ORIGINAL RESEARCH

Efficacy of Chitosan Scaffolded Calcium Silicate-based Cements for Treating Internal Resorption Defects with Perforation: *In Vitro* Study

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Abstract

Aim: The present study aimed to evaluate the efficacy of chitosan scaffold combined with calcium silicate cements in the management of internal resorption with perforation.

Materials and methods: Internal resorption cavities were simulated in 20 human permanent maxillary incisors that were then divided into two groups: group I – biodentine and group II – chitosan scaffold combined with biodentine. The samples were evaluated for the mineralization activity at the end of the 7th day and 14th day using scanning electron microscopy–energy dispersive X-ray (SEM–EDX) analysis. The data were recorded, tabulated, and then statistically analyzed.

Results: From the SEM–EDX analysis, the mean score of calcium and phosphorus ion uptake by the material was obtained. Statistical analysis by nonparametric Mann–Whitney test showed that there was statistically significant difference in calcium ion uptake at the end of the 7th day ($p = 0.016$) and at the end of 14th day ($p = 0.043$) between the group biodentine and group chitosan scaffold combined with biodentine ($p < 0.05$).

Conclusion: In this present study, the use of chitosan scaffolds combined with biodentine showed a statistically significant difference in the mineralization activity when compared with pure biodentine. These scaffolded biomaterials exhibited greater potential for mineralization *in vitro* which can be efficiently used for the management of teeth with internal resorption with perforation. Further clinical trials are required for the understanding of their behavior in real-world scenarios.

Clinical significance: Calcium silicate cements have often exhibited defective hard tissue barrier formation and hence there is a pressing need to search for newer biomaterials that can overcome these shortcomings. Scaffolded biomaterials provide a controlled microcellular environment for bioactivity, and they were found to be efficient in the remineralization of tooth structure. The present study findings indicate that these chitosan scaffolds can be efficiently used in combination with calcium silicate cements for the management of internal resorption with perforation to enhance the treatment outcome.

Keywords: Biodentine, Calcium silicate cements, Chitosan scaffold, Internal resorption, Perforating resorption, Scanning electron microscope.

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Introduction

The etiology and clinical presentation of root resorption are diverse, thereby making it a challenge for clinicians during diagnosis and treatment planning.

Resorption is a physiologic or pathologic process that results in a loss of dentin, cementum, or bone.[1] Root resorption in primary dentition is physiological but in permanent dentition, it does not occur naturally and is invariably inflammatory in nature. Resorption occurs when developmental precementum or predentin are damaged and inflammation of the adjacent soft tissues allows for clastic cell invasion.[2] So, the perplexities of root resorption need to be considered and managed efficiently for successful outcome.

This study is focused on internal resorption as its occurrence was estimated to be anywhere between 0.01 and 55%.[3] Among them, internal root resorption with perforation defects affects the outcome of endodontic treatment because of the weakened tooth structure, and the repair process is more challenging.[4]

A bioactive material is required to reinforce the weakened tooth structure. Calcium silicate cements are known for its bioactivity. Among the calcium silicate cements, biodentine is found to have the potential to serve as a bioactive dentin substitute. Biodentine is a unique tricalcium silicate-based inorganic cement that releases calcium ions that provides an alkaline environment that is conducive for osteoblastic activity. Biodentine shows deposition of apatitic structures that increase the marginal sealing of the material.[5]

There were a few disadvantages that have been documented with these calcium silicate cements, like low washout resistance, decreased bioactivity over time, decreased fracture resistance, etc.[6] There has been a search for newer biomaterials that overcome the defects caused by calcium silicate cements. So, with the advancements evolving in the field of tissue engineering and regenerative medicine, efforts have been made for the

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regeneration of the tooth structure, which primarily includes the use of scaffold.

