ORIGINAL RESEARCH

Clinical Radiographic and Histological Evaluation of Zinc Bioactive Glass as a Pulpotomy Medicament in Primary Molar Teeth: An *In Vivo* Study

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Abstract

Background: In the past 20 years, research into pulpotomy materials has transitioned into the "biological era," marked by the emergence of innovative dental materials such as bioactive glass, which are bio-inductive and supportive of regeneration. This evolution represents significant advancements in dentistry.

Aim: To evaluate the clinical, radiographic, and histological success rate of zinc (Zn) bioactive glass as a pulpotomy medicament for primary molars.

Patients and methods: A total sample size of 40 primary molars was selected from 36 children aged 6–9 years. A regular conventional pulpotomy procedure was followed by the placement of Zn bioactive glass over the radicular orifice. The pulp chamber was filled with reinforced zinc oxide eugenol (ZOE). Patient recall was scheduled at 3, 6, 9, and 12 months, respectively. Further evaluation was done by scanning electron microscopy (SEM). Data were tabulated and subjected to statistical analysis.

Results: The present study shows 100, 97.44, 94.74, and 94.74% clinical success at the end of 3, 6, 9, and 12 months, respectively. The radiographic success was 100, 94.87, 92.11, and 92.11% at 3, 6, 9, and 12 months, respectively. SEM analysis showed a well-defined dentinal bridge formation between Zn bioactive glass and the pulp chamber.

Conclusion: Quite promising clinical, radiographic, and histological results of Zn bioactive glass in the present study show its potential as an additional pulpotomy medicament to the presently existing pulpotomy agents.

Keywords: Pulpotomy, Scanning electron microscope, Zinc bioactive glass.

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Introduction

Pediatric endodontics aims to preserve the pulp of primary teeth until they naturally resorb, with the goal of maintaining proper tooth spacing, preventing detrimental oral habits, preserving esthetics, and ensuring continued chewing function. Pulpotomy is a specialty treatment involving the surgical removal of infected pulp in primary teeth, followed by the application of medication to aid healing of the remaining pulp tissue.[1]

Recent advancements in endodontic materials have emphasized the "biological era," with research focusing on understanding pulp biology, pathophysiology, and healing mechanisms, particularly in the context of pulp regeneration.[1] Stem cell-based regenerative endodontics represents an emerging field with promising potential to transform dental care.[2]

A bioactive substance is one that generates a specific biological response at the interface of a material, resulting in the formation of a link between the tissues and the material.[3] Bioactive glasses (BAGs) are recognized for their biocompatibility, osteoconductivity, osteoinductivity, hemostatic properties, and ability to release therapeutic ions.[1] Despite their advantages, they exhibit high solubility, leading to significant ion release into the surrounding environment, which limits their application in load-bearing scenarios.[4]

Zinc (Zn), following iron, is the second most abundant trace metal in the human body, playing crucial roles in various metabolic and cellular signaling pathways. It interacts with numerous proteins, including structural proteins, transcription factors, and enzymes, supporting essential physiological processes such as growth, neurotransmission, hormone regulation, DNA synthesis, and gene expression.[5]

Zinc is frequently utilized in dentistry due to its ability to inhibit bacterial growth and prevent tooth decay. Examples include zinc oxide eugenol (ZOE), zinc polycarboxylate (ZPC), and zinc oxyphosphate (ZOP).[2]

Cell-biomaterial interactions involve cells producing an extracellular matrix, creating an environment conducive to cell survival. Cell adhesion relies on specific interactions between cellular membrane factors and extracellular matrix, known as focal contacts. These interactions involve cytoskeletal proteins,

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integrins, and other membrane proteins, facilitating cell adhesion and differentiation. The biomaterial surface plays a critical role, as it can chemically interact with matrix proteins, influencing cell adhesion and differentiation.[4]

Depending on cell type, Zn concentration, and bioavailability, Zn's interaction with living tissues can produce specific effects. The biomaterial surface is crucial here, as its chemical interaction with specific matrix proteins can influence cell adhesion and differentiation stability.[4]

Given the limited literature on how Zn affects bioactive glass properties, this study aimed to clinically, radiographically, and histologically evaluate Zn bioactive glass as a pulpotomy medicament in primary molars.

