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Dentin–Pulp Complex Response in Molars of Rats after Occlusal and Cervical Restorations with Conventional Glass Ionomer Cement and Nano-Hydroxyapatite Silica Glass Ionomer Cement

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Abstract: The purpose of this in vivo study was to evaluate and compare the dentin–pulp complex response following occlusal and cervical restorations in rat molars restored with nano-hydroxyapatite silica glass ionomer cement (nano-HA-SiO_2-GIC) and conventional glass ionomer cement (c-GIC). In total, 64 maxillary first molars of 32 male Wistar rats were restored using Fuji IX (c-GIC) and nano-HA-SiO_2-GIC using a split-mouth design. Half of them were reserved for the occlusal type of restoration while the other half was for cervical restorations. After one week and one month, rats were euthanized and were stained with hematoxylin and eosin, Masson's trichrome, and Brown and Brenn techniques for histological examination. Parameters such as disorganization of the pulp tissue, inflammatory cell infiltration, detection of bacteria, and tertiary dentin deposition were measured for each group. One week after sacrifice, the odontoblastic layer was disrupted, and moderate inflammation in the pulp area close to the cut dentin was observed in both types of restorations. Nano-HA-SiO_2-GIC showed significantly superior properties when assessed based on tertiary dentin formation as compared to c-GIC. One month after sacrifice, there was no evidence of disruptions of the odontoblast layer, which exhibited a normal palisade appearance in both groups. In terms of inflammation, the pulp tissue recovered in almost all cases except one of c-GIC, but a few cases of the nano-HA-SiO_2-GIC group still displayed mild-to-moderate inflammatory reactions, especially of the occlusal type. Both c-GIC and nano-HA-SiO_2-GIC exhibited favorable responses in terms of biocompatibility. Nano-HA-SiO_2-GIC exerted more inflammation but encouraged better tertiary dentin formation compared to c-GIC.

Keywords: dentin–pulp complex; nano-HA-SiO_2-GIC; conventional GIC; tertiary dentin deposition

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1. Introduction

The dentin in combination with pulp forms the dentin–pulp complex (DPC), which is derived from the same source during embryogenesis and hence has interconnected roles [1]. The pulp reacts via three basic mechanisms [2] in response to a bacterial provocation, such as caries, or any physical insult, such as cavity preparation or trauma: firstly, by decreasing the dentinal permeability by intratubular deposition of whitlockite crystals or sclerotic dentin, thereby blocking the irritants; secondly, via the deposition of tertiary dentin, which may be reactionary if deposited by the same existing odontoblasts or reparative if formed by

newly differentiated odontoblasts; and thirdly, via the help of the pulpal immune response, which provides humoral and cellular challenges to invading pathogens. Therefore, a DPC attempts to preserve the physical and biological integrity of the tooth by making it disease-free [3].

The relationship of the restorative material's interaction with the tooth structure seems to be affected by several elements, comprising the configuration of the restorative agent and leaching of any constituents that may or may not promote the DPC's protective mechanism of tertiary dentin formation [4]. Therefore, a large variety of materials possessing varying configurations, characteristics, and endorsements for use in clinics are being familiarized in the field of dentistry.

One of the widely used restorative materials in restorative dentistry is glass ionomer cement (GIC). It has been extensively used because of its highly acceptable chemical bonding competency, cosmetic properties, fluoride release, and low thermal expansion [5]. An investigation displayed no change in pulpal response when conventional GIC (c-GIC) was compared to calcium hydroxide, which supports the adequate biocompatibility of c-GIC concerning pulpal tissue [6]. On the contrary, many failures are observed after its clinical use due to bacteriostatic properties, high dissolution after water sorption, weakness in mechanical strength, especially low fracture toughness, and wear resistance [7]. Furthermore, surveys [8] indicate that the continuous and constant release of fluoride is insufficient to provide protection from bacterial attack, which generally leads to the development of secondary caries below restorations [9].

Over the last two decades, researchers added different compounds with antibacterial properties to confront and surpass such problems. Recently, the employment of nano-components for dental materials such as nano-chitosan [10] and poly-ethylenimine nanoparticles [11], which were found to offer an improvement in antibacterial properties at the cost of a compromise in biocompatibility, is becoming well-accepted in the field of dentistry [11,12]. Recently, nano-metallics—for instance, silver (Ag), titanium oxide (TiO_2), zirconium oxide (ZrO_2), and copper (Cu)—were incorporated to improve GIC's mechanical strength. They revealed encouraging outcomes [13,14], but some nano-metals, such as TiO_2, were not able to improve the bonding of GIC with the tooth [15], with an additional compromise of unpleasing aesthetics. Similarly, some investigators have incorporated a few other nanofillers—for example, bioactive glass and niobium pentoxide—into GIC and revealed that they simply resulted in minor improvements in terms of mechanical strength and radio-opacity [16,17].

The inclusion of bioactive nano-silica within c-GIC enhanced its bioactivity properties, which may prevent marginal gap formation [18]. Nano-hydroxyapatite (HAp) is a mineral form of calcium apatite, which is found naturally and is analogous to dental hard tissue structure. The most exceptional feature of HAp is its biocompatibility. For the same reason, it has been utilized as a filler in many restorative materials. Apart from its promising bioactive properties, it has been shown to have the potential to repair enamel demineralization [19], and when incorporated with GIC, it displays better tensile and flexural strength compared to c-GIC [20].

Owing to the above-mentioned results for nano-silica and nano-hydroxyapatite, Rahman et al. merged them as a filler in c-GIC and found that the addition of the nano-HA-silica composite provided an increase of ~73% in the hardness values compared to the c-GIC [21]. Other researchers have found a better exchange of ions between tooth structure and nano-HA-GIC [22] and nano-HA-SiO_2-GIC [23], advocating that higher remineralization was achievable when compared to c-GIC. Furthermore, the cytotoxicity evaluation of the nano-HA-SiO_2-GIC on human dental pulp stem cells (DPSCs) demonstrated a favorable response [24]. Another study by Hii et al. showed that nano-HA-SiO_2-GIC exhibited good biocompatibility on DPSCs, which was comparable to c-GIC. Also, nano-HA-SiO_2-GIC encouraged the attachment and spreading of DPSCs with prominent filopodia, which indicates the good activity and viability of these cells [25].

Despite many studies with promising results for the mechanical, chemical, and in vitro biological evaluations of nano-HA-SiO_2-GIC, to our knowledge, no in-vivo study has been performed to test and compare the biocompatibility of nano-HA-SiO_2-GIC and c-GIC in terms of the dentin–pulp complex response. Hence, this study aimed to assess the dentin-pulp complex response to nano-hydroxyapatite-silica-GIC occlusal and cervical restorations in Wistar rats' molar teeth as compared to conventional GIC in terms of pulp tissue disorganization, infiltration of inflammatory cells, bacterial detection, and deposition of tertiary dentin.

2. Materials and Methods

2.1. Materials

Conventional restorative GIC (Fuji IX, GC Corp Tokyo, Japan. batch number 1712221) and locally produced nano-hydroxyapatite-silica GIC (nano-HA-SiO_2-GIC) were used in this study. The nano-HA-SiO_2 powder was prepared using a one-pot method as described by [21]. Powder of nano-HA-SiO_2 was measured and mixed with c-GIC powder at a proportion by weight of 10%. This mixture was ground by mortar and pestle for 10 min and mixed with the liquid with the same powder/liquid proportions.

2.2. Operative Procedures

The subjects of this study were 32 healthy, two months old, male Wistar rats weighing around 180–220 g, which were divided randomly into two experimental groups (n = 16 per group) for the occlusal and cervical types of restoration. The protocol of this study was submitted and approved by Institutional Animal Care and Use Committee (IACUC), (USM/IACUC/2020/(122-1053). All procedures were carried out as per ethical standards for the care and use of the animals and conformed to the ARRIVE guidelines [26]. The rats were housed in cages (4–5 rats per cage) and placed in ventilated racks at 24 ± 2 °C with a relative humidity of 60 ± 15% and a normal photoperiod (12–12 h light–dark cycle). During the experimentations, the rats were provided with a standard diet of rat pellets and filtered water ad libitum.

