

Nanoleakage At The Zirconomer Improved-Dentin Interface After Incorporation of Nano-Hydroxyapatite: An In-Vitro Study And Implications For Pediatric Dentistry

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Abstract:

Objective: To compare nanoleakage at the dentin-restorative interface of Zirconomer Improved with and without incorporation of 5% w/w nano-hydroxyapatite (n-HA).

Materials and Methods: Thirty-two extracted human premolars were prepared and restored with Zirconomer Improved either without n-HA or after incorporation of 5% w/w n-HA. The modified powder was homogenized by planetary ball milling. After 1000 thermocycles between 5 °C and 55 °C, specimens were sectioned, exposed to 50% ammoniacal silver nitrate for 24 h, developed under fluorescent light, and examined by environmental scanning electron microscopy (ESEM) with backscattered-electron imaging and energy-dispersive X-ray spectroscopy (EDX). Sixteen specimens were reported per group. Nanoleakage was expressed as silver weight percentage.

Results: Mean silver weight was $3.08 \pm 0.59\%$ for Zirconomer Improved without n-HA and $1.86 \pm 0.56\%$ for the n-HA-modified material. The mean difference was 1.22% (95% CI, 0.23 to 2.21), with a statistically significant difference ($p = 0.030$).

Conclusion: Under the conditions of this in vitro experiment, the group receiving Zirconomer Improved powder modified with 5% w/w n-HA showed lower EDX-derived silver signal than the unmodified control.

Keywords: nanoleakage; silver nitrate; nano-hydroxyapatite; Zirconomer Improved.

Introduction:

Dental caries is one of the most common diseases affecting humans and can occur in both primary and permanent dentitions throughout life. [1,2]. The long-term success of restorative treatment depends, among other factors, on the ability of the restorative material to establish a durable seal with the tooth structure and resist leakage at the tooth-restoration interface [3]. Marginal leakage may result from dimensional changes, inadequate material adaptation, and differences in the properties of the restorative material and tooth tissues. Such defects can permit the movement of fluids, ions, and microorganisms along the interface, potentially contributing to marginal discoloration, postoperative sensitivity, recurrent caries, and pulpal irritation [4,5].

Glass ionomer cements (GICs) are widely used restorative materials in pediatric dentistry, Zirconia-reinforced glass ionomer cements were developed to improve the mechanical properties of conventional GICs while maintaining their characteristic fluoride release and chemical interaction with tooth tissues [4,6]. Zirconomer Improved is a newer zirconia-reinforced GIC formulation designed to provide enhanced strength, durability, and esthetic properties while retaining the clinically desirable characteristics of GICs [7,8]. Further modifications of GIC formulations have also been investigated to optimize their physicochemical, mechanical, and interfacial properties [6-9].

Hydroxyapatite (HAp) has attracted considerable attention in dental applications because it is the major inorganic component of human teeth and has a chemical composition and structure similar to the mineral phase of natural dental tissues [10,11]. In particular, nanosized hydroxyapatite (nHAp) has gained increasing research interest because of its favorable physicochemical and mechanical properties, biocompatibility, and potential applications in caries prevention and restorative dentistry. These characteristics have led to the incorporation of nHAp into various dental materials, including GIC-based formulations [12]. However, pure HAp has a relatively porous structure and limited mechanical properties, which may result in reduced strength and increased brittleness when used alone in load-bearing applications. [13].

Previous studies have investigated the incorporation of hydroxyapatite into GICs and reported changes in their physicochemical and mechanical behavior [14,15]. The incorporation of nHAp may also influence the interaction between restorative materials and dental tissues because of its similarity to the mineral phase of tooth structure. However, the experimental formulations, concentrations, and investigated outcomes have varied considerably among studies. Consequently, the effect of nHAp incorporation on the interfacial sealing of zirconia-reinforced GICs remains insufficiently characterized. [16,17].

Interfacial sealing is a critical property of restorative materials because defects or pathways at the tooth-restoration interface may allow the movement of fluids and ions [18]. Microleakage is commonly evaluated using in vitro techniques such as dye penetration, tracer or isotope methods, scanning electron microscopy (SEM), and electrical conductivity measurements [16]. Organic dyes are among the most frequently used methods because they are relatively simple, inexpensive, and safe. Silver nitrate has also been widely used as a tracer for evaluating interfacial leakage. Because silver ions can penetrate very small interfacial spaces and can subsequently be detected by electron microscopy and elemental analysis, silver nitrate staining can provide detailed information regarding nanoscale pathways at the tooth-restoration interface [16,17,19].