Patients and Methods

The current *in vivo* study was carried out in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College, Khammam, Telangana, from August 2021 to September 2022, in collaboration with the Department of Physics, NIT Warangal, Telangana, regarding the preparation of Zn bioactive glass and Virtue Meta Sol, Hyderabad, Telangana, for scanning electron microscopy (SEM) analysis. Ethical clearance was obtained from the Institutional Review Board and Ethics Committee. Forty primary molars were randomly selected from 36 children, aged 6–9 years, for pulpotomy treatment, based on clinical and radiographic criteria.

Inclusion Criteria

- Teeth with ICDAS clinical codes 5 and 6 and radiographic RC-5.[6]
- Vital tooth with healthy periodontium.
- Pain, if present, should be neither spontaneous nor persistent.
- Absence of clinical signs or symptoms that indicate nonvital tooth, such as draining sinuses, soft tissue swelling, tooth mobility, or tenderness on percussion.
- Following the pulpotomy, the tooth should be restorable.
- Children who were scheduled to undergo serial extraction.

Exclusion Criteria

- Demonstration of internal resorption.
- Any interradicular bone loss if present.
- Presence of an abscess or fistula in relation to the tooth.
- Radiographic evidence of calcific globules in the pulp chamber.
- Caries penetrating the floor of pulp chamber.
- A tooth that is about to naturally exfoliate.

The standard pulpotomy procedure was performed. The teeth were anesthetized using lidocaine 2% with epinephrine 1/80,000 (Becain-ADR) and isolated with a rubber dam (GDC Company). Before exposing the pulp, the cavity outline was shaped, and all the marginal caries were removed. The coronal pulp was exposed using a sterile No. 6 round carbide bur (Prima Classics). To facilitate coronal pulp excavation, the access cavity was extended to the boundaries of the pulp horns. A sterile, sharp spoon excavator (GDC Company) was used to amputate the coronal pulp. The pulp was amputated at the root canal entrance. To prevent dentinal chips from entering the radicular pulp, the pulp chamber was irrigated with saline (Fresenius Kabi India Pvt Ltd). To aid in hemostasis, sterile cotton pellets were placed over the amputated pulp stumps after irrigation. After achieving complete hemostasis, Zn bioactive glass powder (NIT, Warangal) was mixed with saline to obtain a thick paste on a glass slab using a cement spatula. This mixture was then transferred directly into the pulp chamber with a plastic filling instrument and placed on the pulp stumps. The remaining pulp chamber was filled with a thick paste of reinforced ZOE (Prevest DenPro Limited). To ensure standardization, a stainless steel crown (3M ESPE) was selected as the most effective long-term restoration, as it provides a reliable seal against microleakage. Therefore, all the teeth were restored with stainless steel crowns, considering the patient's age.

Clinical and radiographic examinations were performed at 3, 6, 9, and 12 months posttreatment to assess for spontaneous pain, draining sinus, swelling or abscess, tooth mobility, premature exfoliation, interradicular radiolucency, periodontal ligament widening, periapical radiolucency, and internal root resorption. The results were collected and statistically analyzed. If one or more of the above signs or symptoms were present, the treatment was considered a failure; however, pulp canal obliteration was not deemed a failure. During the follow-up period, teeth that were extracted to balance the arch underwent histological examination, confirming the success of the treatment.