2.3. Cavity Preparation and Restoration

For cavity preparation and restorative procedures, the rats were sedated by giving an intra-peritoneal injection of Ketamine (90 mg/kg) and Xylazine (5 mg/kg) for the interventions. A surgical table with a customized orthodontic wire loop was adapted to maintain the oral cavity open during the dental procedure, and buccal mucosa was kept retracted with the help of a tweezer as shown in Figure 1a. Cavity preparation was conducted after performing oral antisepsis using 75% ethanol and sterile distilled water.

There were two types of cavity preparations (occlusal and cervical) and two types of restorations (c-GIC and nano-HA-SiO_2-GIC) with two intervals (1 week and 1 month). Eight teeth (n = 8) were designated to each time interval and material, $8 \times 2 \times 2 \times 2 = 64$ tooth cavities. Two tooth cavities per rat using a split-mouth design with two intervals required 32 rats, which was carried out using the model followed by previous studies [27]. The sample size comprised the quantity of prepared restorative cavities, following the methodology of previous studies [28,29]. An example of cervical cavity preparation and restoration of rats' molars is shown in Figure 1b–d.

The maxillary first molars were used with a split-mouth design in which c-GIC was restored on the right side, whereas the left side was employed for nano-HA-SiO_2-GIC for both occlusal and cervical types of restorations using simple random sampling. A cavity of ~0.5 mm in depth and 0.5 mm in width (measured with a periodontal probe) was prepared with a carbide bur of 0.5 mm diameter (H1.FG.005; Komet, Gebr Brasseler GnbH & Co KG, Lemgo, Germany) with highspeed contra-angle at 30,000 rpm (TC Motor 3000, France) under a stereomicroscope at (X16 BMK-4/LED 5X Hobbyist/Dental Non-Modular Stereo Microscope, Meiji Techno, Saitama, Japan). Precautions were taken to minimize overheating by replacing the bur after each four-cavity preparation. Small gauze was used to dry the

cavity walls after preparation and each set of restorations (c-GIC and nano-HA-SiO_2-GIC) was then placed into the respective cavities. Throughout the intervention period, all the rats were observed with a facial grimace scale which is used for coding pain and distress in experimental animals.

Figure 1. (**a**) Customized design wire loop and tweezer in place for mouth retraction. (**b**) Bur in position for cervical cavity preparation on a molar. (**c**) Prepared cervical cavity (**d**) Cervical restoration in place.

2.4. Animal Sacrifice and Histopathological Procedure

Rats were euthanized through carbon dioxide gas overdose inhalation after one week and one month of intervention for both occlusal and cervical types of restorations. The maxilla was dissected and fixed in 10% neutral buffered formalin at room temperature for a day [30], then demineralized in a 20% formic acid solution. This procedure was repeated every other day until the endpoint was achieved. The maxilla was then dissected vertically and passed through a series of ethanol concentrations to dehydrate it (70, 80, 90, and 100 percent). Afterward, the samples were embedded in paraffin blocks and were sectioned along the long axis of the tooth in a buccolingual direction at every 50 µm until the area that contained full pulp and exposed dentinal area of the 1st and 2nd molar was observed. The

serial sections of 4 µm were stained with hematoxylin and eosin (H&E) stain. The following sections underwent Masson's trichrome to assess tertiary dentin formation and soft tissue organization and Brown and Brenn staining for bacterial detection, respectively [29].

2.5. Histological Assessment of Dentine Pulp Complex (DPC) Response

The stained slides were blindly examined under the light microscope (Nikon E100, Tokyo, Japan) by two trained investigators based on parameters such as pulp tissue disorganization, infiltration of inflammatory cells, bacterial detection, and deposition of tertiary dentin as shown in Table 1.

Table 1. Criteria for scoring of dentin-pulp complex response after occlusal and cervical restorations modified from Li et al. [27] and Costa et al. [31].

Score	Histological Characterization
A	**Pulp tissue disorganization**
0	Normal tissue
1	Odontoblastic layer disorganized but normal central pulp
2	Disorganization of pulp tissue morphology
3	Pulp necrosis
B	**Inflammatory cell infiltration**
0	None or few scattered inflammatory cells present in the pulp area corresponding to the pulpal/axial wall, characteristic of normal connective tissue
1	Mild inflammatory cell infiltrate with polymorphonuclear (PMNs) or mononuclear leukocytes (MNLs)
2	Moderate inflammatory cell infiltrate involving the coronal pulp
3	Severe inflammatory cell infiltrate involving the coronal pulp or characterizing abscess
C	**Bacterial detection**
0	Absent or few scattered bacteria next to the area of pulp exposure or newly formed tertiary dentin
1	Presence of bacteria with a clearly visible amount in the coronal pulp
D	**Tertiary dentin deposition**
0	No or very mild tertiary dentin deposition **(0–49 µm = Grade 0)**
1	Initial tertiary dentin deposition extending to not more than one-half of the cavity floor **(50–99 µm = Grade 1)**
2	Moderate tertiary dentin deposition extending to more than one-half of the cavity floor but not completely closing the cavity floor **(100–249 µm = Grade 2)**
3	Intense tertiary dentin deposition, extending entirely along the cavity floor **(250–500 µm = Grade 3)**

Measurements of the remaining dentin thickness (RDT) and the tertiary dentin formation (TDF) were performed for each slide image using Image-J software (Java-based image processing program, Madison, WI, USA). RDT denotes to the thickness of the dentin left after cavity preparation which is between the pulp chamber and the cavity floor (excluding the tertiary dentin which was newly formed). The TDF was considered and calculated as the tertiary dentin thickness which is newly formed after the cavity preparation is performed (between the pulp and the regular dentin). The same parameters for the calculation of RDT and TDF were used for occlusal and cervical types of restorations. An example of RDT and TDF in a rat tooth restoration is shown in Figure 2a. The effects of c-GIC were

assessed as a positive control group and TDF in second molars was taken as the negative control. An example of tertiary dentin formation in 2nd molars is shown in Figure 2b.

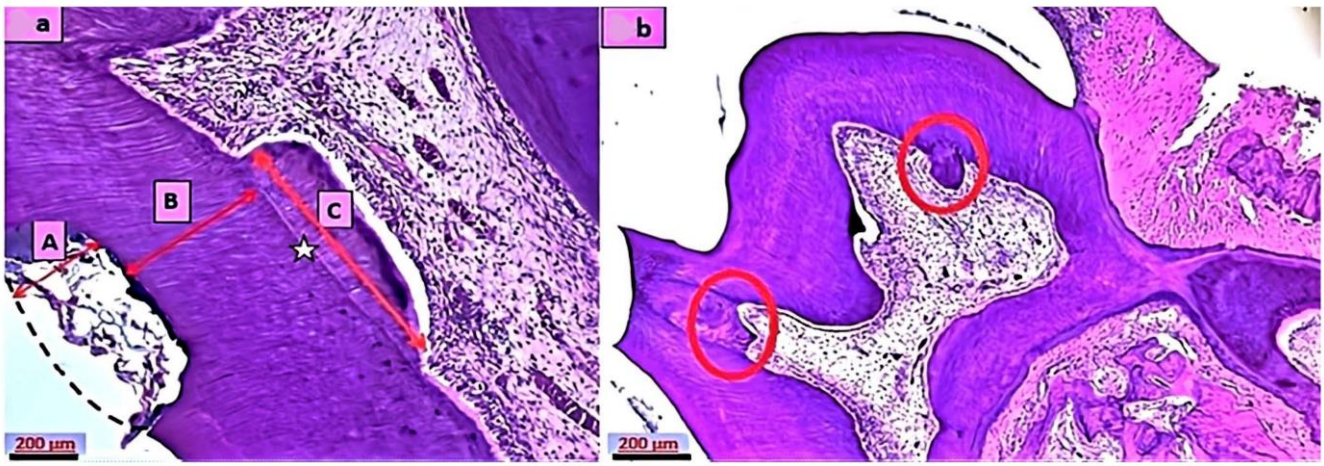


Figure 2. (**a**): (A) Restoration. (B) Remaining dentin thickness (RDT). (C) Tertiary dentin, star indicates calcio-traumatic line (H&E, 10×). (**b**): An example of the 2nd molar having some tertiary dentin formation in the occlusal (left circle) and cervical (right circle) area (H&E).

