeared more fibrotic than 7 days, with more defined and organized collagen fiber bundles forming a capsular structure (arrows) and a persistent vascular component. The inflammatory infiltrate, mainly consisting of neutrophils (N), was diffusely present. 63 days (E and F): Reactive tissue presented as an organized fibrous capsule well-defined by collagen fibers (arrows), with a persistent vascular component and scarce inflammatory cells.



HE; A, C, and E = x4; B, D, and F = x40; T=Polyethylene tube; MM=Skeletal muscle.

Figure 2. ChemFil: 7 days (A and B): Reactive tissue showed richness in fibroblasts, producing delicate collagen fibers (arrows) forming a capsular structure. Leukocytes were predominantemente macrophages, com algumas células gigantes multinucleadas contendo partículas cristalinas (P), neutrófilos e eosinófilos ocasionais. 21 days (C and D): Perimaterial reactive tissue appeared with well-organized fibers. Macrophages and fibroblasts (F) were present, with occasional polymorphonuclear cells. Some multinucleated giant cells with a discreet vascular component were observed at the material interface (GC). 63 days (E and F): Tissue was thin and fibrotic with well-organized bundles (arrows). Birefringent crystalline polyhedral particles (P) were commonly found among fibroblasts. There was no inflammatory infiltrate, only occasional cells.

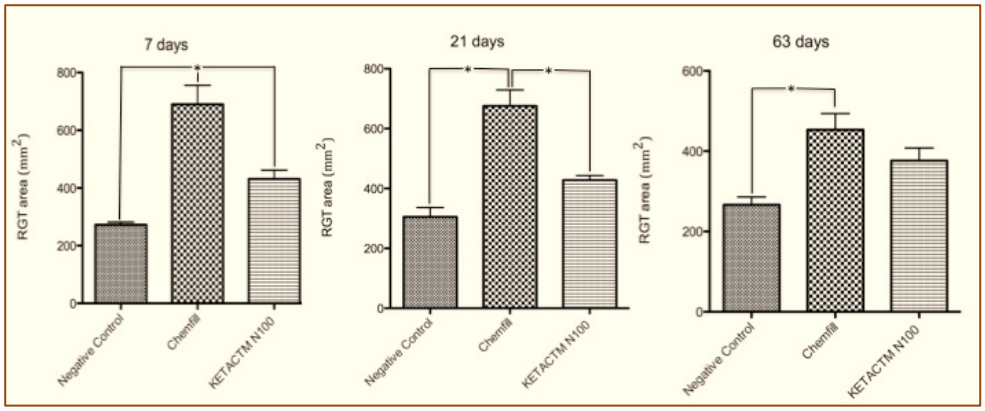


HE; A, C, and E = x4; B, D, and F = x40; T=Polyethylene tube; MM=Skeletal muscle; M=Macrophage; DM=Dermis.

Figure 3. Ketac N-100: 7 days (A and B): Reactive tissue was reduced but well-organized, with collagen fibers arranged in well-established bundles resembling a fibrous capsule (arrows). Macrophages still exhibited monocyte morphology (M); some had small basophilic particles at the material interface (MT). Polymorphonuclear leukocytes were occasional and associated with small areas of edema. 21 days (C and D): The reactive tissue showed capsular organization characterized by regular and parallel orientation of collagen fibers (arrows). Macrophages were mainly at the material interface and appeared laden with basophilic and birefringent particles in their cytoplasm. Polymorphonuclear cells were rare. 63 days (E and F): The perimaterial inflammatory reaction was significantly reduced, characterized by fibroblasts and organized collagen fibers forming a capsular structure (arrows). Some macrophages were laden with material.

Quantitative Microscopic Analysis

The quantitative microscopic analysis revealed significant differences among groups at various time points. On the 7th day, ChemFil exhibited the highest granulomatous tissue values, whereas the empty tube control had the lowest, with a statistically significant difference ($p<0.05$). This pattern persisted on the 21st day, but no significant difference was observed between the Ketac N100 group and the empty tube ($p=0.098$). At 63 days, a statistically significant difference was observed between the control group and ChemFil ($p<0.05$). Figure 4 presents a histogram of the granulomatous tissue area in mm^2 across different time points for the materials used, indicating statistically significant differences.



*Indicates a statistically significant difference.

Figure 4. Histogram of the area values in mm^2 observed at different periods for the materials used.

Polymerization Shrinkage Tensile Test

The results (Table 3) were analyzed for each material at initial and final times, excluding ChemFil®, which undergoes a chemical setting. A significant increase in polymerization shrinkage stress values was observed for all materials between the initial and final times ($p<0.001$). Additionally, a paired t-test comparing values at 40 seconds and the final stage for each material showed a significant difference ($p<0.001$), indicating increased polymerization stress over time.

Table 3. Distribution of data obtained in the polymerization shrinkage tensile test (values in MPa).

