

# Agents in maintaining oral hygiene

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**AGENTS IN MAINTAINING ORAL  
HYGIENE**

GRADUATE THESIS

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## **AGENTS IN MAINTAINING ORAL HYGIENE**

### **Abstract**

Maintaining oral hygiene is a daily challenge for the oral cavity. The use of certain agents such as toothpaste, manual and electric toothbrushes, mouthwashes, and units for interdental cleaning aids in keeping a stable oral microbiome and prevents the occurrence of oral diseases like caries or periodontitis. This thesis will highlight the most common and most effective oral hygiene tools, their differences, designs, types, and ways of usage to maintain oral hygiene. It will also discuss how different parameters of a manual toothbrush and the amount of toothpaste can affect oral health and microbiome.

**Keywords:** oral hygiene; maintaining oral hygiene; manual toothbrush; toothpaste

# **SREDSTVA ZA ODRŽAVANJE ORALNE HIGIJENE**

## **Sažetak**

Održavanje oralne higijene svakodnevni je izazov za usnu šupljinu. Upotreba određenih sredstava kao što su pasta za zube, ručne i električne četkice za zube, vodice za ispiranje usta te sredstva za čišćenje međuzubnih prostora pomaže u održavanju stabilnog oralnog mikrobioma i sprječava pojavu oralnih bolesti poput karijesa ili parodontitisa. Ovaj će rad istaknuti najčešće i najučinkovitije alate za oralnu higijenu, njihove razlike, dizajn, vrste i načine upotrebe za održavanje oralne higijene. Također će se raspraviti o tome kako različiti parametri ručne četkice za zube i količina paste za zube mogu utjecati na oralno zdravlje i mikrobiom.

**Ključne riječi:** oralna higijena; održavanje oralne higijene; ručna četkica za zube; pasta za zube

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The human mouth hosts a complex ecosystem of bacteria, some beneficial and others potentially harmful. For instance, *Streptococcus salivarius* is a naturally occurring bacterium in the oral cavity that helps combat cariogenic and periodontal infections, contributing to the maintenance of healthy teeth. However, beneficial bacteria like *Streptococcus salivarius* require proper oral hygiene practices to function effectively. Neglecting dental care, such as skipping brushing or flossing, impedes these bacteria from controlling harmful pathogens.

Poor oral hygiene leads to a proliferation of detrimental bacteria, resulting in issues like bad breath, tooth decay, and an increased risk of systemic diseases. Additionally, certain medications, such as decongestants and antihistamines, reduce saliva production, thereby compromising the mouth's natural defence mechanism against infections (1).

The 2022 World Health Organisation (WHO) Global Report on Oral Health Status found that oral diseases affect almost 3.5 billion people worldwide, with three-quarters of those impacted living in middle-income countries. Globally, approximately 2 billion individuals have cavities in their permanent teeth, and 514 million children have cavities in their primary teeth (2).

Diet and lifestyle choices also play a crucial role in oral health. Consuming sugary foods, acidic beverages, and smoking can damage teeth and gums over time. A balanced diet rich in water, fresh fruits, and vegetables is essential for maintaining oral health. Avoiding smoking is critical as it not only dries out the mouth but also heightens the risk of periodontal infections and oral cancer (1, 2).

**2. LITERATURE REVIEW**

## 2.1 Historical perspective on oral hygiene

Care for oral hygiene was already evidently found in cavemen from 1.2 million years ago, with the discovery of wooden pieces inside their interdental spaces indicating the use of a toothpick. In an excavation done in 1847 by a British archaeologist named Henry Layard, 660 tablets with cuneiform writing were unveiled, dating back to the time of Enlil-Bani (1798 – 1775 BCE). The writings on the tablets contained instructions on the use of toothpicks. In addition, they described "curative incantations" for oral diseases and treatments for halitosis and excessive salivation.

Since ancient times, people have dreaded the tooth worm, a mythical creature believed to cause dental pain. Consequently, early civilisations such as the Indians, Babylonians, Egyptians, and Greeks advised cleaning teeth with abrasive substances. Ancient Indian methods included using a mixture of crushed charcoal and salt, applied with a twig-brush. A Babylonian cuneiform tablet describes a mixture of red salt and juniper for dental care. In the 4th century BCE in Greece, Hippocrates recommended using a cloth or woollen ball dipped in honey to apply tooth powder. Galen, a 2nd-century Greek physician who served several Roman emperors, suggested using dried radish powder or finely ground white glass mixed with the essential oil of Indian spikenard (muskroot) for oral hygiene (3).

Tools like toothpicks became less popular due to their increased risk of damaging the periodontal tissue. In the 20th century, tooth powder has been replaced with a softer toothpaste that included ingredients such as borax, sodium bicarbonate, charcoal, and soap, making the paste foamy. Comparing today's toothpaste with the ancient ingredients, there are a lot of similarities. The major improvement lies in the added fluoride, increasing its effectiveness and ability to prevent tooth decay (3,4).

The modern concept of mouthwash emerged towards the close of the 19th century, coinciding with the growing recognition of the bacterial causes behind dental decay, gum disease, and bad breath. These early formulations were tailored with specific ingredients to combat bacterial, fungal, or inflammatory aspects of oral health conditions.

The origin of the first invention of a toothbrush is not totally clear, but it reaches back to the year 1223 when a Japanese Zen master described Chinese monks that were using toothbrushes made of horse hair attached to a bamboo or bone handle (3).

In the 18th century, a comprehensive work dedicated to oral hygiene and health maintenance appeared. The concept of Oral Hygiene truly emerged in the mid-19th century. This period saw the unification of various tools, mouthwash solutions, and cleaning methods into a specialised field, driven by the growing oral health industry. A systematic approach to understanding tissue breakdown and the mechanisms of oral diseases led to the development of oral health products with specific ingredients designed to address issues like enamel and dentine demineralization, caries prevention, tooth sensitivity reduction, plaque prevention, and halitosis elimination.

Initially, these products were sold by dentists or pharmacists. However, as the dental industry expanded in the mid-19th century, more items were marketed through dental suppliers rather than directly by dentists. Products like toothbrushes, toothpaste, mouthwash, floss, toothpicks, and tongue cleaners became integral to daily oral hygiene routines. In 1955, the introduction of the cavitron added a new dimension to dental office care.

