

ON DENTAL BIOFILM FROM ORTHODONTIC ELASTIC LIGATURE

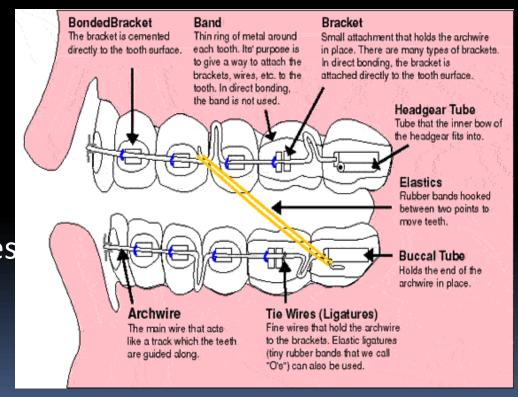
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FIXED ORTHODONTIC APPLIANCE

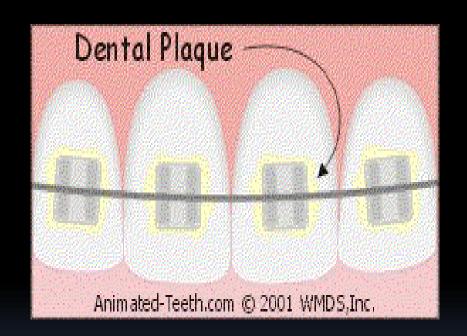
 Appliance that temporarily cemented or bonded on the patient teeth which can't be removed by patient (Daljit, 2008).

- 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 αl., 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)

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)

OBJECTIVES

- To compare the antimicrobial effects of four commercially available mouth washes on dental biofilm isolated from orthodontic elastic ligature.
- To assess bacterial morphology before and after treatment with mouthwashes under Scanning Electron Microscope (SEM).



MATERIALS

- Experimental components of fixed orthodontic appliance:
 - Elastic ligature
- Mouth washes being used:
 - Colgate plax
 - Listerine
 - Oradex
 - Oral B
- Sterilized distilled water (control)



Table 1: The composition of the mouth washes.

Mouth washes	Composition
Colgate	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
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, preserve 21%w/w, purified water, glycerin, flavor, resultation fluoride agents
Oradex	Chlorhexidine gluconate 0.12%w/v



OVERVIEW METHODOLOGY

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



Study of antimicrobial properties of the mouth washes

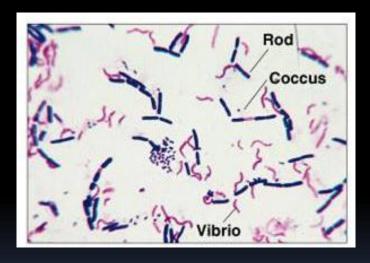
Assessment of antibacterial activity

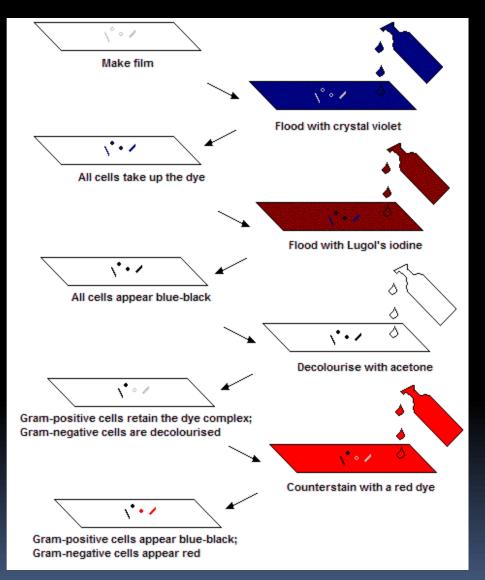
Minimal
Bactericidal
Concentration
Test

Assessment of bacteria morphology

Scanning Electron Microscope

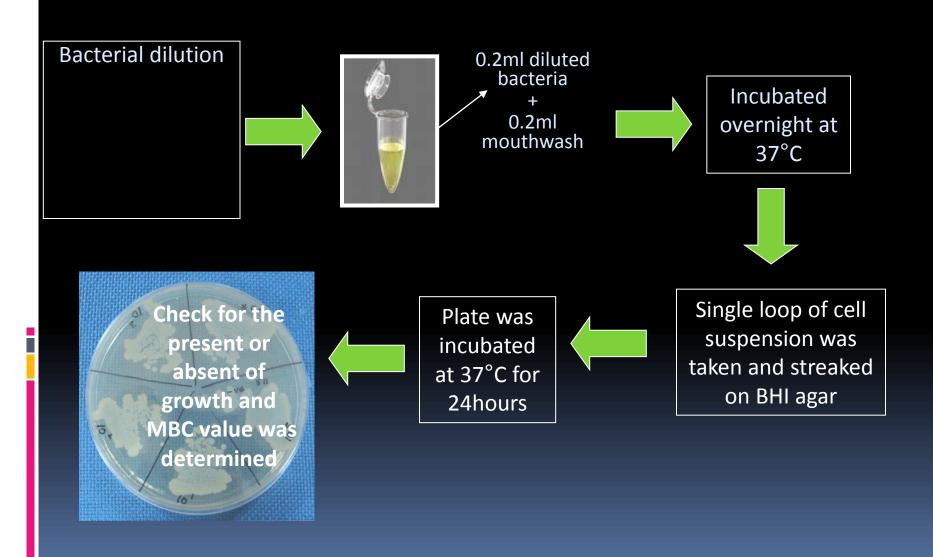
GRAM STAIN identification of bacteria)





(www.bmb.leeds.ac.uk/.../classification/Gram.html)