Following the pulpotomy procedure, eight representative samples from the 40 cases, selected for serial extraction, were extracted at 3, 6, 9, and 12 months, respectively. These samples were then prepared for histological analysis using SEM. All extracted teeth were preserved in 10% buffered formalin for 48 hours. Each specimen was rinsed in a sodium phosphate buffer, concentrated to 0.05 M at pH 7.4, and dehydrated through a series of alcohol concentrations (10–100%, 10 minutes each). The samples were then air-dried. Afterwards, they were mounted on stubs with carbon tape and prepared for gold sputtering. Finally, the samples were coated with a gold alloy (40% gold) for examination under a SEM.

The specimens were examined at magnifications ranging from 500× to 5000×. In terms of morphology, hard tissue barriers were classified as amorphous when tubules were absent or minimal; mixed when tubules occupied a proportionate area relative to the amorphous dentin; and tubular when tubules were predominant or entirely present. The hard tissue barriers were further categorized as central (at the center of the exposure), peripheral (surrounding the canal walls), or centroperipheral (when the hard tissue barrier formation was complete).

Results

Statistical analysis was carried out using IBM SPSS for Windows version 23.0 (SPSS, Chicago, IL, USA). The proportions of variables at the 3, 6, 9, and 12-month intervals were expressed as percentages. The Chi-squared test was applied to compare the proportions between the baseline and subsequent months to evaluate the presence or absence of pathology.

Table 1 and Figure 1 shows the clinical evaluation at 3, 6, 9, and 12-month intervals. After 3 months, none of the cases exhibited any clinical signs or symptoms of pathology, indicating clinical success rates of 100%. After 6 months, one case showed draining sinus and abscess, with a clinical success rate of 97.44%. After 9 months, two cases showed draining sinus and abscess, with a clinical success rate of 94.74%. The clinical success rate was 94.74% after 12 months, as two cases showed draining sinus and one case showed mobility.

Table 2 and Figure 2 represents the radiographic evaluation at 3, 6, 9, and 12-month intervals. After 3 months, a 100% success rate was observed without any signs or symptoms. After 6 months, two

Table 1: Clinical evaluation at 3, 6, 9, and 12 months

		3 months		*6 months*		*9 months*		*12 months*	
Clinical parameters		*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Pain	Present	0	0.00	0	0.00	0	0.00	0	0.00
	Absent	39	100.00	39	100.00	38	100.00	38	100 00
Draining sinus	Present	0	0.00	1	2.56	2	5.26	2	5.26
	Absent	39	100.00	38	97.44	36	94.74	36	94.74
Swelling/abscess	Present	0	0.00	1	2.56	2	5.26	2	5.26
	Absent	39	100.00	38	97.44	36	94.74	36	94.74
Mobility	Present	0	0.00	0	0.00	0	0.00	1	2.63
	Absent	39	100.00	39	100.00	38	100.00	37	97.37
Premature exfoliation	Present	0	0.00	0	0.00	0	0.00	0	0.00
	Absent	39	100.00	39	100.00	38	100.00	38	100 00
Overall success		39	100.00	38	97.44	36	94.74	36	94.74
Chi-square value				3.031		6.129		4.108	
p-value				0.553 NS		0.19 NS		0.392 NS	

Chi-squared test, $p < 0.05$ set as statistically significant; NS, nonsignificant

Table 2: Radiographic evaluation at 3, 6, 9, and 12 months

		3 months		*6 months*		*9 months*		*12 months*	
Radiographic parameters		*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Interradicular radiolucency	Present	0	0.00	2	5.13	3	7.89	3	7.89
	Absent	39	100.00	37	94.87	35	92.11	35	92.11
PDL widening	Present	0	0.00	2	5.13	3	7.89	3	7.89
	Absent	39	100.00	37	94.87	35	92.11	35	92.11
Periapical radiolucency	Present	0	0.00	0	0.00	0	0.00	0	0.00
	Absent	39	100.00	39	100.00	38	100.00	38	100.00
Internal resorption	Present	0	0.00	1	2.56	1	2.63	2	5.26
	Absent	39	100.00	38	97.44	37	97.37	36	94.74
Overall success		39	100.00	37	94.87	35	92.11	35	92.11
Chi-square value				2.27		4043		5.241	
p-value				0.518NS		0.257NS		0.155NS	

