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RESEARCH ARTICLE

THE EFFECTS OF ANTIBACTERIAL AGENTS ON THE DENTAL BIOFILM (EXPERIMENTAL STUDY)

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ABSTRACT

The condition of the oral and oropharyngeal cavities is inextricably linked to the general health and well-being of an individual. Colonization by pathogenic microorganisms or an imbalance of the physiological microbiome in the oral cavity can play an essential role in the development of biofilms as well as many common oral and oropharyngeal conditions, as diverse as dental caries, periodontal diseases and diseases gingivitis. The objective of our work was to test the efficacy of Eludril ® and Betadine ® on Streptococcus Mitis by determining their minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) and to evaluate the action of these drugs. Two molecules face bacterial adhesion on dental surfaces as well as on implant surfaces.The results of our study showed an Eludril® MIC of 0.12 µg / ml and a MBC ® with an interval of [0.25-0.125] µg/ml, the MIC of Betadine was 20 µg/ml with a MBC of one interval. of [40-20] µg / ml. Both antiseptics were effective on bacterial adhesion on enamel surfaces as well as on titanium surfaces, the results also showed the importance of surface characteristics and more specifically the influence of surface roughness on bacterial adhesion. To conclude, the use of mouthwashes with Eludril® or Betadine ®has shown an important role in the fight against the formation of biofilms whatever the surface and an optimal microbial activity against S. mitis strains.

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INTRODUCTION

The oral cavity represents a medium where bacteria, viruses and fungi coexist together on the tooth surfaces, prosthetic surfaces, mucous membranes and the tongue. Bacteria are the largest part of this medium, more than 500 species, these bacteria gather to form the dental biofilm on the surface of the teeth. Each species has developed specific properties to colonize different oral sites subjected to constant changes of conditions, to fight competitors and to resist external aggressions (host immune system, physico-chemical shocks, mechanical friction) **(**Guillaume *et al*., 2013**)**. This dental biofilm and its progression can be at the origin of several periodontal diseases as well as the dental caries or even the loss of the teeth, these diseases are controlled by mechanical means but the addition of chemical means like the antibacterial agents will disturb the Bacterial balance and biofilm formation either by inhibiting bacterial multiplication or by destabilizing adhesion to tooth surfaces. The origin of research in microbiology is often associated with the observations of Antone Van Leeuwenhoek who, in the seventeenth century and thanks to a microscope of his invention, highlighted the presence of microscopic organisms on the surface of his teeth. As well as the invention of several other methods such as the scanning laser with a common focus that allows to study the biofilm in its natural state, so we could study the mechanisms of adhesion of bacteria on the various soft and hard tissues of the oral cavity (Roux Agnès et Jean-Marc GHIGO Bacterial biofilms, 2006) Finally, in the 1980s, the work of William Costerton demonstrates that most of the microbial biomass is fixed on surfaces and constitutes heterogeneous populations embedded in an extracellular matrix rich in water, sugars and proteins (Roux Agnès et Jean-Marc GHIGO Bacterial biofilms, 2006).

The main objective of this work are

- Evaluate the effectiveness of antibacterial agents on the dental biofilm and this will be in the form of an experimental study that is to say that we will choose an example of an antibacterial agent and evaluate its effectiveness on a sample biofilm that will be in the form of a bacterial strain isolated from the oral cavity.
- Identify the bacterial strain by several methods on which will be tested the chosen antiseptics.
- Evaluate the efficacy of the two antiseptics chosen by determining the MIC/MBC for the strain identified.

- Evaluate the effectiveness of two mouthwashes on two different surfaces (teeth and titanium).

MATERIALS AND METHODS

Type of study: This is an experimental study with prospective aims.

Place of study: The study was carried out in the laboratory of bacteriology-virology and hospital hygiene at CHU-IBN ROCHD of Casablanca.

Duration of the study: The study took place over a period of two months from May to July **2017**.

Materials used:

Reagents:

- Columbia Blood Agar (Ref 43 041) Schaedler Broth (possibly).
- Active ingredients in the form of mouthwash:
- Chlorohexidine: Eludril®
- Polyvidone iodine: Betadine®
- Bacterial strain used: Streptococcus S.Mitis 2.

