

# Evaluation of three different concentrations of Chlorhexidine for their substantivity to human dentin

Harpreet Singh, Pooja Kapoor<sup>1</sup>, Jaidev Dhillon<sup>1</sup>, Mandeep Kaur

Departments of Conservative Dentistry and Endodontics and <sup>1</sup>Orthodontics and Dentofacial Orthopaedics, Gian Sagar Dental College and Hospital, Patiala, Punjab, India

## Abstract

**Aim:** To evaluate the substantivity of different concentrations of Chlorhexidine (CHX) to dentin disks prepared *in-vitro*  
**Materials and Methods:** Sixty dentin disks were prepared from extracted human third molars and divided into three groups (each containing 20 disks). All the disks were partially demineralized, as per standard procedure. Group A specimens were then treated with 10 microliters of 0.02% Chlorhexidine, Group B specimens with 10 microliters of 0.2% Chlorhexidine, and Group C specimens were treated with 10 microliters of 2% Chlorhexidine. They were then incubated in 1 ml of Phosphatebufferedsaline PBS (pH 7.4). The substantivity was evaluated after 24 hours and one week of incubation. CHX concentration in the eluates was spectrophotometrically analyzed. **Results:** A significant amount of CHX 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. **Conclusion:** Both 0.02% and 0.2% Chlorhexidine can be clinically recommended when being used for prolonging the durability of resin-dentin bond.

**Key words:** Bond strength, chlorhexidine, concentration

## INTRODUCTION

Chlorhexidine (CHX), is a golden molecule that has been used in dentistry since many years because of its antimicrobial properties.<sup>[1]</sup>

It is a bisbiguanide and has been used in the prevention and management of periodontal disease since decades.<sup>[2]</sup> Also, 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.<sup>[3]</sup>

Among the wide horizon of usage of this molecule, one interesting application in adhesive dentistry is the role of CHX in stabilizing the organic matrix of the resin-dentin bond. Studies have proven that CHX has a substantial role in the preservation of the resin-dentin bond.<sup>[4]</sup>

This property of CHX probably relates to its anti MMP-2, MMP -8, and MMP 9 activity.<sup>[5]</sup> The clinical efficiency of CHX 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.<sup>[6]</sup> This property of substantivity of CHX helps in the formation of a more stable hybrid layer, thus contributing to the success of bonded restorations.<sup>[7]</sup>

Considering the importance of this property of CHX in the field of restorative dentistry, it seems necessary to evaluate the concentration of CHX, which can be clinically used in this field. The aim of our study is to evaluate the substantivity of CHX in three different concentrations.

## MATERIALS AND METHODS

Sixty 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.

### Address for correspondence:

Dr. Harpreet Singh,  
Department of Conservative Dentistry and Endodontics, Gian Sagar Dental College and Hospital, Patiala, Punjab, India.  
E-mail: hsgentledental@gmail.com

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After the removal of the debris, the roots and pulp tissue were removed. 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 kept at approximately 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.<sup>[8]</sup>

The disks were then divided into three groups. Group A disks were treated with 10 microliters of 2% CHX, which was applied for 20 seconds; Group B disks were treated with 10 microliters of 0.2% CHX; while Group C disks were treated with 10 microliters of 0.02% CHX. The disks were then transferred to 2 ml plastic centrifuge tubes having 1 ml of PBS, and incubated at 37°C.<sup>[9]</sup> One ml of PBS solution was taken from each tube and spectrophotometric analysis was done at 260 nm after one hour, 24 hours, and one week of incubation, to estimate the concentration of CHX (in percentage) in the solution.

## OBSERVATIONS AND RESULTS

The substantivity of CHX for all treated substrates expressed as a percentage is shown in Table 1. Higher substantivity was observed for specimens in Group A and Group B, as compared to Group C; with both groups performing significantly better than Group C ( $P < 0.05$ , statistically significant). No significant difference was found between Group A and Group B. However, numerically, Group B was seen to be better than Group A.

## DISCUSSION

The substantivity of CHX is considered as one of its significant features, for which it has been used in dentistry since decades. The use of CHX in the field of periodontics, for its antibacterial action, is considered

to be the gold standard worldwide.<sup>[10]</sup> Significant research has also been done to evaluate its role in the field of endodontics as an irrigant and intracanal medicament.<sup>[11]</sup>

However, of late, its use to stabilize the resin-dentin bond is being researched upon so that it can be used in day-to-day clinical practice.<sup>[12]</sup>

It has been advocated that since CHX has the potential to bind to both organic and inorganic components of the dentin, in clinical usage, when it 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.

The substantivity of CHX 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,<sup>[13]</sup> the mode of interaction of CHX to the organic component of dentin is believed to be via binding to the Type-I collagen. It is necessary to mention here that salivary glycoproteins have an additional role in the retention of CHX in the oral cavity, thus adding to its efficiency as an oral antimicrobial agent.<sup>[14]</sup>

As CHX binds to both the inorganic and organic components of dentin, it would be wise to only partially and not completely demineralize the dentin, before applying CHX, and therefore, this protocol was applied in our study.

The binding of CHX to the dentin matrix component inhibits the collagen bound proteases, and thus, exhibits its antiproteolytic action, which in turn enhances the life span of the adhesive bonded restorations.<sup>[15]</sup>

Over a period of time, different concentrations of CHX have been tested for usage in adhesive dentistry, for enhancing the bond strength.<sup>[7]</sup> The objective of this study was to compare three different concentrations of CHX (0.02, 0.2, and 2%) in terms of their usage in enhancing the bond strength of dentin bonds. The substantivity of all the three concentrations was measured by spectrophotometric analysis.<sup>[9]</sup>

The results of our study revealed that both 0.02 and 0.2% CHX performed better, with higher substantivity, as compared to 2% CHX, and these results were statistically significant. The better performance of 0.2% solution can be attributed to the anti MMP-2, MMP-8, and MMP-9 activity of CHX at this concentration and its ability to form

**Table 1: Percentage of chlorhexidine that remains bound**

Specimen	24 hours	One week
Group A (0.02% CHX)	80.7	80.6
Group B (0.2 CHX)	81.3	81.2
Group C (2% CHX)	68.3	68.1

CHX: Chlorhexidine

a relatively stable monolayer of retained CHX.<sup>[16]</sup> However, there was no significant difference in the performance of 0.02 and 0.2% CHX, although numerically, the scores were better for the latter. The rationale behind such a good performance of CHX, even at such a low concentration (0.02%), needs further exploration.

## CONCLUSION

Within limits, it can be concluded from our study that both 0.02 and 0.2% CHX possess significant substantivity, and thus can be used clinically to enhance the stability of a dentin-adhesive interface when applied to the partially demineralized surface, after acid etching.

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