By 2017, the global oral care market had reached \$28 billion, with projections estimating it would grow to \$40.9 billion by 2025 (3).

## **2.2 Oral microbiome and the impact on health**

The oral microflora has a wide spectrum of microorganisms that are sensitive to the environment in which they live. We can differentiate the bacteria into the core microbiome, which is existent in many parts of the human body, and a variable microbiome that develops in response to the lifestyle and the individual genotype of a person. The oral cavity is a complex biological environment in which the bacteria settle on specific surfaces that they colonise such as the gingival sulcus, the tongue, the cheek, the hard and soft palates, the floor of the mouth, the throat, the saliva, and the teeth. The main composition of bacterial microflora consists of gram-positive and gram-negative bacteria. Gram-positive bacteria are:

- a. *Cocci* - Abiotrophia, Peptostreptococcus, Streptococcus, Stomatococcus
- b. *Rods* – Actinomyces, Bifidobacterium, Corynebacterium, Eubacterium, Lactobacillus, Propionibacterium, Pseudoramibacter, Rothia.

And gram-negative:

- a. *Cocci* – Moraxella, Neisseria, Veillonella
- b. *Rods* – Campylobacter, Capnocytophaga, Desulfobacter, Desulfovibrio, Eikenella, Fusobacterium, Hemophilus, Leptotrichia, Prevotella, Selemonas, Simonsiella, Treponema, Wolinella.

Besides the bacterial member in the composition, the oral microflora also includes microorganisms such as fungi, viruses, and protozoa. The most common protozoa found are Entamoeba gingivalis and Trichomonas tenax, which mainly obtain food by absorbing dissolved organic material. Among fungi, Candida species are the most frequently seen. A study by Ghannoum et al. on twenty healthy individuals identified 85 different fungal genera, with the most common being Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus (5).

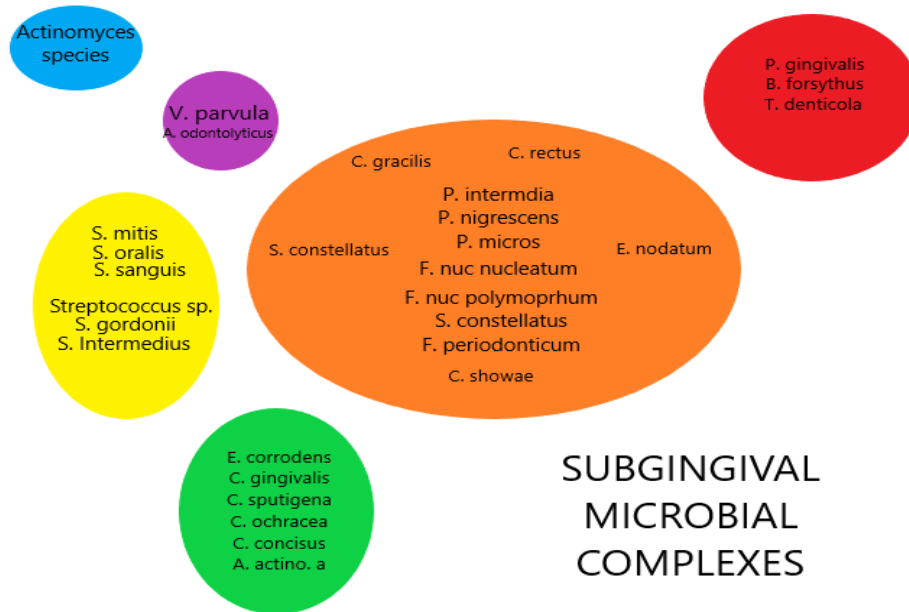
### **2.2.1 Role of oral microbiome in health and disease**

The oral microbiome usually forms a biofilm, which is essential for maintaining oral balance, protecting the mouth, and preventing diseases. Understanding the microbiome's identity and interactions is crucial for knowing the key players involved (7).

Microbial varieties and groups in the human body are vital for various physiological, metabolic, and immunological functions. They aid in digestion and nutrition, energy generation, development of the host mucosa and immune system, fat storage regulation, detoxification of environmental chemicals, skin, and mucosal barrier functions, and maintaining immune balance between pro-inflammatory and anti-inflammatory processes. These communities also promote colonisation resistance and prevent the invasion and growth of pathogens (5).

Increased sugar intake creates acidic conditions in the oral environment causing the demineralization of the tooth layers. The biofilm bacteria metabolise fermentable carbohydrates, resulting in the production of primarily lactic acid as organic acids. These organic acids accumulate in the fluid phase of the biofilm, leading to a decrease in pH and demineralization of the tooth's surface layer. As a result, the enamel porosity increases, causing the spaces between the crystals to widen and the surface to soften. This creates an opportunity for the acids to penetrate deeper into the tooth structure and demineralise the subsurface layers (10).

With the disruption of the homeostasis of the oral microorganism, periodontal disease can be initiated, leading to weakening and damaging of the supportive soft tissue holding the tooth in place. If left untreated, it can result in tooth loss (6, 9). The dysbiotic disease periodontitis appears due to a shift from gram-positive to gram-negative bacteria in the subgingival space. The imbalance of bacteria can be categorised and more certainly depicted within the red complex which can be seen in the following figure:



**Figure 1:** Red complex. Adapted from Mohanty et al. (11).

The group of bacteria known as the red complex, which emerge at a later stage of biofilm formation, consists of species that are recognised as periodontal pathogens. These species include *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (formerly known as *Bacteroides forsythus* or *Tannerella forsythensis*). The red complex represents a significant component of the mature community within biofilms found in areas affected by advancing periodontitis (12).

### 2.2.2 Impact on general health

There are several diseases that are directly linked to oral health conditions such as periodontitis. Heart diseases like endocarditis can result from the spreading of bacteria through the bloodstream, causing an infection of the endocardium. There is also a linkage between periodontitis and birth complications causing premature birth and low birth weight. Besides spreading towards the heart, bacteria can also spread into the lungs, causing pneumonia.

Whilst oral health can impact general health, the reverse can also be true. Conditions like diabetes reduce the probability of acting against infections in the oral cavity. Mucosal lesions are a common appearance in the oral cavity in patients who have HIV/AIDS and autoimmune diseases like Sjogren's syndrome will strongly impact the salivary glands of the oral cavity, causing dry mouth (13).

