

## The Effect of *Salvadora Persica* Extract (Miswak) and Chlorhexidine Gluconate on Human Dentin: A SEM Study

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### Abstract

Bacterial plaque is solely responsible for the initiation and progression of periodontal diseases. There are different mechanical and chemical methods available for the maintenance of oral health through plaque control. Toothbrushes and miswak (chewing sticks) are widely used for the mechanical removal of plaque. Chlorhexidine gluconate (CHX) is one of the best-proven anti-plaque agents.

The aim of this study was to evaluate the effects of CHX and miswak extract on healthy and periodontally involved human dentin. Sixteen human premolars recently extracted for orthodontic and periodontal reasons were used in the study. Teeth were free from caries, cervical restorations, or erosions. The dentin disc specimens were prepared and half of them were etched with 6% citric acid for 120 sec. Both etched and unetched were further treated with CHX and 50% miswak extract and prepared for Scanning Electron Microscopic (SEM) examination. It was concluded that CHX 0.2% and miswak extract 50% had a similar effect on dentin in the control group. Miswak extract removed more smear layer as compared to CHX. Further research is needed *in vivo* to compare the effects of CHX and miswak extract on periodontally involved teeth and teeth with dentinal hypersensitivity.

**Keywords:** Chlorhexidine gluconate, CHX, miswak extract, SEM, human dentin, periodontally involved dentin

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## Introduction

Bacterial plaque is the primary etiology but not solely responsible for the initiation and progression of periodontal diseases. There are different methods available for the maintenance of oral health. These are mainly mechanical and chemical. Toothbrushes and dentifrices are widely used for cleaning the teeth. The traditional toothbrush or chewing stick called "Miswak" has been used since ancient history.<sup>1</sup>



Miswak (chewing sticks) were used by the Babylonians some 7000 years ago; they were later used throughout the Greek and Roman empires and have been used by Jews, Egyptians, and in the Islamic empires. It is believed that this precursor to the modern day toothbrush was used in Europe until about 300 years ago.<sup>2</sup> Today, miswak is being used in Africa, South America, Asia, the Middle East including Saudi Arabia, and throughout the Islamic countries.<sup>3</sup> It has different names in different societies, for instance, miswak, siwak, or arak are used in the Middle East; miswak in Tanzania; datan and miswak in India and Pakistan.<sup>1</sup> The use of the chewing stick is deeply rooted in many cultures. In the Middle East, the most common source of chewing sticks is the Arak (*Salvadora persica*) tree. In West Africa the lime tree (*Citrus aurantifolia*) and the orange tree (*Citrus sinensis*) are used. The roots of Senna (*Cassia vinnea*) were used by black Americans and those of African laburnum (*Cassia sieberiana*) were used in Sierra Leone. Neem (*Azadirachta indica*) is widely used in the Indian subcontinent.<sup>4</sup>

Many studies have demonstrated the antibacterial, anti-caries, anti-periodopathic, and anti-fungal properties of aqueous extracts of various chewing sticks.<sup>5,6,7,8</sup> There are only three studies comparing the antimicrobial effects due to the age of the miswak.<sup>9,10,11</sup> The relative accessibility and popularity of chewing sticks in the Middle East and Africa as an oral hygiene tool make it a cost effective agent for plaque control in such communities.<sup>12,13</sup>

Chlorhexidine gluconate (CHX), a cationic bisbiguanide, is the best-known and most widely used member of the bisbiguanide class. The efficacy of CHX in significantly reducing plaque and gingivitis (compared with placebos) when used twice daily as a supplement to tooth brushing is well established. The mechanism of action of CHX is due mainly to the rupture of the bacterial cell wall and precipitation of the cytoplasmic content.