The scaffolds provide an appropriate microenvironment and facilitate good regenerative outcomes. Chitosan presents superior properties such as good biocompatibility, sustained drug release, biodegradable, and antimicrobial effects.[7] Also, it is proved that chitosan-based scaffolds significantly promoted periodontal ligament cell attachment and osteoblast-related gene expression *in vitro*.[7]

The literature is scarce regarding the use of scaffolds in management of internal resorption defects. Hence, this study aimed to evaluate the mineralization activity and to analyze the morphological characterization of the mineralized chitosan scaffold combined with calcium silicate-based cements when these scaffolded biomaterials are used in the restoration of the internal resorption cavity with perforation.

Materials and Methods

The study protocol was approved by the Institutional Ethics Committee (IEC), Ref. No.: CSP/22/MAY/110/327, and the study was conducted following the guidelines of Helsinki declaration.

Sample-size Determination

Based on previously published studies, sample size was calculated using G*Power 3.1.9.2, which indicated that 10 samples per group (total = 20 samples) would provide 80% power in determining a statistically significant difference among the efficacy of the two groups intervened. The α was set at 5%, and effect size was 1.2.

Methodology

The study was conducted at the institution Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India, from October 2022 to December 2022. Twenty permanent human maxillary incisor teeth without caries, restorations, cracks, or open apex which were extracted due to periodontal reasons, were collected and stored in saline. Using a low-speed, water-cooled diamond disc, teeth were then decoronated to obtain a standardized root length of approximately 15 mm. Standardized access cavity was prepared. The working length of the teeth was determined using K-file (Mani K files 21 mm, Prime Dental Products, India) size #10, and it was calculated 1 mm to be shorter after the initial examination of the file from the apex. Biomechanical preparation of the root canals was then carried out using ProTaper gold (DENTSPLY Maillefer, Ballaigues, Switzerland) rotary files upto F3 size, and the canal was irrigated with 3% NaOCl for 1 minute after each instrumentation.

Simulation of Internal Resorption in Teeth

Using a high-speed water-cooled size 4 round bur, approximately 5–6 mm from the coronal surface, perforating internal resorption cavities were prepared in the middle third of the roots (Fig. 1). The canal was irrigated with 5 mL of 17% EDTA to remove the smear layer and then flushed with 5 mL of normal saline solution. Root canal disinfection was done with 5 mL of 3% NaOCl and finally flushed with 5 mL of distilled water.

Cold Compaction of Gutta-percha in the Apical Region

The apical part of the root canal below the resorption cavity was filled with gutta-percha master cone size F3 (DiaDent ProT, Canada) and AH plus sealer (DENTSPLY Maillefer, Ballaigues, Switzerland). A heated plugger (Buchanan Hand Pluggers, Kerr Dental, United States) was used to condense the gutta-percha only in the apical part, and the remaining gutta percha was removed.

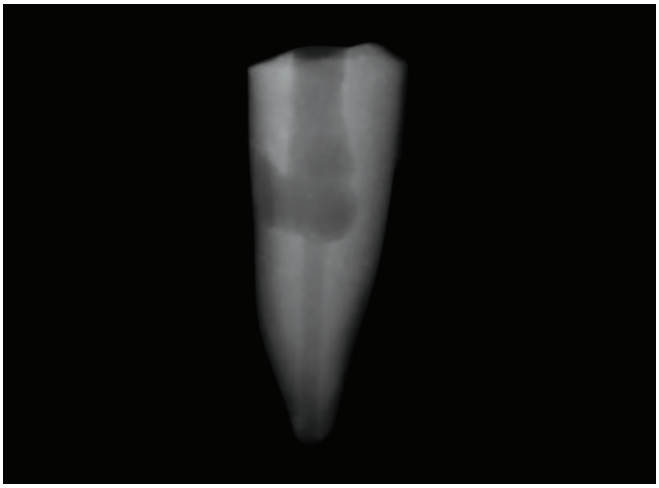


Fig. 1: Simulation of the internal resorption cavity

Groups

Twenty prepared samples were randomly distributed into two groups as 10 per group: group I – Biodentine and group II – Chitosan scaffold combined with Biodentine.