## 2.3 Mechanical agents for oral hygiene

Biofilm is continuously formed in the oral cavity, which can be controlled and removed by actively applying mechanical force. There are different toothbrushes that can be used for this cause. Mechanical plaque control encompasses various techniques like manual toothbrushes, smart toothbrushes (including power-driven, sonic, and ultrasonic toothbrushes), solar-powered toothbrushes (ionic toothbrushes), disposable toothbrushes, and laser toothbrushes, as well as interdental cleaning aids. The field of mechanical plaque control has seen continuous improvements in quality through technological advancements (11).

### 2.3.1 Manual toothbrush

The manual toothbrush is the most popular type of oral hygiene agent. Different elements come into play in the design of the toothbrush, including the bristle material, fibre length and diameter, the total number of fibres, the length of the brush head, the trim design of the brush head, the number and arrangement of bristle tufts, the angulation of the brush head to the handle, and the handle design.



**Figure 2:** Different manual toothbrush types

Toothbrushes can be classified into three groups based on the hardness of their bristles such as soft, medium, and hard. Each of the classifications has a different diameter: soft = 0.16 mm to 0.22 mm; medium = 0.23 to 0.29 mm; hard = 0.30 mm. The material used for the bristles to remove the plaque efficiently is synthetic nylon which has a length of 11 to 12 mm (14).

Techniques for toothbrushing that are used are the Bass method, Stillman's, Charters', Fones technique, Leonard's technique, Horizontal scrub technique, and Modified Stillman's technique.

1) *Bass Method:*

The recommended brushing technique involves keeping the brush head parallel to the occlusal plane, ensuring it covers about 3 – 4 teeth at a time, starting from the back of the dental arch. The bristles should be positioned at a 45-degree angle to the long axis of the tooth and placed at the gum line. Gentle vibratory pressure is then applied using short, back-and-forth movements, which helps to effectively dislodge plaque and food particles from the teeth and gums.

2) *Stillman's Method:*

Like the Bass method, this brushing technique involves placing the bristles at a 45-degree angle to the tooth. However, unlike the Bass method, the bristles are positioned half in the sulcus (the space between the tooth and the gum) and half on the gingiva (gum). The brushing stroke used in this method is the same as in the Bass technique, ensuring effective plaque removal and gum stimulation.

3) *Charters' Method*

In this brushing technique, the bristles are placed at a 45-degree angle to the gum line, with the bristle tips directed coronally (towards the crown of the tooth). The bristles are then activated by applying mild vibratory strokes, allowing the bristle ends to reach and clean interproximal areas (between the teeth). This method ensures effective removal of plaque and debris from the gum line and between the teeth.

4) *Fones Technique*

Here the brush is placed against a group of teeth and moved in a circular motion 4 – 5 times for each group. This guarantees thorough cleaning through the employment of gentle, circular motions to eliminate plaque and debris from the surfaces of the teeth.



5) *Leonards's technique*

The bristles of the toothbrush are placed at a 90-degree angle to the surface of the teeth. The position of the teeth has to be edge to edge while the brush filaments have to be perpendicular to the long axis of the teeth. With up and down strokes on the tooth surface and a slight rotation, plaque will be removed.

6) *Horizontal scrub technique*

The bristles are placed perpendicular to the tooth axis while soft horizontal scrubbing movements are performed with the brush.

7) *Modified Stillman's technique*

The bristles are directed apically at an oblique angle to the tooth's long axis, positioned on the cervical portions of the teeth and partly on the nearby gingiva. They are then moved using short, back-and-forth motions while being gradually shifted toward the coronal part, repeating this process for 20 strokes.

8) *Hirshfeld's brushing technique*

This technique is similar to the Charters' method, the only difference is that the upper and lower jaw will remain occluded. It involves little circular motions that target the gingival crevice.

9) *Smith-Bell (physiologic) brushing technique*

Here bristles are positioned at the level of the incisal edge and moved toward the gingiva creating a frictional effect like during the action of chewing fibrous food (16).

### 2.3.2 Dental Floss

A dental agent for the interdental spaces is the dental floss. Additionally, it aids the toothbrush in removing dental plaque from all tooth surfaces. It is manufactured with different materials. It is available with flavours, as waxed and unwaxed, thin, tape, and meshwork (14).



**Figure 3:** *Left:* Dental floss for bridges; *Middle:* Waxed dental floss; *Right:* Dental floss with flavour.

### 2.3.3 Powered mechanical plaque removal

Since the 1960s, powered or electric toothbrushes have garnered significant attention. The primary reason for using powered brushes is that many patients struggle to effectively remove plaque due to insufficient manual dexterity. Powered toothbrushes aim to reduce the need for dexterity by incorporating automatic movement of the toothbrush head (14).

Like manual toothbrushes, there are also different types of electric toothbrushes that do not just have different sizes and shapes but also different mechanisms of action:

1. "Side to side action" refers to a brushing motion where the brush head moves laterally from one side to the other.