Methods

Removal of the bacterial strain: During our work in the microbiology laboratory CHU-IBN ROCHD, we did not have a reference strain streptococcus Mitis ATCC, faced with this situation, we performed a mouth sample for the purpose to isolate a strain of streptococci that is the subject of our work equipment. A swab sample was taken using a swab, the seeding was done on CNA medium (Columbia agar 5% of sheep blood with Colistin and Nalidixic acid (Figure 1) by quadrant method. then the box was incubated in a jar containing CO2 Gaspack in a temperature of 37 ° for 24 hours.

Phenotypic identification: After 18h incubation, we started on small hemolytic α colonies not exceeding 1μm in diameter. Every positive culture must be observed in a macro and microscopic manner, and macroscopic observation consists in detecting colony size, shape and color. Microscopic examination is based on the observation of fresh bacteria to determine their mobility and the Gram stained state by way of example for the distinction of Gram-positive and Gram-negative bacteria, the bacterial form as well as their grouping mode.

Gram stain: The principle of Gram staining is based on the realization of a smear and the application of the different staining steps as shown in Figure 2:

- Staining with Crystal Violet for 1 min, then rinsing with water.
- Lugol etching (iodine-iodine iodine solution) and allow to act for 1 min, then rinse with water.
- Discoloration with alcohol / acetone by applying a few drops on the obliquely inclined blade, and let act. Rinse thoroughly with water.
- Fuchsin recolor by putting a few drops on the smear. Leave on for 30 seconds. Wash gently with demineralised water. Dry the blade on a hot plate at 50 ° C.

Biochemical identification

Catalase test: The catalase search is a fundamental test for Gram-positive Cocci because it allows the distinction of streptococcal staphylococci. The test involves depositing a drop of hydrogen peroxide (H2O2) on a slide, using a plastic handle; we put one or more colonies isolated from the strain to be tested. We then observe the appearance of an effervescence indicating the degradation of oxygenated water through the enzyme catalase.

API 20 Strep: API 20 Strep is a standardized system combining 20 biochemical tests that have a high discriminating power. It makes it possible to make a diagnosis of group or species for most streptococci, enterococci and for the most common related germs.

Principle: The API Strep Gallery contains 20 microtubes containing dehydrated substrates for demonstration of enzymatic activities or fermentation of sugars. The enzymatic tests are inoculated with a dense suspension, made from a pure culture, which reconstitutes the media. The reactions produced during the incubation period result in spontaneous color changes or revealed by the addition of reagents. The fermentation tests are inoculated with an enriched medium (containing a pH indicator) which rehydrates the sugars. Fermentation of carbohydrates causes acidification resulting in a spontaneous turn of the color indicator. The reading of these reactions is done using the Reading Table and the identification is obtained using the Analytical Catalog. After a 24hour shoot, we proceeded to identify the seed using the API 20 Strep gallery according to the manufacturer's recommendations.

The biomaterials used in the study: The biomaterials used as support for the experiment are:

- **First box:** Enamel. We prepared enamel fragments from permanent natural teeth that we extracted and cut longitudinally into several samples at a length of 5 mm, 3 mm width, 2 mm thickness these samples consist essentially of enamel, the superficial layer of the tooth that comes into direct contact with the bacteria during the formation of the dental biofilm.
- **Second box:** Titanium - Titanium: the essential alloy in the composition of the implants, a light and resistant alloy and especially biocompatible with the oral cavity, the samples were prepared using a disc carried on mandrel to a length of 5 mm, 3mm of width, 2 mm thick. The samples thus prepared were disinfected with (Hexanios G + R, Laboratoire Anios) for 15 minutes and then sterilized in a wet autoclave at 120 ° C. for 40 minutes.

Choice of antiseptic solutions: The choice of solutions depends on several factors to note:

- The microbial target: prefer a broad spectrum antiseptic.
- Intensity of the antimicrobial action: desired bactericidal effect.
- Action time: interest of a residual action.
- Field of application.
- Stability of the product in the mouth.

- Solubility of the product.
- Quality of the packaging.
- Tolerance.
- Undesirable effects: extrinsic stains and burning sensation.
- Cost.

The two antiseptics compared in this study were:

- Chlorhexidine (CHX)
- Lapolyvidone iodine, (PVP-I).