Group I

Biodentine (Septodont, USA) was manipulated according to the manufacturer's instructions. Biodentine was placed incrementally into the resorption cavity and condensed using hand pluggers until the coronal extent of resorption cavity.

Group II

Preparation of Chitosan Scaffold:

Chitosan (CHN) (Sigma-Aldrich) and gelatin (GLN) solutions (1% w/v) were prepared in 1% acetic acid. CHN/GLN solutions were mixed with 0.1% glutaraldehyde (GA) at 10:1 (v/v) ratio followed by agitation for 30 seconds. The addition of GA stabilizes the structure of the films and scaffolds by forming cross-linking between polymers. Finally, the obtained mixture was sonicated for 30 minutes to eliminate air bubbles. For 3D porous scaffold, 400 mL of the CHN/GLN solution was added into a mold and frozen at –4°C for 24 hours and then dried at –80°C for 2 days using a freeze dryer (FDU2200, Tokyo Rikakikai Co., Japan). The scaffolds were further dried in dry oven at 60°C for 3 days to remove remnant acetic acid. The obtained films and scaffolds were soaked in 70% ethanol for 24 hours and then stored in a deep freezer at –80°C after washing with ultrapure water. In such a way, chitosan scaffolds were prepared by freeze-drying method. The scaffolds were then loaded with biodentine and placed inside the simulated resorption cavity.

The samples of both the groups were then covered with a wet gauze, and placed inside the incubator at 37°C with 100% humidity for 1 hour to allow for the setting of the material. Later, backfilling of Gutta-percha was done for all the samples (Fig. 2). Then the teeth were stored in phosphate buffer saline solution. The solution was changed for every 3 days.

Mineralization activity analysis: The samples were subdivided into two groups in such a way that five samples were evaluated at the

end of 7th day, and 5 samples at the end of 14th day of each group. The weight percentage of calcium ions uptake by the samples was estimated using scanning electron microscopy–energy dispersive X-ray analysis (SEM–EDX) (ThermoFisherScientific, United States). The elemental composition was calculated on the surface of the sample without any modifications. The SEM (ThermoFisherScientific, United States) images were captured at 4000× magnification to analyze the morphological characterization of the mineralized surface.