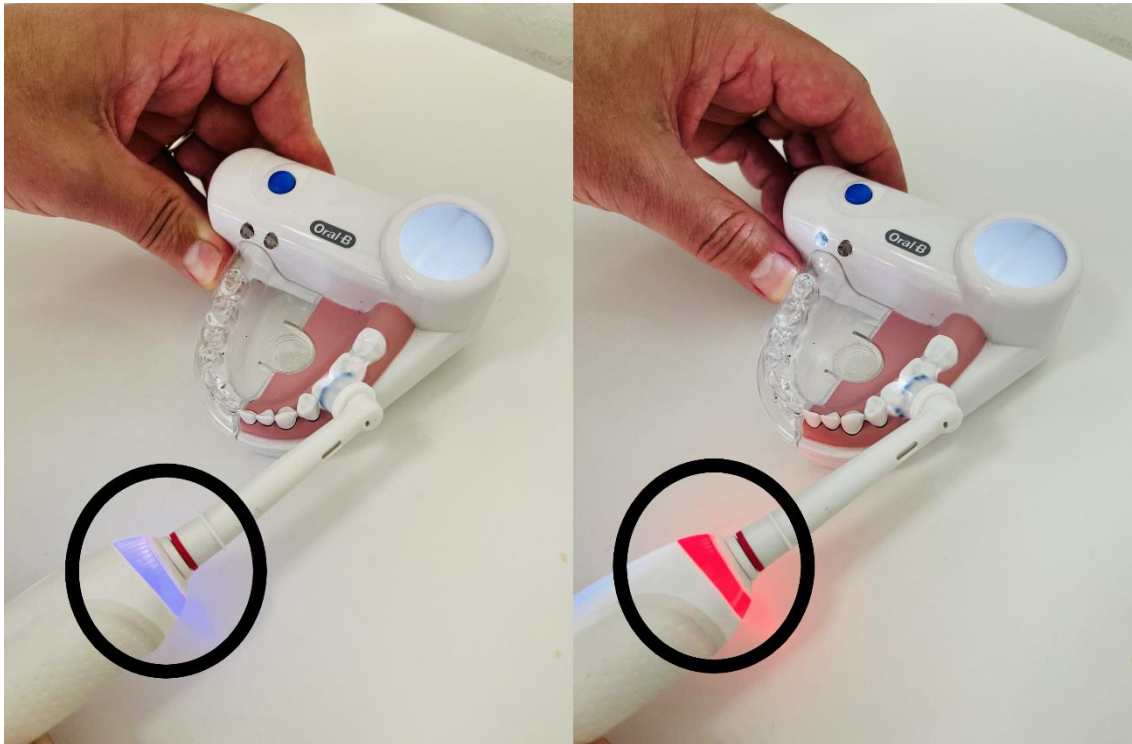
2. "Counter oscillation" describes a brushing technique where adjacent groups of bristles (typically six to ten) rotate independently, with each group rotating in the opposite direction to its neighbour.
3. "Rotation oscillation" denotes a brushing method where the brush head rotates back-and-forth.
4. "Circular" signifies a brushing motion where the brush head rotates in a circular manner.
5. "Ultrasonic" indicates a brushing action where the bristles vibrate at frequencies higher than 20 kHz.
6. "Ionic" characterises a brush designed to electrify the tooth surface, aiming to disrupt the attachment of dental plaque (16).



**Figure 4:** Different types of toothbrushes

Some electric toothbrushes, such as the iO Series 9 electric toothbrush from Oral-B, have integrated magnetic technology which aids the user in cleaning their teeth. Some of those features are a round brush head, smart pressure sensor, interactive colour display, smart brushing modes, and AI brushing recognition (15).

The smart pressure sensor can be seen in the picture below:



**Figure 5:** Smart pressure sensor changing colour depending on pressure; blue = normal pressure; red = excessive pressure.

#### 2.3.4 Interdental brush

Like dental floss, interdental brushes also aid in removing plaque in the interdental spaces. They are constructed with a metal wire core on which soft nylon filaments are connected in a twisted formation. Interdental brushes come in different sizes which are differentiated by colours and different shapes, such as straight and angled. Replacing the wire in the core with rubber can be done to mitigate possible harmful effects of the metal wire (17).

## 2.4 Chemical agents for oral hygiene

### 2.4.1 Antibacterial mouthwash

Mouthrinses are utilised for several purposes: to freshen breath, aid in the prevention or control of tooth decay, decrease plaque, prevent, or diminish gingivitis, slow down the formation of tartar (hardened plaque) on teeth, or achieve a combination of these outcomes. Most mouthrinses can be obtained without a prescription.

Ingredients most often found in mouthrinse are water, alcohol, cleansing agents, flavouring ingredients, and colouring agents. Additionally, they carry active ingredients like antimicrobials that reduce biofilm on the teeth and act against halitosis. Furthermore, fluoride aids in reducing minor lesions on tooth enamel, making teeth more resistant to decay, while astringent salts can temporarily mask bad breath, and odour neutralizers work by chemically inactivating odour-causing compounds like zinc salts, ketone, terpene, and ionone (18).

*Chlorhexidine* (CHX) is a widely utilised antiseptic mouthwash that was developed in the 1940s in the UK and originally used as a disinfectant before its antiplaque ability was discovered in 1976. It is favoured by both dental professionals and the general population, thanks to its antimicrobial properties (18, 19). It is the most potent chemotherapeutic antibacterial agent for reducing *Streptococcus mutans* and the biofilm of the oral cavity (19).

### 2.4.2 Dentifrice

Dentifrice, also known as toothpaste, is used as an aiding compound for the removal of dental plaque, freshening the breath, and whitening the tooth. The most common ingredients of toothpaste are an abrasive agent, a binder, a surfactant, and a humectant; some of them also contain sodium bicarbonate. *Abrasives* are materials used for abrading, grinding, or polishing. They eliminate substances sticking to the surface of the teeth without causing scratches, restoring their natural shine. The prevention of separation of powder and liquid elements is provided by *binders*. They mainly keep the toothpaste moist so it will not dry out by retaining moisture. *Humectants* help in the prevention of water loss and provide hardening of the paste inside the tube. Additionally, *sweeteners* for taste, *colouring agents* for an attractive appearance, and *preservatives* for preventing growth of microorganisms inside the toothpaste are added (20).



**Figure 6:** Different toothpastes

### 2.4.3 Fluoride

The compound fluoride is used for the long-term prevention of tooth demineralization as an ingredient in toothpaste, water, milk, mouthrinses, tooth gels, and varnishes. The common fluoride concentration in toothpaste ranges from 1000 to 1500 parts per million (ppm). It should be mentioned that enamel defects can occur when fluoride concentrations are too high which will result in fluorosis in developing teeth (21).

Fluoride can operate through four mechanisms of action:

- 1) Inhibition of demineralization with the formation of fluoroapatite crystals which have a higher resistance to acidic damage.
- 2) Increased speed of newly formed fluoroapatite crystals by connecting calcium and phosphate ions.
- 3) Impeding the release of acid by the interference of the production of phosphoenol pyruvate which is an important substance in the glycolytic pathway for bacteria.
- 4) Fluoride ions are continuously attached to dental tissue, oral mucosa, and dental biofilm and cause the decrease of demineralization and improve remineralization (22).