Chi-squared test, $p < 0.05$ set as statistically significant; NS, nonsignificant

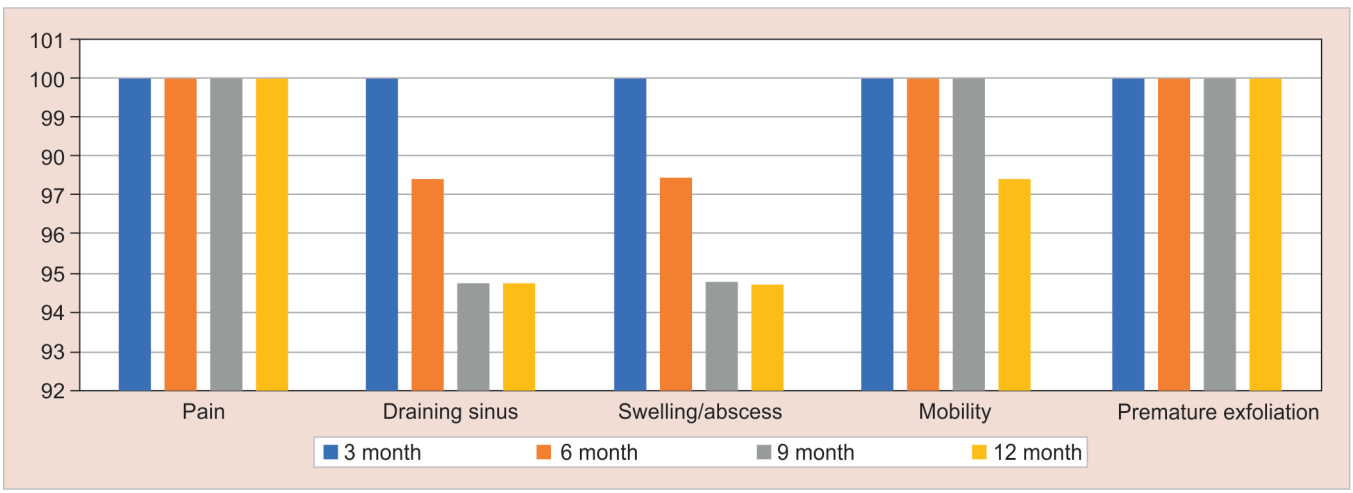


Fig. 1: Clinical evaluation at 3, 6, 9, and 12 months

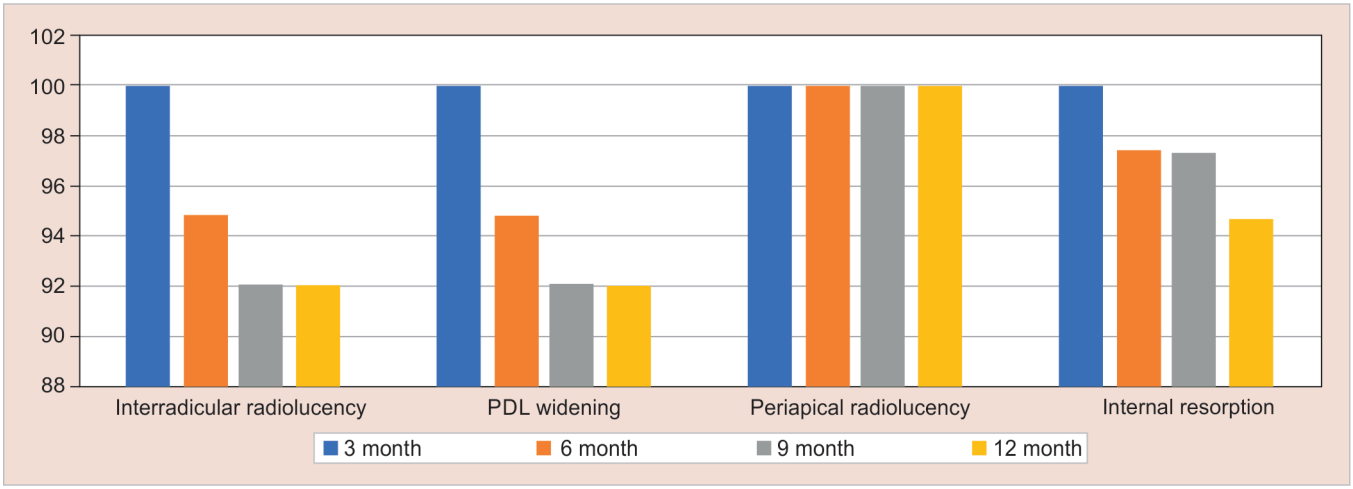


Fig. 2: Radiographic evaluation at 3, 6, 9, and 12 months

cases showed interradicular radiolucency and PDL widening, and one case showed internal resorption, with a radiographic success of 94.87%. After 9 months, three cases showed interradicular radiolucency and PDL widening, and one case showed internal resorption, with a radiographic success of 92.11%. After 12 months, three cases showed interradicular radiolucency and PDL widening, and two cases showed internal resorption, with a radiographic success of 92.11%.

Scanning electron microscopy findings at 3 months (Fig. 3A) suggest that both specimens showed newly formed dentin with multiple channels, interlacing coarse collagen bundles, and immature, odontoblast-like cells, indicating the beginning of the hard tissue barrier formation. At 6 months (Fig. 3B), out of two specimens, one presented with a mixed type of hard tissue barrier with equal proportions of dentinal tubules and amorphous dentin, and the other presented hard tissue with an amorphous pattern. The localization of the hard tissue barrier was centroperipheral, indicating the completion of hard tissue barrier formation. At 9 months (Fig. 3C), both specimens presented with a mixed type of hard tissue barrier. The localization