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



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

Bacteria sample before and after treatment were placed on nuclear pore



Fixed with the 4% glutaraldehyde in 0.1M cacodylate buffer



Sample was osmicated in 2% Aq OsO4 for an overnight



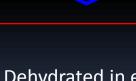
Dehydrated in 100% Ethanol 2X for 15 minutes



Dehydrated in ascending series of Ethanol (30,35, 50, 70, 80, 95%)



Washed in distilled water for 15 minutes



Dehydrated in ethanol and acetone mixture with different ratio
(3:1, 1:1, 1:3)
-15 minutes each-



Immersed in pure acetone 3X for 20 minutes



Critical point drying process about 2 hours



The nuclear pore Paper containing bacteria sample was mounted on stubs with double sided tape



Coated with gold in Sputter coater



Viewed under SEM-10kV General view - 3500xs Specific magnification - 10000xs





a) Identification of the isolated bacteria

Table 1: The identification of isolated bacteria from orthodontic elastic ligature under light microscope under magnification 1000x

Sample	Gram stain	Morphology
Elastic ligature	Gram positive	Cocci

b) Assessment of antibacterial activity – MBC test

Sample: Elastic ligature (Gram positive cocci)

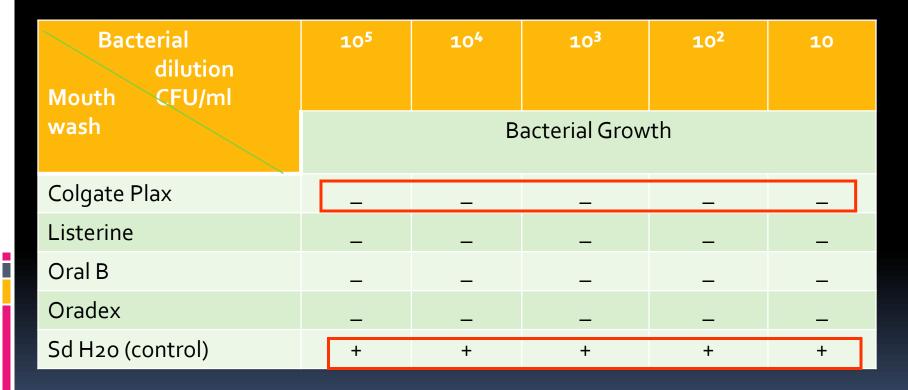
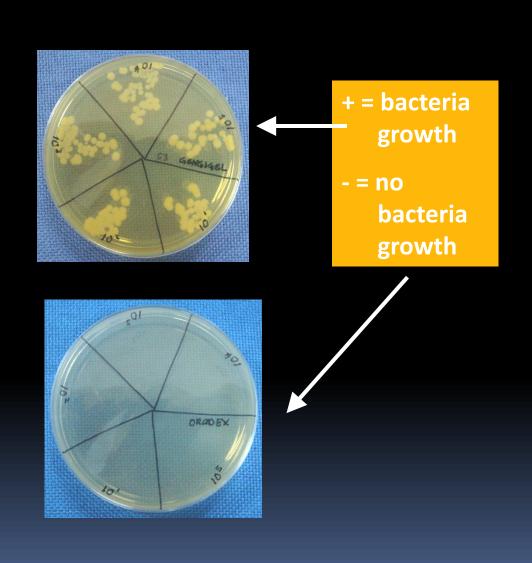


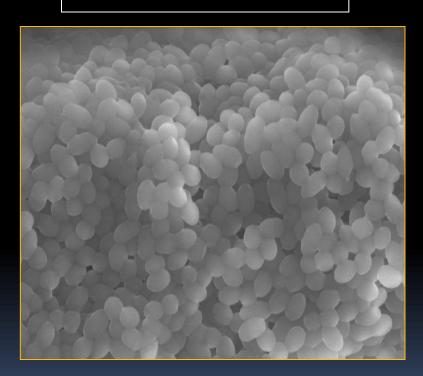
Table 2: The presence and absence of the bacteria growth in various mouth washes with different concentration of bacteria seen on agar plate.



c) Assessment of bacteria morphology - SEM

Sample: Elastic ligature (Gram positive cocci)

Before treatment



15.0kV Mag: 15000x

After treatment

Isoldædatedteatsansæspleated treateithworka OBadex Magnification: (X2000, 15KV)



DISCUSSION

In our experiments, most of the commercially available mouth washes exhibited a strong antibacterial activity against all tested microorganisms. This was well expected because the major chemical constituent in the mouth washes contain antibacterial properties like cetylpyridinium chloride, sodium fluoride, alcohol and chlorhexidine gluconate.

In our study, Oradex exhibited a strong antibacterial activity against tested isolated bacteria. This was well expected because the major chemical constituent in the Oradex was 0.12%w/v chlorhexidine gluconate which contain antibacterial properties.

- The result obtained in this present study was consistent with previous findings which proved that antibacterial effects of chlorhexidine is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering bacterial osmotic equilibrium.
- Chlorhexidine was proven to disrupt sugar transportation in acidogenic organisms of the oral streptococci (Keevil *et αl.*, 1983) and this enables chlorhexidine to control plaque organisms that cause dental caries

- Oradex cause the bacterial cell wall to rupture and bursting of the cells. None of the bacteria can be seen on the SEM pictures for mouth washes after treatment which indicated the effectiveness of the mouth washes in killing all the oral bacteria.
- The presence of active ingredients in the product causing alterations in bacteria osmotic equilibrium that leads to change in shape and morphology (Greenstein et al., 1986).



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.

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H N A K