#### **2.4.4 Antibiotics**

In dental medicine, antibiotics are commonly used for the therapy of odontogenic and non-odontogenic acute and chronic infections, in prophylactic scenarios to prevent infections in high-risk patients such as those suffering from systemic diseases like endocarditis, and in cases of preventing infections on surgical sites in oral surgery (23). Although antibiotics are generally not used specifically for oral hygiene, they do count as a first-generation antiplaque agent, reducing plaque from 20% to 50% in the oral cavity (24).

### **2.5 The efficacy of different designs of a manual toothbrush**

The efficacy of plaque removal depends on different design parameters of manual toothbrushes, which in turn has a potential effect on oral hygiene. In a published study from 2023, eight types of manual toothbrush designs underwent a robot test on replicated human teeth.

#### **2.5.1 Parameters**

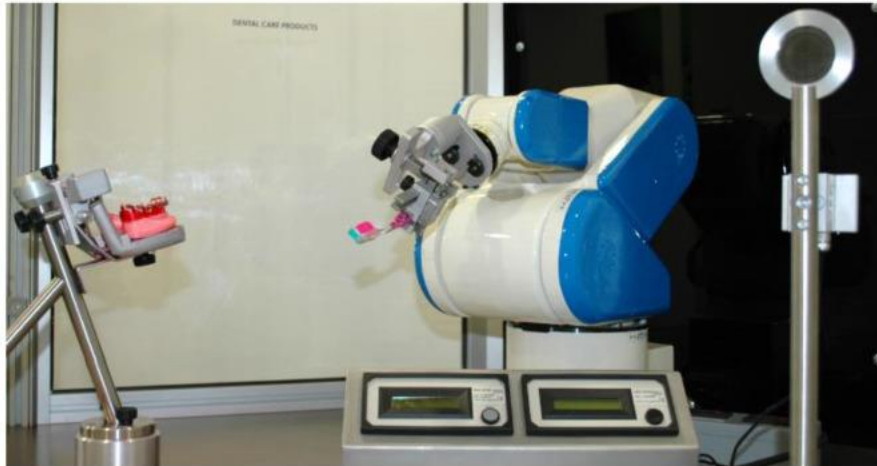
Design parameters were:

- (1) Head-size
- (2) Filament-diameter
- (3) Cutting-height
- (4) Hardness
- (5) Interdental-height

The movements that were tested were horizontal, rotating, and vertical and each test was repeated five times.

### 2.5.2 Determination of tooth surfaces

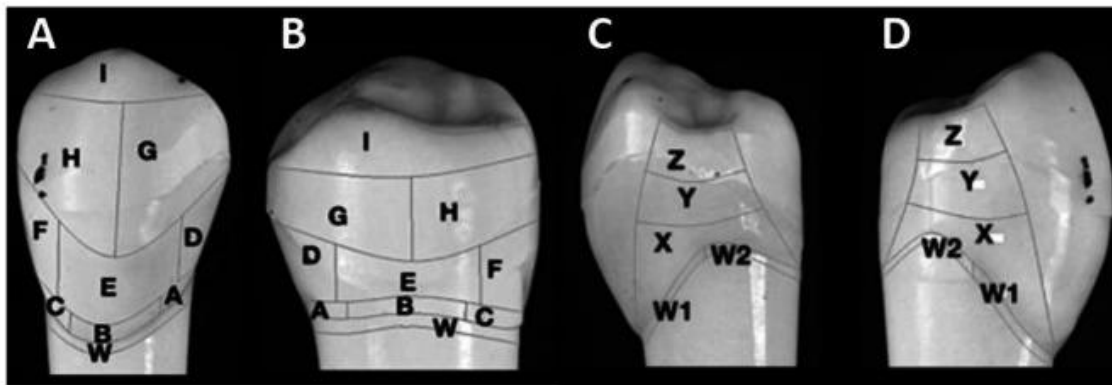
A method called automatic plaque planimetry (APP) was used to determine how well plaque was removed. This involves analysing 30 specific areas on the teeth. Each tooth is rotated in front of a high-definition camera that helps a computer process the images.



**Figure 7:** Robot toothbrushing set-up. Taken with permission of the publisher: ORMED Institute for Oral Medicine at the University of Witten/Herdecke (25).

The amount of plaque (a sticky film of bacteria) on each tooth is measured before and after brushing. This measurement is done for seven different parts of the tooth surfaces: all surfaces facing the cheeks (buccal surfaces), all surfaces facing the tongue (lingual surfaces), specific risky areas near the gum line and between the teeth, surfaces closest to the centre of the mouth (mesial surfaces), surfaces farthest from the centre of the mouth (distal surfaces), and an overall average reduction in plaque across all 30 areas. Areas below the gum line (W fields) are included in the overall measurements but are not shown separately in the results.



