

Bioactive Glass S53P4 versus Chlorhexidine Gluconate as Intracanal Medicament in Primary Teeth: An *In-vivo* Study Using Polymerase Chain Reaction Analysis

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Abstract:

Background: Bacteria have long been recognized as the primary etiology for pulpal and periapical lesions, which necessitates the elimination of bacteria from the root canal system. In primary teeth, irrigation and debridement is the main protocol required to disinfect the canal. Biomechanical preparation cannot be vigorously done on the primary teeth due to anatomical barrier such as thin and flared roots. This calls for the use of an effective intracanal medication that will assist disinfection of root canal system. Aim of the study was to examine the *in-vivo* susceptibility of root canal bacteria to chlorhexidine (CHX) gluconate-1% gel and bioactive glass (BAG) S53P4 when used as intracanal medicaments using polymerase chain reaction (PCR).

Methodology: PCR (analysis used oligonucleotide primers of *Escherichia coli*) was used to detect and compare the microbial load reduction after medication of 14 teeth for a week with either CHX gel - 1% or BAG S53P4. The pre and post microbial load was checked in the form of colony forming units. When analysis was done, a statistically significant difference was observed between the two groups.

Results: The study revealed that both medicaments caused a considerable amount of microbial load reduction. BAG S53P4 caused much more reduction than CHX 1% gel. Statistical analysis showed a significant difference between the two groups.

Conclusion: BAG S53P4 has superior antibacterial property as compared to CHX 1% gel.

Key Words: Bioactive glass S53P4, chlorhexidine - 1% gel, polymerase chain reaction

Introduction

One of the crucial points in endodontic therapy is to disinfect root canal before root filling¹ because of the role of bacteria and their by-products in both the initiation and perpetuation of pulpal and periapical disease. The majority of bacteria found in the root canal microflora may be simply removed by the mechanical action of endodontic instruments. Nevertheless, because of the anatomical complexities of primary root canals, organic residues and bacteria located in the dentinal tubules cannot be sufficiently cleaned even after meticulous mechanical procedures.²

The irrigants used during the endodontic procedures have a role in eliminating these bacteria and their by-products up to a certain extent, i.e., although chemomechanical preparation has an important cleaning effect, it cannot eliminate all the bacteria from the root canal system. The remaining bacteria may multiply during the period between appointments, often reaching the same level that it was at the start of the previous session, in cases where the canal is not dressed with a disinfectant between visits. This calls for the use of an effective intracanal medication that will assist disinfection of the root canal system.³ Calcium hydroxide has been considered the "gold standard" as an intracanal medicament, but now it has been proved that this material is not equally effective against all the bacteria.⁴ The various medicaments compared in various *in-vitro* studies are active point (medicated gutta-percha with chlorhexidine (CHX) diacetate), calcium hydroxide plus point (medicated gutta-percha with calcium hydroxide), calcium hydroxide, 1% CHX gel, bioactive glass (BAG) (S53P4),⁵ camphorated paramonochlorophenol, camphorated phenol.⁶ Thus, the present study was undertaken to compare the *in-vivo* efficiency of CHX gluconate gel and BAG S53P4 using polymerase chain reaction (PCR) using oligonucleotide primers of *Escherichia coli* for detection of microbial load in the root canal.

Methodology (Figures 1-3)

The study group consisted of 14 patients requiring pulpectomy in a primary second molar. The ethical clearance was taken from the Ethical Committee of Our Institute. The selected teeth were asymptomatic, showed no response to sensitivity testing and were non-vital. Standard access cavity preparations were made in all the 14 teeth and then samples (pre-medicament) were collected with the help of absorbent



Figure 1: Teeth specimen.



Figure 2: Bioactive glass S53P4.



Figure 3: Polymerase chain reaction machine.

points. Consecutively, 4 absorbent points were placed in the canal for a minute each. The absorbent points were then placed in the vial containing TE buffer (transport media) and were sent for the PCR analysis (hot start PCR). All the teeth were then instrumented after working length determination. Instrumentation was done with K-file system and 2.5% NaOCl. Then the teeth were medicated for 1 week. The 14 teeth were randomly divided into 2 groups. Group 1 consisted of 7 teeth which were treated with 1% CHX gel for a week. Hexigel was placed in the canals with absorbent points. Group 2 consisted

of 7 teeth which were treated with BAG S53P4. BAG S53P4 was mixed with saline and then placed in the canals with absorbent points. BAG S53P4 is marketed in the particle size of 70-710 μm which is not ideal to be used as a medicament. Hence, the crystalline powder was transferred into a sterile glass mortar and pestle, crushed into smaller size and then passed through a sieve having mesh size of 45 μm . The final particle size was 45 μm , suitable to be used as a medicament.