In all rat molars, mild tertiary dentin is usually formed in occlusal and cervical aspects due to the continuous tooth grinding nature of the rats and subsequently mild deposition of tertiary dentin [32]. Hence, we considered Grade 0 as tertiary dentin thickness ranging from 0 to 49 µm (which was the maximum TDF observed in all 2nd molars in our study) and considered normal, hence Grades 1, 2, and 3 were considered actual deposition of TDF induced by a particular restoration which was categorized as Grade 1 with 50–99 µm of TDF, Grade 2 with 100–249 µm and Grade 3 with 250–500 µm of TDF as assessed with Image J analysis.

2.6. Statistical Analysis

All tests were performed using the Statistical package for social sciences (SPSS) version 22. Two-way ANOVA was carried out to calculate a significant difference in the remaining dentin thickness (RDT) and dentin–pulp complex response resulting from both types of materials being tested. One-way ANOVA was carried out to evaluate any significant difference in the tertiary dentin formation (TDF). The value of significance was set at $p < 0.05$ for all the tests.

3. Results

3.1. Dentine Pulp Complex Response

Table 2 shows the mean scores for each parameter for occlusal restorations and Table 3 shows the mean scores for each parameter for cervical restorations using c-GIC and nano-HA-SiO_2-GIC.

3.1.1. Pulp Tissue Disorganization

For occlusal restorations, both groups (c-GIC and nano-HA-SiO_2-GIC) generally displayed odontoblastic layer disruption in areas related to the cut dentinal tubules. At 1 week, 80% of the teeth exhibited Grade 1 pulp tissue disorganization in both c-GIC and nano-HA-SiO_2-GIC groups with central pulp being normal. An example of a c-GIC case is shown in Figure 3A–C). At 1 month interval, almost 100% showed a normal palisade appearance of odontoblast in both c-GIC and nano-HA-SiO_2-GIC groups. However, in the cervical type at 1-week and 1-month intervals, both kinds of restorations had Grade '0' pulp tissue disorganization, indicating a milder response as compared to that of the occlusal group. In

general, a slightly higher mean score for nano-HA-SiO_2-GIC was observed as compared to the c-GIC group at both 1-week and 1-month intervals.

Table 2. Mean score and SD of dentin–pulp complex reaction for occlusal c-GIC and nano-HA-SiO_2-GIC at each experimental time interval.

Study Parameters	Time Interval	c-GIC (n = 8)	Nano-HA-SiO_2-GIC (n = 8)	Two-Way ANOVA $p \leq 0.05$
Pulp tissue disorganization	1 week	1.00 (0.53)	1.12 (0.64)	0.317
	1 month	0.25 (0.46)	0.62 (1.06)	0.180
Inflammatory cell infiltration	1 week	0.87 (0.83)	1.25 (0.70)	0.083
	1 month	0.00 (0.00)	0.85 (1.06)	0.059
Bacterial detection	1 week	0.00 (0.00)	0.00 (0.00)	NA
	1 month	0.00 (0.00)	0.00 (0.00)	NA
Tertiary dentin formation	1 week	0.62 (0.74)	0.75 (0.46)	0.564
	1 month	1.00 (0.53)	1.25 (0.46)	0.157

Table 3. Mean score and SD of dentin–pulp complex response for cervical c-GIC and nano-HA-SiO_2-GIC at each experimental time interval.

Study Parameters	Time Interval	c-GIC (n = 8)	Nano-HA-SiO_2-GIC (n = 8)	Two-Way ANOVA $p \leq 0.05$
Pulp tissue disorganization	1 week	0.75 (0.88)	0.75 (0.88)	1.000
	1 month	0.75 (1.03)	0.50 (0.75)	0.157
Inflammatory cell infiltration	1 week	0.50 (0.75)	1.00 (0.53)	0.046 *
	1 month	0.70 (0.75)	0.25 (0.46)	0.170
Bacterial detection	1 week	0.00 (0.00)	0.00 (0.00)	NA
	1 month	0.12 (0.35)	0.00 (0.00)	0.351
Tertiary dentin formation	1 week	0.75 (0.70)	0.87 (0.83)	0.317
	1 month	2.00 (0.92)	2.37 (0.91)	0.083

* indicates significant difference between cGIC and nano-HA-SiO2-GIC ($p \leq 0.05$).

3.1.2. Inflammatory Cell Infiltration

In the occlusal restorations, at a 1-week interval, almost 75–80% of the molars restored with both c-GIC and nano-HA-SiO_2-GIC showed no or mild inflammation, while 20–25% of the samples underwent moderate inflammation as shown in Table 3. However, at a 1-month interval, all teeth in the c-GIC group showed no inflammation, which was not observed in the nano-HA-SiO_2-GIC group, in which 30% of the restored molars still showed mild inflammation, as shown in Figure 4A–C. For cervical restorations, at 1 week, most of the samples of c-GIC and nano-HA-SiO_2-GIC restorations showed mild to moderate inflammation, but at 1-month intervals, almost all samples showed no inflammation. In general, again nano-HA-SiO_2-GIC showed higher scores than c-GIC in terms of inflammation at 1-week and 1-month intervals.

3.1.3. Bacterial Detection

Only one sample from the c-GIC group of cervical restoration showed evidence of bacterial detection, as shown in Figure 5. All other samples of both groups displayed no bacterial detection when observed with Brown and Brenn staining.

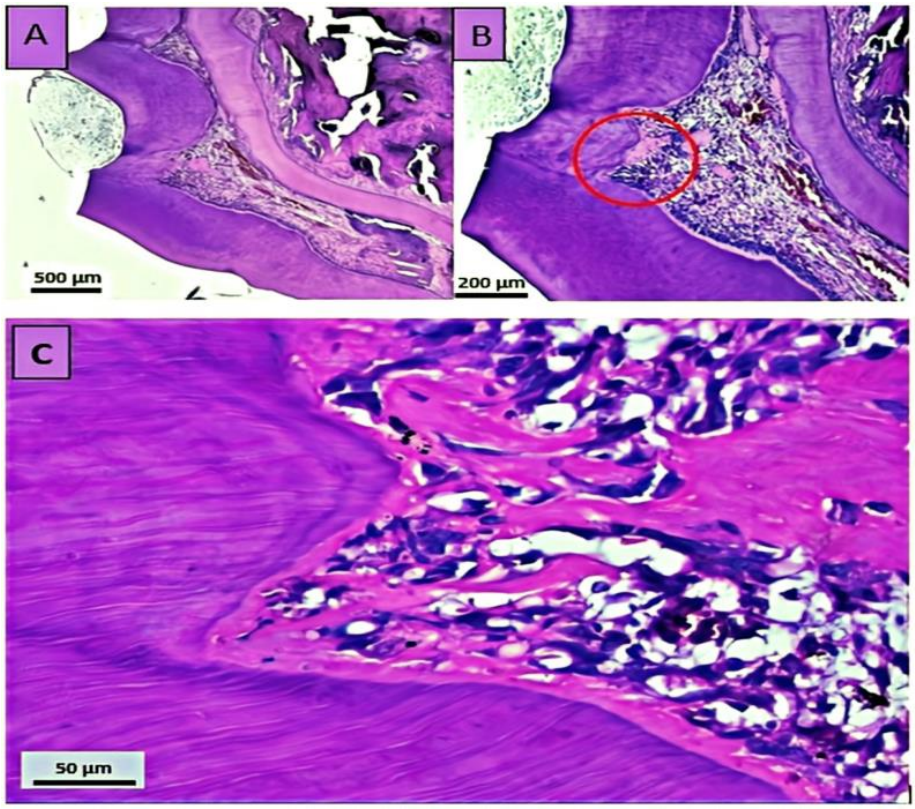


Figure 3. (**A**–**C**) (4×, 10× and 40×): Microscopic view of c-GIC occlusal restoration showing Grade 1 pulp tissue disorganization after 1 week of interventional period in the area encircled as red in (**B**) (H&E).

