IN VITRO: EVALUATION OF DIFFERENT MOUTH WASHES ON DENTAL BIOFILM FROM OF ORTHODONTIC LIGATURE

S.F. Hussain, F. H. AL-BAYATY, N. R.Abdul Rahim, M. A. Abdulla & Hasnah bt Belgum

1ST IID FACULTY OF DENTISTRY 19/10/2012

ABSTRACT

Clinical study has shown that patients who get the orthodontic treatment are more susceptible for having the enamel white spot formation (Eliades et al., 1995) (Badawi et al., 2003)
Since the advent of fixed orthodontic appliance for adult patientsand its use has become increasingly popular, bringing about the need to address questions regarding microorganism adherence and biofilm development (Menzaghi, 1991; Lee, 2000)
Objectives: To compare the antimicrobial effects of commercially available mouth washes and new herbal mouth wash (Miswak) on dental biofilm isolated from orthodontic ligatures and to assess bacterial morphology before and after treatment with mouthwashes.
Material and method: Four commercially available mouth washes was included in this study (Oradex, Listerine, Oral B, and Colgate plax), one new herbal mouth wash (Miswak aqueous and alcohol extracts) and sterilized distilled water was used as a control. Orthodontic elastic and stainless steel ligatures were collected from 25 patients after 3 week of orthodontic treatment. Bacteria isolated then the gram stain used to identify the bacteria attached to orthodontic ligatures. Antimicrobial activity assessed by Minimal Bactericidal Concentration test (MBC) to determine the lowest concentration of bacteria inhibited by the fixed amount of tested mouthwashes after an overnight aerobic and anaerobic incubation at 37°C. Assessment of bacteria morphology performed with Scanning Electron Microscopy (SEM) before and after treatment with mouth washes.
Results: Only cocci gram positive bacteria identified from ligature of all samples. MBC showed absence of bacteria growth in all tested mouthwashes. SEM examination of bacteria showed variable alterations in the morphology of the isolated bacteria.
Conclusion: This study showed that Miswak ethanol extract and commercially available mouth washes exhibit strong antimicrobial activity against gram positive cocci. While oradex represents significant morphological changes comparing to other mouth washes. On the other hand Miswak aqueous extract seems to have weak antibacterial effects against all tested bacteria samples.

OBJECTIVES

1. To compare the antimicrobial effects of four commercially available mouth washes on dental biofilm isolated from orthodontic elastic ligature.

2. To assess bacterial morphology before and after treatment with mouthwashes under Scanning Electron Microscope (SEM).

NOVELTY

1. No previous study investigate and compare antimicrobialthe effect of the four commercially available mouth washes and herbal mouth wash miswak alcoholic and aqeous extract on dental biofilm isolated from orthodontic elastic ligature.
2. The salvardora persica (Miswak) mouth wash formulated by our researchers. and not exist in the market in Malaysia

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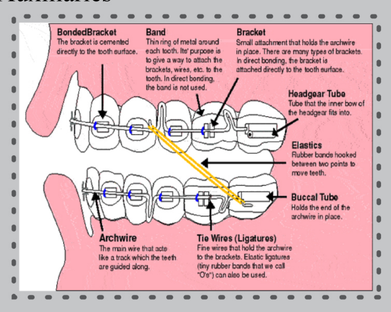
INTRODUCTION

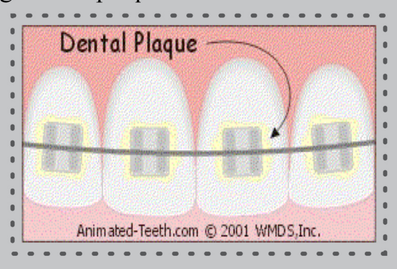
Fixed Orthodontic Appliance

Appliance that temporarily cemented or bonded on the patient teeth which can't be removed by patient (Daljit, 2008).
Components:
- Brackets
- Separators
- Archwires
- Elastomeric modules
- Auxillaries



Dental Biofilm Bacteria

Definition: community of bacteria and their extracellular polymers that are attach to a surfaces of appliance.
It can lead to enamel decalcification, dental caries and periodontal disease.
Eg: dental plaque



Mouth Washes

- Also well-known as mouth rinse.
- According to Oxford Medical Dictionary, a mouthwash is defined as an aqueous solution with antibacterial, astringent, or deodorizing properties used for the rinsing of the mouth and teeth.
- Come with different active ingredients and flavor (Almas et al., 2005).
- It manufactured in two forms:
  - Spray - Wash

Clinical study has shown that patients who get the orthodontic treatment are more susceptible for having the enamel white spot formation (Eliades et al., 1995).