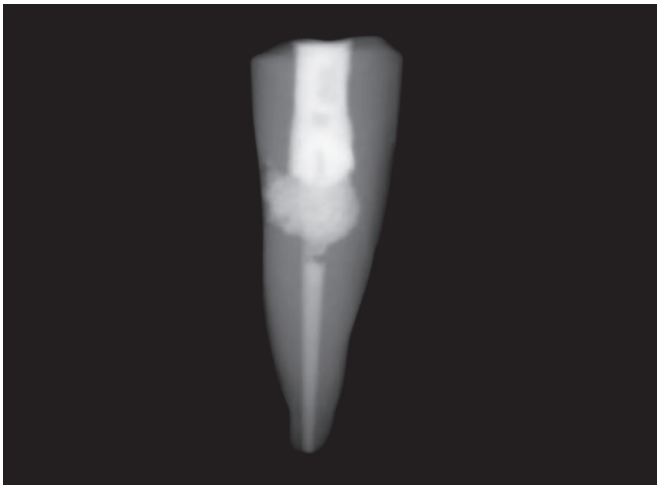


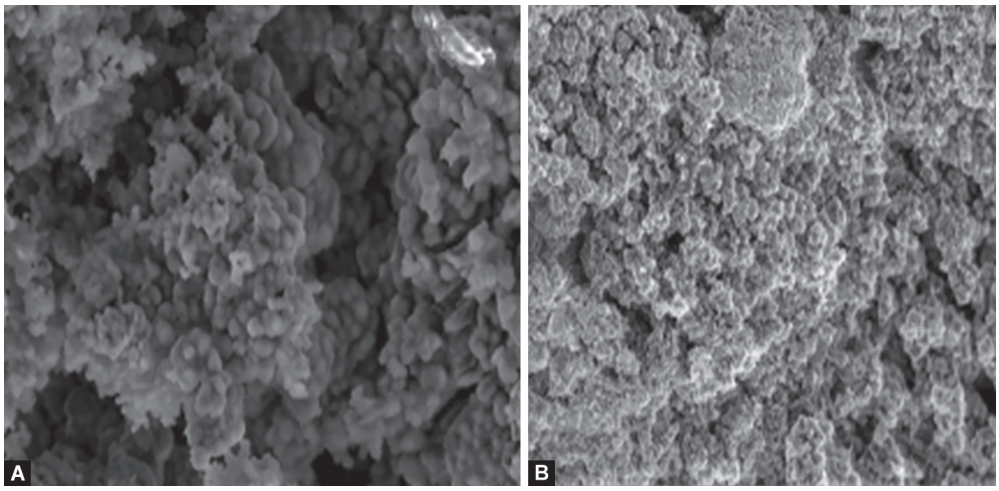
Fig. 2: Condensation of the material and backfill of gutta-percha

Statistical Analysis

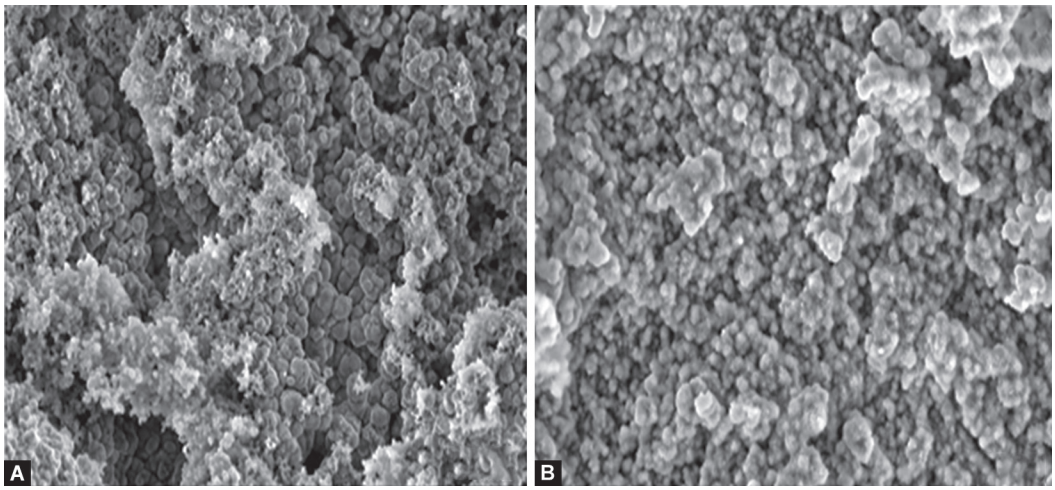
The results of the study were analyzed using SPSS software version 16.0 (IBM, India). The mean and standard deviation were used to describe the results of the experimental groups and subgroups. Mann–Whitney test was used for intergroup comparison and Wilcoxon signed-rank test was used for intragroup comparison. The mean difference was set at p-value < 0.05.

Results

The SEM image of biodentine at the end of the 7th day appeared like tangled branches of a tree, which revealed the presence of irregular apatite-like structures over the regular surface (Fig. 3A). The SEM image of the chitosan scaffold combined with biodentine at the end of the 7th day exhibited a regular but nonuniform configuration throughout the sample surface at the end of the 7th day (Fig. 3B). At the end of the 14th day, biodentine showed some irregular apatite-like structures (Fig. 4A), whereas chitosan scaffold



Figs 3A and B: SEM image at the end of the 7th day at 4000× magnification of (A) Biodentine; and (B) Chitosan scaffold with biodentine



Figs 4A and B: SEM image at the end of the 14th day at 4000× magnification of (A) Biodentine; and (B) Chitosan scaffold with biodentine