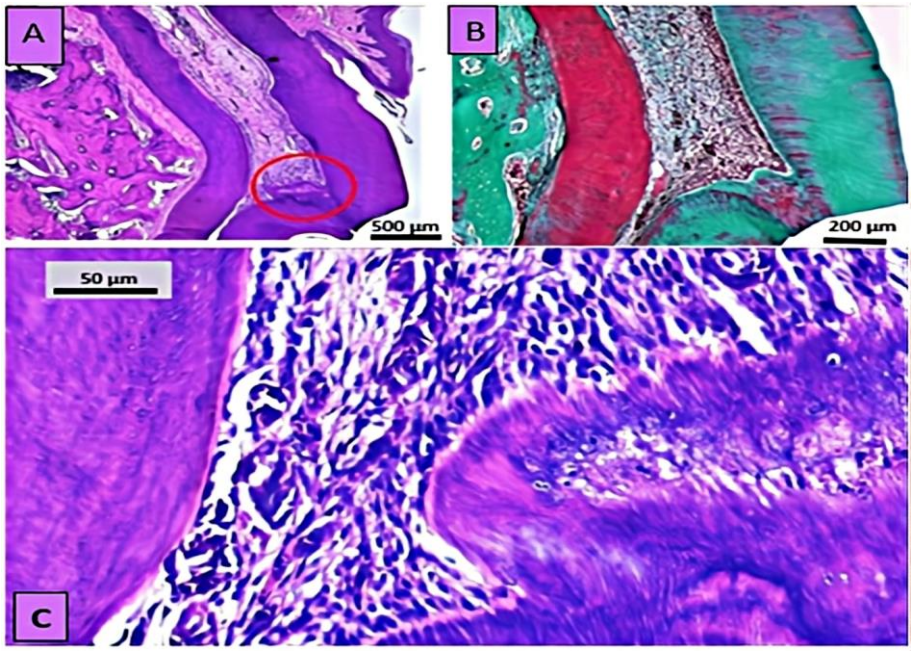


Figure 4. (**A**–**C**) (4×, 10×, and 40×): Microscopic view of occlusal restoration with nano-HA-SiO_2-GIC showing Grade 1 inflammation at 1 month of sacrifice in the area encircled as red in (**A**) ((**A**,**C**) = H&E stain, (**B**) = trichrome stain).

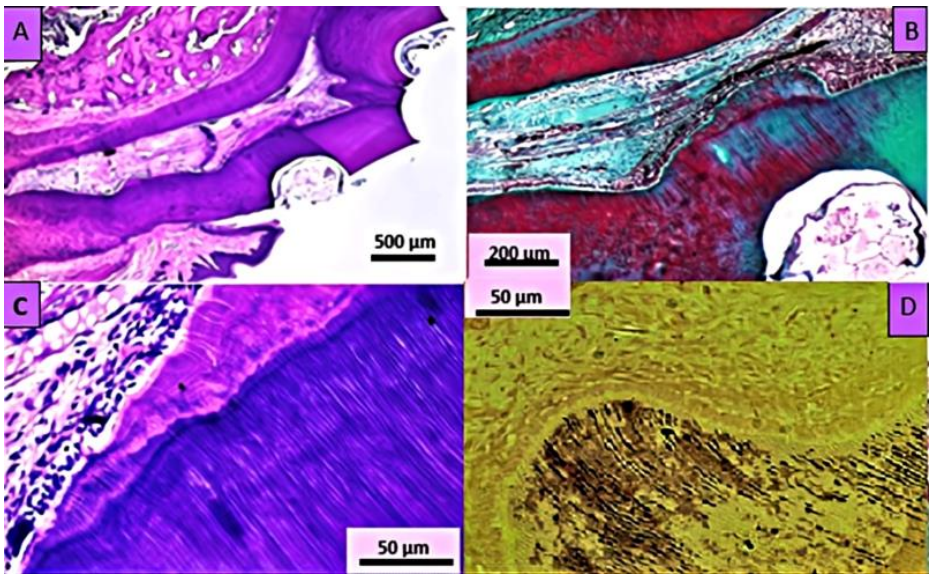


Figure 5. (**A**–**D**) (4×, 10×, and 40×): Microscopic view of cervical restoration with c-GIC at a 1-month interval (H&E and trichrome showing new dentin formation). (**D**) shows the bacterial detection under Brown and Brenn staining.

3.1.4. Residual Dentin Thickness and Tertiary Dentin Formation

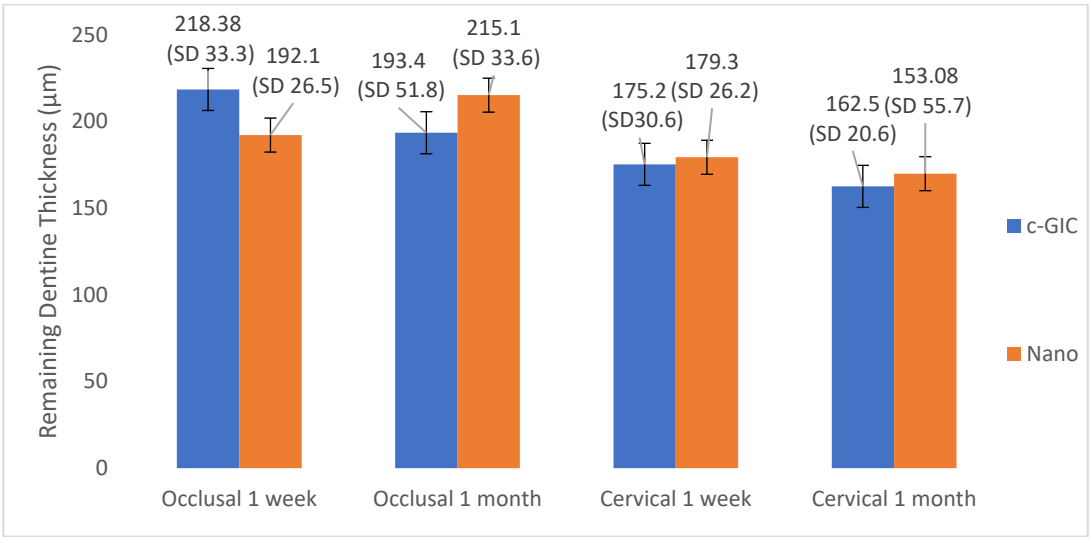
Figure 6a indicates the remaining dentin thickness (RDT) in all the cavities of c-GIC and nano-HA-SiO_2-GIC occlusal and cervical restorations at different time intervals, which shows that all cavities had a RDT of ~200 µm, which is appropriate for investigating the pulp complex response to any restorative materials. For occlusal cavities, in both groups of restorations, RDT was in the range of 192–218 µm and for cervical cavities, RDT was in the range of 163–179 µm. Morphometric evaluation indicates that neither specific factors (type of material and time of being euthanized) nor their interrelation were statistically significant (statistical data not shown). Therefore, all cavities prepared on occlusal and cervical aspects had almost comparable RDTs, hence the reproducibility of the procedures we performed was demonstrated.

A significant difference in results was detected when the tertiary dentin thickness (TDT) of both c-GIC and nano-HA-SiO_2-GIC in first molars was compared with second molars (which was taken as negative control). These results in Table 4 and Figure 6b show that both types of restoration (c-GIC and nano-HA-SiO_2-GIC) had a reasonable inductive influence on the deposition of tertiary dentin as compared with second molars. Although nano-HA-SiO_2-GIC showed higher TDF compared to c-GIC, the result was not statistically significant. Also, there was more TDF in cervical restorations as compared to occlusal restorations, which is observed in Figure 6b.