Chlorhexidine (CHX): Chlorhexidine is the reference antiseptic most used in periodontology during the attack phase of the treatment by its antibacterial and anti-inflammatory action. This molecule has a broad antibacterial spectrum, it would be bacteriostatic at low dose and bactericidal at high dose. In fact, at a low concentration, the cell membrane of the bacterium will be damaged, while at a high concentration proteins and nucleic acids will precipitate, its action is very powerful on gram-positive bacteria, in particular streptococci. - It has a significant remanence on the dental surfaces and mucous but there is the appearance of bacterial resistance related in particular to prolonged use. This is why its use should be limited to a maximum of two weeks in attack treatment. To evaluate the ability of chlorhexidine to inhibit bacterial growth in a culture medium study, the concentration used can range from 0.2% to 2% for an exposure time of 5 min.

Polyvidone iodine PVP-I: The activity of PVI is very fast ranging from a few seconds to a few minutes, it is due to its strong oxidative properties, its mechanism of action is reflected in reacting with the cell membrane of bacteria as well as with nucleotides and cause to inside the cell a reaction with enzymes in the respiratory chain and a blockage of cytoplasmic proteins. The antibacterial activity is good on both Gram-positive and Gram-negative bacteria after a contact of 120 seconds. It is also noted that the bactericidal action increases with the decrease of the concentration.

Determination of MIC and MBC

- The purpose of this method is to determine the lowest concentration of the tested antimicrobial that inhibits the growth of the bacterium tested (the MIC, usually expressed in µg / ml or mg / liter). However, the IJC does not always represent an absolute value. The "true" MIC is a point between the lowest concentration that prevents the growth of the bacteria and the lowest concentration following the test. Determinations of MICs by the serial dilution method therefore show a variation in a dilution inherent in the method.
- MBC is the lowest concentration of antiseptic that leaves only 0.01% or less survivors of the initial inoculum after 18 hours of incubation at 37 ° C. It characterizes the bactericidal effect of an antiseptic. This value can be measured at a fixed time, often with 18 hours of bacterial / antiseptic contact. Calculation techniques are based on counts that compare the number of bacteria before the inoculum and after the action of antiseptics. They are more often carried out in a liquid medium.

Determination of the MIC

Preparation of the bacterial suspension: The bacterial suspension will be prepared from a freshly incubated Streptococcus culture medium, by taking an isolated colony, a sample using a swab and placing it in 2.5 ml of distilled water prepared at 0.5 McFarland. (108 CFU / ml). After a 1/100 dilution in liquid MH-II culture medium, the bacterial suspension will have a concentration of 106 CFU / ml.

The microdilutions on a microplate: (Figure 4)

- The test was performed for each antiseptic Eludril and Betadine.
- A volume of 100 µl of MH-II medium was dispensed into each well of the microplate using a micropipette calibrated in lines 3 and 6 (figure 6).
- 100 µl of the antiseptic stock solution were added to the first well of each line 3 and 6, a cascade dilution followed from the wells of the stock solutions to the last wells with a dilution factor of ½.
- 100 µL of these antiseptic dilutions are transferred to line number 1 and 4 respectively (figure 6) to obtain another dilution of ½ of the antiseptic.
- 100 µl of bacterial suspension were added to the number 1 and 4 lines of the plate for a final bacterial concentration with the antiseptic of 5 × 10 5 CFU / ml.
- Column 11 will be dedicated to the Positive control with 200µl of bacterial suspension to see the bacterial viability, Column 12 will be the negative control containing only MH-II only to control sterility (figure 6).
- The microplates are then placed in the oven at 37 ° C for 24 hours.
- After 24 hours of incubation, we note the results of each test, the highest dilution giving a clarity identical to the negative control, represents the minimum inhibitory concentration.

Determination of the MBC

- After determining the value of the initial MIC of the first plate prepared for each antiseptic, we proceed to the determination of the MBC from the wells where there is no turbidity.
- Single agar plates were prepared for sowing them from the determined wells of the microplates.
- Sampling is done directly from the wells determined with a calibrated loop of 1µl, the seeding of the culture dishes is made with a separation fir.
- After preparation of the boxes of the two antiseptics, these boxes are placed in the oven at 37 ° C for 24 hours.
- After 24 hours of incubation, a count of bacterial colonies appeared is performed and according to this counted number, the value of the MBC is determined for each antiseptic used.
- While knowing that the determination of the value of the MBC is based on the principle of a bacterial growth which is lower than 0.01%, therefore, the bactericidal activity of an antiseptic is measured by putting in contact the germs and the product to be tested for a period of 5 minutes.
- According to AFNOR standards, the minimum bactericidal concentration is the lowest concentration at which the product is able to reduce by at least 105 fold (= 5log reduction) the number of living cells after 5 minutes of germ-antiseptic contact.