Early studies used 10 ml of a 0.2% solution for a total of 20 mg CHX per use. There are studies supporting the effectiveness of 0.12% CHX using 15 ml of this solution.<sup>15,16,17</sup> The total amount of CHX per use was essentially the same, and the clinical findings with the two formulations were similar: plaque reductions in the 50-55% range and reduction in gingivitis of about 45%. The major side effects of CHX are a brown staining of the teeth and tongue, formation of supragingival calculus, taste alteration, and oral desquamation in children.<sup>18,19,16,20</sup> Allergic reactions have been reported in some patients, especially Asians.<sup>21</sup>

When CHX is used following brushing, one should allow at least 30 minutes after tooth brushing and before rinsing with this chemical because of an



interaction (and possible activation) between various positively charged dentifrice detergents and the cationic CHX.<sup>22</sup> Also, an antagonistic interaction can occur with the anionic fluoride ion in mouthrinses, toothpastes, and stannous fluoride products. The 30-minute interval is intended to minimize the diminution in activity of CHX.<sup>21</sup>

To date, there are no studies comparing miswak extract and CHX solution on the dentinal root surface. The aim of this study was to compare the dentin surface changes after the application of 50% aqueous extract of miswak (*Salvadora persica*) and 0.2% CHX on etched and unetched healthy and periodontally involved dentin.

### Materials and Methods

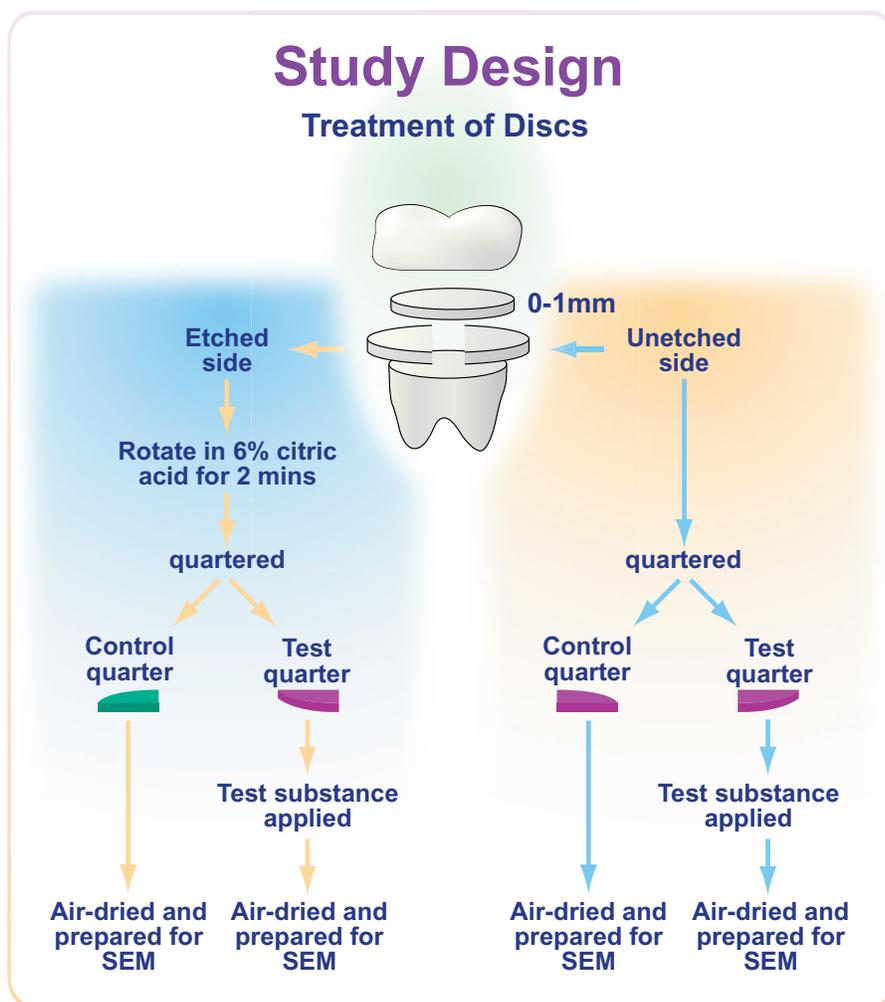
#### Study Design

Sixteen human premolars recently extracted for orthodontic (eight) and periodontic reasons (eight)

were used in the study. Teeth were free from caries, cervical restorations, or erosions and were stored in tubes containing saline. The anatomical crown (including 1 mm of the coronal portion of the root) was removed with a water coolant high-speed bur. To remove the cementum and expose the underlying dentin, the root surface was thoroughly planed and flattened with a 15 mm grit diamond (Perioset, Intensive Philadelphia, PA) used under continuous water coolant at 20,000 to 30,000 rpm. The root was then sectioned mesiodistally to obtain a 1 mm thick dentin disc. (Figure 1)