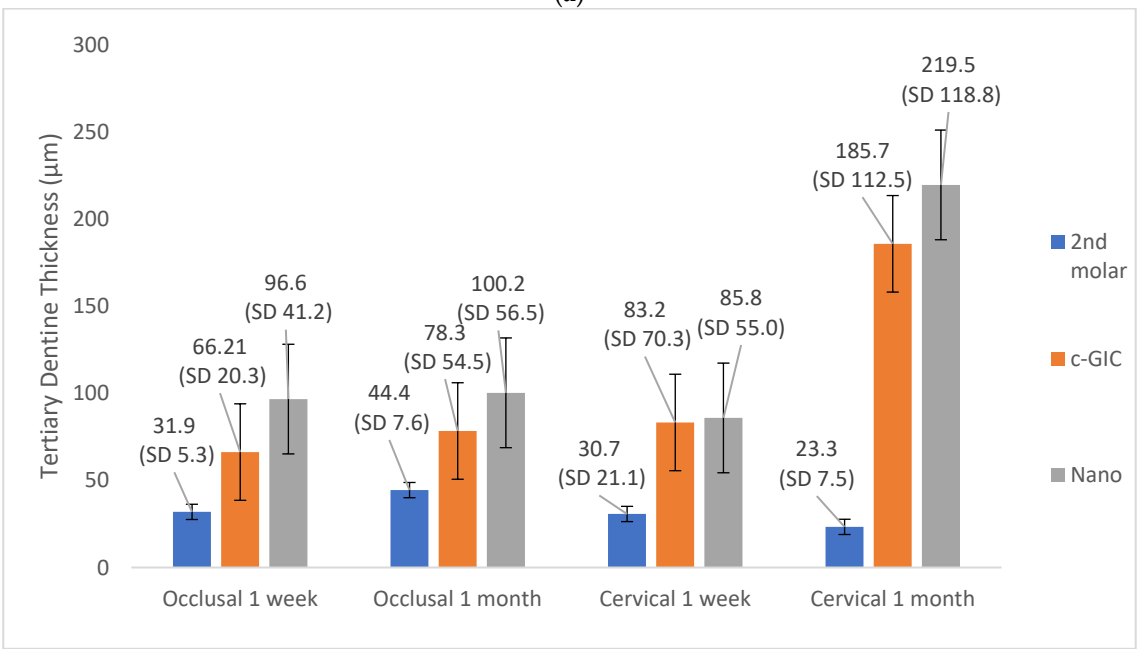
Table 4. Comparisons of tertiary dentin thickness of nano-HA-SiO_2-GIC, c-GIC, and 2nd molar (negative control) on various days using one-way ANOVA.

	Occlusal 1 Week	**Occlusal 1 Month**	**Cervical 1 Week**	**Cervical 1 Month**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Nano-HA-SiO_2-GIC	96.66 (41.24)	100.10 (56.53)	85.82 (17.69)	219.59 (118.85)
c-GIC	66.21 (43.15)	78.29 (54.55)	83.30 (70.34)	185.72 (112.51)
2nd molar	31.97 (5.30)	44.48 (7.79)	30.70 (21.18)	23.38 (7.51)
	p: 0.005 *	p: 0.070	p: 0.075	p: 0.001 *

* indicates significant difference between cGIC and nano-HA-SiO2-GIC ($p \leq 0.05$).



(**a**)



(**b**)

Figure 6. (**a**): Comparison of RDT values shown by c-GIC and nano-HA-SiO_2-GIC in both cervical and occlusal restorations in different interventional periods. (**b**): Comparison of TDT values among 2nd molar, c-GIC, and nano-HA-SiO_2-GIC for both materials at different time intervals.

At the 1-week interval of occlusal restorations, 80% of the teeth restored with nano-HA-SiO_2-GIC showed Grade 1 deposition of tertiary dentin, whereas samples of the positive control c-GIC group showed 50% of Grade 1 tertiary dentin deposition ($p > 0.05$). Teeth that underwent a 1-month intervention showed almost similar results for both types of restorations. With regards to the cervical type of restorations, both materials exhibited similar responses at the 1-week interval, but at the 1-month interval, nano-HA-SiO_2-GIC showed a better response than c-GIC in which 60% of the teeth exhibited Grade 3 TDF, whereas this grade was only observed in 30% of c-GIC-restored teeth. It was also noted that the nano-HA-SiO_2-GIC group displayed better deposition of tertiary dentin formation as shown in Figure 7. This is in comparison to the c-GIC group in terms of thickness and extension of the tertiary dentin deposition along the cavity wall, as shown earlier in Figure 6b.

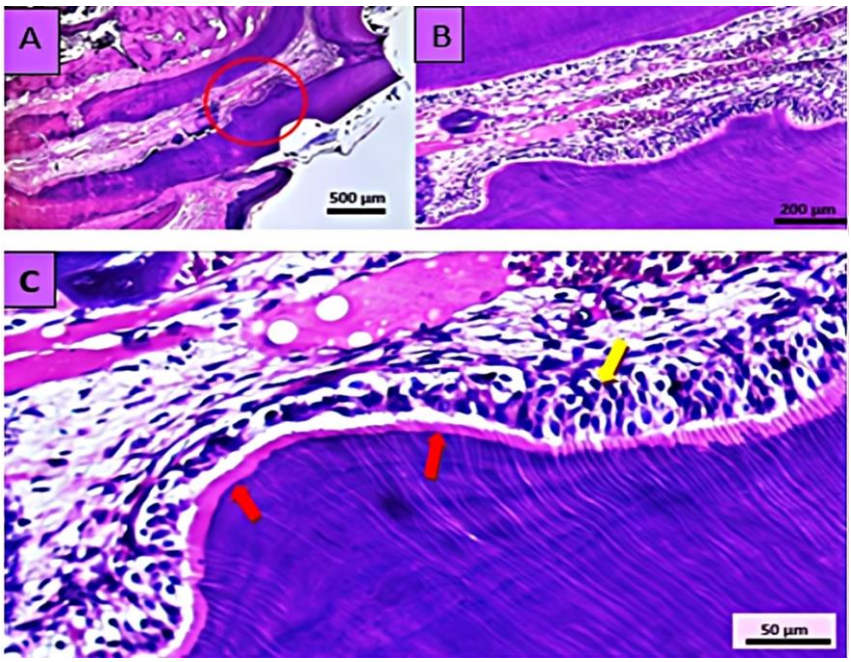


Figure 7. (**A**–**C**) (4×, 10× and 40×) (where (**B**,**C**) are magnified view of red encircled area of (**A**): Microscopic view of cervical restoration with nano-HA-SiO_2-GIC. A very organized continuation of dentinal tubules can be seen with a normal palisade appearance (yellow arrow) of odontoblasts on either side of newly formed tertiary dentin, with dilated blood vessels. A clear distinctive pre-dentin can also be seen (red arrows).

4. Discussion

Since secondary caries at the cavo-surface margins is the prime reason for the failure of any restoration, restorative materials that are biocompatible and promote tertiary dentin formation are highly desirable. This study inspected a potential bioactive novel nano-hydroxyapatite-silica-glass ionomer cement in an animal model and investigated the reaction of the dentin–pulp complex in tooth cavities of rats.

Our study outcomes provide evidence that nano-HA-SiO_2-GIC exerts mild to moderate dentin–pulp complex reactions comparable to conventional GIC and therefore attains adequate biocompatibility. The methodology used in this study permits us to characterize: (i) the different effects of occlusal and cervical types of restoration on dentin–pulp complex; (ii) the differentiation of tertiary dentin which is formed due to normal physiological processes (such as tooth grinding and attrition) with the tertiary dentin that is formed because of biological effects of the material; and (iii) the reproducibility of the cavity preparation in molars of rats by using Image J analysis. Judgment between TDF resulting from normal physiological effects and what is consequential from the restorative material is a critical point, mostly explored by the investigation of longer duration observation that is the 1-month interventional period [27,29]. Rat molar teeth are an excellent model for studying dentin–pulp complex response after restoration due to their anatomical and genetic similarities to humans [27,33].