Evaluation of the effectiveness of each of the two antiseptics on two different materials: To evaluate antiseptics on two different materials, we chose to test the effectiveness of mouthwash ELUDRIL and BETADINE on teeth and titanium fragments using as bacterial strains oral streptococci widespread in the oral cavity. Bacterial adhesion will be observed on biomaterials used after seeding streptococci in culture dishes using a swab in the presence of tooth and titanium samples, of identical length and thickness, and after incubation with 37 ° C for 24 hours. A macroscopic observation will be made in a first place using a magnifying glass and then a microscopic observation using an optical microscope. Experimental protocol: To test the effect of mouthwashes chosen on the adhesion of streptococci, we will use two boxes:

- **First box:** three enamel samples seeded on the surface from a 0.5 McFarland-adjusted bacterial suspension prepared from a strain of streptococcus, incubated on single agar for 24 hours, and then moved into tubes:
- **First tube:** this tube will serve as a control, therefore, it must contain only the bacterial suspension with the fragment of enamel and 1ml of distilled water.
- **Second tube:** this tube will contain a fragment of enamel mixed with 1ml of distilled and vortexed water in order to detach the bacteria present on the surface, with 1ml of Eludril mouthwash for a contact time of 5 min.
- **Third tube:** will contain a fragment of enamel mixed with 1ml of distilled and vortexed water, supplemented with 1 ml of the Betadine mouthwash for a contact time of 2 min.

- **Second box**: three titanium samples seeded on the surface by the same bacterial suspension in a single agar medium, incubated for 24 hours at 37 ° C, and moved in tubes as the previous ones.
- **First tube**: control containing the bacterial suspension with the titanium fragment and 1 ml of distilled water.
- **Second tube**: a titanium fragment mixed with 1 ml of distilled water with 1 ml of the Eludril mouthwash for a contact time of 5 min.
- **Third tube**: a titanium fragment mixed with 1ml of distilled and vortexed water, with 1ml of the Betadine mouthwash for a contact time of 2 min.

The comparison will be done to be able to measure the number of bacteria eliminated by the antiseptic solution and the number of bacteria in suspension. It will also be for the spectrum of action of antiseptics.

After preparation of the tubes, we counted the number of bacteria remaining after the action of each antiseptic and this will be done in comparison with the control solution. For this we will take a volume of 1 .mu.l of each tube and we will seed it in appropriate media to be able to count the colonies after incubation of 24 hours.

RESULTS

Identification of the bacterial strain: The result of the culture showed smooth colonies with a greenish color evoking a character of partial hemolysis (Figure 9).

Phenotypic identification:

Results of the Gram stain:

Gram staining showed gram + cocci, violet in color, grouped in a chain in favor of streptococci.

Biochemical identification

The catalase test results

The so-called catalase positive bacterium shows effervescence on the blade when in contact with hydrogen peroxide. The bacterium that does not have the catalase enzyme does not emit bubbles, the preparation remains intact and therefore the test is considered negative, which is the case for the bacterial strain tested in our study. We can conclude that this is a streptococcus.

Results of the API 20 STREP gallery: After adding the appropriate reagents, the API 20STREP gallery gave the following profile: After the incubation of the API20 STREP gallery, we found a reference code of 0040400 with a good identification corresponding to the bacterial species of Streptococcus mitis 2. (id 97%) (figure 12)

MIC and MBC results: The evaluation of the bactericidal efficacy of antiseptics tested in our study: Chlorhexidine (Eludril) and Polyvidone Iodine (Betadine) was carried out by determining the minimal inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) by the method microdilutions, obeying the strictest possible standards AFNOR.

Results of the CMI and MBC of Eludril: The MICs and MBCs of Eludril® were determined relative to an initial solution containing chlorhexidine digluconate at 0.5 ml / 100 ml. After reading the microplates made, we noticed a disappearance of the bacterial growth in the 5th column, that is to say at a concentration corresponding to a 1/32 dilution.