Groups	Mean	SD
Ketac N100 40s	0.324	0.0410
Ketac N100 final	0.562	0.0425
Vitremer 40s	0.229	0.0313
Vitremer final	0.405	0.0461
Compoglass 40s	0.389	0.0359
Compoglass final	0.673	0.0673
Z350 40s	0.405	0.0346
Z350 final	0.626	0.0503

Figure 5 presents the mean polymerization shrinkage stress at 40 seconds, showing significant differences between all materials (ANOVA, $p<0.001$). Tukey's post hoc test revealed significant differences between all groups ($p<0.001$), except between Compoglass and Z350 ($p=0.748$).

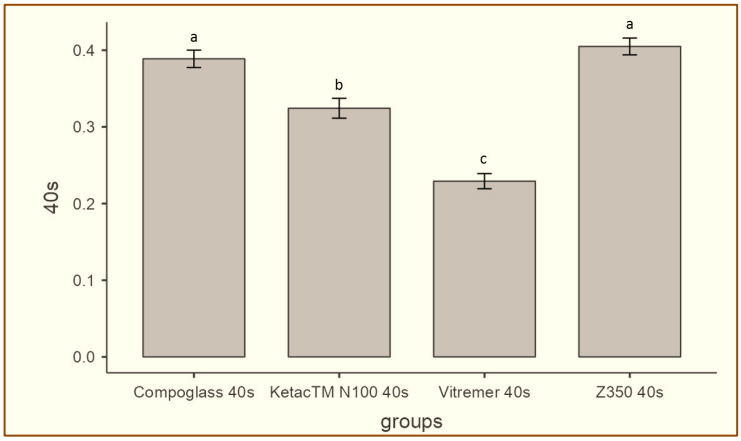


Figure 5. Distribution of polymerization shrinkage tensile test at 40 seconds.

At the final time, similar patterns were observed (Figure 6), with significant differences between all groups (ANOVA, $p<0.001$), except between Compoglass and Z350 ($p=0.202$). An important difference was observed between Ketac N100 and Z350 ($p=0.046$). The overall order of polymerization shrinkage stress was as follows: Vitremer < Ketac N100 < Z350 ≈ Compoglass.

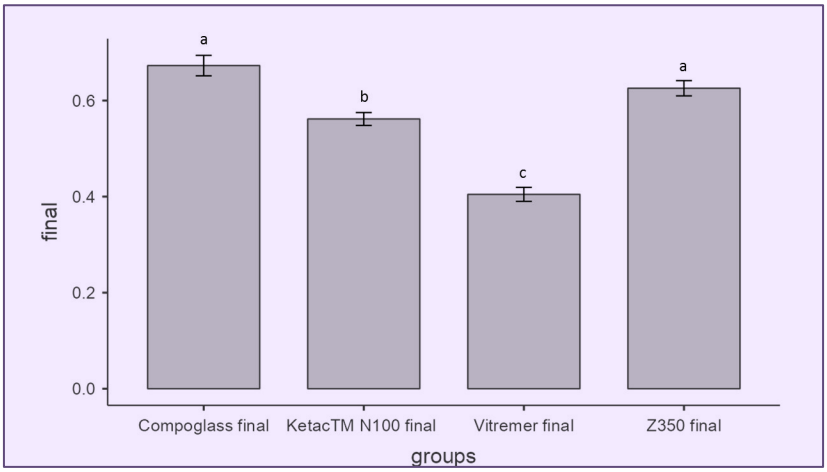


Figure 6. Final distribution of polymerization shrinkage tensile test at the final time (after 5 minutes).

Discussion

Isogenic BALB/c mice are genetically similar animals, ensuring a homogeneous response pattern to the same stimuli [11]. Polyethylene tubes are justified because they do not irritate adjacent tissues; thus, the reaction at the tube opening is related solely to the material's toxicity [12]. Since toxic and inflammatory reactions exhibit the same characteristics in all tissues, implants assess the biological response to the implanted material [13], providing information on the material's compatibility with connective tissue and the biological activity of surrounding tissues.

This study's experimental periods followed the standards ISO 10993-1994 recommended for implantation tests (7, 21, and 63 days). Regarding the biological response, if it is favorable at 60 days, it is unlikely that a subsequent inflammatory reaction will occur unless there is material deterioration or microbial contamination [13,14].

In the 7- and 21-day periods, the experimental groups exhibited more pronounced inflammation than at 63 days, during which repair characteristics were observed, with thin reactive tissue and organized collagen

fibers. However, although no significant difference was observed between the two evaluated glass ionomers (conventional and NRMGI) at 63 days, there was a considerable difference, with lower values for Ketac N100 NRMGI. At 7 and 21 days, inflammation was significantly present in terms of perimaterial granulomatous tissue and the presence of polymorphonuclear leukocytes and areas of edema. In the glass ionomer groups, crystalline birefringent particles associated with macrophages or multinucleated giant cells were observed in all periods.