Regarding pulp tissue disorganization, the response of both types of restorations at 1-week intervals in both occlusal and cervical type restorations was more restricted to the odontoblastic layer disruption in the pulp area corresponding to the cut dentinal tubules. However, after a 1-month interval, the normal palisade arrangement of cell bodies of odontoblasts in the outermost zone of the pulp was restored. A study by Ohshima [34], who utilized the same animal model, reported that odontoblasts returned to their normal arrangement, although they were observed to be severely injured right after the preparation of cavities, which was also observed in our study. The morphological design of our study does not permit us to differentiate whether these odontoblast cells were previously existing

or newly differentiated odontoblast cells from undifferentiated ecto-mesenchymal cells from the core of the pulp. However, this is more common in various attacks [2]. In our study, we did not observe any case of pulpal necrosis in both types of restoration, which is in line with the study by Korner et al. [35] who also observed that high fluoride-releasing materials such as GIC and silver diamine fluoride did not cause any necrosis.

There are two main causes of inflammation after any cavity preparation followed by restoration. Firstly, the inflammation occurs due to abrupt movement of the dentinal fluid caused by cutting dentin, which usually resumes its normal status in 8 to 19 days [36]. Secondly, inflammation occurs due to the response of the restorative material, which may be because of discharge of some unknown components. For GIC, the unreacted groups have been shown to have mild to moderate cytotoxic effects on cell cultures [37], mainly depending on the type of additives being added in GIC [38]. Ribeiro et al. [39] reported that at the seventh day, a mild inflammation of the pulp related with local tissue disruption was witnessed in the majority of the teeth filled with c-GIC, and this was also observed in our study. A study [28] to access pulp response in a rat tooth model using Fuji Lining LC and Fuji IX showed no response or slight inflammation in most of the samples with mild disorganization of pulp tissue throughout a seven-day interval. This contrasted with our study results in which we found more inflammation and moderate levels of tissue disorganization. The reason for this difference might be because they used different lining and base materials whereas cavities made in our study were deeper, which might explain the results observed.

In the above-mentioned study, after 30 days, the pulp tissue in all groups recovered and exhibited a normal manifestation. However, we observed slightly more inflammation in the nano-HA-SiO_2-GIC group as compared to c-GIC in both occlusal and cervical restorations. Reasons might be ascribed to its nanoscale. When converted to the nanoscale, many materials may show considerable toxicity at the cellular level [5,40,41]. Since the nanomaterials are comparable in size to proteins, DNA, viruses, and biological molecules, part of their effects may lie in the interaction between living things and the environment. Nevertheless, this mechanism of interaction is still not very clear, and necessitates further investigation in the future.

Hii et al. [25] performed a biological evaluation of nano-HA-SiO_2-GIC on human dental pulp stem cells (DPSCs). At the highest concentration of 200 mg/mL, they observed a moderate level of cytotoxicity to DPSCs. This moderate level of cytotoxicity was believed to be attributed to the formation of a component/by-product that leached out from the cement. An earlier study [42] on nano-HA-SiO_2-GIC showed detection of a high degree of cross-linking of silyl species between the glass particles in the GIC matrix and nano-silica. As a result, during the setting of nano-HA-silica-GIC, lesser glass particles were free to react with the polyacrylic acid (PAA), thereby allowing this unreacted free PAA to be released after the setting of the nano-HA-silica-GIC, and hence causing more inflammation in the pulp tissue [21], as observed in our study.

Another interesting point to be mentioned here is that whilst the subsequent inflammatory process will cause the break-down of tissue, low-grade inflammation potentially induced by mechanical trauma and mild necrosis may encourage regenerative mechanisms, including angiogenesis and stem cell differentiation [43]. This may explain our results for nano-HA-SiO_2-GIC having more inflammation but at the same time better tertiary dentin formation.

For the detection of bacteria, our study revealed some promising findings whereby no bacteria were observed in both groups (except for one) which is in line with findings of Six et al. [29]. However, it is difficult to achieve any association between inflammation of the pulp and the presence of bacteria based on a smaller number of subjects. The absence of bacterial contamination under both types of restoration based on GICs supports two possible explanations. First, the barrier of the tertiary dentin layer, self-repair processes, and alteration of blood vessels, result in prompt healing of the pulp. Moreover, the slow release

of fluoride from both c-GIC and nano-HA-SiO_2-GIC may also constrain the penetration of bacteria into the dentin.

The morphometric data of remaining dentin thickness (RDT) analyzed in our study, in terms of the dimension of the rats' molars, established that a reasonable residual dentin thickness of ~200 µm was equivalent in both restoration types (c-GIC and nano-HA-SiO_2-GIC) and in both groups of occlusal and cervical restorations which is adequate for determining the biological response, as mentioned by Li et al. [27]. Thus, this parameter in terms of RDT was consistent. So, our study data indicate that a valuable evaluation can be performed between the two types of restoration (c-GIC and nano HA-SiO_2-GIC) and two groups (occlusal and cervical), and hence, the reproducibility of rat molars for restorations was supported. Also, the shape and width of the upper first molars of Wistar rats are appropriate to deliver enough histological sections, permitting us to assess the results and conclude meaningful assessments [32]. These time intervals were selected because a study performed on the pulps of rat molars showed that the inflammatory reaction subsided in 8 days, and tertiary dentin formation was initiated in 2 weeks, resulting in a proper hard tissue dentinal bridge formation in 2 consecutive weeks [27].

In our study, the negative controls showed some deposition of tertiary dentin which was in the range of 31–44 µm (Grade 0) because of attrition. It can be suggested that some tertiary dentin formation was seen in occlusal aspects (beneath the cuspal tips) of the molars with and without restoration due to the continuous tooth grinding nature of the rats and subsequent adaptation to the masticatory stresses resulting in deposition of tertiary dentin which is also reported by Lovschall et al. [44]. For that purpose, morphometric analysis was carried out to compare the TD thickness of the first molar and second molar, and the different grading system mentioned above was utilized (Table 1). Also, this point emphasizes the importance of having another parameter included in the study, which was cervical restorations. In cervical restorations, the same grading system mentioned above was followed, as we also observed some TDF in second molars in the cervical area, which was in the range of 21–30 µm (Grade 0). This physiological cervical dentin deposition was also observed by Lovschall et al. [44] as a discrete deposition of asymmetrical dentin in the mesio-cervical area of the pulp corresponding to the exposure of dentinal tubules associated with cervical root dentin. In both occlusal and cervical restorations, the grading and scoring criteria we used differentiates between the TDF due to attrition and TDF due to restoration with biomaterials.

GIC is known to have good outcomes in terms of tertiary dentin formation. As mentioned in a clinical trial [45], when GIC was evaluated as an atraumatic restorative technique, the tertiary dentin was found in almost 90% of the teeth inspected, though the degree of deposition was small. This was almost observed in our study occlusal group at 1-week duration, in which four out of eight teeth in the c-GIC group, and to a greater extent in the nano-HA-SiO_2-GIC group, in which six out of eight teeth showed some level of tertiary dentin formation. Our findings of early tertiary dentin formation by both groups are in line with a study by Oshima [34], who reported that after three days of cavity preparation, odontoblasts that were newly differentiated replace the damaged odontoblasts and ultimately by five days after cavity preparation, they began to produce reparative dentin. Also, an immunoelectron-microscopic study by Hirata and co-workers demonstrated that a large amount of osteocalcin mRNA expression was found in odontoblasts 3 days post cavity preparation [46]. Osteocalcin is a major non-collagenous protein of the dentin matrix which forms the basis of newly formed tertiary dentin. In our study, at a 1-month interventional period, all samples of both types of restorations showed a Grade 2 level of tertiary dentin formation.

Concerning cervical restorations, almost all first molars from both c-GIC and nano-HA-SiO_2-GIC groups revealed a favorable response of the dentin–pulp complex in terms of tertiary dentin formation. However, results were better for nano-HA-SiO_2-GIC restorations, in which the extent of the newly formed tertiary dentin was similar to the thickness and extent of the cavity, which is an encouraging outcome. A study by Hii et al. [47] found that

nano-HA-SiO_2-GIC extracts promote an early odontogenic differentiation of human dental pulp stem cells (DPSCs). This supports the idea that nano-HA-SiO_2-GIC may have a better inductive influence on early differentiation and ultimately deposition of tertiary dentin from ecto-mesenchymal cells of the dental pulp.

