Title:

1. Effect of Miswak-Black Silica Toothbrush on **Color Stability and Surface Roughness** of Resin Composite, Resin-Modified Glass Ionomer, Enamel Surface: An In-vitro and In-vivo Study
2. Assessment of the **Remineralization Potential** of Miswak-Black Silica Toothbrush: An In-vitro study

Merged title: Effect of Miswak-Black Silica Toothbrush on the **Color Stability** and Surface **Roughness** of Resin Composite, Resin-Modified Glass Ionomer, and Enamel Surface, and Its **Remineralization** Potential: An In-vitro and In-vivo Study

Introduction:

Toothbrushing with a fluoridated toothpaste is the most common method for dental plaque removal. Abrasive particles-containing toothpastes are useful to eliminate dental plaque. Unfortunately, these particles may lead to a dental/toothbrush abrasion. Which is influenced by a number of factors, such as the force used when brushing, the frequency of brushing and length, brush type particularly filament stiffness, and the abrasively of the toothpaste used (Bhola et al., 2023; Hamza et al., 2020). Toothbrush abrasion is particularly undesirable for resin composite restorations due to its negative effect on aesthetics and biology, leading in decreased gloss and discoloration (Alex & Venkatesh, 2024).

The World Health Organization (WHO) has endorsed Salvadora persica sticks, commonly known as Miswak, as a highly effective tool for oral health. This is due to the mechanical action provided by their softwood fibers and the therapeutic effects of their natural chemical compounds (Al-Ahmari et al., 2022). Jassoma *et. al.,* in 2019, reported a decrease in oral bacteria populations and plaque scores with the use of Miswak (Jassoma et al., 2019). This was agreed with different clinical and laboratory studies (Bhat et al., 2012; Sulaiman et al., 2020; Varma et al., 2018).

Miswak sticks are usually pencil-sized, 10 -25 cm long, and 0.5-1.5 cm in diameter (Al-Ahmari et al., 2022). A newly introduced Miswak black silica-combined toothbrush (Shinyei Kaisha, Japan) was marketed for easier and more practical used. According to the manufacturer's instructions, the Miswak black silica-combined toothbrush is designed to be used without toothpaste. Despite its ease of use and promising performance, to the best of the author's knowledge, only one study has investigated the effects of this Miswak-black silica toothbrush.

❖ The aims of this study are:

a. To measure antibacterial effect of Miswak-black Silica toothbrush compared to soft manual toothbrush (combined in-vivo and in-vitro)

b. To evaluate the surface roughness of human enamel, resin composite, and resin modified glass ionomer samples after toothbrushing simulation with Miswak-black Silica toothbrush or Colgate soft manual toothbrush (in-vitro)
c. To investigate the color stability of human enamel, resin composite and resin modified glass ionomer samples after toothbrushing simulation with Miswak-black Silica toothbrush or Colgate soft manual toothbrush (in-vitro)
d. To assess the remineralization potential of Miswak-Black Silica Toothbrush on tooth structure (in-vitro)

❖ Methodology:

Evaluation of the antibacterial effect (Combined in-vivo and in-vitro):

Following the methodology of Thamke et al, a total of 50 participants who meet the inclusion criteria listed in table.1 will be included in the study (Thamke et al., 2018). After a thorough clinical examination, study flowchart will be explained to each participant and informed consent will be taken (Fig.1). Every participant will be given a number to easily compare their samples' results. Relevant data will be documented and saved properly. Participants confidentially will be assured and no personal data will be shown.

Table.1: inclusion and exclusion criteria of study participation:

Inclusion criteria	Exclusion criteria
• Age of 18–35 years • Tooth brushing frequency of two times daily	Patients with • Open carious lesions • Plaque index scores of >2 • Severe gingivitis (gingival index score >2) • Throat infections • Irregular brushing frequency • Smokers • Medically compromised

