Comparative evaluation of substantivity of two biguanides - 0.2% polyhexanide and 2% chlorhexidine on human dentin

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Abstract

Background: Substantivity is one of the desirable characteristics of root canal irrigants. Among conventional endodontic irrigants, only chlorhexidine (CHX) is known to exert significant substantivity on root canal dentin. This study explored the substantivity activity of a polymeric biguanide-poly hexamethylene biguanide (PHMB).

Aim: The aim of this study was to determine the substantivity of a polymeric biguanide - 0.2% PHMB and compare it with that of 2% CHX.

Materials and Methods: To evaluate and compare the substantivity of 0.2% PHMB and 2% CHX on root canal dentin, dentin disks were prepared and substantivity after 1 h, 24 h, 7 days and 21 days was measured using spectrophotometry.

Statistical Analysis Used: The data so obtained were analyzed, and the intergroup comparison was made using unpaired t-test.

Results: The results of this study indicated that 0.2% PHMB exerts significantly greater substantivity than 2% CHX on human dentin.

Conclusion: Within the limitations, this study supports the use of 0.2% polyhexanide as an endodontic irrigant based on its property of substantivity.

Keywords: Chlorhexidine; endodontic irrigants; poly hexamethylene biguanide; polyhexanide; substantivity

INTRODUCTION

Micro-organisms are the known cause of root canal infections.[1-4] The role of chemo-mechanical preparation in eradicating microflora from infected root canal space and establish a sterile condition has been emphasized on.[5-10] However, to date, by no means, bacteria can be completely eliminated from the root canal system. All our methods of chemomechanical preparation aim to lessen the microbial load as much as possible.

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In these circumstances, it seems logical to incorporate a long-acting antimicrobial agent in endodontic therapy to eliminate the residual bacteria and/or to counteract the bacterial leakage and thus prevent reinfection. Substantivity refers to the prolonged association between the material and a substrate. It is not just a simple deposition of the material on the substrate.

Among intracanal medicaments, the gold standard is calcium hydroxide (Ca(OH)₂). Its antimicrobial activity is linked to the hydroxyl ions release under aqueous conditions. These hydroxyl ions are powerful antioxidants and mediate bactericidal effect via damage to cytoplasmic membranes, proteins, and bacterial DNA. Ca(OH)₂ is bactericidal against a wide spectrum of endodontic pathogens but has relatively weak activity against Enterococcus faecalis (E. faecalis). Ballal et al. showed that the antimicrobial potential of Ca(OH)₂ against E. faecalis reduces rapidly as time elapses, possibly due to dilution of the chemical.

Amongst conventional root canal irrigants, chlorhexidine (CHX) is known to exert a residual antibacterial effect on root canal dentin for up to 3 weeks. The aim of this study was to determine the substantivity of a polymeric biguanide-0.2% poly hexamethylene biguanide (PHMB) and compare it with that of 2% CHX.

MATERIALS AND METHODS

Preparation of test specimens
Eighty dentin disks, of 1 mm [Figure 1a] were obtained by decoronation of the crown and required apical resection of the selected teeth using a diamond disk, at slow speed (<100 rpm) with water coolant. To ensure standardization, dentin disk thickness was measured by means of a vernier caliper (GDC, India) [Figure 1b], and pulpal lumen of 1 mm diameter was obtained using an ISO #FG 57 straight fissure bur (SS white, USA).

The dentin disks were serially treated in an ultrasonic bath with each of the following solutions for 4 min to eliminate smear layer-5% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid liquid. Following this, the dentin disks were autoclaved at 121°C for 15 min at 15 psi above atmospheric pressure. Thereafter, the dentin disks were randomly assigned to two equal groups (n = 40) on the basis of irrigant used. The irrigation solution for the two groups consisted of 0.2% PHMB solution and 2% CHX (Prevest Denpro Limited, India), respectively.

Evaluation of substantivity of 0.2% PHMB and its comparison with 2% CHX:

The dentin disks were treated as follows:

Group I (n = 40) – Treatment with 0.2% PHMB

Group II (n = 40) – Treatment with 2% CHX.

A fresh solution of 0.2% PHMB was prepared in deionized double distilled water using 98% w/v of PHMB in powdered crystalline form (Sinobio chemistry Ltd., China). The dentin disks were treated with 10 µl of 0.2% PHMB and 2% CHX for 20 s in groups one and two, respectively. The treated dentin disks were moved to 2 ml centrifuge tubes containing 1 ml of PBS solution. Thereafter, the tubes were incubated at 37°C. After each test period, centrifugation was done for 2 min. One ml of Phosphate buffered saline solution was then withdrawn from the tube using a micropipette and subjected to spectrophotometry. The spectrophotometric analysis (BioMate 3, Thermo Fisher Scientific, India) was carried out at 260 nm for CHX and 598 nm for PHMB.

The concentrations of the irrigants were determined based on UV-absorbances versus test irrigants, on a standard curve. The substantivity of 2% CHX or 0.2% PHMB to dentin was expressed in percentage based on their applied dose.

The values were submitted to statistical analysis with unpaired Student’s t-test to find which material differed from the others at different intervals.

![Figure 1](image1.png)

**Figure 1:** (a) Prepared dentin disk specimen. (b) Measurement of dentin disk using Vernier caliper

![Figure 2](image2.png)

**Figure 2:** Bar diagram comparing substantivity of 2% chlorhexidine and 0.2% poly hexamethylene biguanide
RESULTS

The following observations were made from the descriptive statistics represented in Table 1 and Figure 2.

The mean substantivity for 0.2% PHMB at incubation period of 1 h was (73.73 ± 1.41), at 24 h was (76.32 ± 2.78), at 7 days was (77.74 ± 1.33) and at 21 days was (69.98 ± 6.89).

