Original Article

Effect of *Salvadora persica* Extract (Miswak) on the Dentinal Tubules of Sound Root Dentin: Scanning Electron Microscope Study

Sahar Khunkar, Amal I. Linjawi[1]

Departments of Restorative Dentistry and [1]Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background/Purpose: Root dentin is vulnerable to a higher risk of demineralization than coronal enamel. This study aimed to evaluate the effect of miswak extract on the dentinal tubules of sound root dentin. **Materials and Methods:** Twenty bovine root dentin blocks, approximately 2 mm × 3 mm × 3 mm (width × length × depth) in dimensions, were prepared from freshly extracted sound bovine incisors. The sample was divided into two groups: control and miswak group. The control group had sound root dentin block and the miswak test group was treated with miswak extract 20% for 24 h. The two groups of all specimens were subjected to ultrasonication for 10 min. Scanning electron microscope images were analyzed for surface typography. **Results:** Fifty percent of the control group had surface particles (SPs), while the other 50% had no SPs. For the dentinal tubules, all (100%) the control group had a mixture of opened and partially opened dentinal tubules. On the other hand, for the miswak group, all (100%) the sample had SPs and blocked dentinal tubules. **Conclusion:** Miswak showed total blocking of the dentinal tubules compared to the control group. This might indicate that miswak has a role in reducing dentinal hypersensitivity of exposed root dentin.

Keywords: Dentinal tubules, miswak, root dentin, *Salvadora persica*, ultrasonication

Introduction

Miswak is a chewing stick used in the Arab countries for tooth cleaning. It was used by the Babylonians 7000 years ago. "Miswak tree" is a natural toothbrush in traditional medicine. Miswak is derived from a plant species of "*Salvadora persica*" that belongs to the family Salvadoraceae. Toothbrushing using miswak is a constant practice in many Muslim countries.[1-4] It also became recommended by the World Health Organization since 1987.[5] A variety of vehicles have been used to deliver its extract such as mouth rinse,[6-9] dentifrices,[10,11] and gel.[12]

In addition to its mechanical effects, miswak was proven to have a variety of biological effects.[1,13-15] Studies have shown that rinsing with miswak extract caused a significant and immediate rise in the pH of the plaque. Thus, miswak was proposed to have a great potential in the prevention of caries.[15] The aqueous extract of miswak was also found to have an antibacterial activity against a number of bacteria including *Streptococcus mutans* and anaerobic streptococci.[2,7,14,16] Other proven effects of miswak include antifungal,[17] antioxidant, and anticariogenic effect.[18-21]

The biological and cleansing effects of miswak have been attributed to various chemicals found in its composition. The chemical composition of miswak consists of organic and inorganic compounds. The organic components include saponin, flavonoid, an alkaloid, and a herbal steroid named benzyl 2–4 isothiocyanate.[1,22,23] The inorganic compounds include sulfated compounds, silica, calcium, fluoride, Vitamin C, oxalate, and tannic and gallic acids.[13,24]

In 2001 and 2002, Almas evaluated the effect of 25% and 50% aqueous extract of miswak with a pH of 6.0 on unetched and etched root dentin for both sound and periodontally

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Address for correspondence: Dr. Amal I. Linjawi, Department of Orthodontic, Faculty of Dentistry, King Abdulaziz University, P.O. Box 80209, Jeddah 21589, Saudi Arabia.
E-mail: ailinjawi@kau.edu.sa

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involved dentin using scanning electron microscope (SEM). He found that miswak caused opening of the dentinal tubules for both sound and etched root dentin. However, on the etched dentin, miswak also occluded some of the dentinal tubules.[25,26] The chemical analysis for such phenomena was further tested by Wassel and Sherief in 2019.[27] They found that miswak-containing varnishes due to their release of high concentration of Ca^{++} and PO_4^{-} ions in addition to F^{-} ions showed a better effect than 5% NaF in remineralizing enamel lesions.[27]

Dentinal hypersensitivity treatment is a major goal within oral health-care clinic. Recent researches are being conducted to find materials that can reduce the permeability of the dentin which achieved through partially or fully blocking the dentinal tubules.[28]

To our knowledge, there are very limited studies that evaluated the miswak extract and its effect on the dentinal tubules of the root dentin. Thus, the objective of this experimental study is to investigate the effect of miswak extract on the dentinal tubules of sound root dentin. The null hypothesis tested was that miswak extract does not have an effect on the dentinal tubules compared to a control.

Materials and Methods

Study design

This is a laboratory study to assess the effect of miswak on the dentinal tubules of sound root dentin. The study was approved by the Ethical Committee of the Faculty of Dentistry at King Abdulaziz University (Ethical approval No. 103-06-19).

Specimen preparation

Twenty root dentin blocks were prepared from freshly extracted sound bovine incisors with approximate dimensions of 2 mm × 3 mm × 3 mm (width × length × depth). The blocks were cut under water cooling using a low-speed diamond saw (Isomet, Buehler, IL, USA). The blocks were then embedded in self-curing acrylic resin (Shade A2, UNIFAST II; GC, Tokyo, Japan), and the surfaces of the dentin were then polished with 800, 1200, and 2000 grit silicon carbide (SiC) papers (Sankyo, Saitama, Japan). The polished surfaces were then covered by acid-resistant nail varnish (Shiseido, Tokyo, Japan) leaving an exposed window with an approximate dimension of 1.5 mm × 2.5 mm for treatment[29] [Figure 1].