of the hard tissue barrier was centroperipheral in one specimen and peripheral in the other. At 12 months (Fig. 3D), both specimens presented with a mixed type of hard tissue barrier with centroperipheral localization.

Discussion

Recent advancements in pulp biology have shifted focus from conventional inert materials for replacing diseased pulp tissues to regenerative techniques. This marks a significant paradigm change toward regenerative vital pulp therapy, particularly for primary teeth.[7]

Bioactive glasses can be synthesized using either standard melt-quenching or sol–gel methods.[3] When in contact with body fluids or simulated body fluids, BAGs undergo rapid ionic dissolution and glass degradation through the exchange of H^+ ions with Na^+ and Ca^{2+} from the glass network. Hydrolysis of silica groups during ion exchange generates silanol groups (Si–O–H), increasing the local pH. As pH rises, further degradation of the silica network occurs, resulting in orthosilicic acid and $Si(OH)_4$ forming a negatively charged gel layer on the surface. This gel layer serves as a matrix for hydroxyapatite precipitation.[8]

Advanced biomimetic properties have been incorporated into glass by modifying the chemical composition and concentration of glass constituents, allowing adaptation for specific therapeutic applications.[9] Recently developed BAGs, known as HZ glasses, resemble Hench's 45S5 composition but include higher levels of Zn (5–20% as ZnO).[5]