A study performed by Yap et al. [48] observed the effects of the incorporation of nano-hydroxyapatite (nano-HAp) in GIC and found it to have better mechanical properties. Moreover, nano-HAp crystals enhanced bonding strength to dentin [49]. Also, Nano-silica has been reported to have high mechanical strength and good thermostability [18] which may also have some positive influence on our study results related to nano-HA-SiO_2-GIC which is a merger of nano HAp and nano silica in GIC.

The nano-HA-SiO_2-GIC was found to have improved hardness when combined with c-GIC due the higher content of nano-silica, which results in denser cement to produce stronger GIC [50]. When tested for genotoxicity, Ghani et al. [51] reported that there was no notable rise in the number of revertant colonies related to the upsurge in the concentrations of nano-HA-SiO_2, and hence was concluded to be non-genotoxic material and had no mutagenic potential. Murugan et al. [52] recommended the integration of nanohydroxyapatite into c-GIC, which exhibited promising enhancements in the mechanical features, flexural strength, antibacterial properties, and a decrease in the microleakage of the c-GIC, therefore making it one of the most appropriate additives to be utilized. However, further research needs to be performed to authenticate the prospective use of nano HA SiO_2 GIC in clinical human trials.

5. Conclusions

The nano HA is comparable to c-GIC, which serves as the control, and also shows more tertiary dentin deposit, but slightly more inflammatory cell response.

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References

1. Li, Z.; Liu, L.; Wang, L.; Song, D. The effects and potential applications of concentrated growth factor in dentin-pulp complex regeneration. *Stem Cell Res. Ther.* **2021**, *12*, 357. [CrossRef]
2. Berman, L.; Hargreaves, K. *Cohen's Pathways of the Pulp-E-Book*; Elsevier Health Sciences: Amsterdam, The Netherlands, 2020.
3. Zhang, L.; Chen, Z. Autophagy in the dentin-pulp complex against inflammation. *Oral Dis.* **2018**, *24*, 11–13. [CrossRef]
4. Ferracane, J.; Cooper, P.; Smith, A. Can interaction of materials with the dentin-pulp complex contribute to dentin regeneration? *Odontology* **2010**, *98*, 2–14. [CrossRef]
5. Amin, F.; Rahman, S.; Khurshid, Z.; Zafar, M.; Sefat, F.; Kumar, N. Effect of Nanostructures on the Properties of Glass Ionomer Dental Restoratives/Cements: A Comprehensive Narrative Review. *Materials* **2021**, *14*, 6260. [CrossRef]
6. Mickenautsch, S.; Yengopal, V.; Banerjee, A. Pulp response to resin-modified glass ionomer and calcium hydroxide cements in deep cavities: A quantitative systematic review. *Dent. Mater.* **2010**, *26*, 761–770. [CrossRef]
7. Scholtanus, J.; Huysmans, M. Clinical failure of class-II restorations of a highly viscous glass-ionomer material over a 6-year period: A retrospective study. *J. Dent.* **2007**, *35*, 156–162. [CrossRef]
8. Farrugia, C.; Camilleri, J. Antimicrobial properties of conventional restorative filling materials and advances in antimicrobial properties of composite resins and glass ionomer cements—A literature review. *Dent. Mater.* **2015**, *31*, e89–e99. [CrossRef]
9. Berg, J.; Croll, T. Glass ionomer restorative cement systems: An update. *Pediatr. Dent.* **2015**, *37*, 116–124.
10. Kumar, R.S.; Ravikumar, N.; Kavitha, S.; Mahalaxmi, S.; Jayasree, R.; Kumar, T.S.; Haneesh, M. Nanochitosan modified glass ionomer cement with enhanced mechanical properties and fluoride release. *Int. J. Biol. Macromol.* **2017**, *104 Pt B*, 1860–1865. [CrossRef]
11. Beyth, N.; Houri-Haddad, Y.; Baraness-Hadar, L.; Yudovin-Farber, I.; Domb, A.; Weiss, E. Surface antimicrobial activity and biocompatibility of incorporated polyethylenimine nanoparticles. *Biomaterials* **2008**, *29*, 4157–4163. [CrossRef]
12. Zhou, J.; Xu, Q.; Fan, C.; Ren, H.; Xu, S.; Hu, F.; Wang, L.; Yang, K.; Ji, Q. Characteristics of chitosan-modified glass ionomer cement and their effects on the adhesion and proliferation of human gingival fibroblasts: An in vitro study. *J. Mater. Sci. Mater. Med.* **2019**, *30*, 39. [CrossRef] [PubMed]
13. Paiva, L.; Fidalgo, T.; da Costa, L.; Maia, L.; Balan, L.; Anselme, K.; Ploux, L.; Thiré, R. Antibacterial properties and compressive strength of new one-step preparation silver nanoparticles in glass ionomer cements (NanoAg-GIC). *J. Dent.* **2018**, *69*, 102–109. [CrossRef] [PubMed]
14. Kantovitz, K.; Fernandes, F.; Feitosa, I.; Lazzarini, M.; Denucci, G.; Gomes, O.; Giovani, P.; Moreira, K.; Pecorari, V.; Borges, A.; et al. TiO_2 nanotubes improve physico-mechanical properties of glass ionomer cement. *Dent. Mater.* **2020**, *36*, e85–e92. [CrossRef] [PubMed]
15. Moheet, I.; Luddin, N.; Rahman, I.; Kannan, T.; Ghani, N.N.A.; Masudi, S. Modifications of Glass Ionomer Cement Powder by Addition of Recently Fabricated Nano-Fillers and Their Effect on the Properties: A Review. *Eur. J. Dent.* **2019**, *13*, 470–477. [CrossRef] [PubMed]
16. Sasaki, J.; Kiba, W.; Abe, G.; Katata, C.; Hashimoto, M.; Kitagawa, H.; Imazato, S. Fabrication of strontium-releasable inorganic cement by incorporation of bioactive glass. *Dent. Mater.* **2019**, *35*, 780–788. [CrossRef] [PubMed]
17. Garcia, I.; Leitune, V.; Balbinot, G.; Samuel, S.; Collares, F. Influence of niobium pentoxide addition on the properties of glass ionomer cements. *Acta Biomater. Odontol. Scand.* **2016**, *2*, 138–143. [CrossRef] [PubMed]
18. Mabrouk, M.; Selim, M.; Beherei, H.; El-Gohary, M. Effect of incorporation of nano bioactive silica into commercial Glass Ionomer Cement (GIC). *J. Genet. Eng. Biotechnol.* **2012**, *10*, 113–119. [CrossRef]
19. Huang, S.; Gao, S.; Cheng, L.; Yu, H. Remineralization potential of nano-hydroxyapatite on initial enamel lesions: An in vitro study. *Caries Res.* **2011**, *45*, 460–468. [CrossRef]
20. Moshaverinia, A.; Ansari, S.; Movasaghi, Z.; Billington, R.; Darr, J.; Rehman, I. Modification of conventional glass-ionomer cements with N-vinylpyrrolidone containing polyacids, nano-hydroxy and fluoroapatite to improve mechanical properties. *Dent. Mater.* **2008**, *24*, 1381–1390. [CrossRef]
21. Rahman, I.; MASUDI, S.; Luddin, N.; Shiekh, R. One-pot synthesis of hydroxyapatite–silica nanopowder composite for hardness enhancement of glass ionomer cement (GIC). *Bull. Mater. Sci.* **2014**, *37*, 213–219. [CrossRef]
22. Tamilselvi, R.; Dakshinamoorthy, M.; Arumugam, K.; Sathyapriya, B.; Lakshmanan, P. Effect of CPP ACP and Nano Hydroxyapatite Incorporated GIC on Remineralization of Dentin. *Indian J. Public Health Res. Dev.* **2019**, *10*, 936–941. [CrossRef]
23. Moheet, I.; Luddin, N.; Rahman, I.A.; Masudi, S.; Kannan, T.; Ghani, N.N.A. Analysis of Ionic-Exchange of Selected Elements between Novel Nano-Hydroxyapatite-Silica Added Glass Ionomer Cement and Natural Teeth. *Polymers* **2021**, *13*, 3504. [CrossRef] [PubMed]
24. Noorani, T.; Luddin, N.; Rahman, I.; Masudi, S. In vitro cytotoxicity evaluation of novel nano-hydroxyapatite-silica incorporated glass ionomer cement. *J. Clin. Diagn. Res. JCDR* **2017**, *11*, ZC105. [CrossRef] [PubMed]
25. Hii, S.; Luddin, N.; Kannan, T.; Rahman, I.A.; Ghani, N.N.A. The Biological Evaluation of Conventional and Nano-Hydroxyapatite-Silica Glass Ionomer Cement on Dental Pulp Stem Cells: A Comparative Study. *Contemp. Clin. Dent.* **2019**, *10*, 324–332. [PubMed]
26. Sert, N.P.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.; Baker, M.; Browne, W.; Clark, A.; Cuthill, I.; Dirnagl, U.; et al. The ARRIVE guidelines 2019: Updated guidelines for reporting animal research. *bioRxiv* **2019**, 703181. [CrossRef]
27. Li, F.; Wang, P.; Weir, M.; Fouad, A.; Xu, H. Evaluation of antibacterial and remineralizing nanocomposite and adhesive in rat tooth cavity model. *Acta Biomater.* **2014**, *10*, 2804–2813. [CrossRef]