After 1 week the patients were recalled, the teeth reopened, the medicament was removed, followed by saline irrigation. This was followed by post medicament sampling. This was done in the same way as the pre-medicament sample was collected and sent for the PCR analysis. The teeth were then obturated with Zinc-Oxide Eugenol. The analysis used oligonucleotide primers of *E. coli* for detection of microbial load in the root canal. The evaluation of pre- and post-medicament microbial load in the form of colony forming units and the percentage reduction in each of the 14 teeth was carried out. Statistical analysis was done for comparison between the two groups using Mann-Whitney test and $P < 0.01$ was considered as statistical significant values.

Results

The microbial load in the post medicament sample after treatment with CHX gel in Group 1 (CHX 1% gel) was considerably reduced by 82.17% (Table 1) and in Group 2 (BAG S53P4) was reduced by 93.5% after 1 week (Table 2) (Graph 1). Statistical analysis showed a statistical significant difference between the two groups ($P < 0.01$).

Discussion

Bacteria and their by-products play an essential role in the initiation and perpetuation of pulpal and periapical diseases.³ The successful outcome of endodontic treatment depends on root canal disinfection.⁵ Although chemomechanical preparation has an important cleaning effect, it cannot eliminate all the bacteria from the root canal system. These remaining bacteria grow and multiply within the root canal and can reach the same level that was present before the commencement of treatment if no antibacterial dressing is given between the endodontic visits.³ Thus, intracanal medication is a valuable adjunct to chemomechanical preparation in the disinfection of the root canal system, reducing the endodontic microbiota, and therefore favoring periapical tissue repair. Among the various intracanal medicaments calcium hydroxide ($\text{Ca}[\text{OH}]_2$) has been considered the gold standard because of its consistent antibacterial activity.⁴ However, Kim and Kim,⁷ carried out a literature review and concluded that $\text{Ca}(\text{OH})_2$ has a wide range of antimicrobial effects against common endodontic pathogens, but it is less effective against *Enterococcus faecalis* and *Candida albicans*. The addition of vehicles or other agents might contribute to the antimicrobial effect of $\text{Ca}(\text{OH})_2$. Gomes *et al.*⁸ also in an *in-vitro* study demonstrated that $\text{Ca}(\text{OH})_2$ is not effective against all bacterial species found in

Table 1: Group 1 - CHX 1% gel.

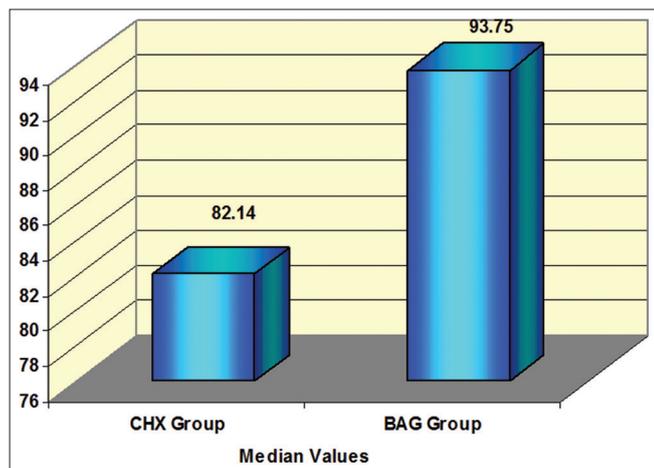
Sample	Colony forming units		% reduction
	Before	After	
1	180000000	30000000	83.33
2	510000000	120000000	76.47
3	19000000	3600000	81.05
4	410000000	79000000	80.73
5	5200000	720000	86.15
6	140000000	25000000	82.14
7	1000000000	170000000	83
Mean	323457143	61188571	81.84
Median	180000000	30000000	82.14

CHX: Chlorhexidine

Table 2: Group 2 - BAG S53P4.

Sample	Colony forming units		% reduction
	Before	After	
1	44000	3000	93.18
2	27000	2800	89.63
3	350000	21000	94
4	15000	1100	92.67
5	300000	13000	95.67
6	32000	1900	94.06
7	40000	2500	93.75
Mean	115428.6	6471.429	93.28
Median	40000	2800	93.75

BAG: Bioactive glass



Graph 1: Comparison of microbial load between the chlorhexidine and bioactive glass groups.

root canals Zehnder *et al.*⁴ demonstrated the effect of aqueous calcium hydroxide and BAG S53P4 (BAG) powder suspension on standardized bovine dentin blocks infected with *E. faecalis* and concluded that calcium hydroxide was ineffective, but BAG suspension eliminated the infection in the sampled dentin layers after 5 days. Prabhakar and Kumar⁹ carried a study to compare the effect of enamel and dentin powder on the antibacterial efficacy of a commercially available BAG and concluded that among the various materials evaluated, though BAG exhibits antimicrobial efficacy, the addition of powdered enamel and dentin in aqueous suspension definitely enhanced this property.