#### Treatment

The dentin disc was divided into two halves, etched and unetched groups. One half of the etched half disc was rotated in 6% citric acid for 120 sec and then quartered. Control and test quarters were prepared accordingly. (Figure 1)



**Table 1. Treatment modalities of the root dentin.**

Group	Specimens	Citric Acid Etched pH 2.5	Test Material	pH	Quantity	Duration (sec)	No.
1	Healthy (Control)	Etched	-	2.5	5 ml	120	4
		Unetched					4
2	Periodontally Involved (Control)	Etched	-	2.5	5 ml	120	4
		Unetched					4
3	Healthy (Experimental)	Etched	CHX	6.1	5 ml	120	4
		Unetched	CHX				4
4	Periodontally Involved (Experimental)	Etched	CHX	6.1	5 ml	120	4
		Unetched	CHX				4
5	Healthy (Experimental)	Etched	Miswak	6.0	5 ml	120	4
		Unetched	Miswak				4
6	Periodontally Involved (Experimental)	Etched	Miswak	6.0	5 ml	120	4
		Unetched	Miswak				4

The dentin specimens were etched and unetched. They were etched with 6% citric acid (pH 2.5). Following etching, CHX 0.2% (pH 6.1) or 50% aqueous extract of miswak (pH 6.0) were used. The pH of each solution (citric acid, CHX, and miswak extract) was tested with a hand-held, battery operated pH meter (Model 594100 Cole Parmer Instrument Company, Chicago, IL). A total of 48 specimens were obtained and prepared as per study design. The 48 dentin specimens were divided into 6 groups of healthy and periodontally involved discs of 8 specimens each. (Table 1)

### SEM Preparation

All specimens were fixed in glutaraldehyde (pH 7.3) and then prepared for Scanning Electron Microscopy (SEM) after fixation; dehydration was done in a graded series of ethanol and with 100% acetone use as a final step. Each of the specimens was mounted on aluminum stubs coated in gold with a sputter technique. The specimens were examined by the SEM (SEM, Jeol, Japan) operated at 25 KV and with a tilt angle of between 0-30 degrees. Twelve micrographs were prepared, one from each subgroup of 4 specimens.

### Results

The appearance of each of the specimens within a conditioning regimen were similar. The results are presented in Table 2.

#### Control Group

1. More dentinal tubules are open in periodontally involved dentin as compared to healthy dentin in both etched and unetched specimens. (Figures 2, 3, 4, 5)

#### Healthy vs Periodontally Diseased Dentin

2. CHX and miswak solutions have a similar effect on healthy dentin. (Figures 6, 8, 10, 12)
3. Miswak opened more dentinal tubules in periodontally involved dentin. (Figure 11)

#### CHX vs Miswak Extract

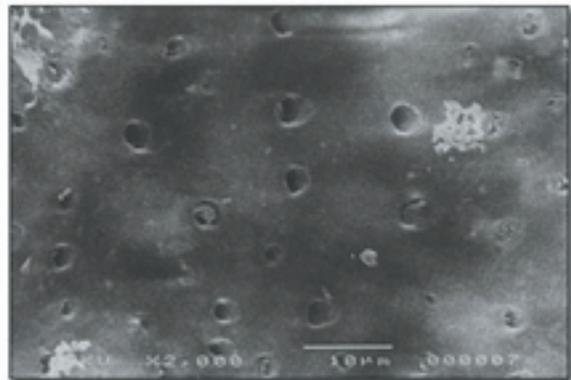
4. CHX partially occluded dentinal tubules in both etched specimens. (Figures 6, 7)
5. Miswak extract opened more dentinal tubules in healthy etched and periodontally involved etched specimens. (Figures 10, 11)

