

Original Article**EFFECT OF MISWAK EXTRACT ON HEALTHY HUMAN DENTIN: AN IN VITRO STUDY**

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هدفت هذه الدراسة مقارنة شكل سطح الجذر بعد تطبيق خلاصة المسواك في الكحول ، الماء ، والخلول الملحي وذلك من حيث طريقة التطبيق وزمن التعرض .

دلت النتائج على أن خلاصة المسواك ذات ٢.٥ إلى ٢.٩ pH قادرة على إزالة طبقة اللطخة من على سطح العاج ذا الأقية العاجية المفتوحة . وهذا على النقيض من تفرش سطح العاج بخلاصة المسواك المائية التي تتصف بدرجة pH أعلى والتي بحدود ٦.٣ إلى ٦.٦ ، وكذلك بالنسبة لخلاصة المسواك الملحية . إذ كانت لهم القدرة على إزالة طبقة اللطخة ، لكن بشكل جزئي ودون كشف الأقية العاجية . لا يمكن الاستنتاج بأن درجة pH لها تأثير حاسم لتقرير حدوث التخرش .

The purpose of this study was to compare the texture of dentin surfaces after the application of saline, aqueous and alcohol-derived miswak extracts using different modes of application and exposure times. It was concluded that alcohol derived miswak extract at pH of 2.5 to 2.9, selectively removed the smear layer from the dentin surface exposing the dentinal tubules. This result was in contrast to burnishing dentin surface with aqueous - miswak extract of higher pH 6.3 to 6.6 or with saline, both of which partially removed the smear layer without dentinal tubules exposure. It cannot be excluded that the pH of the solutions used is an important factor in determining whether etching occurs.

Introduction

New connective tissue attachment to demineralized root surfaces has been reported to occur in various animals models¹³ and in humans.⁴⁵ Root surface conditioning by topical application of acidic solutions has been shown to remove the smear layer resulting from root instrumentation and any remaining root surface contaminants.^{6,7} Citric acid or tetracycline hydrochloride demineralization of radicular dentin uncovers and widens the orifices of dentinal tubules and exposes the dentin collagen matrix. In this manner, a matrix which supports migration and proliferation of cells involved in periodontal wound healing is provided.^{8,9} However, the benefit of such acid surface demineralization has also been questioned.¹⁰

The relative availability and popularity of chewing sticks in the Middle East and Africa in oral hygiene regimen make them cost effective agents for plaque control in these communities.

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Recently, ethanol extract of miswak has been shown to have a stronger microbial inhibitory effect on different microorganisms than the aqueous extract.¹¹ However, there has been little baseline information comparing the effect of aqueous and alcohol extracts of miswak on root dentin.

The purpose of this study was to compare instrumented human dentin treated with saline, aqueous extract of miswak and alcohol extracts of miswak *in vitro* using scanning electron microscopy.

Materials and Methods**Selection and Preparation of Specimens**

Twenty human premolars recently extracted for orthodontic reason were used. 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) and the most apical segment of the root, 2 to 3 mm coronal to the

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apex, were removed with a water cooled high speed bur. To remove the cementum and expose the underlying dentin, each root surface was thoroughly planed and flattened with a 15 urn grit diamond¹ used under continuous water coolant at 20.000 to 30.000 rpm. The root was then sectioned bucco-lingually parallel to its axis. Two root surface blocks (3x3 mm square) were obtained from each section. This yielded a total of 72 dentin specimens (8 specimens were discarded).

Preparation of Miswak Extracts

To prepare the aqueous extract, the chewing sticks were cut into small pieces and ground to powder form in a ball mill. The powder was weighed into 10 gm portions and placed in a sterile screw capped bottle to which 100 ml of sterile deionized distilled water was added. The extract was allowed to soak for 48 hours at 4°C before the mixture was centrifuged at 2000 rpm for 10 minutes.¹² The supernatant was passed through a 0.45 mm membrane filter. The extract was prepared at 5, 10 and 25% concentration (v/v) with pH of 6.6, 6.4 and 6.3, respectively.

