Original Article

Evaluation of rise in pH and oxygen release at the site of simulated external root resorption cavities using different oxygen-releasing biomaterials: An *in vitro* study

Bollineni Swetha, Duvvuri Lakshmi Malini, T. Murali Mohan, B. Devi Priya, O. Asha Reddy[1], G. Haripriya

Department of Conservative Dentistry and Endodontics, Government Dental College and Hospital, Vijayawada, Andhra Pradesh, [1]Department of Conservative Dentistry and Endodontics, Balaji Dental College, Telangana, India

Abstract

Context: External inflammatory root resorption has rapid onset and progresses aggressively. It leads to cementum loss, which allows communication between the infected pulp and the periodontium through the denuded dentinal tubules. Primary management strategy includes adequate chemomechanical debridement and lesion arrest for which maintaining alkaline pH and aerobic conditions is essential for healing and repair of the resorption defect.

Aims: The aim of this study is to evaluate rise in pH and oxygen release at the site of simulated external root resorption cavities using different oxygen-releasing biomaterials.

Materials and Methods: In 40 extracted single-rooted teeth access opening and chemomechanical debridement were done. Cavities simulating resorption defect are prepared on the roots. The samples are divided into four groups ($n = 10$) based on the biomaterial used. After placing the biomaterial, the root apices were sealed. Half of the samples from each group were tested for oxygen release using dissolved oxygen meter and the other half for rise in pH using pH meter at 7, 14, 21, and 28 days.

Statistical Analysis: The pH values were analyzed using Friedman 2-way analysis of variance (ANOVA) and Kruskal–Wallis test. Oxygen release was measured using the two-way and repeated-measures ANOVA.

Results: Calcium peroxide group showed the highest mean pH and oxygen release than other groups at any given point of time.

Conclusions: Incorporating oxygen-releasing biomaterials such as calcium peroxide and perfluorodecalin into intracanal medicaments, such as calcium hydroxide, creates an alkaline and oxygen-enriched milieu in the periapical tissues.

Keywords: Calcium hydroxide; calcium peroxide; external root resorption; intracanal medicament; oxygen release; perfluorodecalin

Address for correspondence:
Dr. Bollineni Swetha,
Department of Conservative Dentistry and Endodontics,
Government Dental College and Hospital, Vijayawada,
Andhra Pradesh, India.
E-mail: gudimellaharipriya@gmail.com

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INTRODUCTION

Root resorption is an intricate yet intriguing pathological process that significantly impacts the structural integrity of permanent teeth. This condition, characterized by the progressive degradation of dentin and cementum, poses

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a formidable challenge in the field of dentistry. Despite the strides in dental research and practice, understanding its etiology, pathophysiology, and effective management strategies is a captivating enigma in the field of endodontics.

According to the source, root resorption can be categorized into two primary forms: internal resorption which pertains to the pulp and external resorption involving the periodontal ligament (PDL). Notably, external root resorption exhibits a higher incidence and is multifactorial in its etiology.[1] Its development necessitates the confluence of two crucial factors: mechanical damage to the PDL and cementum, or their structural loss, in conjunction with various stimulating factors, such as an infected root canal system, traumatic injury, developmental anomalies, and a range of other triggers.[2] Its rapid progression ultimately leads to tooth loss. Hence, as soon as a conclusive diagnosis is reached, it is essential to intervene and arrest its advancement.[3] The primary objective of the treatment is to eradicate the infection source, establishing a conducive environment that favors periapical healing.[4,5]

Recent studies reveal that the pH and hypoxic environment in the surrounding periodontium, created by majority of the stimulating factors, play a pivotal role in the progression of resorption by fostering osteoclastogenesis.[6,7] Human clastic cells are quite sensitive to changes in oxygen tension, with their activity accelerated by 2–4 times under hypoxic conditions. Concurrently, the presence of an acidic environment also stimulates osteoclast activity and inhibits alkaline phosphatases, further amplifying the resorption process.[8]

While the long-term usage of calcium hydroxide medicament was proven effective in the management of external inflammatory resorption,[9] this method is not without its inherent limitations. Despite the capacity of hydroxyl ions from calcium hydroxide to neutralize the acidic environment,[10] it still lacks the ability to mitigate the hypoxic environment. In addition, the treatment regimen necessitates multiple visits for changing intracanal dressings, and this, in turn, relies on the compliance and motivation of the patient.[11]

Hence, there remains a need for a more efficacious approach to expedite the healing process and facilitate the formation of hard tissue at sites affected by external root resorption.