**Table 2. Effects of CHX and miswak extract on root dentin.**

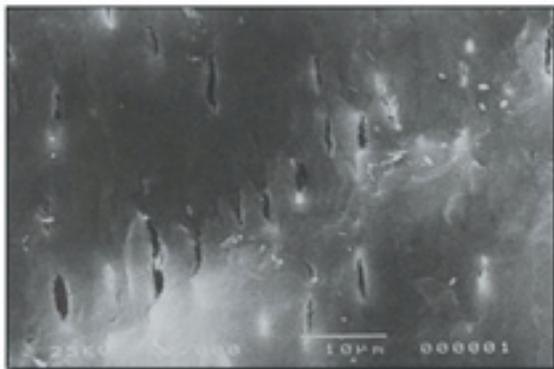
Dentin Specimen	Control Group Citric Acid		Experimental Group			
			CHX		Miswak	
	E	UE	E	UE	E	UE
Healthy	SL PODT GD C	SL GD	SL GD DTO	SL GD	GD ODT DTPO	GD PODT ODT
Periodontally Involved	ODT PODT GD	ODT PODT DTPO GD	ODT PODT DTO	SL GD	GD PODT ODT	DTO GD

**Key:**

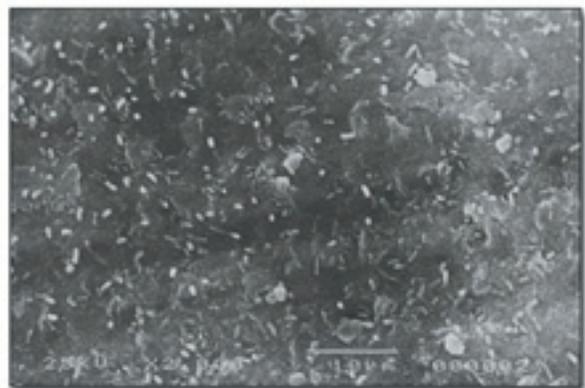
- E = Etched
- UE = Unetched
- C = Cracks
- GD = Grinding debris
- SL = Smear layer
- ODT = Opened dentinal tubules
- PODT = Partially opened dentinal tubules
- DTO = Dentinal tubules occluded
- DTPO = Dentinal tubules partially occluded



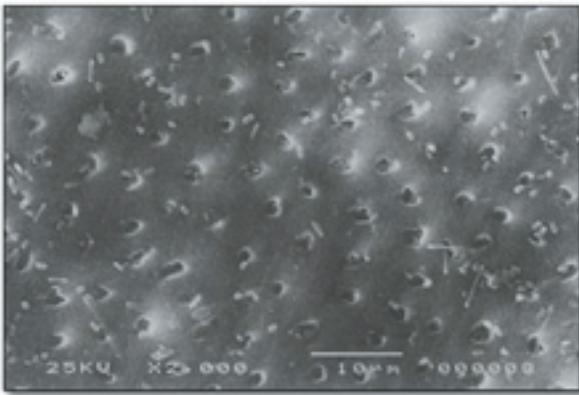
**Figure 3: SEM photomicrograph of dentin surface shows opened dentinal tubules, partially opened dentinal tubules, and grinding debris. (Periodontally involved etched)**



**Figure 2: SEM photomicrograph of dentin surface shows smear layer, partially opened dentinal tubules, grinding debris, and some cracks. (Healthy etched)**



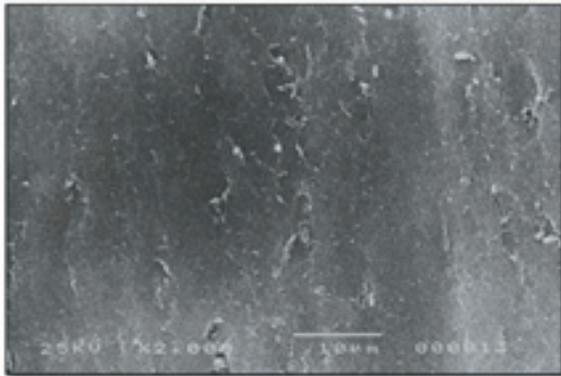
**Figure 4: SEM photomicrograph of dentin surface shows smear layer and some grinding debris. (Healthy unetched)**