Therefore the MIC value found in the presence of streptococci mitis is 0.12ug / ml. After obtaining the value of the Eludril MIC, similar volumes were taken from the 5th column to the 3rd, then we seeded the simple agar media in order to determine the value. of MBC, and after 24 hours of incubation, we noticed that the use of the mouthwash was very effective because there was no visible bacterial growth on the boxes used

Results of the MIC and MBC of Betadine: The MICs and MBCs of Betadine were determined in relation to an initial solution containing PVP-I at 10 g / 100 ml. After reading the microplates made, we noticed a disappearance of the bacterial growth in the 8th column, that is to say at a concentration corresponding to a dilution of 1/128. So the value of the MIC found in the presence of streptococcus mitis is 20ug / ml. After sampling from the 8th column to the 6th column and seeding the agar plates, we noticed that the bacterial growth disappeared from the 7th column so we can say that the value of the CMB for the Betadine is between two concentrations 1/128 and 1/64, at this value we noticed a bacterial growth less than 0.01% and almost invisible to the naked eye.

Figure 1. CNA culture medium before seeding

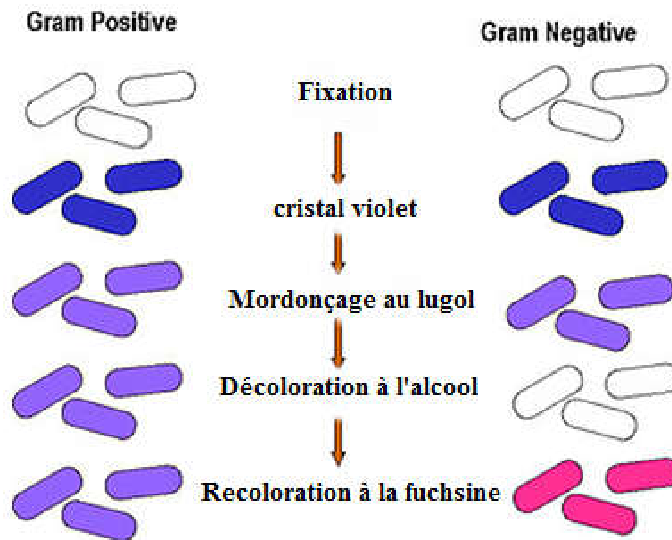

Figure 2. Principle of GRAM coloring (3)