Table 1: Descriptive statistics on intergroup comparison of calcium ion uptake by the experimental groups at the end of the 7th day and 14th using Mann–Whitney test

	Group	*N*	*Mean rank*	*Sum of ranks*
Ca – 7 days	Biodentine	5	3.20	16.00
	Chitosan	5	7.80	39.00
	Total	10		
Ca – 14 days	Biodentine	5	3.80	19.00
	Chitosan	5	7.20	36.00
	Total	10		
Test statistics[b]		Ca – 7 days	Ca – 14 days	
Mann–Whitney *U*		1.000	4.000	
Wilcoxon *W*		16.000	19.000	
Z		–2.402	–1.996	
Asymp. sig. (2-tailed)		0.016	0.043	
Exact sig. [2*(1-tailed sig.)]		0.016[a]	0.043[a]	

[a]Not corrected for ties; [b]Grouping variable

Table 2: Descriptive statistics on intergroup comparison of phosphorus ion uptake by the experimental groups at the end of the 7th day and 14th using Mann–Whitney test

	Group	*N*	*Mean rank*	*Sum of ranks*
P – 7 days	Biodentine	5	5.20	26.00
	Chitosan	5	5.80	29.00
	Total	10		
P – 14 days	Biodentine	5	6.00	30.00
	Chitosan	5	5.00	25.00
	Total	10		
Test statistics[b]			P – 7 days	P – 14 days
Mann–Whitney *U*			11.000	10.000
Wilcoxon *W*			26.000	25.000
Z			–0.313	–0.522
Asymp. sig. (2-tailed)			0.754	0.602
Exact sig. [2*(1-tailed sig.)]			0.841[a]	0.690[a]

[a]Not corrected for ties; [b]Grouping variable

combined with biodentine showed a uniform regular pattern of apatite-like structures (Fig. 4B).

The mean score of weight percentage of calcium ion was found to be greater in group chitosan scaffold combined with biodentine at the end of the 7th and 14th day, and the Mann–Whitney test showed there was a statistically significant difference between the two groups ($p < 0.05$) (Table 1). The mean score of weight percentage of phosphorus ion was found to be similar for both the groups at the end of the 7th day and 14th day, and the Mann–Whitney test showed that there was no statistically significant difference between the groups ($p < 0.05$) (Table 2). Group I biodentine showed no statistically significant difference between calcium and phosphorus ion at the end of the 7th day and 14th day ($p < 0.05$) on statistical analysis by Wilcoxon signed-rank test (Table 3). Group II chitosan scaffold combined with biodentine showed a statistically significant difference between the phosphorous ion, whereas no difference in the calcium ion at the end of the 7th day and 14th day on statistical analysis using Wilcoxon-signed rank test ($p < 0.05$) (Table 4).

Table 3: Descriptive statistics on comparison of calcium and phosphorous ion within the experimental group biodentine at the end of the 7th day and 14th day using Wilcoxon-signed rank test

	Ca – 14 days Ca – 7 days	*P – 14 days P – 7 days*
Z	–1.214[a]	–0.405[b]
Asymp. sig. (2-tailed)	0.225	0.686

[a]Based on negative ranks; [b]Based on positive ranks

Table 4: Descriptive statistics on comparison of calcium and phosphorous ion within the experimental group chitosan at the end of the 7th day and 14th day using Wilcoxon-signed rank test

	Ca – 14 days Ca – 7 days	*P – 14 days P – 7 days*
Z	–0.674[a]	–2.023[b]
Asymp. sig. (2-tailed)	0.500	0.043

[a]Based on negative ranks; [b]Based on positive ranks

Inference

At the end of the 7th and 14th day, chitosan scaffold combined with biodentine showed significantly higher uptake of calcium ions than in the pure biodentine group. Also, significantly higher weight percentage of phosphorous ion was observed in the group with chitosan scaffold combined with biodentine at the end of the 14th day when compared with pure biodentine group.

