Analysis of Different Concentrations of Chlorhexidine for Their Substantivity Antimicrobial Activity to Dentin Discs
Nishu Vakil1*, Kimpreet Kaur2, Abhishek Singh3, Balbir Kaur4

1Dental Surgeon, Department of Periodontology, Indira Gandhi Government Dental College, Jammu
2Consultant Periodontist, 3Associate Professor, Department of Community Medicine, SHKM, Government Medical College, Mewat, Nalhar, Haryana 122107, India
4Professor and Head, Department of Forensic Medicine, NMCTH, Biratnagar, Nepal

*Corresponding author: Dr. Nishu Vakil
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Abstract

**Background:** To better simulate and tap the potential clinical application of Chlorhexidine, it seems necessary to evaluate the concentration of Chlorhexidine, which can be clinically used in dental practice. **Aim:** To assess the substantivity of of different medicaments in chlorhexidine-treated human root dentin. **Methods:** This prospective study was conducted on forty five patients. They were divided into three groups. Group I specimens were then treated with 10 microliters of 0.02% Chlorhexidine, Group II specimens with 10 microliters of 0.2% Chlorhexidine, and Group III specimens were treated with 10 microliters of 2% Chlorhexidine. They were then incubated in 1 ml of Phosphate buffer ed saline. The substantivity was evaluated after 24 hours and one week of incubation. **Results:** Highest substantivity was seen for Group II at 24 hours (84%) as compared to at one week (83.9%). Substantivity in Group I was a little lower than that seen in Group II. Substantivity was seen for Group I at 24 hours (83.1%) as compared to at one week (82.8%). Highest substantivity was observed for Group II then Group I, as compared to Group C; with both groups performing significantly better than Group C. **Conclusion:** On the basis of findings of this study, it can be stated that 0.02% and 0.2% Chlorhexidine can be used in dental practice to enhance the stability of a dentin-adhesive interface. Further larger controlled trials are warranted to support our findings.

**Keywords:** Chlorhexidine, substantivity, dentin discs, different concentrations.

**INTRODUCTION**

There must be therapeutic measures available effectively preventing the reinfection and potentially improving the outcome of endodontic treatment. Chlorhexidine gluconate is a broad-spectrum antibacterial agent [1, 2] whose antimicrobial efficacy equals that of the conventional root canal irrigants and medicaments [3, 4]. Unlike the conventional medicaments, the positively charged molecules of Chlorhexidine can adsorb onto the dentin and prevent microbial colonization on the dentin surface for some time.

A number of studies have demonstrated Chlorhexidine has a substantial role in stabilizing the organic matrix of the resin-dentin bond and the preservation of the resin-dentin bond [5]. The efficiency of Chlorhexidine in bonding is attributed to its property of substantivity, which ensures its release and availability at the site of delivery, for a considerable period of time [6]. Substantivity of Chlorhexidine helps in the formation of a more stable hybrid layer, thus contributing to the success of bonded restorations [7].

To better simulate and tap the potential clinical application of Chlorhexidine, it seems necessary to evaluate the concentration of Chlorhexidine, which can be clinically used in dental practice. Thus, this study was planned to assess the substantivity of different medicaments in chlorhexidine-treated human root dentin.

**METHODS**

The study was conducted at a tertiary care teaching dental hospital of northern India. In this prospective study forty five extracted non-carious mandibular third molars were collected after the informed consent of the patients and were stored in 0.9% sodium chloride containing 0.02% sodium azide at 4°C for 20 days. Roots and pulp tissue were removed after the removal of the debris. The enamel and cementum was removed using diamond points and water as a coolant. A diamond saw was used to make
dentin disks from the remaining tooth portions. The disk size was 5 mm in diameter and 2 mm in thickness. The disks were dried and then immersed in distilled water, at 37°C, for one week, and their wet mass was recorded. The part of the disk that was initially in contact with the pulp chamber was covered with two layers of nail varnish and dried. The dry mass was also recorded. All the disks were partially demineralized using etching liquid, 37% phosphoric acid, for 15 seconds and then washing it off with distilled water for 60 seconds.

Three groups were formed.