Preparation of 20% miswak aqueous extract

The preparation of the miswak aqueous extract was done as followed in the protocol published by Sofrata *et al*.[15] The sticks of miswak were dried at room temperature for a couple of days, then cut into small pieces, and ground in a house grinding machine or ball mill to make it powder. The preparation of 20% concentration of miswak aqueous extract (pH 4.2) was done by adding 20 g of powdered miswak into 100 ml sterilized and deionized water and kept for about 48 h at 4°C. The mixture was then centrifuged at 2200 rpm

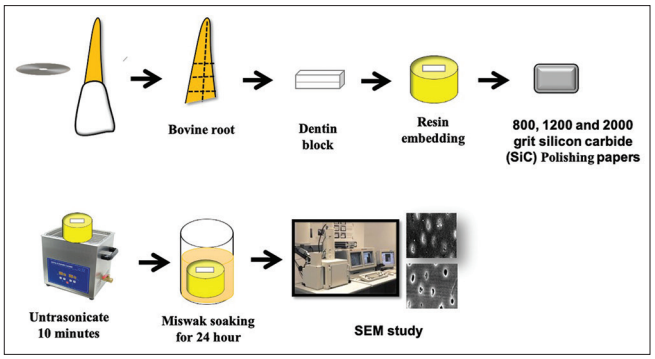


Figure 1: Schematic drawing showing steps of miswak treatment of bovine dentin for 24 h

for 10 min. To remove possible bacteria contamination, the supernatant was passed through Millipore filters (0.45 μm pore size; Sigma-Aldrich Chemie GmbH, Germany). The extract was then stored in a sterilized bottle at 4°C and used within 1 week.[30]

Specimen treatment

The collected specimens were divided into two groups; control and miswak test group. The control group had sound root dentin block, and the miswak test group was treated with miswak extract 20% for 24 h. The two groups of all specimens were subjected to ultrasonication for 10 min.[28] The schematic illustration of experimental steps is shown in Figure 1.

Scanning electron microscope preparation

All specimens were prepared for SEM assessment by dehydrating them in ascending grades of ethanol. Then, they were immersed in hexamethyldisilazane for 10 min and placed on filter paper in a covered glass vial for 24 h at room temperature for drying and fixing. Specimens were then coated by gold-sputter coating (SC-701AT, Elionix, Tokyo, Japan) and examined from the top surface using SEM (JSM-5310 LV; JEOL, Tokyo, Japan) at ×2000 magnification with an accelerating voltage of 20 kV.

SEM was done for the observation of the dentinal tubules to examine if there is any precipitation of inorganic substances from miswak inside the dentinal tubules. Twenty micrographs were obtained, ten from each group.

Statistical image analysis

SEM images were analyzed for surface typography. The surface typography characteristics were described using the scale developed by Almas[26] with slight modifications as follows:

- Surface particles (SPs)
- Opened dentinal tubule (ODT)
- Partially ODT (PODT)
- Blocked dentinal tubule (BDT)
- Partially BDT (PBDT).

Descriptive statistics for the percentages of the surface typography characteristics were calculated and compared.

Results

The surface typography characteristics for each group are shown in Figure 2. In the control group, all specimens (100%) had no smear layer (SL), 50% had SPs, while the other 50% had no SPs (SL). For its dentinal tubules, all (100%) the control group had a mixture of opened (ODT) and partially opened (PODT) dentinal tubules. On the other hand, in the miswak group, all specimens (100%) had no SL, 100% had SPs, and 100% had BDTs.

A representative SEM image of the control group is shown in Figure 3. The images showed no SL, no SPs, ODTs, and PODTs of the control group.

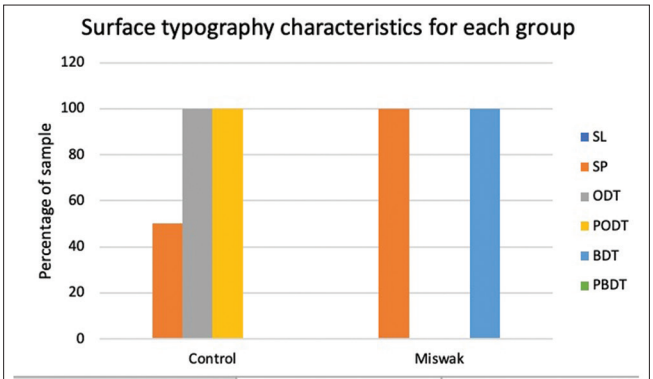
A representative SEM image of the miswak group is shown in Figure 4. The images showed no SL, presence of SPs, and BDTs of the miswak group.