To prepare the alcohol extract, roots of the Arak tree were cut into small pieces and then powdered also in a ball mill. An extract (percolate) was prepared from 1 kg powder using 96% ethanol by the percolation method. The mix was percolated 6 times using 2.5 liters of 96% ethanol each time. The resulting percolate was then concentrated and the ethanol solvent completely removed at low temperature and reduced pressure. The yield of the extract obtained was found to be 6% (w/w). The stock solution of miswak extract was prepared by dissolving 1.0 gm of the miswak extract in 5 ml sterilized ringer solution. The 5, 10 and 25% miswak concentration were prepared by mixing with ringer solution.¹³ The pH values were 2.9, 2.7 and 2.5, respectively.

Treatment

The solutions used for treatment of the root dentin were saline (pH5.1), aqueous extract of miswak at 5, 10 and 25 per cent concentrations with pH 6.6, 6.4 and 6.3, respectively,

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and alcohol extract of miswak at 5, 10 and 25 per cent concentrations with pH 2.9, 2.7 and 2.5, respectively. The pH of each solution (saline, aqueous and alcohol extracts of miswak) was tested with a hand-held, battery operated pH meter (Model 5941.00 Cole Parmer Instrument Company, Chicago, IL). The 72 specimens were randomly divided into 8 groups of nine specimens each. Group 1 was soaked in saline, group 2 received burnishing with saline for 60 seconds. Groups 3, 4 and 5 received burnishing with aqueous extract of miswak for 120 seconds. Groups 6, 7 and 8 received burnishing with alcohol extract of miswak for 120 seconds (Table I).

SEM Preparation

All specimens were prepared for scanning electron microscopy (SEM). After fixation, dehydration was done in a graded series of ethanol and with 100% acetone as a final step. Each of the sectioned pieces was mounted on aluminum stub, coated in gold with a sputter technique. The specimens were examined in the scanning electron microscope⁵ operated at 10 to 20 KV and with a tilt angle of between 0 and 30 degrees.

Table 1. Treatment modalities of the root dentin.

Group	Surface Treatment	Concentration	pH	Duration (in seconds)	Number
1	Soaked in saline	-	5.1	30	9
2	Burnished with saline	-	5.1	60	9
3	Burnished with aqueous extract of miswak	5%	6.6	120	9
4	Burnished with aqueous extract of miswak	10%	6.4	120	9
5	Burnished with aqueous extract of miswak	25%	6.3	120	9
6	Burnished with alcohol extract of miswak	5%	2.9	120	9
7	Burnished with alcohol extract of miswak	10%	2.7	120	9
8	Burnished with alcohol extract of miswak	25%	2.5	120	9

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Results

The appearances of each of the specimens within a conditioning regimen were generally similar. The results are presented in Table II.

Saline Treated Root Surfaces

An amorphous irregular coating characteristically covered the whole surface, and obscured the dentinal tubules. Examination revealed a thin mineralized smear layer covering the root surface (Fig. 1). Specimens burnished for 60 seconds with a saline solution revealed undulating ridges perpendicular to the direction of the bur. The smear layer was partially removed with some orifices of dentinal tubules almost completely covered, whereas others were only partially occluded (Fig. 2).

Aqueous Miswak Extract Treated Root Surfaces

The surfaces of samples burnished with different concentrations for 120 seconds showed partial removal of the smear layer. Most of the orifices of dentinal tubules were partially occluded with surfaces debris. Occasional cracks appeared in the layer covering some dentinal tubules orifices (Fig. 3). Others specimens revealed a flat dentin surface with few exposed dentinal tubules. Many dentinal tubules had slit-shaped orifices (Fig. 4).

Alcohol Miswak Extract Treated Root Surfaces

The dentin surface treated with a 5, 10 and 25% concentrations for 120 seconds showed a similar appearance. The dentin surface was readily visible in all burnished specimens as evidenced by lack of smear layer and abundance of patent dentinal tubules (Fig. 5). All of the dentinal tubules were exposed and did not reveal any intensified peritubular dentin at the surface.