Figure 3. Preparation of samples for sterilization

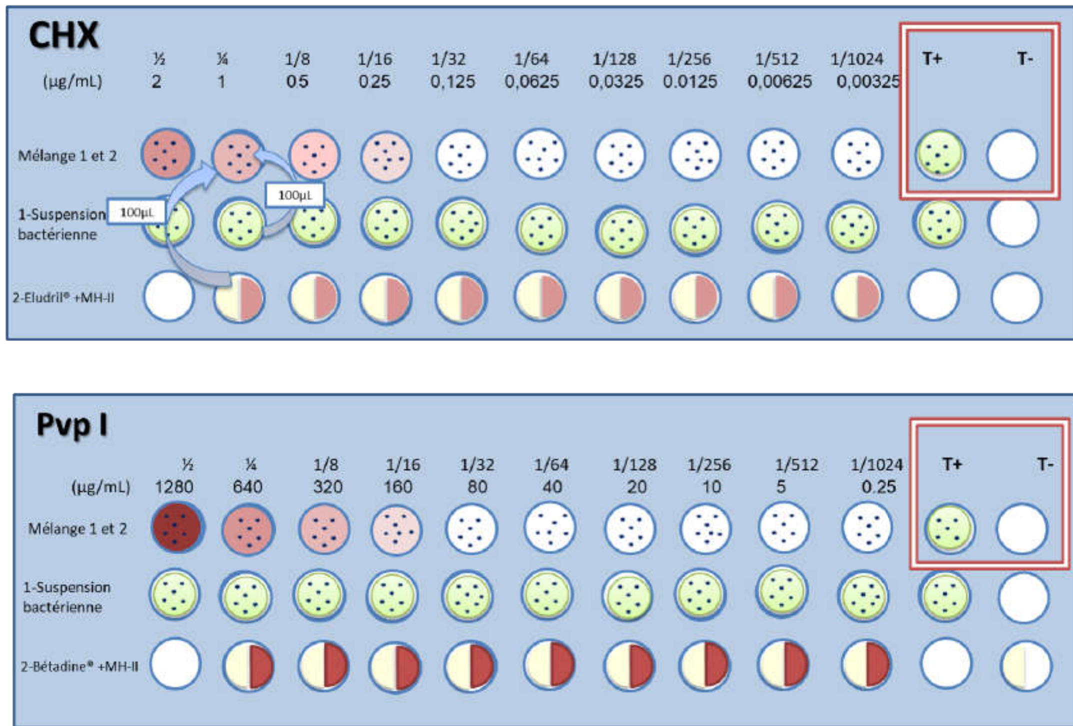

Figure 4. The microdilutions of the two antiseptics

Figure 5. Preparation of agars for the determination of MBC

Figure 6. Preparation of the two boxes for incubation

Figure 7. Preparation of the tubes after incubation

Figure 8. Inoculation of boxes from prepared tubes

Figure 9. A box of CNA after seeding.

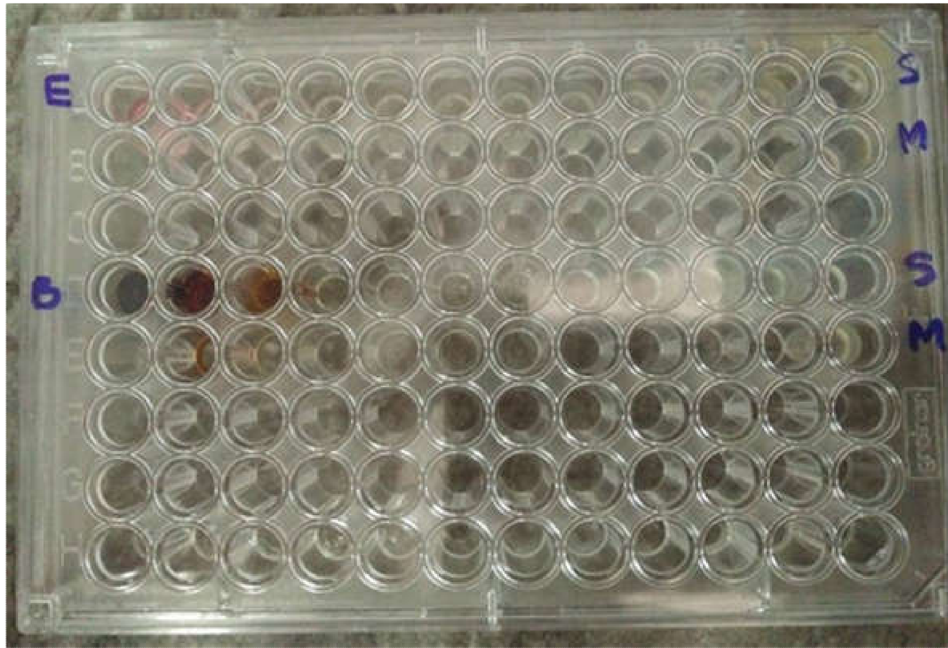

Figure 10. Microplate showing the MICs of the 2 baths of mouth on S. mitis

Figure 11. API20 STREP Gallery after incubation

Figure 12. Colonies formed after 24 hours of incubation of the sample box

Figure 13. Absence of colony formation after the use of each antiseptic after 24 hours

Table 1. MIC/MBC Results from baths of mouth against S.mitis

Antiseptics / CMI/MBC	Eludril® (µg/ml)	Bétadine® (µg/ml)
CMI (µg/ml)	0.12	20
MBC (µg/ml)	[0.25-0.125]	[40-20]

- Columns from 1 to 10 represent dilutions of mouthwash (reason of ½) respectively corresponding to dilutions of ½ up to 1/2048 in the medium of culture MH.
- Columns 11 and 12 respectively represent the positive and negative controls.
- The MIC of Eludril® corresponds to a dilution of 1/32 (5th column), while that of Betadine® is 1/128 (8th column).

Results of the evaluation of the efficiency of antiseptics on enamel and on titanium: After the incubation period of 24 hours, the results of the seeding are read from the 3 tubes prepared to test the effectiveness of Eludril and Betadine on email and on titanium. Remember that these tubes that we prepared contained:

- **First tube:** control containing the bacterial suspension with the titanium fragment with 1 ml of distilled water.
- **Second tube:** a titanium fragment mixed with 1ml of distilled water with 1ml Eludril mouthwash for a contact time of 5 min.
- **Third tube:** a titanium fragment mixed with 1ml of distilled water, with 1ml of Betadine for a contact time of 2 min.