Therefore, the present in vitro study specifically investigated nanoleakage at the Zirconomer Improved-dentin interface. The study compared silver nitrate penetration between unmodified Zirconomer Improved and Zirconomer Improved modified with 5% w/w nano-hydroxyapatite (nHAp). The null hypothesis was that there would be no significant difference in nanoleakage between the two groups.

Materials and Methods:

1. Materials

The materials used in the study, their composition, manufacturer, and batch numbers, are listed in **Table 1** and displayed in Figure 1.

Table 1. Materials used in the study

Material Name	Composition	Manufacturer	Batch No.
Zirconomer Improved (Fig. 1.A)	Zirconia-reinforced glass ionomer Powder: Fluoroaluminosilicate glass; zirconium oxide, pigment, and others. Liquid: ▪ Polyacrylic acid solution and tartaric acid.	Shofu Dental, Tokyo, Japan.	02210480
Nanoparticles Hydroxyapatite (Fig.1.B)	Particles: powder ▪ Calcium phosphate compound $\text{Ca}_{10}(\text{PO}_4)_2\text{OH}_2$	Nanogate lab Egypt	HAp20223



Figure 1. The materials used in the study, (A) Zirconomer-Improved Glass-ionomer, (B)

2. Methods

2.1 Characterization of Nano-hydroxyapatite:

Hydroxyapatite nanoparticles were synthesized using a modified wet chemical method. A calcium chloride solution was gradually combined with ammonium hydrogen phosphate under constant stirring, and the pH was adjusted to 10 with ammonia. The mixture was kept at 40 °C for four hours, then stirred at room temperature for an additional 16 hours to produce a white hydroxyapatite precipitate [20,21]. The nanoparticles were characterized with a high-resolution transmission electron microscope (TEM; JOEL JEM-2100, 200 kV). TEM analysis showed that the hydroxyapatite nanoparticles have a needle shape with an average width of 10 ± 5 nm and an average length of $80 \text{ nm} \pm 10 \text{ nm}$ (Fig. 2A). Nano-hydroxyapatite-modified zirconia-reinforced glass ionomer was prepared by adding 5% w/w nHAP powder to Zirconomer Improved, then milling the mixture for 30 minutes at 200 rpm in a planetary ball mill to ensure uniformity (Fig. 2B).

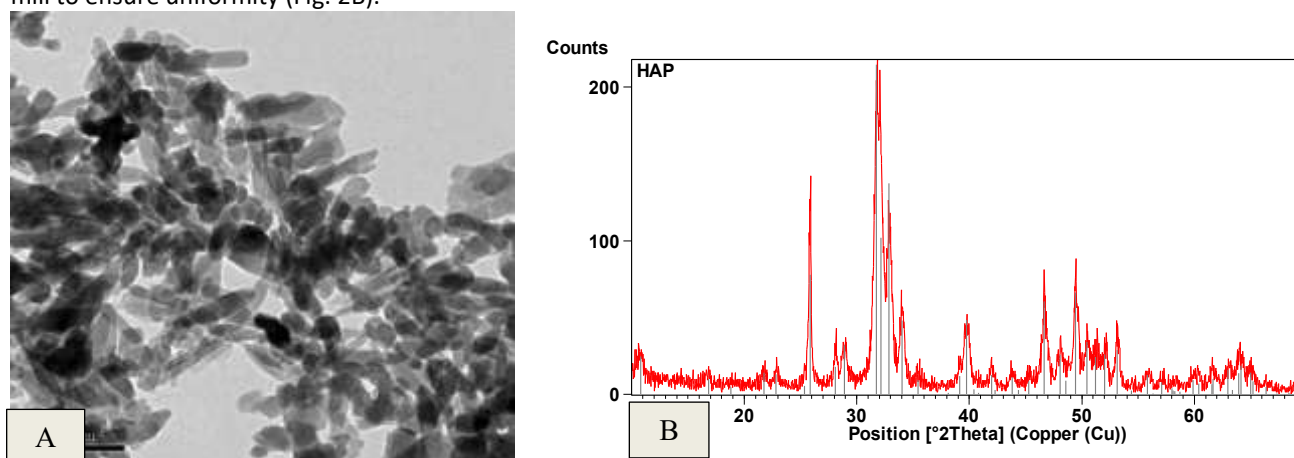


Figure 2. (A) TEM image showing synthesized needle-shaped nanohydroxyapatite particles (B) XRD Spectrum of Zirconomer Improved glass-ionomer displaying characteristic peaks of crystalline HAP.