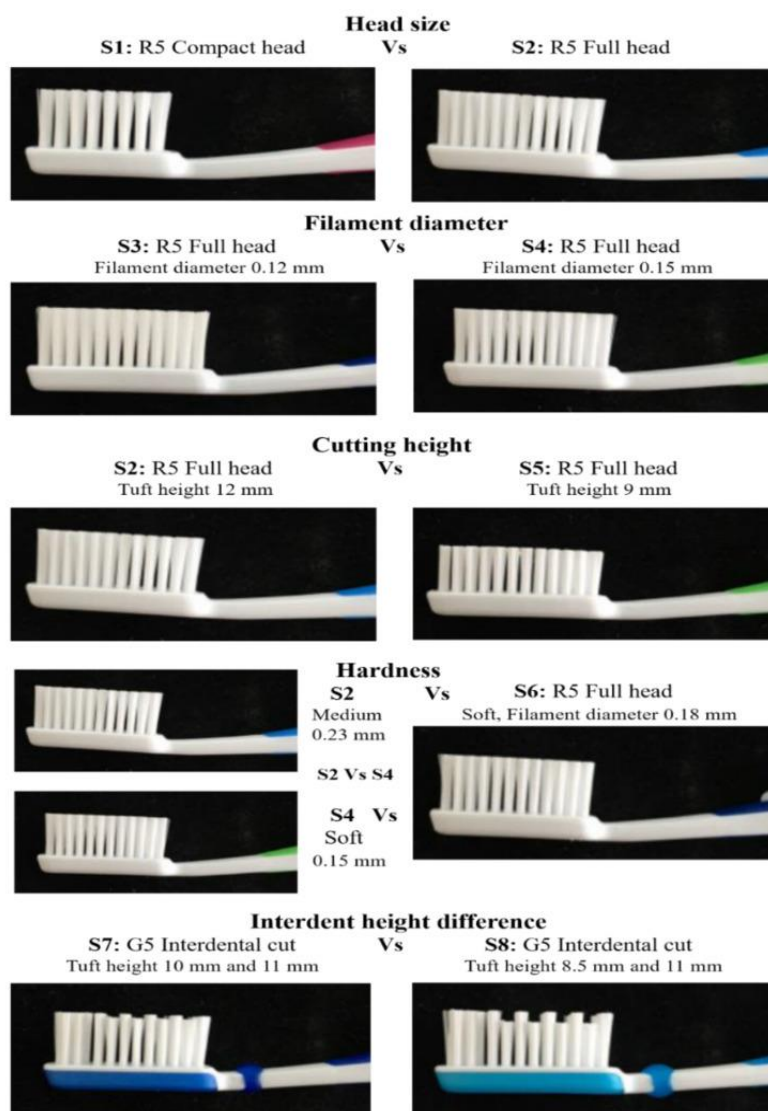


**Figure 8:** Variables of tooth surfaces. Taken with permission of the publisher: ORMED Institute for Oral Medicine at the University of Witten/Herdecke (25).

The automated plaque detection fields covered seven different tooth surface variables: (A) buccal sites (towards the cheek), (B) lingual sites (towards the tongue), (C) mesial sites (between teeth, closer to the front), (D) distal sites (between teeth, closer to the back), ABCDF fields (risk areas near the gum line and between teeth), and the total mean plaque reduction across all 30 tooth sites as can be seen in Figure 8.

### 2.5.3 Toothbrush designs and calculation of distribution

The toothbrushes were compared by their head size, filament diameter, tuft height, hardness, and interdental height difference. Eight manual toothbrushes from the company Dr Best with rounded nylon filaments were used. Each of them featured different properties that can be seen in the picture below:



**Figure 9:** Eight Dr Best® toothbrushes, Haleon (Formerly GSK Consumer Healthcare, Brentford, UK) with differing properties. Taken with permission by ORMED Institute for Oral Medicine at the University of Witten/Herdecke (25).

For the statistical analysis and testing, the ORMED Institute used the Kolmogorov-Smirnov test to check if the data from 13 tooth surface variables followed a normal distribution. The results of this test showed that 4 out of 13 variables, which were buccal, lingual, buccal W, and total, did not follow a normal distribution. For the remaining non-normally distributed variables, the Wilcoxon-Mann-Whitney U-test (WMW) was applied, which is a suitable test for unknown distributions. As an additional test, the Levene test and t-test helped to check if the variances of the toothbrush groups were equal. Finally, the significance level for cleaning efficacy between brushes was set at a value of 5%.

#### 2.5.4 Statistical Analysis

##### *Cleaning efficacy for head size*

S1 performed better in horizontal movements while S2 performed better in vertical movements. Both toothbrushes were similar in plaque removal with rotating movements.

##### *Cleaning efficacy for filament diameter*

S3 is significantly more efficient in removing simulated plaque than S4 across different brushing movements.

##### *Cleaning efficacy for cutting height*

The comparison between cutting heights showed that S2 (the larger cutting height brush) was significantly better at removing simulated plaque compared to S5 (the smaller cutting height brush). The difference in efficacy between S2 and S5 was statistically significant ( $p < 0.05$ ).

##### *Cleaning efficacy for interdental height*

The overall performance in horizontal movements of S7 in interdental plaque removal showed a better removal in comparison to S8. In rotating and vertical movements, the S8 brush with a larger interdental cut removed a higher percentage of plaque in the interdental area compared to S7.

##### *Cleaning efficacy for hardness*

Each of the toothbrush hardness was compared to the other: S2 vs S4; S2 vs S6; and S4 vs S6.

For horizontal movements, the soft S4 brush outperformed the medium hard S2 brush in all tooth fields, while the soft S6 brush was better in two fields compared to S2's better performance in four fields, though only two of these were significantly better.

In rotating movements, the soft S4 brush was generally more effective than the S2 brush. The soft S6 brush showed greater efficiency overall and in three specific fields compared to the S2 brush.

For vertical movements, there were no significant differences between the S2 and S4 brushes. The S2 brush was more efficient in three areas compared to one for the S6 brush, though there was no significant advantage in the total area.

Overall, the soft S6 brush was statistically more effective in plaque removal, particularly in rotating movements. When comparing the two soft brushes, the S4 brush performed significantly better in the Buccal and Lingual areas and most movements, while the S6 brush was superior in the Mesial and Distal areas. For total area, the S6 brush had a significant advantage only in rotating movement (25).

### **2.5.5 Overview of results**

In this study, the parameters of a toothbrush have been tested and simulated with three different types of movements simultaneously. The highest efficiency was shown to be the rotational movement in all design parameters. The optimum parameters for a toothbrush are: a compact head, a small filament diameter (0.12 mm), a larger tuft height (12 mm), a soft brush (0.15 mm or 0.18 mm), and a greater difference between tuft heights (8.5 and 11.0 mm).

The study found that the soft S4 brush was generally more efficient than the medium hard S2 brush in all areas. For manual toothbrush head size, the full head S2 brush was more effective in vertical movements, while the compact S1 brush was better in horizontal movements; both were similar in rotating movements. This suggests that a brushing technique is important and that combining different movements might be beneficial for specific dental issues, though caution is needed with horizontal movements in dental erosion.

The findings of this in vitro study on robotic brushing indicate that longer, softer filaments or those with varying lengths are more effective in removing simulated plaque compared to shorter and harder filaments typically found in standard toothbrushes with a larger, flat trim and stiff bristles. These results have the potential to enhance toothbrush design and performance through valuable insights gained from this research (25).

## **2.6 Efficacy of toothpaste amount on cleaning effect**

The toothpaste amount plays an important role in achieving the optimum dental care for the removal of plaque as shown by a 2023 study reviewing the efficacy of toothpaste amount on the cleaning effect (26).