28. Rendjova, V.; Gjorgoski, I.; Ristoski, T.; Apostolska, S. In vivo study of pulp reaction to glass ionomer cements and dentin adhesives. *Prilozi* **2012**, *33*, 265–277.
29. Six, N.; Lasfargues, J.; Goldberg, M. In vivo study of the pulp reaction to Fuji IX, a glass ionomer cement. *J. Dent.* **2000**, *28*, 413–422. [CrossRef]
30. Trongkij, P.H.; Sutimuntanakul, S.; Lapthanasupkul, P.; Chaimanakarn, C.; Wong, R.H.; Banomyong, D. Pulpal responses after direct pulp capping with two calcium-silicate cements in a rat model. *Dent. Mater. J.* **2019**, *38*, 584–590. [CrossRef]
31. De Souza Costa, C.A.; Giro, E.M.A.; do Nascimento, A.B.L.; Teixeira, H.M.; Hebling, J. Short-term evaluation of the pulpo-dentin complex response to a resin-modified glass-ionomer cement and a bonding agent applied in deep cavities. *Dent. Mater.* **2003**, *19*, 739–746. [CrossRef]
32. Von Böhl, M.; Ren, Y.; Kuijpers-Jagtman, A.; Fudalej, P.; Maltha, J. Age-related changes of dental pulp tissue after experimental tooth movement in rats. *PeerJ* **2016**, *4*, e1625. [CrossRef]
33. Dammaschke, T. Rat molar teeth as a study model for direct pulp capping research in dentistry. *Lab. Anim.* **2010**, *44*, 1–6. [CrossRef]
34. Ohshima, H. Ultrastructural changes in odontoblasts and pulp capillaries following cavity preparation in rat molars. *Arch. Histol. Cytol.* **1990**, *53*, 423–438. [CrossRef]
35. Korwar, A.; Sharma, S.; Logani, A.; Shah, N. Pulp response to high fluoride releasing glass ionomer, silver diamine fluoride, and calcium hydroxide used for indirect pulp treatment: An in-vivo comparative study. *Contemp. Clin. Dent.* **2015**, *6*, 288–292.
36. Sveen, O.; Hawes, R. Differentiation of new odontoblasts and dentine bridge formation in rat molar teeth after tooth grinding. *Arch. Oral Biol.* **1968**, *13*, 1399–1409. [CrossRef]
37. Stanislawski, L.; Daniau, X.; Lauti, A.; Goldberg, M. Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements. *J. Biomed. Mater. Res.* **1999**, *48*, 277–288. [CrossRef]
38. Lan, W.; Lan, W.; Wang, T.; Lee, Y.; Tseng, W.; Lin, C.; Jeng, J.; Chang, M. Cytotoxicity of conventional and modified glass ionomer cements. *Oper. Dent.* **2003**, *28*, 251–259. [PubMed]
39. Ribeiro, A.; Sacono, N.; Soares, D.; Bordini, E.; de Souza Costa, C.; Hebling, J. Human pulp response to conventional and resin-modified glass ionomer cements applied in very deep cavities. *Clin. Oral Investig.* **2020**, *24*, 1739–1748. [CrossRef] [PubMed]
40. Padovani, G.C.; Feitosa, V.P.; Sauro, S.; Tay, F.R.; Durán, G.; Paula, A.J.; Durán, N. Advances in Dental Materials through Nanotechnology: Facts, Perspectives and Toxicological Aspects. *Trends Biotechnol.* **2015**, *33*, 621–636. [CrossRef] [PubMed]
41. Feng, X.; Chen, A.; Zhang, Y.; Wang, J.; Shao, L.; Wei, L. Application of dental nanomaterials: Potential toxicity to the central nervous system. *Int. J. Nanomed.* **2015**, *10*, 3547.
42. Shiekh, R.; Rahman, I.A.; Luddin, N. Modification of glass ionomer cement by incorporating hydroxyapatite-silica nano-powder composite: Sol–gel synthesis and characterization. *Ceram. Int.* **2014**, *40*, 3165–3170. [CrossRef]
43. Cooper, P.; Takahashi, Y.; Graham, L.; Simon, S.; Imazato, S.; Smith, A. Inflammation–regeneration interplay in the dentine–pulp complex. *J. Dent.* **2010**, *38*, 687–697. [CrossRef]
44. Lovschall, H.; Fejerskov, O.; Josephsen, K. Age-related and site-specific changes in the pulpodentinal morphology of rat molars. *Arch. Oral Biol.* **2002**, *47*, 361–367. [CrossRef] [PubMed]
45. Gotjamanos, T. Pulp response in primary teeth with deep residual caries treated with silver fluoride and glass ionomer cement ('atraumatic'technique). *Aust. Dent. J.* **1996**, *41*, 328–334. [CrossRef] [PubMed]
46. Hirata, M.; Yamaza, T.; Mei, Y.; Akamine, A. Expression of osteocalcin and Jun D in the early period during reactionary dentin formation after tooth preparation in rat molars. *Cell Tissue Res.* **2005**, *319*, 455–465. [CrossRef]
47. Ching, H.S.; Ponnuraj, K.T.; Luddin, N.; Rahman, I.A.; Ghani, N.N.A. Early Odontogenic Differentiation of Dental Pulp Stem Cells Treated with Nanohydroxyapatite–Silica–Glass Ionomer Cement. *Polymers* **2020**, *12*, 2125. [CrossRef]
48. Yap, A.; Pek, Y.; Kumar, R.; Cheang, P.; Khor, K. Experimental studies on a new bioactive material: HAIonomer cements. *Biomaterials* **2002**, *23*, 955–962. [CrossRef]
49. Wang, R.; Wang, Q.; Wang, X.; Tian, L.; Liu, H.; Zhao, M.; Peng, C.; Cai, Q.; Shi, Y. Enhancement of nano-hydroxyapatite bonding to dentin through a collagen/calcium dual-affinitive peptide for dentinal tubule occlusion. *J. Biomater. Appl.* **2014**, *29*, 268–277. [CrossRef]
50. Moheet, I.; Luddin, N.; Rahman, I.A.; Masudi, S.; Kannan, T.; Ghani, N.A. Evaluation of mechanical properties and bond strength of nano-hydroxyapatite-silica added glass ionomer cement. *Ceram. Int.* **2018**, *44*, 9899–9906. [CrossRef]
51. Ghani, N.; Sazri, A.; Yee, C.; Luddin, N.; Ponnuraj, K. Genotoxicity evaluation of locally produced nano-hydroxyapatite-silica: An in vitro study using the bacterial reverse mutation test. *Dent. Med. Res.* **2019**, *7*, 12. [CrossRef]
52. Murugan, R.; Yazid, F.; Nasruddin, N.; Anuar, N. Effects of Nanohydroxyapatite Incorporation into Glass Ionomer Cement (GIC). *Minerals* **2021**, *12*, 9. [CrossRef]