Discussion

Dentin hypersensitivity is a major goal for oral health care, especially in elderly patients.[28] The blockage of the dentinal tubules is proposed to reduce dentin permeability and thus can help to reduce teeth sensitivity problems.[28] Removal of the SL from exposed root dentin surface was also found to be important to allow for the treatment material to reach the dentinal tubules as well as for gingival attachment healing after scaling and root planning.[31,32] Miswak was reported to have a great potential as an oral cleansing and antimicrobial agent.[1-15] However, limited studies assessed its actual effect on the dentinal tubules. The current study evaluated the effect of miswak extract on the dentinal tubules of sound root dentin under SEM findings showed that 20% miswak aqueous extract causes removal of the SL and blockage of the dentinal tubules when applied to sound root dentin compared to the control group. In support to the current findings, in 2001, Almas evaluated the effect of 25% aqueous extract of miswak on human dentin using SEM.[25] He found that miswak caused partial removal of SL and occlusion of dentinal tubules in dentin specimens burnished with miswak solution compared to specimens soaked in miswak extracts.[25] In 2002, Almas also evaluated the effect of miswak but using 50% miswak aqueous extract on etched and unetched human dentin using SEM. He found that miswak removes more SL and opens the dentinal tubules compared to the control group.[26] From the current study and the two studies of Almas, it might be hypothesized that, with lower the concentration of miswak, the more the blockage of the dentinal tubules will occur.

The current study also showed that all samples of the 20% miswak group had full blockage of the dentinal tubules. On the other hand, all the control groups had fully and PODTs. Our speculation for the full blockage of the dentinal tubules with the application of miswak could be due to the presence of organic substances and inorganic ions such as Ca^{++}, PO_4^{-}, sterols, fluoride, trimethylamine, chloride, salvadorine, silica, vitamin C, sulfur, tannins, saponins, and flavonoids.[4] In support to our findings, Wassel and Sharif conducted an elemental analysis to assess the Ca^{++}, PO_4^{-}, and F^{-} ion release from natural products including miswak using SEM and energy-dispersive X-ray. They found that miswak containing varnishes showed high Ca^{++}, PO_4^{-}, and F^{-}.[27]

The research and clinical interest to miswak appears to increase due to its multiple mechanical and biological effects in addition to its chemical properties. The effect of miswak on blocking the dentinal tubules, as confirmed in the current study, could propose the role of miswak in reducing dentinal hypersensitivity of exposed root dentin. Thus, further studies are needed to assess such hypothesis.



SEM characteristics	Control	20% Miswak
SL = Smear layer	100% No SL	100% No SL
SP = Surface Particles	50% SP 50% No SP	100% SP
DT = Dentinal Tubules	100% ODT + PODT	100% BDT

Figure 2: Surface typography characteristics for each studied group (n = 10 for each group); SL: Smear layer, SP: Surface particles, ODT: Opened dentinal tubules, PODT: Partially opened dentinal tubules, BDT: Blocked dentinal tubules, PBDT: Partially blocked dentinal tubules

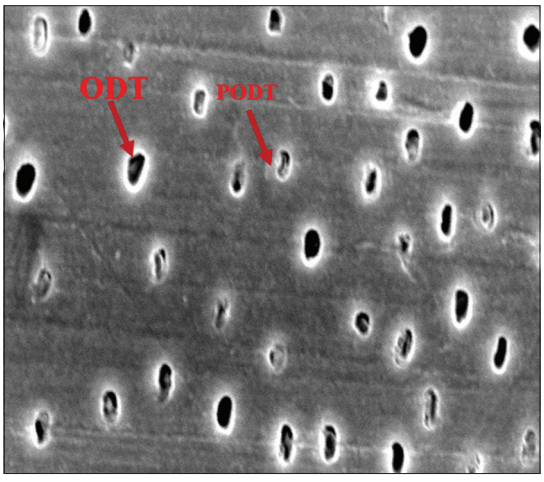


Figure 3: Representative scanning electron microscope image illustrating a control group with no smear layer, no surface particles, opened dentinal tubules, and partially opened dentinal tubules

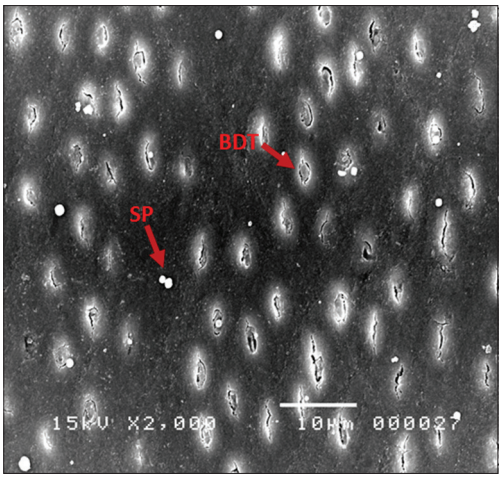


Figure 4: Representative scanning electron microscope image illustrating a miswak group with no smear layer, presence surface particles, and blocked dentinal tubules occluded

Conclusion

The findings of the current study showed that 20% aqueous extract of miswak caused total blocking of the dentinal tubules compared to the control group. This might indicate that miswak has a role in reducing dentinal hypersensitivity of exposed root dentin.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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