### **2.6.1 Overview of method and toothpaste amounts**

In this study, 32 extracted human molars were tested. For preparation of the toothpaste appliance, the teeth were stored and covered in epoxy resin and etched with 1% hydrochloric acid solution to facilitate the adhesion of polyphenols in the staining media. After that, saturated sodium carbonate solution was added for a duration of two minutes, followed by 1% phytic acid solution for one minute.

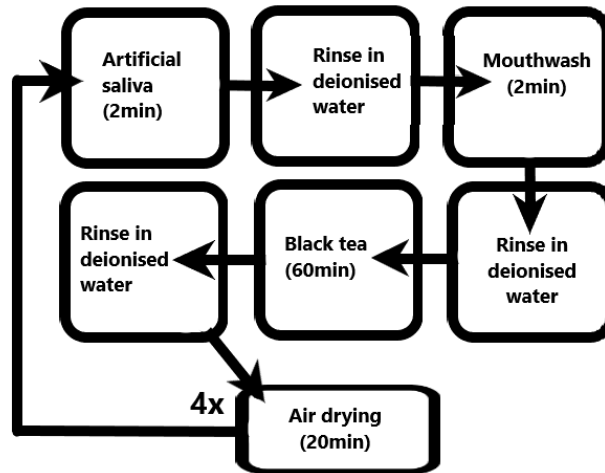
For the purpose of testing, four groups were established. Each group had a different toothpaste amount which was based on recommendations of the studies by the European Academy for Paediatric Dentistry (27).

The first group includes a sample with a full length of toothpaste on the brush, with the maximum amount being 1.00 grams which represents the recommended amount for children above 6 years. The second group comprises a slightly lesser amount of toothpaste sample, specifically 0.50 grams. This group is also suggested for children above 6 years of age.

For children between ages 2 and 6, the study specifies a pea-sized amount of toothpaste, quantified as 0.25 grams. Finally, for children under 2 years, the recommended amount is even smaller, being described as a grain of rice-sized amount, or 0.125 grams of toothpaste. These groups help differentiate the efficiency of various toothpaste amounts in removing plaque across different age ranges (27).

The toothpaste that was used had a relative dentine abrasion value of  $60.19 \pm 1.35$  and each of the four toothpaste samples were diluted into 1 mL of water which aids in creating a simulation of saliva during the brushing.

To achieve proper teeth staining to see the stain removal at the end of the procedure, the 32 teeth samples underwent a staining process that was adapted according to the modified protocol from Lath et al. (28). The steps are shown in the following picture:



**Figure 9:** Staining procedure. Adapted from Sandra et al. (28).

The V8-brushing simulator aided in acting as a sample holder and simulating the toothbrush movements. Prior to brushing, the toothbrushes (Signal Kinder Milchzahn toothbrush, for children aged 0–6 years, Unilever, Germany) were moistened with deionized water for 5 seconds. The toothbrushes were moved in a back-and-forth motion over the sample surfaces with these settings: Brushing load 1.5 N and brushing frequency 2.5 Hz. Samples were brushed with horizontal movements for durations of 10, 30, 60, 120, 180, and 300 seconds using a toothpaste slurry with specific dilution ratios (1:1, 1:2, 1:4, and 1:8). After brushing, the samples were thoroughly rinsed in deionized water.

### 2.6.2 Photographic documentation and colour measurements

For a better overview of the staining differences, photographs were taken before and after the brushing with the help of a reflex camera (EOS 600D, Canon Germany GmbH, Germany). Colorimetric measurements have been done by calculating the stain removal initially, after staining, and after brushing for which the following formulas have been used:

$$\text{Stain removal / \%} = \frac{\Delta E_1}{\Delta E_2} \cdot 100$$

**$\Delta E_1$ :** colorimetric difference between brushed and stained specimen

**$\Delta E_2$ :** colorimetric difference between initial situation and stained specimen

$$\Delta E 1 = \sqrt{\Delta L_1^2 + \Delta a_1^2 + \Delta b_1^2}$$

$$\Delta E 2 = \sqrt{\Delta L_2^2 + \Delta a_2^2 + \Delta b_2^2}$$

$\Delta L 1 = L$  after brushing –  $L$  after staining

$\Delta L 2 = L$  initial –  $L$  after staining

$\Delta a 1 = a$  after brushing –  $a$  after staining

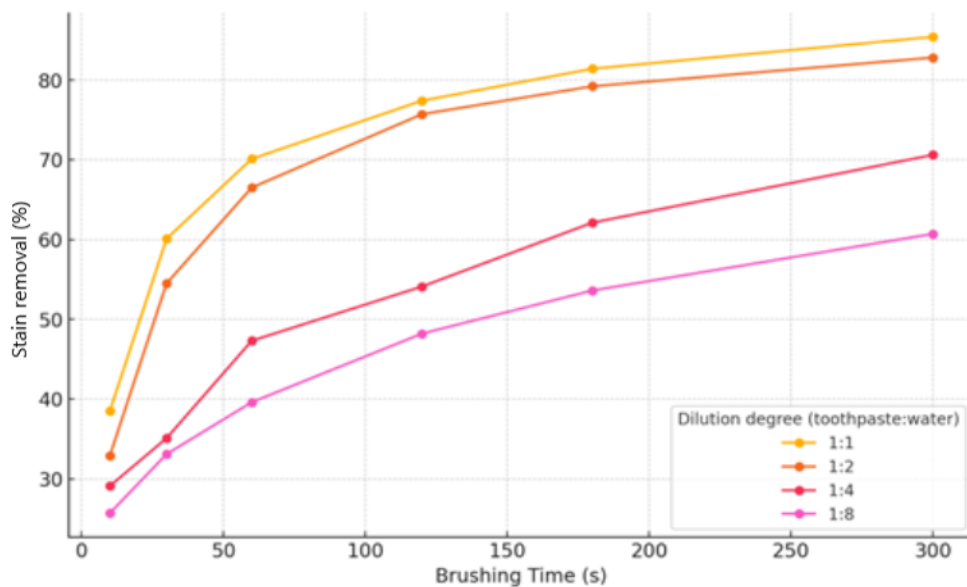
$\Delta E 2 = a$  initial –  $a$  after staining

$\Delta b 1 = b$  after brushing –  $b$  after staining

$\Delta b 2 = b$  initial –  $b$  after staining

For testing the normal distribution, post hoc Tukey test and Levene’s test were used for analyses of homogeneity of variance. A significance level was set to  $\leq 0.05$ .