Discussions

This study was conceived to provide a newer approach for the management of internal resorption defects with perforation. The standardized internal resorption cavities with perforation which simulate this condition clinically and radiographically were prepared in root samples and used for the experimental study.

In clinical situations, the teeth with active resorption may present with profuse bleeding from the granulomatous and inflamed pulpal tissues that impair visibility in the initial stages of treatment. The communication resulting from perforated resorptive defects may also be associated with mild hemorrhage when attempts are made to dry the canal after chemomechanical preparation. Furthermore, the irregularly concave nature of resorption defects makes them inaccessible to direct mechanical debridement.[8]

A hard-setting bioactive material is preferred to reinforce the thin and weakened tooth structure in the resorption site. Incorporating the benefits of a strong restorative material with bioactivity is essential for a long-term successful outcome.

Calcium silicate cements have pronounced bioactivity that provides additional prospects in the rehabilitation of resorbed teeth even if root walls are perforated. Mineral trioxide aggregate (MTA) has long been considered as gold standard material for mineralization as it is biocompatible and has superior sealing properties. It is well-tolerated in the periapical tissues, and when used as a root-end filling material in the absence of infection, it supports almost complete regeneration of the adjacent periodontium.[9,10] These are some of the desirable properties expected in the context of perforation repair because of the unintentional extrusion of the material when a perforating internal resorptive defect is repaired in an orthograde manner.[10] But the teeth treated with MTA exhibited discoloration, whereas those treated with biodentine maintained color stability.[11]

Biodentine is a calcium silicate-based cement created as dentin support with additional advantages over MTA like increased adhesion of stem cells, greater ability to produce apatite-like crystals, lesser setting time, and reduced tooth discolouration.[9–11] Moreover, biodentine has better material handling properties compared to MTA, the use of which is more time-consuming and technically difficult.[12] Biodentine has an initial setting time of less than 12 minutes and exhibits higher mechanical properties with excellent sealing ability.[13] Biodentine was found to have better physicochemical and biological properties when compared with other tricalcium silicate cements.[14] Since, biodentine has been proved to have superior properties and enhanced bioactivity when compared with MTA, biodentine was preferred for the present study.

Although these calcium silicate cements are biocompatible and bioactive, the rate of biomineralization in response to the body tissue fluids varies and defects are observed in the formed hard-tissue barrier. Tunnel defects have also been demonstrated in hard-tissue barriers generated over pulp wounds associated with both calcium hydroxide (CH) and calcium silicate-based cements. The primary difference between the two agents is that CH products are absorbable over time and become dimensionally unstable. The slow disintegration of the CH after mineralized tissue formation can allow microleakage, permitting a slow ingress of microorganisms through defects.[15]

In recent times, the use of scaffold for the regeneration of tooth structure has found to be efficient. Scaffolds are 3D porous constructs that provide a cellular microenvironment needed for tissue engineering.[16] Dental tissue engineering is expected to regenerate damaged or lost components of a tooth.[16] Bioactivity enables the scaffold to integrate into the surrounding tissues, and the biomineralization is marked by hydroxyapatite (HAP) formation on the scaffold surface.[17]

Scaffold biomaterials can be naturally derived or synthetically derived like polymers, ceramics, and composites. Various scaffolds have been investigated for dentine regeneration, and among them chitosan has exhibited better potential for biomineralization.[18] Chitosan is a natural polysaccharide derived from the exoskeleton of crustaceans. It is a deacetylated form of chitin. Chitosan is found to be biodegradable, nontoxic, and biocompatible. Chitosan is antibacterial in nature, mucoadhesive, and possesses wound-healing capacity.[19] There were only a few studies that tested the bioactivity of these scaffold biomaterials in a tooth model. Furthermore, the potential of improving the bioactivity of chitosan scaffold by incorporating mineral .trioxide aggregate was explored and found to enhance the mineralization activity.[20]

The use of a chitosan scaffold combined with biodentine might facilitate greater mineralization potential, and this combination can be used effectively in the treatment of internal resorption with perforation. There is a very evident scarcity in the literature on the use of scaffolds in the management of resorption defects. Hence, this study aimed to evaluate the efficacy of chitosan scaffold combined with biodentine in the treatment of simulated internal resorption.