Recently, oxygen-releasing biomaterials are gaining popularity in the field of medicine for tissue engineering, regeneration therapy, and for the treatment of ischemic tissues. These materials, by releasing oxygen at the site of application for extended durations, serve the dual purpose of reducing the risk of necrosis and promoting the process of healing.

Notable examples for such oxygen-releasing biomaterials include solid and liquid inorganic peroxides and perfluorocarbons.[12] Among these, calcium peroxide stands out as a prominent choice due to its recognized safety, biocompatibility, and ready availability among the category of inorganic peroxides. It has a long history of applications as an oxygen-releasing compound in the domain of regenerative medicine.[13] In the field of dentistry, calcium peroxide has been used for enamel bleaching and remineralization of incipient lesions.[14] The dissolution of calcium peroxide in water generates both oxygen and hydrogen peroxide, with the release rates being contingent upon factors such as temperature and pH. Specifically, higher pH and temperature levels are associated with reduced H_2O_2 production and increased O_2 release.[15]

Perfluorocarbons, characterized by their chemical and biological inertness, also have a longstanding legacy in serving as oxygen carriers in a diverse range of biomedical applications.[16] Among them, Perfluorodecalin distinguishes itself through its remarkable oxygen storage capacity by being able to dissolve substantial quantities of oxygen. Consequently, its potential applications extend to scenarios where there is a high demand for oxygen.[17,18]

Considering the limitations associated with conventional usage of calcium hydroxide medicament for treating external inflammatory root resorption (EIRR), it is time we look into better alternatives. Accumulating evidence indicates that oxygen-releasing biomaterials, as previously mentioned, have demonstrated success in the medical field for the healing of chronic and ischemic wounds, as well as tissue regeneration. Consequently, there is potential for their application in dentistry, particularly in cases such as external resorption where an elevated oxygen supply is critically needed.

The primary objective of this current study is to assess the impact on pH levels and the release of oxygen at simulated external root resorption sites on employing various oxygen-releasing biomaterials.

MATERIALS AND METHODS

Forty permanent teeth with a single root and single root canal and no previous endodontic treatment were collected. All the collected teeth were cleaned of debris and soft-tissue remnants and were disinfected by immersing in 5.25% sodium hypochlorite for 1 h. The samples were carefully rinsed in distilled water and stored in normal saline until being used. All the teeth were decoronated using a diamond disk at an equal length of 14 mm from the apex. Full-length radiographs of each tooth were taken in buccolingual and mesiodistal directions to confirm the presence of a single canal as well as the similarity of the canal shape and root dentin dimensions in all samples. To simulate EIRR lesion, a cavity with a diameter of 1.4 mm and a depth of 0.7 mm were prepared using a 1.4-mm high-speed round carbide bur on the buccal surface of each root, 5 mm from the apex. To eliminate the smear layer, the created cavities were filled with 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 1 min, followed by 3 mL of 5.25% sodium hypochlorite, and then, rinsed with distilled water.[19]

The working length was confirmed by taking a radiograph with a # 15 k-file. The canals were prepared using protaper rotary system in the sequence till F3 file. After preparation, the root canals were irrigated using 5 ml of 17% EDTA followed by 5.25% sodium hypochlorite to remove the smear layer. Distilled water was used as a final irrigant.[20] After through drying, the apical ends were sealed with sticky wax and two coats of nail varnish was applied throughout the external root surface leaving the lesion area.

The teeth were randomly categorized into four groups ($n = 10$) based on the intracanal medicament being placed. All the canals were dried using paper points before placing the medicament.

1. Control group: The first group served as a control group where no medicament was placed
2. Calcium hydroxide group: The root canals were filled with calcium hydroxide (RC Cal, Prime dental)
3. Calcium peroxide group: Calcium hydroxide combined with calcium peroxide in the ratio of 1:1 (w/w)
4. Perfluorodecalin group: Calcium hydroxide combined with perfluorodecalin in the ratio of 1:1 (w/v).

After placing the medicament, radiographs were taken to ensure proper filling of the canal with medicament. Finally, the access cavities were sealed with modeling wax and were stored in vials containing 7.5 ml normal saline solution at 37°C for the rest of the period of the study.

The pH and oxygen release were measured for each group at 24 h, 7 days, 14 days, and 21 days using digital pH meter (in a similar way as that of Nadar *et al*.[21]) and dissolved oxygen meter, respectively.

Statistical analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). pH values were analyzed using Friedman 2-way analysis of variance (ANOVA) and Kruskal–wallis test. Oxygen release was measured using the 2-way and repeated measures ANOVA. $P < 0.005$ was considered statistically significant.