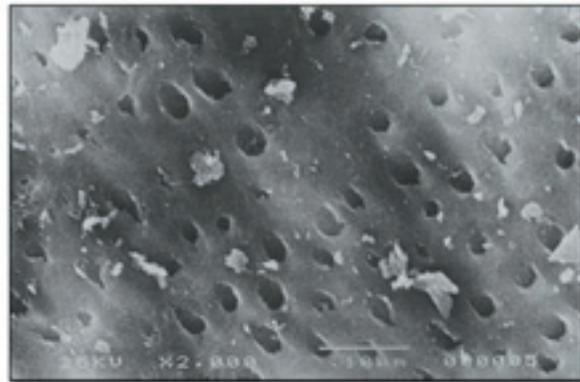
**Figure 5:** SEM photomicrograph of dentin surface shows opened dentinal tubules, partially opened dentinal tubules, dentinal tubules partially occluded, and some dentinal tubules occluded as well. (Periodontally involved unetched)



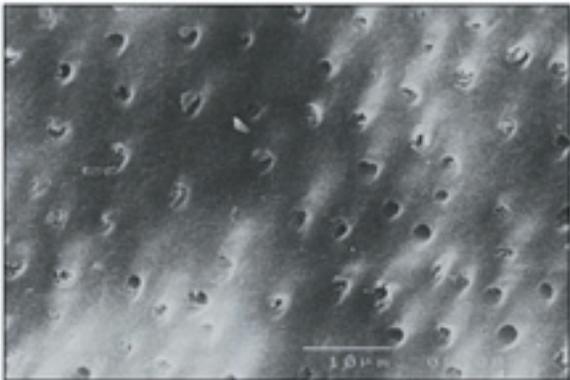
**Figure 8:** SEM photomicrograph of dentin surface shows smear layer and grinding debris. (Healthy unetched and CHX)



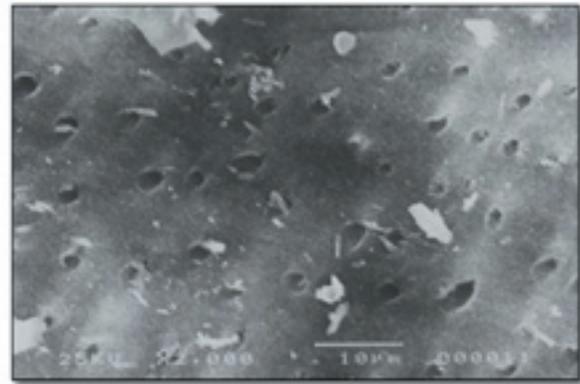
**Figure 6:** SEM photomicrograph of dentin surface shows smear layer, grinding debris, and dentinal tubules occluded. (Healthy etched and CHX)



**Figure 10:** SEM photomicrograph of dentin surface shows grinding debris, opened dentinal tubules, and dentinal tubules partially occluded. (Healthy etched and miswak)



**Figure 7:** SEM photomicrograph of dentin surface shows opened dentinal tubules, partially opened dentinal tubules, and some dentinal tubules occluded. (Periodontally involved etched and CHX)



**Figure 11:** SEM photomicrograph of dentin surface shows grinding debris, partially opened dentinal tubules, and opened dentinal tubules. (Periodontally involved etched and miswak)

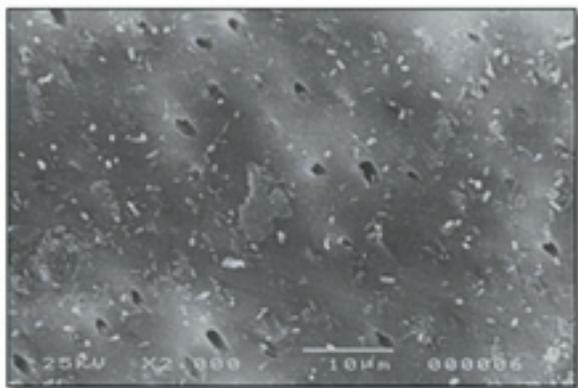


Figure 12: SEM photomicrograph of dentin surface shows grinding debris, partially opened dentinal tubules, and opened dentinal tubules. (Healthy unetched and miswak)

## Discussion

This study investigated utilizing a SEM examination of root dentin after application of CHX and miswak extract. The root dentin was either etched or unetched by using citric acid on both healthy and periodontally involved surfaces.