There are various *in vitro* methods used to reproduce and scientifically interpret the biomineralization process of dental tissues in response to bioactive materials. One such method is the EDX, which was used to interpret the mineralization process in this study.

The tooth model was immersed in phosphate buffer saline solution for the interpretation of bioactivity. In the SEM analysis, biodentine showed some protruding irregular structures and also the apatite layer appears to be rod-like structure at the end of the 7th day (Fig. 3A). These results were found to be in accordance with the previous study done by James Ghilotti et al.[21] The surface analysis by SEM of the tooth model containing the chitosan scaffold combined with biodentine exhibited a regular but nonuniform configuration at the end of the 7th day (Fig. 3B). In this study, at the end of the 14th day, biodentine showed some irregular apatite-like structures (Fig. 4A), whereas chitosan scaffold combined with biodentine showed uniform regular apatite-like structures (Fig. 4B), which revealed the formation of HPA. The EDX spectra were analyzed at the end of the 7th day and 14th day. The biomineralization of the scaffold was appreciated in the EDX spectra by the higher percentage of elements Ca and P. On intergroup comparison, the group chitosan scaffold combined with biodentine showed a statistically significant difference in the calcium ion uptake at the end of the 7th day – 0.016 ($p < 0.05$) and at the end of the 14th day – 0.043 ($p < 0.05$). Phosphorous ion uptake was predominantly evident in the experimental group chitosan scaffold combined with biodentine after the end of the 14th day with a statistically significant value $p < 0.05$. The calcium and phosphorous ion uptake by the biodentine group showed no statistically significant difference at the end of

the 7th and 14th day. Also, few elements were found in sparse. Na, Mg, and Cl elements were found in the chitosan group whereas, in the biodentine group, trace elements of zirconia and alumina were evident. By the end of the 14th day, trace elements were found in lesser amounts, while calcium and phosphorous elements were seen predominantly. This might be due to the increase of mineralization activity and the formation of HPA that was found to be in accordance with the study done by Leonor et al. on chitosan microparticles.[22] During the initial stage of calcium and phosphate precipitation, the crystals were probably amorphous calcium phosphate.[22] As the mineralization process progresses, octacalcium phosphate (OCP) crystals were formed, which eventually transformed into HAP. The calcium phosphorous ratio obtained in the current study during the duration of 2 weeks suggested that HAP formation on the scaffold was due to the transformation from OCP. Also, the present study results were found to be in accordance with the proposition that HAP formation has intermediate phases like amorphous calcium phosphate, brushite, and OCP.[23] Impurities such as Na and Cl were formed over the mineralized tooth surface, which were found to be the products of ionic substitutions of Ca, phosphate, and hydroxide of the HAP.[24]

In this study, chitosan scaffold combined with biodentine showed enhanced bioactivity with the formation of apatite-like structures at the end of the 7th day and 14th day when immersed in mineralization solution. Thus, it can be stated that this novel method can be used in the management of internal resorption defects with perforation to reinforce the tooth structure and provide an optimum seal for a long-term successful outcome. The limitations of this study were that the oral environment cannot be simulated completely *in vitro* and the short duration of the study. Clinical trials with this chitosan scaffold combined with biodentine will help to assess the potential of this scaffold for the management of tooth resorption in a more definitive manner.

Conclusions

The efficient management of internal resorption with perforation remains a challenge. Chitosan scaffolds have showed superior properties with enhanced mineralization potential. The use of chitosan scaffolds combined with biodentine showed a statistically significant difference in the mineralization activity when compared with biodentine alone. These scaffolded biomaterials exhibit greater potential for mineralization *in vitro*, and future clinical trials would be the next step to integrate this innovative technology into everyday clinical practice.

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