The treated surface appeared relatively smooth with a large number of wide open dentinal tubules. Tubules appeared to be widened into funnel shapes due to the removal of peritubular dentin (Fig. 6).

Discussion

This present study evaluated SEM characteristics of dentinal surfaces following burnishing application of aqueous and alcohol miswak extracts applied in different concentrations and for different periods of time.

The instrumented saline control surface presented an irregular coating with no dentinal tubules orifices. This characteristic appearance was probably due to the presence of a surface smear layer. The presence of a smear layer has been described on root surface instrumented with periodontal curettes.⁶ This smear layer consists of organic and inorganic materials in particles varying in size from less than 1 μ m to

Table II. Effects by the various agents on scaled root dentin.

Group	Saline Soaking	Saline Burnish Incj	Aqueous Extract of Miswak (120 Sees)			Alcohol Extract of Miswak (120 Sees)		
	(30 Sees)	(60 Sees)	5%	10%	25%	5%	10%	25%
Smear Layer	P	PR	PR	PR	PR	R	R	R
Exposed dentinal tubules	Ne	Pe	Pe	Pe	E	E	E	E
Cracks (small)	-	-	-	Yes	Yes	-	Yes	Yes
Degree of occlusion of dentinal tubules	CO	CO	PO	PO	PO	NO	NO	NO

P: Present; PR: Partially removed; R: removed; Ne: Not exposed; Pe: Partially exposed; CO: Completely obstructed; PO: Partially occluded; NO: Not occluded.

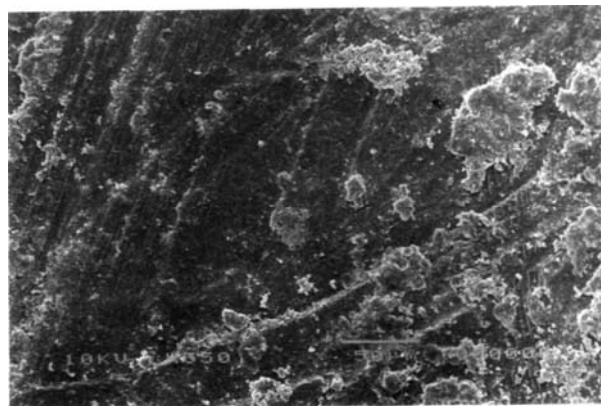


Figure 1. SEM photomicrograph of dentin surface soaked with saline for 30 seconds. Presence of amorphous uniform smear layer covering dentin surface. The surface is interrupted by multiple scratches (original magnification x 350).

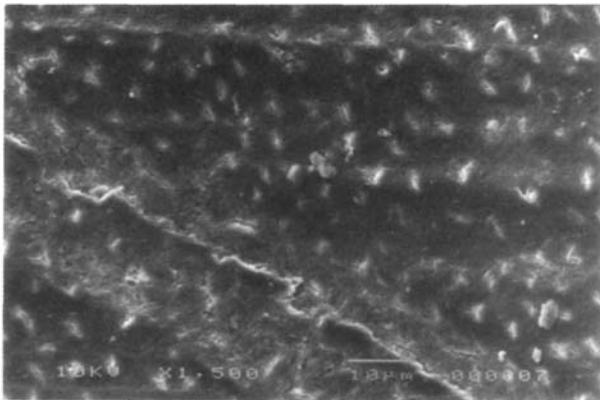


Figure 2. SEM photomicrograph of dentin surface burnished with saline for 60 seconds. Smear layer is partially removed. Note intensification of peritubular dentin (original magnification x 1500).