Glass ionomer-based materials showed moderate perimaterial inflammatory reactions at 7 and 21 days. The toxicity of conventional glass ionomer is attributed to the presence of metallic ions and the low pH of the material when manipulated [15]. On the other hand, NRMGI contains 2-hydroxymethyl methacrylate (HEMA) molecules, which can be released into the tissue [16]. No difference was observed in microscopic evaluations at 7 and 21 days. At 63 days, the NRMGI group showed better results than conventional ionomers, but the perimaterial region exhibited tissue repair characteristics [12,14,15].

Based on the results, the lower tissue reaction is attributed to NRMGI being light-cured and consequently less soluble than conventional glass ionomers. Moreover, most of the NRMGI particles are nanometric, potentially reducing the material's impact on connective tissue, resulting in better outcomes.

Specific mechanical properties that were once restricted to resins are now observed in modified glass ionomers, including polymerization shrinkage. This factor promotes the formation of gaps and microleakage at the resin/restoration interface and is directly related to the clinical failure of restorations [16]. However, many studies report improvements in the mechanical properties of resin-modified glass ionomers compared to conventional ones, such as hardness, tensile strength, and compression resistance [17].

Methods have been developed to determine the polymerization shrinkage forces of resins [18]. The polymerization shrinkage verification used was initially described by Condon and Ferracane [19]. The tension was measured at two intervals: immediately after polymerization (t40s) and after 5 minutes (t5). These intervals were chosen based on the polymerization shrinkage occurring and generating tension of approximately 70-85% of the total immediately after activating visible light in resins and resin-modified glass ionomers. After 5 minutes, the shrinkage reaches between 92 and 95% of the total. Thus, we agree with the statement, as we found a pattern of higher polymerization tensions at the final time ($p<0.001$).

We used different materials with distinct characteristics: conventional glass ionomer cement (GIC), resin-modified glass ionomer cement (RMGIC), compomer, and composite resin. It was observed that the traditional GIC, in terms of polymerization shrinkage tension, was close to zero, as it does not contain resin particles and, therefore, does not undergo shrinkage. Moreover, the more resin added to the material, the greater the shrinkage.

A previous study reported that combining composite resin and glass ionomer coating materials can reduce residual stresses during polymerization shrinkage and loading. However, Gerdolle et al. [9] conducted polymerization shrinkage tests and revealed that the compomer Compoglass F, used in our studies, behaved very similarly to the P60 resin and Filtek, unlike Fuji II LC, which is consistent with our results. Other studies emphasize the improvement in the mechanical properties of resin-modified ionomers compared to conventional ionomers when analyzing mechanical strength, tensile strength, and compression resistance [9,17].

A literature analysis revealed that Ketac Nano did not show superior mechanical properties to conventionally cured micro-filled glass ionomer cement (RMGICs) when subjected to flexural and tensile strength tests. Including zirconia nanoparticles in the composition of glass ionomer cement (GIC) has been associated with improved mechanical properties and reduced porosities. However, studies indicate that this improvement is subject to the specific quantity of added particles, a variability that occurs among different

materials. It is important to note that a significant improvement in this property was not observed concerning polymerization shrinkage, a factor related to microleakage. Additionally, in clinical studies employing resin-modified glass ionomer cement, the durability of the restorations was found to be satisfactory. We agree with the conclusions of these studies, indicating that the observed performance order is Vitremer < Ketac N100 < Z350 ≈ Compoglass.

The compilation of results found in the literature, along with the data obtained in this research, allows us to infer that the polymerization shrinkage of these materials does not significantly impact the durability of restorations. Therefore, it is suggested that the clinical use of these materials is indicated, emphasizing the advantages associated with the fluoride release capability present in these compounds.

Conclusion

The material exhibited a positive tissue response when in contact with the connective tissue of isogenic mice. Additionally, it was observed that the polymerization shrinkage of this material *in vitro* is of intermediate nature, approaching values more closely to those of a resin than a conventional glass ionomer.

Authors' Contributions

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BKBL	https://orcid.org/0000-0002-8598-7777	Conceptualization, Investigation, and Writing - Original Draft.
FKC	https://orcid.org/0000-0002-4837-0583	Methodology, Validation, Formal Analysis, Data Curation, Supervision and Funding Acquisition.
RABS	https://orcid.org/0000-0002-0230-1347	Methodology, Software, Writing - Review and Editing, Visualization and Supervision.
FCPPS	https://orcid.org/0000-0002-1455-1538	Methodology, Software, Formal Analysis, and Supervision.
AC	https://orcid.org/0000-0002-5902-5646	Validation, Resources, and Writing - Review and Editing.
LAB	https://orcid.org/0000-0001-7118-6859	Writing - Review and Editing and Supervision.
PNF	https://orcid.org/0000-0001-8802-6480	Methodology, Validation, Formal Analysis, Writing - Review and Editing, Visualization and Supervision

All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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