The fixed orthodontic appliance remain in the mouth for a relatively long time as a consequence the properties of the fixed orthodontic appliance and the quantity and quality of bacterial accumulation on the fixed orthodontic appliance materials play decisive roles in their failure
(Eliades et al., 1995).

The use of fixed appliances is a significant challenge to the patient for maintaining good oral hygiene to avoid or minimize decalcification of enamel during treatment resulting in higher incidence of white spot lesions in orthodontic patients (Badawi et al., 2003). The same concept can be applied for fixed orthodontic appliance material.

Recent findings have shown that the used of fixed orthodontic appliance such as metallic orthodontic brackets can inflict ecological changes in the oral environment like decreased pH and increased the plaque accumulation which may elevate the Streptococcus mutans colonization
(Ahn et al., 2002)

(Gjermo et al.,1989)

Chlorhexidine has some unpleasant side effects restricting its general use such as :
- bitter taste
- staining of the teeth & composite restorations
- sloughing of the oral mucosa
- Allergic reactions have been reported in some patients especially Asians

In order to overcome such side effects World Health Organization (WHO) advice researchers to investigate the possible use of natural products such as HERB and PLANT EXTRACTS

Salvadora Persica (Miswak) is a medical plant whose roots has been used by many people in Africa, South America Middle east and Asia (Almas, 1993).

Chewing Sticks (MISWAK- Siwak)
The practice of teeth cleaning by chewing stick had been known since antiquity. The precise method for the use of implement spread by Babylonian 5000 B.C. and the fashion ultimately spread throughout the Greek and Romanian empire. (Lewis and Lewis, 1977)

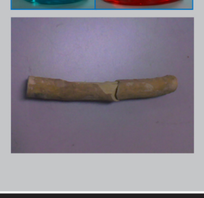
(WHO) has recommended and encouraged the use of these sticks MISWAK- Siwak as an effective tool for oral hygiene. (WHO, 1987)
Due to its mechanical action of the soft wood fibers, and its therapeutic action of a chemical constituent . (Khoory 1983)

PROBLEM STATEMENT

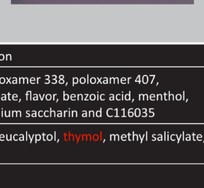
Since the advent of increased orthodontic treatment for adult patients, the use of fixed orthodontic appliance has become increasingly popular, bringing about the need to address questions regarding microorganism adherence and biofilm development (Menzaghi, 1991; Lee, 2000

MATERIALS

Experimental components of fixed orthodontic appliance:
Elastic ligature



Stainless steel ligature



Mouth washes being used:
- Colgate plax
- Listerine
- Oradex
- Oral B
- Herbal mouthwash (Miswak)

Sterilized distilled water (control)

Mouth washes	Composition
Colgate plax	Water, glycerin, sorbitol, propylene glycol, poloxamer 338, poloxamer 407, PEG-40hydrogenated castor oil, sodium benzoate, flavor, benzoic acid, menthol, cetylpyridinium chloride, sodium fluoride, sodium saccharin and C116035
Listerine	Water, ethanol, benzoic acid, poloxamer 407, eucalyptol, thymol, methyl salicylate, menthol and caramel
Oradex	Chlorhexidine gluconate 0.12%w/v
Oral B	Cetylpyridinium chloride 0.053%w/w, sodium fluoride 0.05%w/w, sodium benzoate 0.025%w/w, methylparaben 0.1%w/w, propylparaben 0.01%w/w, purified water, glycerin, flavor, polysorbate 20, sodium saccharin, C142051 and C147005

Table 1: The composition of the mouth washes.

METHODOLOGY

Overview Methodology

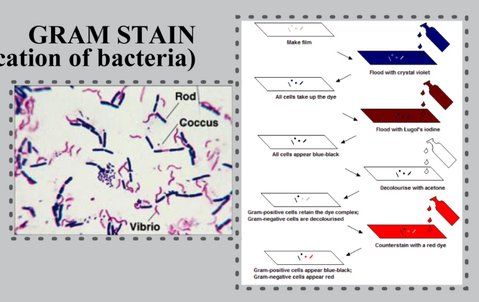
Identification of isolated bacteria attached on components of fixed orthodontic appliance
- GRAM STAIN -

Study of antimicrobial properties of the mouth washes

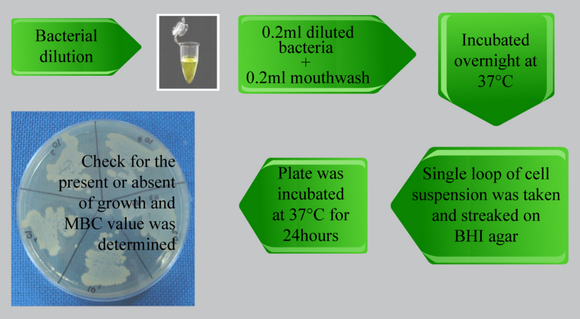
Assessment of antibacterial activity | Minimal Bactericidal Concentration Test