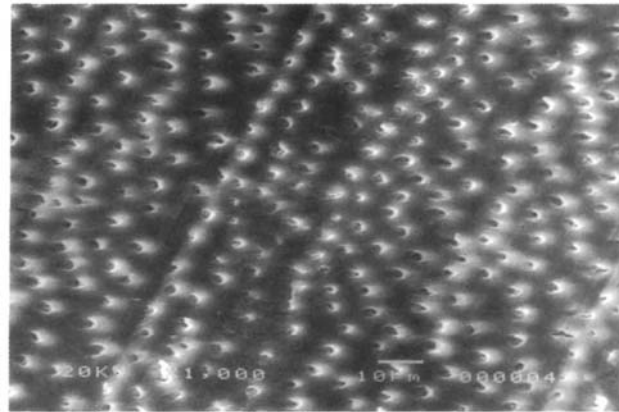


Figure 5. SEM photomicrograph of dentin surface treated with 25% alcohol miswak extract for 2 minutes. The surface is flat with widely exposed dentinal tubules (original magnification x 1000).

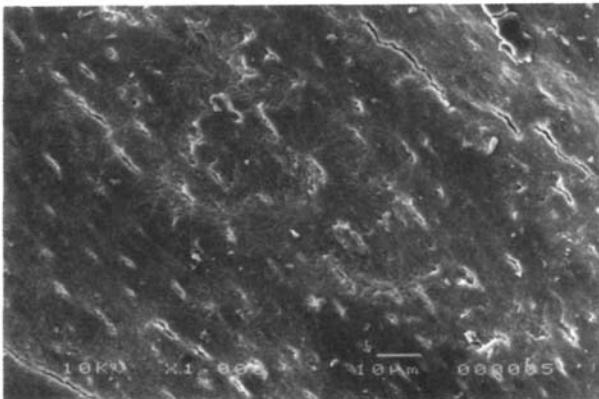


Figure 3. SEM photomicrograph of dentin surface burnished with 5% aqueous miswak extract for 2 minutes. Smear layer is partially removed. Dentinal tubules are partially occluded with debris (original magnification x 1000).

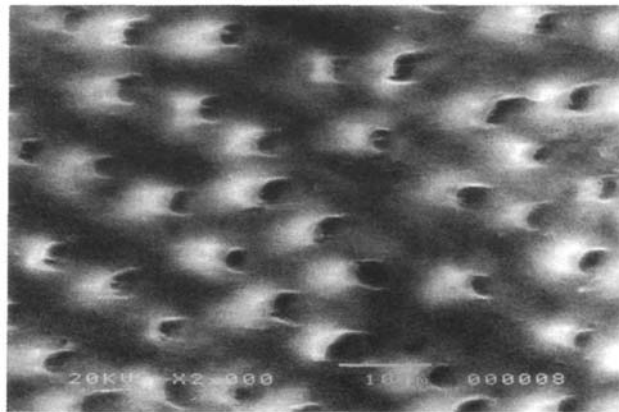


Figure 6. 2000 magnification of Figure 5. Note the wide funnel shaped dentinal tubules widely exposed.

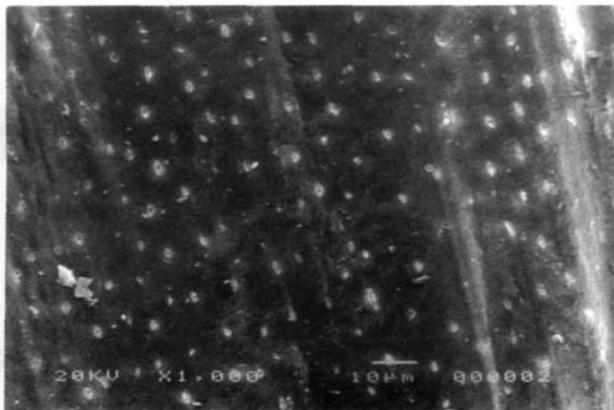


Figure 4. SEM photomicrograph of dentin surface burnished with 25% aqueous miswak extract for 2 minutes. Smear layer is partially removed. Most dentinal tubules are partially occluded. Few dentinal tubules are wide opened (original magnification x 1000).

more than 15 μm .¹⁴ The smear layer is associated with the root surface and is virtually removed by etching solutions.¹⁵

Miswak alcohol extract resulted in the disappearance of the above-mentioned amorphous surface. This finding is consistent with a demineralization effect produced by acid solution application as previously described.^{16,17} In contrast, the burnishing application of saline and miswak aqueous extract demonstrated an incomplete removal of the smear layer. None of the dentinal tubules revealed enlarged orifices. The burnishing effect of the root conditioner on the instrumented tooth surface has been reported to cause a chemical and mechanical removal of the smear layer allowing for

demineralization of the underlying root surface.¹⁸ Saline appears to remove only the superficial portion of the smear layer, leaving dentinal tubules occluded with debris.