A root surface exposed to a periodontal pocket may harbor bacterial cells<sup>22,23</sup> as well as their cytotoxic products<sup>24,25</sup>, thus, representing a poor substrate for plasma protein absorption and subsequent fibrin adhesion. The root surface may then become hypermineralized and may accumulate exogenous organic material.<sup>25</sup> Chemical decontamination or detoxification of diseased root surfaces has been achieved with agents such as EDTA<sup>26</sup>, tetracycline hydrochloride<sup>27</sup>, and citric acid.<sup>28</sup> Etching agents dissolve the smear layer as well as parts of the mineralized root surface exposing collagenous fibers depending on exposure time and agent.<sup>28</sup> However, conflicting results regarding the etching effect have been presented depending on the mode of application.<sup>29</sup> Rubbing of the dentin surface with a cotton pellet soaked in citric acid appears to expose more intertubular fibrils and widen dentinal tubules to a greater extent compared to passive application (without rubbing) of the acid.<sup>30</sup> In the present study, the passive application of citric acid was performed to avoid the mechanical action of rubbing the dentin and creating a smear layer. After mechanical instrumentation of a root surface, a smear layer is usually present.<sup>31</sup> It has been characterized as an amorphous structure obscuring the underlying dentin surface.<sup>31</sup> After acid treatment, the smear layer is removed leaving patent dentinal tubules and varying degrees of dentin matrix exposed.<sup>32,33</sup>

The results of the present study suggest the agents used for conditioning root surfaces affect the surfaces in different ways and may be of importance in periodontal regenerative procedures. Wen et al<sup>30</sup> evaluated the effect of citric acid (pH 1.0) application techniques on freshly extracted teeth and concluded it may be undesirable to apply citric acid using excessive pressure. So in the present study the soaking of dentin was done only in etched healthy dentin and when there was a periodontally involved smear layer and grinding debris was present. Etching did partially remove the smear layer in the control group. When CHX and miswak extract were used, there was less removal of the smear layer in etched dentin by CHX compared to miswak extract. This may be explained by the possible astringent effect of CHX, though the pH of both CHX and miswak extract was similar (6 and 6.1, respectively) in healthy dentin. It was observed that miswak opened more dentinal tubules in periodontally involved dentin as compared to CHX. This may have a clinical implication of enhancing the regenerative opportunity of periodontium and root surface healing.

The clinical interest and relevance of miswak appears to arise from a number of mechanisms in addition to its acidic and antimicrobial properties. The results of the study are limited to physical findings of root dentin changes and do not represent in vivo differences that may result from physiologic effects of miswak extract. The results of the present study are in accordance with previous work regarding the effects of chewing stick extract on healthy and periodontally involved dentin<sup>34</sup> and on healthy dentin.<sup>35</sup> The chemical analysis of *Salvadora persica* has shown it contains trimethylamine, salvadorine, chlorides, fluoride in large amounts, silica, sulphur, vitamin C, and small quantities of tannins, saponins, flavenoids, and sterols.<sup>36</sup> It would be interesting to find out the effect of these individual constituents of miswak on root dentin.

The surface characteristics produced by the two solutions (CHX and miswak) were not significantly different, so the selection of either agent should be based on its other properties. Additional studies in the future may provide results that could justify the clinical application of these agents. The role of both CHX and miswak extract may be explored in subjects with dentinal hypersensitivity.

## Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1.	CHX 0.2% and 50% miswak extract had similar effects on dentin specimens.
2.	Miswak extract removes more smear layer as compared to CHX.
3.	The pH of both CHX and miswak extract may have the same effect on dentin.
4.	Further research is needed in vivo to compare the effects of CHX and miswak extract on periodontally involved teeth and hypersensitivity teeth.

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