2.2 Sample Grouping

Table 2 summarizes the sample grouping and the tests conducted on various material groups.

Table 2. Tests applied to different Material groups

Group	Nano-leakage
Zirconomer (Improved) alone	16
Zirconomer (Improved) with nanohydroxyapatite (nHA)	16
Total	32

2.3 Tooth Preparation

Thirty-two healthy human premolars, extracted for orthodontic purposes (Faculty of Dentistry, Suez Canal University), were used in this study. The teeth were stored in physiological saline to be used within one month of extraction. Each tooth was mounted in cold-cured acrylic resin blocks (1.5 mm apical to the cemento-enamel junction, CEJ), aligned parallel to the molds (Fig. 3A). The occlusal enamel of the crowns was removed at the dentino-enamel junction (DEJ) using a low-speed automated diamond saw under running water on a hard-tissue microtome (IsoMet 4000, Buehler Ltd., Lake Bluff, IL, USA) (Fig. 3B) to expose flat mid-coronal dentinal surfaces. Exposed dentin was examined under a microscope to ensure the absence of residual enamel, pulp exposure, or cracks (Fig. 3C). To prevent dehydration, the teeth were kept covered with damp tissue until used shortly after dentin exposure.

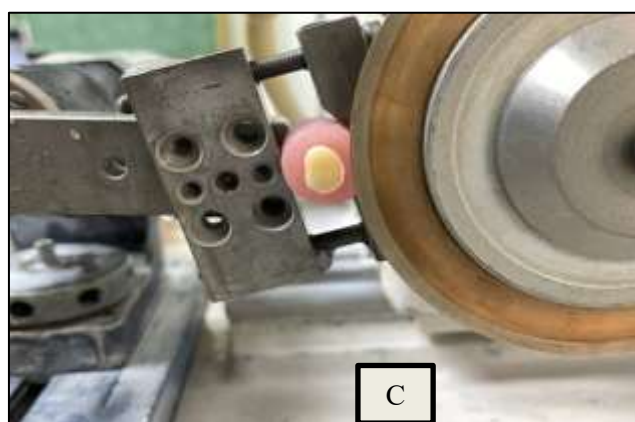
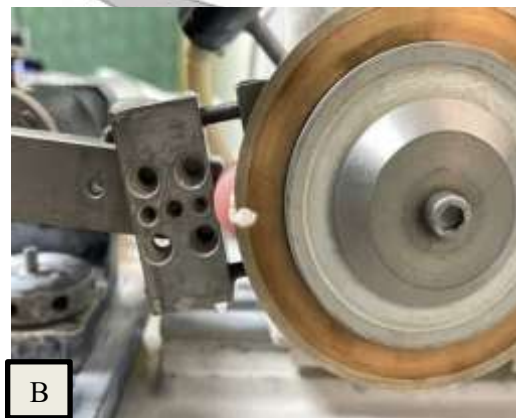


Figure 3. (A) The standard mold used to mount the teeth, (B) Enamel removal in a Microtome (IsoMet 4000, Buehler Ltd., Lake Bluff, IL, USA), (C) Tooth with occlusal enamel removed.

Materials were mixed at a 2:1 powder-to-liquid ratio according to the manufacturer's instructions and placed into 4x4 mm Teflon molds, which were later split open (Fig. 4). Group I: Zirconomer Improved (control), and Group II: Zirconomer Improved modified with nanohydroxyapatite. The specimens underwent artificial aging via thermocycling (1000 cycles between 5 °C and 55 °C), with 10-second dwell times in each bath, to mimic oral temperature fluctuations.