Assessment of bacteria morphology | Scanning Electron Microscope

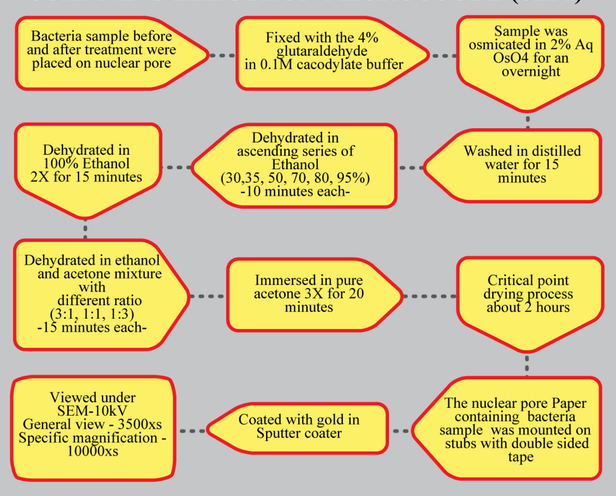
GRAM STAIN (identification of bacteria)



-Assessment of antibacterial activity- MINIMAL BACTERICIDAL CONCENTRATION (MBC)



-Assessment of bacteria morphology- SCANNING ELECTRON MICROSCOPE (SEM)



RESULTS

a) Identification of the isolated bacteria

Sample	Gram stain	Morphology
Elastic ligature	Gram positive	Cocci
Wire ligature	Gram positive	Cocci

Table 1: The identification of isolated bacteria from different components of fixed orthodontic appliance under light microscope under magnification 1000x

b) Assessment of antibacterial activity – MBC test

Sample: Elastic and SS ligature (Gram positive cocci)

Mouth wash / Bacterial dilution CFU/ml	10^5	10^4	10^3	10^2	10
	Bacterial Growth				
Colgate Plax	_	_	_	_	_
Listerine	_	_	_	_	_
Oral B	_	_	_	_	_
Oradex	_	_	_	_	_
Miswak Alcoholic extract	_	_	_	_	_
Miswak Aqueous extract	+	+	+	+	+
Sd H2o (control)	+	+	+	+	+

+ = bacteria growth - = no bacteria growth

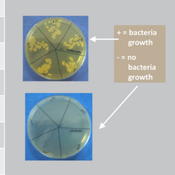
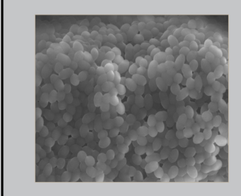


Table 2: The presence and absence of the bacteria growth in various mouth washes with different concentration of bacteria seen on agar plate.

c) Assessment of bacteria morphology - SEM

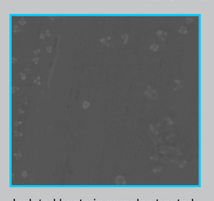
Sample: ligature (Gram positive cocci)

Before treatment

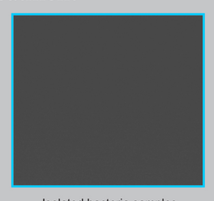


15.0kV Mag: 15000x

After treatment



Isolated bacteria samples treated with Oral B



Isolated bacteria samples treated with Oradex

Magnification: (X2000, 15KV)

CONCLUSION

This study showed commercially available mouth washes (Oradex, Listerine, Oral B, and Colgate Plax) exhibit strong antimicrobial activity against gram positive cocci.
Oradex represents significant morphological changes comparing to other mouth washes.
. Salvadora persica (Miswak) alcohol extract demonstrated strong antimicrobial activity on the other hand Miswak aqueous extract that showed weak antibacterial effects.
Miswak can be as good as the commercially mouthwash in the market.

COMMERCIALIZATION/ USEFULNESS

- Recommendation and advice the orthodontic patients to use Listerine(contain alchohol) , Oral B, Oradex, and Colgate Plax as chemical plaque (dental biofilm) control
- Miswak can be commercialized as new herbal mouth wash with strong antibacterial effect in the market

IMPACT TOWARD SOCIO ECONOMY

- The Miswak mouth wash could be of great benefit to the society because it is cheap and with no side effect.

- The Miswak mouth wash cab be recommended to be use to treat gingival inflammation, oral ulceration and control plaque (dental biofilm) in patient with orthodontic appliance.