The mean substantivity for 2% CHX at incubation period of 1 h was (67 ± 1.88), 24 h was (67.06 ± 3.11), 7 days was (66.51 ± 1.95), and at 21 days was (57.4 ± 5.45).

Intergroup comparison using an unpaired t-test [Table 2] indicated a significant difference in the substantivity values of 0.2% PHMB and 2% CHX at all the incubation periods. The substantivity of PHMB was statistically superior to CHX at all the incubation periods. The results are represented in Table 1.

The results displayed higher substantivity for 0.2% PHMB than 2% CHX at all the time intervals.

DISCUSSION

Biguanides include bis (biguanides) and polymeric biguanides. CHX and alexidine are known bis (biguanides) and have been shown to possess many desirable properties of an endodontic irrigant. Both have antimicrobial properties and exert a substantive residual activity on dentin.[29,30]

PHMB is a polymeric biguanide which was first synthesized by Rose and Swain in 1954.[31] PHMB is a cation with a backbone of polymeric biguanide units. It can be presented as \([-(CH_2)_6.NH.C(=NH).NH.C(=NH).NH-N] \) (n = 2–40; average = 11).[32] PHMB exists as a colorless, odorless solid/powder of >94.2% purity. It is neither corrosive nor irritating. It is highly water-soluble and stable.[33]

PHMB is a safe biocide and is not detrimental to environmental and public health.[34] It has found several antimicrobial applications in medicine, dentistry, and environmental sciences.[35,36] However, its utility in endodontic disinfection has seldom been explored. This study compared the substantivity of two biguanides-CHX and PHMB.

In this study, dentin disks were subjected to treatment with either 0.2% PHMB or 2% CHX for 20 s. This was followed by substantivity determination at four different incubation periods.

Different approaches have been used in dental literature to determine the substantivity of dental materials, including UV spectrophotometry,[32] liquid chromatography,[33] and microbiological assays.[34] In this study, dentin disks were prepared, and UV spectrophotometry was utilized to determine substantivity. Previous studies have successfully utilized this method for the determination of substantivity.[35,36]

Souza et al.[33] determined in a clinical assay using reverse-phase high-performance liquid chromatography, the retention of antimicrobial activity of 2% CHX formulations (gel and liquid) and QMix on root canal dentin. The observations were made at different intervals of time. It was found that all three test agents were retentive after 120 days. After 24 h evaluation period, CHX gel had significantly better substantivity than CHX liquid. However, they did not perform statistically different later. On the contrary, the substantivity of Qmix was significantly less than the two CHX formulations at all the time intervals tested in this study.

Komorowski et al.[57] evaluated the substantivity of CHX against E. faecalis. 0.2% CHX was compared with 2.5% NaOCl and sterile saline. The observations were made up to 21 days. Results indicated that 7 days treatment with CHX was associated with significantly low bacterial colonization of root dentin.

Rosenthal et al.[55] used a similar methodology to evaluate the substantivity of CHX on bovine root canal dentin. The evaluation periods included 1 day, 3 weeks, 6 weeks, and 12 weeks. CHX extracts were associated with all the time intervals tested with the highest values after 1 day and lowest values at 12 weeks.

In a semiquantitative essay employing measurement of zones of inhibition, White et al.[36] found that both 2.0% and 0.12% CHX treated teeth were associated with persisting antimicrobial activity. However, the values for 2% CHX were significantly more than in 0.2% group. In view of the substantivity, CHX was found to be significantly more than the two CHX formulations at all the time intervals tested in this study.

Table 1: Mean and standard deviation of substantivity of 2% chlorhexidine and 0.2% poly hexamethylene biguanide at different time intervals

| Time interval | 1 h       | 24 h      | 168 h/7 days | 21 days   |
|---------------|-----------|-----------|--------------|-----------|
| PHMB          | 73.73     | 76.32     | 77.74        | 69.98     |
| SD            | 1.41      | 2.78      | 1.33         | 6.89      |
| CHX mean      | 67        | 67.06     | 66.51        | 57.4      |
| SD            | 1.88      | 3.11      | 1.95         | 5.45      |

Table 2: Intergroup comparison using Student’s t-test

| Time interval | 1 h       | 24 h      | 168 h/7 days | 21 days   |
|---------------|-----------|-----------|--------------|-----------|
| CHX versus PHMB | 0.000    | 0.000     | 0.000        | 0.000     |

PHMB: Poly hexamethylene biguanide, CHX: Chlorhexidine
to 72 h, the authors suggested that CHX could be used as a potential replacement to NaOCl for endodontic irrigation.

Mahendra et al.\textsuperscript{[9]} conducted a microbiological study to compare the antimicrobial substantivity of varying concentrations of CHX as root canal irrigant. Through observation of time periods of 12 h, 1 day, 2 days, and 3 days, antibacterial substantivity of varying concentrations of CHX were rated in the following order 2% > 1% > 0.1%.

To the extent of the author’s belief, none of the studies published till dates have compared the substantivity of 0.2% PHMB and 2% CHX.

In this study, both the cationic biguanides displayed a strong affinity toward anionic dentin. PHMB was significantly better than CHX at all the test intervals. The exact reason for this difference is not known. However, this could be attributed to the polymeric existence of PHMB.

**CONCLUSION**

Within the constraints of this study, it was concluded that 0.2% PHMB solution shows a statistically higher substantivity than 2% CHX at all the incubation periods. 0.2% PHMB solution could be used as an alternative to CHX in root canal irrigation.

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**Conflicts of interest**

There are no conflicts of interest.

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