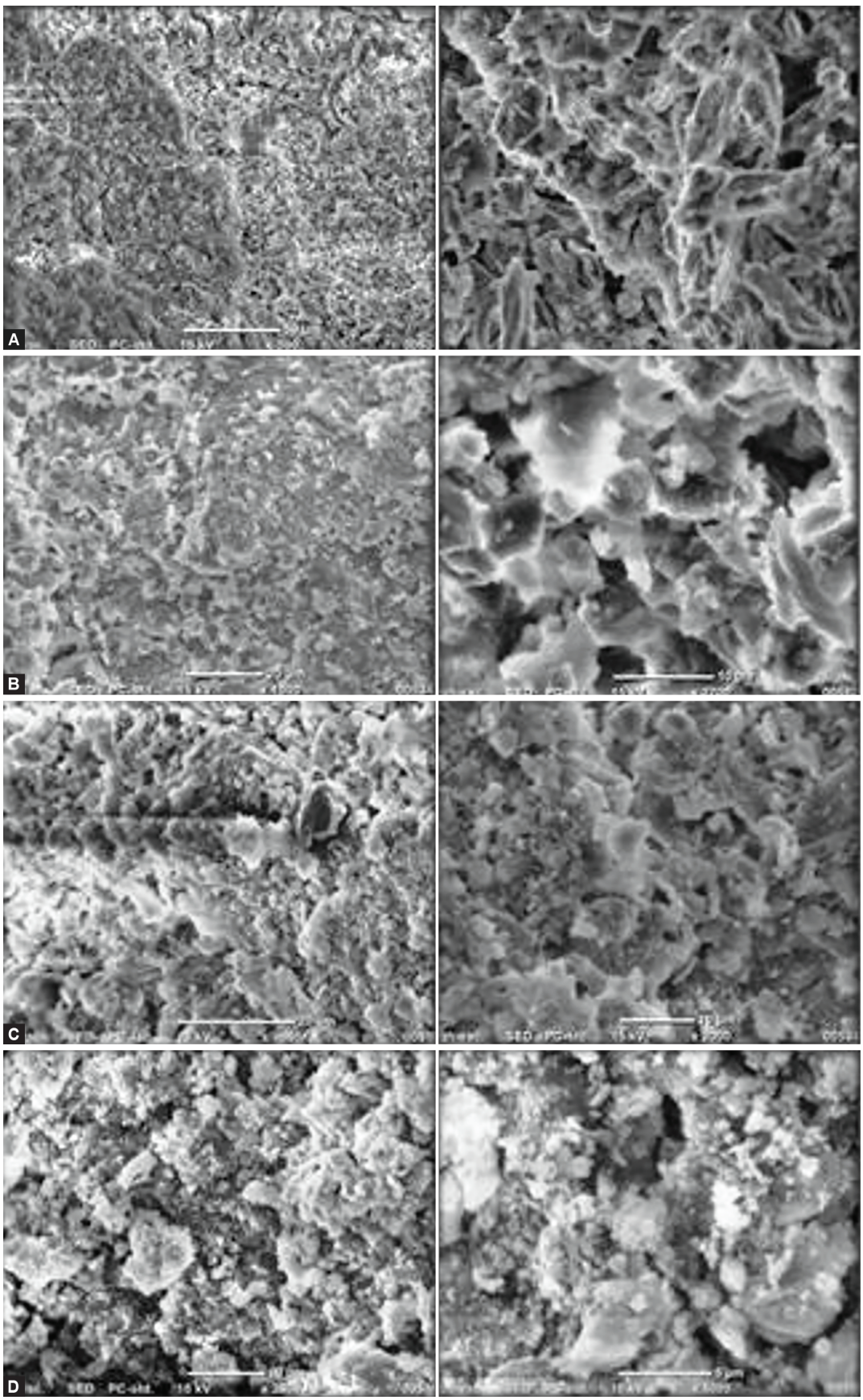
Zinc significantly influences the crystallinity modulation of apatite crystals, reducing the solubility of carbonated and noncarbonated apatite. In BAG structures, ZnO functions as an intermediate oxide, creating covalent bonds between adjacent SiO_4 tetrahedral layers to improve glass stability, or as a network modifier, which reduces surface area and pore size.[2]

Studies show that Zn^{2+} has a dose-dependent impact on the differentiation of mesenchymal stem cells into osteoblasts, highlighting the need to determine safe upper intake levels. Research continues to explore optimal Zn concentrations in various biomaterials, considering factors such as physicochemical characteristics, cell types, and kinetics of Zn release in different physiological environments, which currently limits widespread commercial application.[10]

In the present study, the clinical success rates were found to be 100, 97.44, 94.74, and 94.74% at 3, 6, 9, and 12 months, respectively. Radiographically, the success rates were recorded at 100, 94.87, 92.11, and 92.11% during the same time periods.