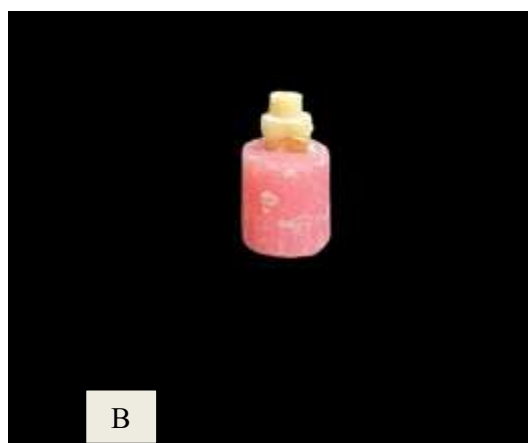
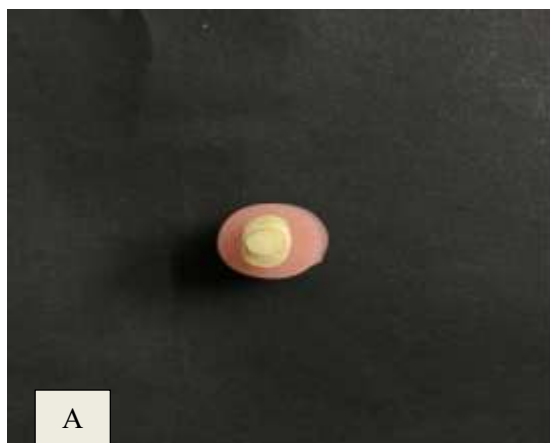


Figure 4. The sample with built-up restoration, (A) Occlusal view, (B). Lateral view.

2.4 Nanoleakage test

Nanoleakage was assessed on specimens prepared as previously described. After preparation, the specimens were cut longitudinally into 1 mm thick beams using a 0.6 mm diamond disc at 2500 rpm with a feed rate of 16.5 mm/min under abundant water coolant (Cool 2 water-soluble anticorrosive cooling lubricant, Buehler Ltd., Lake Bluff, IL, USA) (Fig. 5A). A horizontal cut was made at the CEJ level to obtain beam specimens (Fig. 5B). The specimens were immersed in 50% ammoniacal silver nitrate for 24 hours, rinsed, and placed in a photo-developing solution under fluorescent light for eight hours to reduce silver ions (Fig. 5C). Afterwards, the specimens were thoroughly rinsed for five minutes to remove any residue. Dried specimens were examined under an Environmental SEM (TESCAN VEGA 3) at 1000× magnification using backscattered electron mode and EDX to assess silver penetration at the dentin-restorative interface.

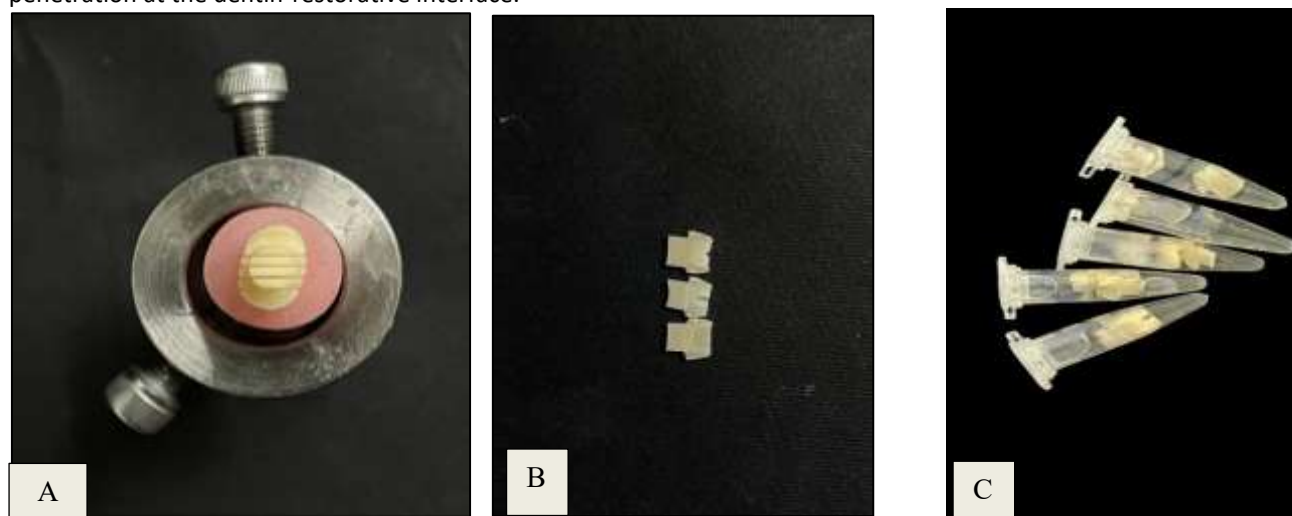


Figure 5. (A) Section of the bonded tooth for nanoleakage testing, (B) The beam obtained after the final cut. (C) The specimens immersed in ammoniacal silver nitrate.