The preparation of the tubes which contained the tooth fragments followed the same steps as the preparation of the titanium. All tubes will be vortexed for 2 minutes to detach the adhering bacteria from the surface of the email and titanium and have them in suspension. The formation of bacterial colonies was greater at the enamel surfaces than at the surfaces of the titanium, knowing that the starting solution was similar for the two tests adjusted to 108 CFU / ml. A control box is prepared for a negative control which contains only 1 µl of the bacterial suspension which is equivalent to 5.10 5 CFU / mL. Exposure to Eludril for 5 min and Betadine for 2 min gave an almost radical reduction in colony levels compared to the control box, so we can say that the use of these two antiseptics is very effective in respecting the indicated contact time. The contact time indicated for the use of Betadine which is 2 min, is sufficient for the solution to eliminate all the colonies present on the surface of the substrates, and this is a duration which is less than that used for Eludril, we can say then that the use of Betadine is more effective than Eludril.

- Control box in the presence of email: a formation of visible bacterial colonies on the streaks in number of 184 colonies. (Figure 12)
- Box of control medium MH in the presence of titanium: a formation of colonies lower than that found in the presence of email since titanium is characterized by a non-retentive surface for bacteria.

The observation of the remaining boxes that have been under the influence of "Eludril" during a contact time of 5min:

- Box in the presence of dental fragments: no colony formed visible to the naked eye.
- Box in the presence of titanium fragments: no colony formed visible to the naked eye.
- The observation of the remaining boxes that were under the effect of "Betadine" during a contact time of 2min:

- Box in the presence of dental fragments: no colony formed visible to the naked eye.
- Box in the presence of titanium fragments: no colony formed visible to the naked eye.

The results thus found show that the inhibition time of the bacterial growth of betadine was 2 min which is less than that used for Eludril which is 5 min. We can then conclude that the use of Betadine has been shown to be more effective in eliminating bacterial growth in the presence of organic as well as metallic material.

DISCUSSION

The oral cavity is home to one of the most complex bacterial ecosystems in the body. Several hundred species of microorganisms coexist in the oral environment. Many authors have tried to quantify this population knowing that 1 mg of dental plaque contains about 100 million bacteria and 1 ml of saliva contains an average number of 750 million bacteria (only a small part of which is culturable on culture medium) (4). This bacterial diversity will require, in order to live and develop in this environment, to find favorable adhesion surfaces, rich and varied nutritive and respiratory conditions, physicochemical factors compatible with this flora and controllable inhibitory factors. To study this adhesion, we chose the most common bacterial species in the oral flora: streptococci, for example, during the eruption of the first lacteal teeth of the child, the appearance of enamel-type hard tooth surface in the oral cavity allows the installation of Streptococcus sanguis and Streptococcus mutans. Streptococcus salivarius is widely distributed on the cheeks and tongue. However, Streptococcus salivarius will have greater attachment to the lining of the dorsal aspect of the tongue than to other mucosal surfaces of the oral cavity (Sixou, 2007).

Properties of antiseptics used: Several studies have been conducted to confirm the utility of using antiseptic solutions in the mouth to reduce bacterial adhesion and have better results in platelet retention. The first antiseptic used in this study was chlorhexidine, which gave very good in vitro results in reducing bacterial growth, and is one of the most widely used antiseptics in dentistry (Jame, 2004). The effectiveness of CHX as an active agent has been tested in several dental fields, in pharmaceutical formulations such as mouth rinses, oral irrigations and slow release devices (5) CHX is a positively charged molecule, electrostatically binding to negatively charged sites on the bacterial membrane resulting in membrane damage leading to leakage of intracellular cytoplasmic organelles (Denton, 1991).

The unique advantage of CHX as an antiplaque agent is its ability to bind to the salivary film (Rolla *et al.*, 1970) in the oral cavity thereby prolonging its retention in the oral cavity (Ben Slama, 2004). The effectiveness of the CHX molecule is related to its concentration, pH, formulation, but also its persistence, due to its binding power on dental surfaces. Its activity persists for several minutes with stable efficiency. The alcohol in which the active ingredient is diluted would potentiate its activity. In addition, it has anti-inflammatory and healing properties (Ben Slama, 2004). The bactericidal spectrum of chlorhexidine was determined, there was a broad spectrum of activity with particularly sensitive gram-positive cocci (MIC: 0.19 to 2.0 µg / ml).