RESULTS

pH

The pH values are presented in Table 1. Calcium peroxide group showed the highest mean pH values than other groups at any given point of time. While calcium hydroxide group and perfluorodecalin group showed similar pH values at 24 h, calcium hydroxide group showed significantly higher pH values than Perfluorodecalin at 7, 14, and 28 days.

Table 1: Mean pH values at various time intervals

Groups	24 h	7 days	14 days	21 days
Group 1: Control	$6.770^{a,A}$	$6.684^{a,A}$	$6.935^{a,A}$	$6.984^{a,A}$
Group 2: Calcium hydroxide	$10.716^{b,A}$	$10.664^{b,A}$	$9.57^{b,B}$	$9.190^{b,B}$
Group 3: Calcium peroxide	$11.305^{c,A}$	$11.246^{b,A}$	$11.103^{c,B}$	$11.009^{c,B}$
Group 4: Perfluorodecalin	$10.710^{b,A}$	$10.053^{a,A}$	$9.451^{d,B}$	$8.879^{d,B}$

Same lower case showed no statistically significant difference in the same column. Same upper case showed no statistically significant difference in the same row

Table 2: Mean values of oxygen release at various time intervals

Groups	24 h	7 days	14 days	21 days
Group 1: Control	$4.6^{A,a}$	$2.30^{B,a}$	$0.70^{C,a}$	$0.11^{D,a}$
Group 2: Calcium hydroxide	$5.34^{A,b}$	$2.38^{B,a}$	$1.01^{C,a}$	$0.136^{D,a}$
Group 3: Calcium peroxide	$6.92^{A,c}$	$4.16^{B,b}$	$2.08^{C,b}$	$0.61^{D,b}$
Group 4: Perfluorodecalin	$5.90^{A,d}$	$2.94^{B,a}$	$1.25^{C,a}$	$0.311^{D,a}$

Same lower case showed no statistically significant difference in the same column. Same upper case showed no statistically significant difference in the same row

Comparing within the groups, for control group did not show any significant difference at any given time interval whereas, for all the other groups, the pH values decreased over time [Figure 1].

Oxygen release

Both calcium peroxide and perfluorodecalin showed significantly higher oxygen release than the control group and calcium hydroxide group [Figure 2]. However, calcium peroxide group showed significantly higher oxygen release than Perfluorodecalin [Table 2].

DISCUSSION

The permeability of dentinal tubules is contingent on the qualitative and quantitative attributes of these tubules, which in turn depend on their spatial arrangement within dentin as well as the age of the patient.[22] These aspects about dentinal tubules are important as they effect the transfer of ions through the tubules. With consideration of these determinants, the teeth used in this study were collected from the individuals aged within 20 and 35 years, and the cavities were positioned 5 mm from the apical region. To mitigate the potential inconsistency arising from variations in radicular dentin thickness among different teeth, all samples were meticulously prepared to maintain a consistent remaining dentin thickness of 1 mm which was ensured using a radiograph. This standardized preparation methodology yielded a justified surface area suitable for the systematic examination of ion diffusion while minimizing discrepancies between the specimens. Although there is a paucity of compelling scientific evidence to support the hypothesis that the smear layer affects the diffusion

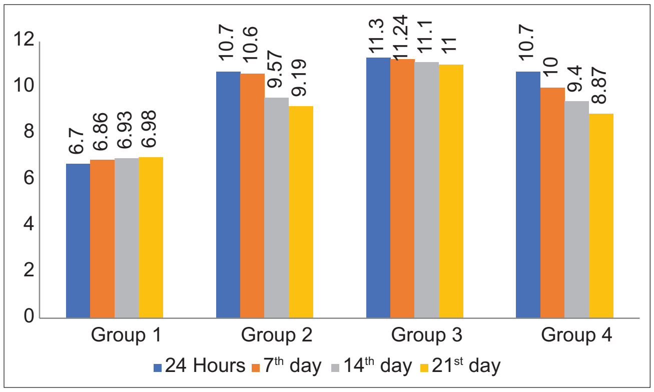

Figure 1: Mean pH values at various time intervals

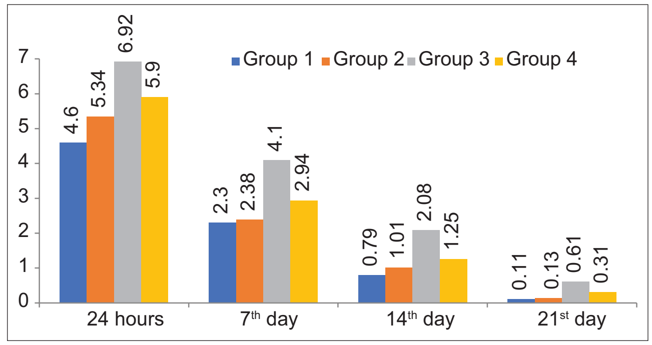

Figure 2: Comparison of oxygen release of all the groups at various time intervals

of ions through the dentinal tubules, it is noteworthy that this study methodically removed the smear layer from both the instrumented root canal walls and external cavities to promote uniformity among the samples.