2.5 Statistical analysis and data interpretation

Data were presented as mean and standard deviation (SD) values. Non-parametric data on nanoleakage were analyzed with the Mann–Whitney U test. The significance level was set at $p < 0.05$. Statistical analysis was conducted using R software, version 4.4.2, for Windows.

Results:

1. Nanoleakage:

The cement with incorporated nanoparticles revealed significantly lower nanoleakage values (1.86 ± 0.56) ($p=0.030$) than the cement without incorporated nHA particles (3.08 ± 0.59), as presented in **Table 3** and **Figure 6**.

Table (3): Intergroup comparison and summary statistics of silver weight (%).

Silver weight (%) (Mean±SD)		Mean difference (95% CI)	Test statistic	p-value
Cement without nano HA	Cement with nano HA			
3.08±0.59	1.86±0.56	1.22 (0.23:2.21)	16.00	0.030*

CI Confidence interval, * significant ($p < 0.05$).

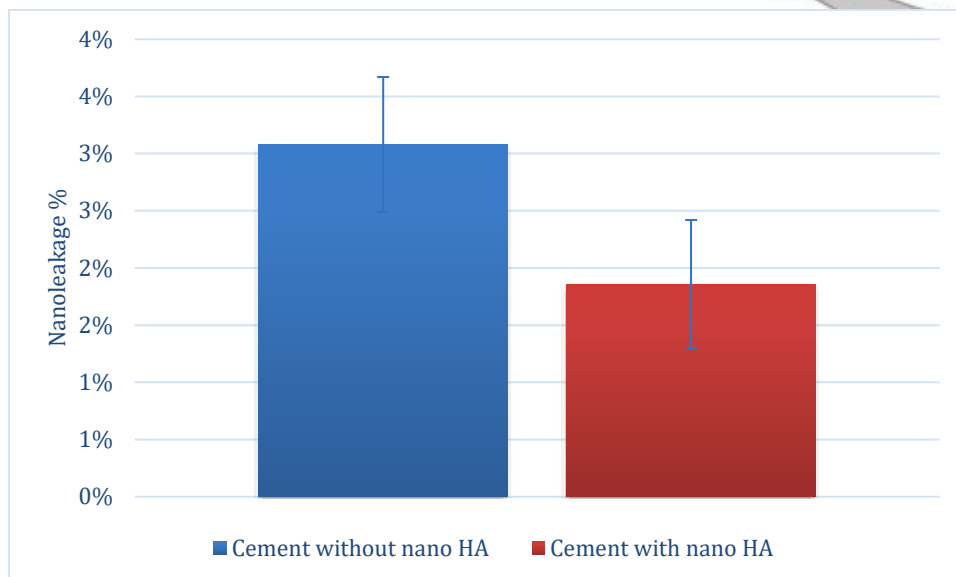


Figure (6): Bar chart showing means and standard deviation values of nanoleakage % measured by depth of silver penetration (nm).