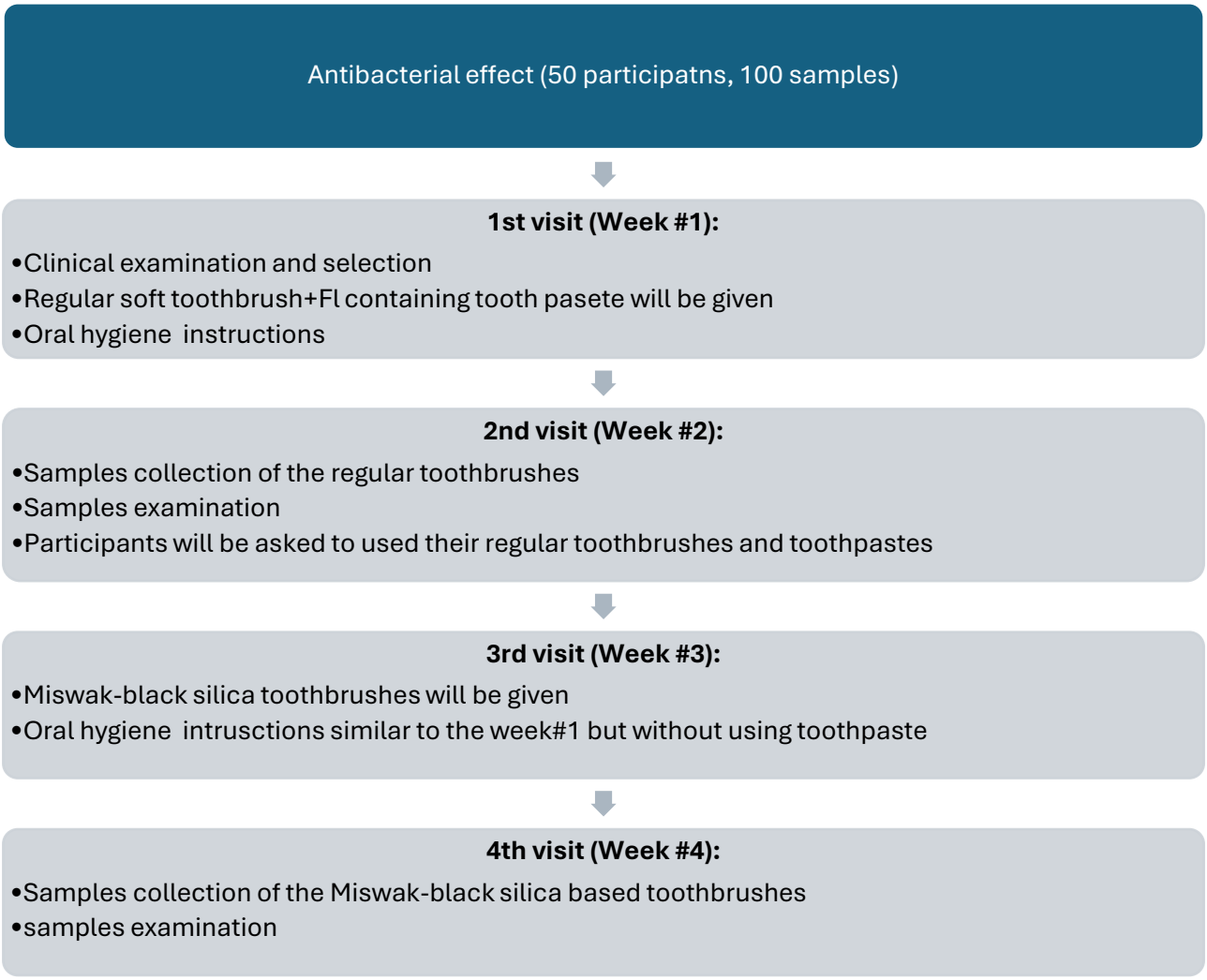

Figure. 1: Clinical Study flowchart

All participants will be given standard brushing instructions and one regular soft toothbrush with fluoride-containing toothpaste, and one Miswak-black Silica toothbrush (twice a day for 30 sec.) and asked to return the brush after one week of usage. For interproximal surfaces, participants will be instructed to maintain their usual oral hygiene routine using standard dental floss once daily.

After a washout period of one week, the same participants will be then provided with Colgate soft manual toothbrush and given the same brushing instructions. They will be asked to return the used soft manual toothbrush after one week of usage.

The bristles of the used toothbrushes will be sectioned using surgical scalpel blades and submerged in 5 ml of saline solution. From this, 0.1 ml will be inoculated onto agar plates.

The agar plate will be used to assess the antibacterial effect of the two toothbrushes. After 48 hours of incubation, colony-forming units (CFU) will be measured to assess bacterial growth. To evaluate the antibacterial efficacy of Miswak-black Silica filaments, the zone of inhibition was compared to soft manual toothbrush following 24 hours of incubation.

- **Evaluation of the surface roughness and color stability of enamel and resin composite samples (In-vitro):**

Sample preparation:

A total of twenty enamel samples, each of 5 mm x 4 mm x 1.5 mm dimension, will be carefully prepared from the intact buccal surfaces of freshly extracted, unerupted human third molars. The samples will be obtained using a diamond disc (Isomet, Buehler Ltd, Evanston, IL, USA) with water cooling to ensure the integrity of the enamel. Moreover, twenty resin composite samples will be prepared using a Teflon mold (5 mm x 4 mm x 1.5 mm) using Tetric N-ceram resin composite material (shade A2, Ivoclar Vivadent AG, Schaan, Liechtenstein) and light-cured following the manufacturer's instructions. Twenty resin modified glass ionomer (GC Fuji II LC) samples will be fabricated also similar to the resin composite samples.

All samples will be immersed in artificial saliva at 37°C for 24 h, and then randomly assigned to two subgroups for each enamel, resin composite, and resin-modified glass ionomer groups (n=10) based on the toothbrush type (Figure 2).