These results are consistent with the findings of Govindaraj et al., who evaluated the efficacy of bioactive glass and hydroxyapatite crystals as pulpotomy materials in primary molars. At the end of their study, both treatment groups achieved 100% clinical success and 84% radiographic success. No clinical symptoms were observed in any of the primary molars treated with bioactive glass throughout the evaluation period. The use of bioactive glass has proven to be superior in providing a long-lasting seal over the vital pulp, as it does not degrade, disintegrate, or resorb over time.[1]

A rapid surface reaction occurs when bioactive glass interacts with physiological fluids, facilitating its binding to collagen fibers. This reaction involves rapid ion exchange and release, leading to the development of silanol groups at the glass-fluid interface by attacking the silica network. Ongoing condensation of silanols results in the formation of a silanol-rich gel layer. The ions released from bioactive glass bind to reactive groups on collagen structures, forming a hydroxyapatite carbonate



Figs 3A to D: Photomicrographs of samples under SEM: (A) 3 months; (B) 6 months; (C) 9 months; (D) 12 months

layer (HCA) on the surface. Given that the dentin matrix is predominantly composed of collagen fibers, the adhesion of bioactive glass particles to this tissue creates a robust chemical bond. This hermetic seal not only prevents microleakage but also serves as a matrix for mineralization, contributing significantly to the observed clinical success when bioactive glass was used as a pulpotomy agent.[11]

In addition, bioactive glass has antibacterial properties because it can increase the pH of aqueous suspensions, which is harmful to bacterial cells.[1] However, after 6 months of observation, radiographs of two primary teeth revealed furcal radiolucency and widened periodontal ligament space. This may be attributed to the body's inflammatory response to the foreign bioactive glass material. The initial inflammatory reaction could be exacerbated by the elevated alkalinity at the application site, caused by the rapid release of Na^+, K^+, Mg^{2+}, and Ca^{2+} ions when bioactive glass interacts with bodily fluids or physiological solutions. The pH will rise to about 10.5 as a result of cation exchange mechanisms that elevate the concentration of hydroxyl ions at the BAG/solution interface.[1]

The current study's findings are consistent with Huang et al., who demonstrated that free Zn ions (0–5 ppm) enhanced the proliferation of human dental pulp stem cells (hDPSCs) and increased alkaline phosphatase (ALP) activity. Additionally, they observed that conditioned medium from Zn-containing bioactive glass (ZnBG-CM) stimulated the synthesis and secretion of odontogenic markers like dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1). Furthermore, treatment with ZnBG-CM upregulated mRNA expression of osteogenic markers including RUNX2, OCN, BSP, BMP-2, MEPE, and ON.[2]

Similarly, Zheng et al. reported that Zn oxide-modified bioactive glass nanoparticles (ZnO-BGN) enhanced osteogenic differentiation of human mesenchymal stem cells (hMSCs), as evidenced by increased ALP activity. They concluded that ZnO-BGN is a promising material for bone regeneration due to its ability to form apatite, unique ion release properties, efficacy, and safety. Consistent with these findings, SEM analysis in the present study revealed well-defined formation of a dentinal bridge between Zn bioactive glass and the pulp chamber.[12]

Although the results of the current study are promising, future long-term clinical trials with larger sample sizes are needed to properly assess the effectiveness of Zn bioactive glass in primary molar pulpotomies.

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