2. Nanoleakage Pattern analysis

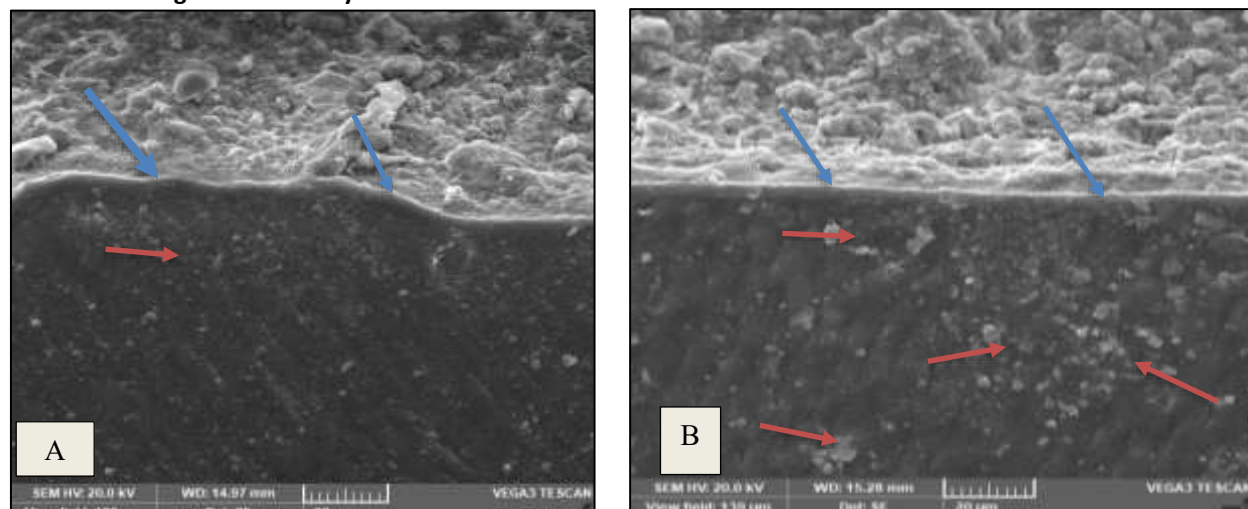


Figure 7. The SEM micrograph shows a well-defined restorative-dentine interface (blue arrows). The (orange arrows) indicate silver nitrate deposition, representing the nanoleakage pathway. (A) Zirconomer Improved reinforced with nano-hydroxyapatite shows minimal silver nitrate penetration (B). Zirconomer Improved shows increased silver nitrate deposition, demonstrating higher nanoleakage.

Discussion:

Glass ionomer cements (GICs) are popular because they adhere well to tooth structure, release fluoride, and are biocompatible, which helps improve restoration durability and reduces treatment complication time [22]. Their self-adhesion to tooth structure eliminates the need for bonding agents and enhances durability [23]. However, GICs have low abrasion resistance, are moisture sensitive, and have poor aesthetics, which restricts their application in areas exposed to high masticatory forces. To address these issues, modified formulations have been developed [24]. Zirconomer Improved, was introduced to provide the strength of amalgam along with the fluoride benefits of GICs [7]. This material contains micronized glass and evenly dispersed zirconia particles for enhanced mechanical performance [25].

Recently, nanohydroxyapatite particles have been incorporated into GIC, significantly improving its physical and biological properties [26].

The clinical effectiveness of restorative materials relies on the durability of their adhesion to dental tissues. A weakened bond leads to compromised interfacial sealing, ultimately causing sensitivity, staining, and caries [27].

This study assessed nanoleakage test after thermocycling. The specimens underwent thermal cycling (5°C to 55°C for 1000 cycles with 10-second intervals) to simulate typical intraoral temperature fluctuations. This approach helps predict in-vivo performance by mimicking long-term marginal deterioration [28].

In this study, Zirconomer Improved with nano-HA showed significantly lower nanoleakage values ($1.86 \pm 0.56\%$) compared to Zirconomer Improved without nano-HA ($3.08 \pm 0.59\%$). This improvement is attributed to the presence of nano-HA particles, which mimic natural apatite, enhance adaptation, and reduce interfacial gaps. These findings are consistent with those of Sapkale et al. (2018) and Akhavan et al. (2013), who reported that nano-HA improves bioactivity and reduces leakage [29,30].

Reducing nanoleakage is essential to prevent saliva and microorganism infiltration. Poor adaptation of the restoration to the tooth can create pathways for microbes, leading to secondary diseases. Therefore, ideal restorative materials should have minimal nanoleakage to achieve long-term clinical success. [31].

One limitation of the study was that it was conducted in vitro with short-term thermocycling. Therefore, the results may not fully reflect the challenging oral environment or the long-term clinical performance of the tested materials. Future research with different n-HA concentrations and longer clinical follow-ups is needed to confirm these findings.

Conclusions:

Within the limitations of this in vitro experiment, Zirconomer Improved modified with 5% w/w n-HA and ball milling showed lower EDX-derived silver weight percentage at the dentin–restorative interface than unmodified Zirconomer Improved.

Declarations

Ethics approval and consent to participate:

This in vitro study was approved by the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University, with approval number (624/2023). The study was conducted in accordance with the ethical standards of the Declaration of Helsinki. This study used extracted or exfoliated teeth with no patient identifiers, and the need for informed consent was waived by the Ethics Committee of the Faculty of Dentistry, Suez Canal University.

Consent for publication

Not applicable

Data availability

All data generated or analyzed during this study are included in this manuscript

Competing Interests:

The authors have no conflicts of interest relevant to this article.

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Authors' contributions

Conception and study design were done by AS, MD, MF and RB. AS carried out the practical work, discussed the practical steps, performed data analysis and wrote the first draft of manuscript. MD, MF revised the manuscript. RB discussed the practical steps, edited and revised the manuscript. All authors read and approved the final manuscript

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