Based on the results of the study, the combination of calcium hydroxide and calcium peroxide (Group 3) has shown notably a heightened release of oxygen and an elevated pH compared to combination of Perfluorodecalin and calcium hydroxide (Group 4) and only calcium hydroxide (Group 2).

Numerous studies in the literature have previously explored the effect on the pH of external root surface in EIRR due to the diffusion of hydroxyl ions through dentinal tubules. Nevertheless, investigations examining the utility of calcium hydroxide as a sealer or intracanal medicament have yielded conflicting findings. Some studies have reported an elevation in alkalinity attributable to hydroxyl ion diffusion,[23] whereas others contend that this effect is neutralized by the buffering capacity of dentinal tubules. Since the buffering influence of dentinal tubules is a universal phenomenon applicable to all medicaments, it was not treated as a confounding factor in the present study.

According to Nerwich *et al*.[24] following the placement of the intracanal medicament, ions exhibit a prompt diffusion into the inner root dentin within hours. However, their migration to the outer root dentin necessitates a span of 1–7 days, with a peak manifestation at approximately 2–3 weeks. Consequently, the present study conducted pH measurements at intervals of 1, 7, 14, and 21 days.

Our findings indicate that the group treated with a combination of calcium hydroxide and calcium peroxide as an intracanal medicament consistently exhibited highest mean pH level in comparison to the other experimental groups at any given time interval.

Upon exposure to moisture, calcium peroxide undergoes catalytic decomposition yielding calcium hydroxide and hydrogen peroxide as its byproducts. Subsequently, the hydrogen peroxide further decomposes to give water and oxygen.

$$CaO_2 + 2H_2O \rightarrow Ca\,(OH)_2 + H_2O_2$$

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

As calcium peroxide incorporated group has generated additional calcium hydroxide, it explains the highest mean of pH compared to other experimental groups in this study. Nevertheless, it is worthnoting that, within this particular group, the mean pH exhibited a decline from day 1 to day 21. This trend aligns with prior research findings and can be attributed to the formation of calcium carbonate. According to Miñana *et al*., exposure to atmospheric CO_2 may reduce the availability of hydroxyl ions through a chemical reaction, forming calcium carbonate crusts.[25]

In addition to the investigation of hydroxyl ion release, several prior studies have explored calcium ion release at sites associated with EIRR as calcium ions were also important for hard tissue formation.[26] Nevertheless, to the best of our knowledge, our study is pioneering in its emphasis on the significance of oxygen at EIRR sites for the promotion of healing and hard tissue formation, as well as the utilization of various oxygen-releasing biomaterials to achieve this objective.

Furthermore, apart from its impact on pH, the application of calcium peroxide also yielded increased oxygen release when compared to the other experimental groups. Being an inorganic peroxide, calcium peroxide readily dissolves in water releasing oxygen and calcium ions, thereby elucidating the highest mean oxygen levels observed. Conversely, Perfluorodecalin, an organic compound with all hydrogen atoms replaced by fluorine atoms, possessed the capacity to release oxygen, but its efficacy in terms of oxygen and hydroxyl ion release was less pronounced due to the presence of strong intramolecular covalent bonds and hydrophobic characteristics when compared to peroxides.[27]

While this study meticulously aimed to replicate *in vivo* conditions, it is important to acknowledge that, in the *in vivo* context, the apex and lateral canals play a significant role in establishing communication between the root canal and periodontium, which can exert influence over the pH of the surrounding tissues. In addition, factors such as pH, temperature, and partial pressure of oxygen in the local environment may also impact oxygen release.[28] Consequently, we recommend that future *in vitro* investigations strive to closely mimic *in vivo* conditions to further enhance the applicability of their findings.

CONCLUSIONS

To summarize, to the best knowledge of the authors, this study constitutes the pioneering investigation to assess the impact of various oxygen-releasing biomaterials on both pH and oxygen release within simulated external root resorption cavities. The study's findings indicate the potential benefits of incorporating calcium peroxide into intracanal medicaments, such as calcium hydroxide, as this collaborative combination appears to foster an alkaline and oxygen-enriched milieu in the periapical tissues. Such an environment has the capacity to facilitate the healing process and promote hard tissue formation by inhibiting osteoclast activity.

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Conflicts of interest
There are no conflicts of interest.

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