Group I: Disks were treated with 10 ml of 2% Chlorhexidine
Group II: Disks were treated with 10 ml of 0.2% Chlorhexidine
Group III: Disks were treated with 10 ml of 0.02% Chlorhexidine

The disks were then transferred to 2 ml plastic centrifuge tubes having 1 ml of Phosphate buffered saline, and incubated at 37°C. One ml of Phosphate buffered saline solution was taken from each tube and analysis was done after one hour, 24 hours, and one week of incubation, to assess the concentration of Chlorhexidine in the solution.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

RESULTS

Highest substantivity was seen for Group II at 24 hours (84%) as compared to at one week (83.9%). Substantivity in Group I was a little lower than that seen in Group II. Substantivity was seen for Group I at 24 hours (83.1%) as compared to at one week (82.8%). Highest substantivity was observed for Group II then Group I, as compared to Group C; with both groups performing significantly better than Group C (Table-1).

Table-1: Concentrations of chlorhexidine that remains bound at various time intervals

<table>
<thead>
<tr>
<th>Study group</th>
<th>Concentration</th>
<th>At 24 hours</th>
<th>At 1 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.02% Chlorhexidine</td>
<td>83.1</td>
<td>82.8</td>
</tr>
<tr>
<td>Group II</td>
<td>0.2% Chlorhexidine</td>
<td>84.0</td>
<td>83.9</td>
</tr>
<tr>
<td>Group III</td>
<td>2% Chlorhexidine</td>
<td>70.6</td>
<td>68.5</td>
</tr>
</tbody>
</table>

Fig-1: Bar chart showing concentrations of chlorhexidine that remains bound at various time intervals

DISCUSSION

Chlorhexidine gluconate is a bisbiguanide and has been used in the control of periodontal disease since long [8]. Due to its broad spectrum antimicrobial activity and specific effectiveness against resistant bacteria such as Enterococcus faecalis, it has been used in the management of chronic endodontic infections as well [9].

Among the wide horizon of usage of this molecule, one interesting application in adhesive dentistry is the role of Chlorhexidine in stabilizing the organic matrix of the resin-dentin bond. Studies have proven that Chlorhexidine has a substantial role in the preservation of the resin-dentin bond. Komorowski et al., [10] used 0.2% Chlorhexidine solution for 7 days. Heling et al., [11] used a biodegradable controlled-release device for the delivery of Chlorhexidine for 7 days, whereas Lenet et al., [12] used both a nondegradable controlled-release device and a gel vehicle for 7 days. The root canals of bovine teeth are considerably larger than those of human teeth; therefore, the volume of Chlorhexidine available to interact with the dentin is relatively large.
Clinically, when Chlorhexidine is applied after acid etching on the prepared tooth surface, it is not washed off, and the dentin bonding agent is applied and the procedure is continued. Chlorhexidine has the potential to bind to both organic and inorganic components of the dentin [12]. The substantivity of Chlorhexidine is related to its bond with both the inorganic and organic components of dentin. Even as bonding to the hydroxyapatite (inorganic) of dentin is believed to be by the formation of a phosphate salt, the mode of interaction of Chlorhexidine to the organic component of dentin is believed to be via binding to the Type-I collagen [13]. It is necessary to mention here that salivary glycoproteins have an additional role in the retention of Chlorhexidine in the oral cavity, thus adding to its efficiency as an oral antimicrobial agent. The binding of Chlorhexidine to the dentin matrix component inhibits the collagen bond proteases, and thus, exhibits its antiproteolytic action, which in turn enhances the life span of the adhesive bonded restorations [14].

We observed that highest substantivity was seen for Group II at 24 hours (84%) as compared to at one week (83.9%). Substantivity in Group I was a little lower than that seen in Group II. Substantivity was seen for Group I at 24 hours (83.1%) as compared to at one week (82.8%). Highest substantivity was observed for Group II then Group I, as compared to Group C; with both groups performing significantly better than Group C. Another study by Singh H et al., [15] is also in concordance with our observations. He observed that a significant amount of Chlorhexidine was found retained on the dentin disks in Group B as compared to Group C. Also, Group A performed significantly better than group C. However, no statistically significant difference was observed between Group A and Group B. Both 0.02% and 0.2% Chlorhexidine can be clinically recommended when being used for prolonging the durability of resin-dentin bond.

CONCLUSION
On the basis of findings of this study, it can be stated that 0.02% and 0.2% Chlorhexidine can be used in dental practice to enhance the stability of a dentin-adhesive interface. Further larger controlled trials are warranted to support our findings.

REFERENCES
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