Stoor *et al.*¹⁰ carried a study to evaluate the effect of BAG S53P4 on the oral microorganisms *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Actinomyces naeslundii*, *Streptococcus mutans*, and *Streptococcus sanguis* and concluded that BAG S53P4 shows a broad antimicrobial effect on microorganisms. There are a number of *in-vitro* studies showing the antibacterial effect of BAGs, but there was no research found about the *in-vivo* antibacterial effect of BAG (BAG S53P4) so far.

The present study was carried to examine the *in-vivo* efficacy of CHX gluconate gel and BAG (BAG S53P4) using PCR analysis. The analysis used oligonucleotide primers of *E. coli* for detection of microbial load in the root canal and found BAG as more effective for microbial load reduction as compared to CHX gluconate gel. *E. faecalis* was selected as test microorganisms because it shows resistance to elimination from root canal and is also associated with etiopathogenesis of persistent apical periodontitis.¹¹

On the contrary to the present study, Krithikadatta *et al.*¹² evaluated the antibacterial efficacy of the four medicaments (2% CHX gel, 2% metronidazole gel, BAG (S53P4) in comparison with calcium hydroxide) against *E. faecalis* in an *in-vitro* study using extracted premolar teeth and concluded that 2% CHX gel was more effective as compared to other medicaments. Atilla-Pektas *et al.*⁵ compared the antimicrobial activities of active point (medicated gutta-percha with CHX diacetate), calcium hydroxide plus point (medicated gutta-percha with calcium hydroxide), calcium hydroxide, 1% CHX gel, and BAG (S53P4) against *E. faecalis* and *S. mutans* in an *in-vitro* study and found that the medicaments containing CHX were effective against both *E. faecalis* and *S. mutans*.

CHX gluconate in the form of a salt i.e., gluconate, acetate or hydrochlorate has been used since the 1950's at different concentrations as an oral antiseptic in the form of a mouthwash, subgingival irrigant, gel, toothpaste, and chewing gum.¹³ It is a cationic bisbiguanide that seems to act by adsorbing onto the cell wall of the microorganisms and causing leakage of intracellular components. The positive CHX molecule interacts with negative phosphate group in the inner cell membrane of the bacteria. At low concentrations of CHX, small molecular weight substances will leak out, resulting in bacteriostatic effect. At higher concentrations, it has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein crosslinking.¹⁴ The optimal antimicrobial action of CHX ranges from pH 5.5 to 7. It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria, bacterial spores, lipophilic virus, yeasts, and dermatophytes. Furthermore, CHX adsorbs to surfaces covered with acidic proteins, such as hydroxyapatite and is gradually released in the form of an active cation

(substantivity), justifying its use as a root canal irrigant and medicament *in-vitro* and *in-vivo*.¹³ In the present study, gel form was preferred because the gel formulation contacts well with the canal wall due its viscosity and thus, the time of contact is increased and the availability of CHX for its antibacterial activity is constantly present.¹⁵

BAG S53P4 was invented by Dr. Hench in 1969 and since then it has been used to treat a variety of medical conditions.¹⁶ The term BAG refers to the ability of these materials to allow hard and soft tissues to directly bond to their surface. BAG is basically a 4 component system of oxides containing SiO₂, Na₂O, CaO, and P₂O₅.⁴ It has been shown in various studies that the BAG paste appears to possess a broad antibacterial effect on microorganisms of both supra and subgingival plaque. Consequently, the BAG paste will have a beneficial effect on oral health from both a cariologic and periodontal point of view.¹⁷ The antimicrobial potential of BAG is largely a function of their ability to raise the pH in aqueous suspension. These high pH levels are not well tolerated by either bacterial or host cells. The other mechanism is that the dentin powder apparently triggers an increased dissolution of BAG particles and due to its complex surface, dentin powder acts as a recipient for ions in solution and thus acts as a catalyst for the dissolution of glass in aqueous suspension. The ionic flow between glass and dentin powder appears to interfere with bacterial viability. Increased silica dissolution from the glass takes place and this suggests that Si exerts an indirect effect by promoting Ca and P precipitations which interferes with the cellular integrity of bacteria. As SiO₂ only dissolve in a high pH environment BAG appears to be an ideal slow release system for these ions because the NaOH dissolved from the glass is highly alkaline. Silica acts as a surfactant at solid - liquid interfaces, and may thus directly inhibit the bacterial viability.⁴

In the present study, BAGS53P4 showed better microbial (*E. coli*) load reduction (93.75%) as compared to CHX 1% gel (82.14%). The probable reasons for less efficiency of CHX as compared to BAG may be due to inhibition of CHX activity by organic part of dentin - Type 1 collagen, acid proteins, glycoproteins, periapical exudate, and dead microbial cells present in the dentinal tubules¹⁸ whereas the action of BAG is enhanced in the presence of increased pH, dentin, and silica.¹⁹

Conclusion

The present study concludes that CHX gel 1% and BAGS53P4 have good antibacterial activity and BAGS53P4 has been proven to be a better intracanal medicament, but it has limitations of being a costly material.

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