The results of the present study suggest that the agents used for conditioning root surfaces, affect the surfaces in different ways and may be of importance in periodontal regenerative procedure. Wen et al¹⁹ evaluated the effects of citric acid (pH 1.0) application techniques on freshly extracted teeth and concluded that it may be undesirable to apply citric acid using excessive pressure.

In the present study, burnishing root surfaces with either saline for 60 seconds or aqueous miswak extract for 120 seconds produced an incomplete removal of smear layer while alcohol derived extract produced a surface free of smear layer. Brannstrom and Johnson²⁰ stated that the smear layer is only removed by demineralizing solutions. This approach may suggest that miswak alcohol extracts have etchant properties. Alcohol derived extract has been the most effective in smear layer removal in this study.

The pH range of alcohol derived extract with different concentrations varied from 2.5 to 2.9 while the one for aqueous solution with different concentrations varied from 6.3 to 6.9. Low pH solutions produced more etching effects in this study. This finding is not consistent with Blomlof and LindsKog²¹ who found that etching at neutral pH with agents such as EDTA have been shown to be equally if not more efficient than to agents with low pH in exposing collagen fibrils on dentin surfaces.

In controlled experimental studies, etching of root surfaces at low pH have been shown to impair periodontal healing²² in comparison with etching at neutral pH.²³ However, in this study, the pH does appear to be a crucial factor in determining whether etching occurs.

The mode of applying miswak extracts did not appear to have any consistent effects on the root surfaces. The lack of surface effects after burnishing with saline and aqueous extract is in contrast with the changes observed with the alcohol miswak extract. It is theorized that the burnishing technique may result in a chemical and mechanical action which enhances the removal of chemically loosened inorganic

material and surface debris exposing underlying dentin to the demineralization action of acid.^{24,25} It should be recalled that in the present study, miswak extract of aqueous and alcohol solution was applied by a cotton pellet using a burnishing technique in order to imitate the chemical use of miswak chewing sticks.

The incomplete removal of the smear layer by saline and aqueous miswak extract may be explained by the astringent action of saline and aqueous solution on the smear layer. This astringent action of both solutions on the effect of smear layer and the surface peritubular dentin had contributed to their constriction. However, the application of alcohol derived extract at different concentration was effective in removing the smear layer. Due to low pH, the weak hydrogen bonds attaching the alcohol to the collagen of the smear layer could be easily broken down leading to the separation of the smear layer from the dentin surface and exposing the tubules.

The clinical interest of miswak appears to arise from a number of mechanisms in addition to its acidic and antimicrobial properties especially for the alcohol derived extract. Although alcohol derived extract demineralizes dentin, the time dependence changes are less than those produced by citric acid. Register and Burdick²⁶ suggested that the range of acid penetration depended upon pH and time of application of the acid solution used. In their dog model, the 2 minutes application period was sufficient if the pH range was from 1.5 to 2.0.

The results of this study are limited to the physical findings of root surface changes and do not represent *in vivo* differences that may result from the physiologic effects of miswak extract. The surface characteristics produced by the two extracts were 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 clinical application of these agents.

Conclusion

Based on the findings of this *in vitro* study, the following conclusions can be drawn:

1. Soaking the root dentin with saline did not result in the disappearance of the smear layer.

2. Burnishing the root dentin with saline and aqueous miswak extract partially removed the smear layer.
3. Alcohol miswak extract resulted in the disappearance of the smear layer.
4. The degree of exposed dentinal tubules was greater in the alcohol extract of miswak group.

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