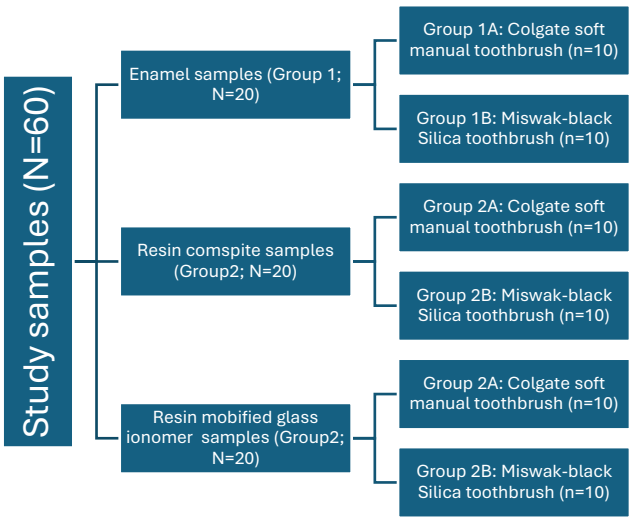

Figure 2: Study flowchart for both surface roughness and color stability tests (N=60, n=10) *(same samples will be used for color stability and surface roughness tests)*

Tooth brushing simulator:

Following the method of Rosentritt et al, toothbrushing will be performed with a toothbrush simulator (ZM-3; SD Mechatronik, Feldkirchen-Westerham, Germany) with a toothbrush simulator (ZM-3; SD Mechatronik, Feldkirchen-Westerham, Germany) (Rosentritt et al., 2023). For sub-groups 1A and 1B, a Colgate soft manual toothbrush will be used with 250 g Colgate fluoride-containing toothpaste. For sub-groups 2A and 2B, a Miswak-black Silica toothbrush will be used without addition of toothpaste. Tooth brushing will be done and in circular movement and load of 250 g under water coolant for 10,000 cycles to simulate one year of toothbrushing for all groups.

Surface roughness evaluation:

The mean surface roughness of the enamel and resin composite samples was assessed using a 3D surface profilometer New View 7200 (Zygo Corporation, Chicago, USA). Pre- and post-toothbrushing, the Ra values (arithmetical mean surface roughness) of all samples will be analyzed. The difference between the post-toothbrushing and pre-toothbrushing Ra values (ΔRa = post – pre) will be calculated to quantify the extent of changes in surface roughness induced by the brushing process.

Color stability evaluation

Same samples used for surface roughness evaluation will be evaluated for color stability using a portable spectrophotometer probe (Vita Easyshade III, Vita Zahnfabrik H. Rauter GmbH, Bad Sackingen, Germany) before and after exposed to simulation toothbrushing.

Measurements will do for all the samples on a gray background. Three color parameters will be evaluated: L, a, and b, where L* represents lightness, in which the higher the L value, the greater the lightness; a* represents red color on positive values and green color on negative values; and b* represents yellow color on positive values and blue color on negative values.

The baseline color parameters (L1, a1, and b1) will be measured three times for each specimen at the same time of day, and the average was used to represent the color coordinates before the application of miswak derivatives and thermocycling. The same assessment will be repeated after exposed to toothbrushing simulation and rinsing with distilled water and blot drying with tissue paper as post-toothbrushing records (L2, a2, and b2).

The mean ΔE_{00} values will be calculated by the equation:

$$\Delta E_{00}=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]\times½$$

Where ΔL* is the difference of L*, Δa* is the variation of a*, and Δb* is the variation of b. An ΔE_{00} value of ≥2.8 is considered to correspond to the perceptible threshold, while a value of ≥4.2 is equivalent to the clinically acceptable threshold for color differences.

- **Evaluation of Remineralization Potential of Miswak-Black Silica Toothbrush (In-vitro):**

Twenty enamel samples will be incubated in a demineralizing solution (Ready-to-use buffer solution [pH 4.00 ± 0.05; Thermo Fisher Scientific, USA]) at 37°C for 96 h. Then, they will be randomly selected for treatment with one of toothbrushing technique using a toothbrush simulator as pervious part.

All enamel samples will be assessed using scanning electron microscopy (SEM), at baseline, after artificial demineralization and after surface brushing using either regular soft toothbrush+ Fl toothpaste or Miswak-black silica toothbrush.

- **Statistical analysis:**

❖ **Initial Estimated Budget:**

Materials	Cost	Quantity	Total Cost
Tetric N-ceram, shade A2, Ivoclar Vivadent AG, Schaan, Liechtenstein	110	7	770
GC Fuji II LC: resin modified glass ionomer	530	7	3710
Colgate soft manual toothbrush	12	100	1200
Miswak black silica-combined toothbrush, Shinyei Kaisha, Japan	27	100	2700
Colgate Fluoride containing tooth paste	11	100	1100
Toothbrush simulator (ZM-3; SD Mechatronik, Feldkirchen-Westerham, Germany) Toothbrush+simulator.pdf	37,000	1	37,000
Laboratory fees	25,000	1	25,000
Publishing process fees	19,000	1	19,000
Others (English editing, **printing**, sources…etc.)	8,000	1	8,000
		Total	124,970

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