Finally, the stain removal (%) was set in dependence of the brushing time and dilution degree.



**Figure 10:** Effect of brushing time and dilution on Stain removal adapted from Sandra et al (26).

Results of this study have shown that the longer the brushing time, the more effective the stain removal was for all dilution levels. The 1:1 dilution removed about 40% of the stain and reached the highest stain removal for all brushing times followed by 1:2, 1:4, and 1:8 dilutions. Within

10 seconds of brushing, the diluted 1:1 toothpaste reached a stain removal of 40% while the 1:8 dilution removed about 40% after 60 seconds of brushing time, which shows that the cleaning efficiency was reduced by a significant amount. The dilution 1:8 achieved a stain removal of around 60% after 300 seconds, while the 1:1 dilution showed the same cleaning efficiency (60%) after only 30 seconds.

The stain removal progression when brushing with a dilution degree of 1:1 and 1:2 showed inconsistent behaviour, depending on the brushing time. Initially, the stain removal increased until the 120-second mark, but after that, the increase was only marginal.

Comparing the two dilution degrees, it was found that at all brushing times, the stain removal was slightly lower for the 1:2 dilution degree, although this difference was not statistically significant. On the other hand, for the 1:1 dilution degree, significantly higher cleaning efficacy was observed compared to the 1:4 and 1:8 dilution degrees, across all brushing times. No statistical significance was found for the 1:4 and 1:8 dilution degrees after 10, 30, 60, and 120 seconds (26).

### **2.6.3 Overview of results**

This study expressed the importance of not only the toothpaste amount but also the way and the time of applying toothpaste. For the total result, the amount of 1.00 g of toothpaste has a significantly higher efficiency in all brushing times in comparison to 0.25 g and 0.125 g. Toothpaste itself has a crucial role in the cleaning efficiency. However, the only parameter that was changed during this study was the amount of toothpaste and not any other parameter such as concentration, the type of toothbrush, toothbrush formulation, and dilution medium, which could also affect the results (26).





The variety of tools that can be used for maintaining oral hygiene can be very specific and slight changes of certain parameters can result in crucial differences that affect the microbiome of the oral cavity as shown by the previously mentioned studies (25, 26).

Even though both the study for toothpaste amount and the study of the impact of manual toothbrush design on plaque removal efficacy were aimed at proving their individual importance, they have also shown that parameters such as technique, movements, and time are important factors that need to be considered during the studies and cannot be neglected (25, 26).

It is important to say that both in vitro studies were simulations and brushing was performed with the help of machines that are more consistent and very precise but also very efficient in doing so (29). It can be argued whether the conditions in which the materials and tools were tested were completely realistic since humans have their individual brushing techniques including frequency and amount of pressure that is applied to their teeth and differing oral microbiomes affected by diet and environment (30, 31, 32). It should be mentioned that an attempt was made to replicate real clinical conditions as closely as possible by using general brushing techniques (horizontal, vertical, circular) (25) and using artificial saliva for diluting toothpaste (26). A study by Tomas et al. showed an important correlation between robot toothbrushing simulation and clinical standardized toothbrushing which enables an efficient, quick, and consistent laboratory testing of tooth cleaning (33).

While toothpaste amount plays an important role, the study mainly focused on suggested ingredient amounts for children above 6, between 2 and 6, and below 2 years of age (26, 27). The ingredients like calcium phosphate (hydroxyapatite) compounds and fluoride that can be regulated for different ages could influence the toothpaste's effectiveness as well and should be further investigated (34, 35).

Brushing itself can remove plaque, as we have seen from the parameter efficacy study, and could be used alone without toothpaste, as shown by this systematic review by Cees et al. (36), but in another study from Tellefsen et al., we see that brushing alone will not remove as much biofilm as brushing in combination with toothpaste (37).



The combined findings from these two in vitro studies highlight important considerations for both toothpaste efficacy and toothbrush design.

The study on the impact of manual toothbrush design on plaque removal efficacy revealed that toothbrushes with longer, softer filaments and variable filament lengths achieved better simulated plaque removal compared to standard toothbrushes with short, hard filaments and a larger head. This suggests that modifying the toothbrush design could enhance cleaning efficiency.

Regarding the influence of the amount of toothpaste on cleaning efficacy, the study demonstrated that a medium-abrasive, hydrated silica-based toothpaste exhibited significantly higher cleaning efficacy with an increased amount of toothpaste, suggesting that higher quantities of toothpaste improve cleaning performance.

Together, these studies provide valuable insights into optimising both toothpaste usage and toothbrush design to improve oral hygiene. Both studies underscore the importance of further clinical studies that are essential to verify the in vitro results to confirm the optimal toothpaste amount for effective oral hygiene and to determine the practical implications of toothbrush design parameters.

The variety and availability of modern oral care products allow us to maintain a healthy oral environment. However, it is not only the use of these products but also the approach to using them that matters. This includes considering factors such as ingredients, application time and method, and product design. Continued research into these factors is essential to discover the most effective ways to maintain optimal oral hygiene.



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Fayas Adel graduated from Wittekind Gymnasium in Lübbecke, Germany, in 2017, earning a diploma that qualified him for university admission. He pursued his passion for dental medicine by enrolling in the School of Dental Medicine at the University of Zagreb in Croatia in October 2018.

Throughout his academic journey, Fayas Adel has amassed significant practical experience through various internships. He interned at the private practice of Dr Niklas Lehmann, a specialist in implantology, in Bad Oeynhausen, Germany, in September 2021, and later at the polyclinic of Dr Alexander Schembri in Msida, Malta, in September 2023. During his studies, Fayas Adel collected valuable knowledge in gaining hands-on experience in restorative dentistry, fixed and mobile prosthodontics, pediatric dentistry, and oral surgery, all under expert supervision.

Furthermore, Fayas Adel has completed 500 working hours at the general healthcare centre Dom Zdravlja MUP-a RH in Zagreb, Croatia, under the guidance of Dr Branimir Bebek.

The extensive clinical exposure has provided him with a robust foundation in dental medicine. As Fayas Adel approaches the completion of his studies, his academic and practical experiences underscore his readiness to make significant contributions to the field.