Exposure of suspensions of various bacterial species to chlorhexidine (0.02%) for 10 minutes at room temperature reduced viable organisms by approximately 99.99% in most cases. The addition of serum to the test system greatly reduced the bactericidal action. A study with Streptococcus mutans revealed that the presence of sucrose (5%) in the growth medium used for the preparation of bacterial suspensions has a profound influence on the bactericidal activity. This is believed to be the result of the adsorption of chlorhexidine to extracellular polysaccharides (Hennessey, 1973). We also used in this study the molecule of polyvidone iodine or betadine, which is a very effective antibacterial agent and the most effective compared to other iodine solutions, the combination of iodine with polyvinyl pyrolidone increases its ability to dissolve in water and in alcohol, also reduces its side effects of local irritation and colorations caused by pure iodine, PVP-I is a hydrophilic polymer that does not have an antibacterial efficacy, but it plays the role of transporter of iodine molecules on the bacterial surface, thanks to its high affinity towards the cell membrane, the targets of iodine are often located on the cytoplasmic membrane and destroying them can take a few seconds (Hemant Gupta, 2014). Povidone iodine is an effective germicide. High dilutions are active in the destruction, within fifteen seconds after its application, of organisms commonly found in the mouth. High dilutions, however, should be freshly made; the loss of color is accompanied by a weakening of the germicidal activity. When povidone-iodine is used to prepare the oral mucosa for local anesthetic injection prior to dental procedures, the risk of direct bacterial infection is completely eliminated. In a previous study, Doran D and al used polyvinylpyrrolidone iodide in ninety-two patients who received a total of 115 injections, and in five cases, bacteria were recovered from the needle injection (Doran D.Zinner, 1961).

Eludril® MIC and MBC: The Eludril mouthwash had an inhibitory effect on S.Mitis at a concentration of 0.12 µg / ml and represents a very effective value for prolonged use of the molecule by minimizing the visible side effects in the oral cavity. The results obtained in our study are close to those found in the literature, which confirms that the most widely used chemical form is chlorhexidine digluconate at concentrations of optimal efficiency between 0.10 and 0.20%. It would be bacteriostatic at low dose and bactericidal at high dose. Its action is very powerful on Gram-positive bacteria, in particular streptococci, its effect has been demonstrated in vitro on the majority of pathogenic germs of the oral cavity. It has variable activity on Gram-negative bacteria, and Lactobacillus would be resistant to it. The same is true of spores, mycobacteria and viruses, with the exception of HIV and some viruses of the herpes group (Ben Slama, 2004). Lamie B.M. and al in 2012 conducted a study in which they evaluated the antibacterial activity of three essential oils on five oral bacteria by determining their MIC and MBC microdilutions. In this study, they used Eludril® and Givalex®, and showed that Eludril® was effective on germs at a dilution of 1/128 (ie 7.8 µg / ml), the difference obtained can be explained by the nature strains used in their study, which are different from those used in ours (the sensitivity of bacteria to antiseptics is not the same), as well as the difference of the media used and methods in the experiment (Lamie, 2012) Another study was carried out by Moradian and al (2013) to test the antibacterial effect of the two medicinal plants in comparison with chlorhexidine at 0.2 µg / ml. They used a method of hydroxyapatite disks on agar and microdilutions.

The MIC of Chlorhexidine for S. mutans was 0.024 µg / ml, a value which is lower than that found in our study, which shows us that S. mutans is less resistant to this molecule than S. Mitis, which means that it will need a low dose of chlorhexidine to inhibit its multiplication (Mordian, 2013). The susceptibility of S. mutans to chlorhexidine has apparently been studied systematically before. Hennessey investigated the susceptibility of a single streptococcal isolate to chlorhexidine and found a MIC value of 0.19 µg / ml, which is a close result of our study, Meurman and al (Jarvinen Helina, 1993) reported. tested the susceptibility of 28 clinical isolates of S. mutans to chlorhexidine and noted that the bacteria showed no resistance to this molecule with respect to the resistance of S. mutans to other antimicrobial agents, the results are in agreement with those of Liebana and al (Jarvinen Helina, 1993), who found that S. mutans remained sensitive for at least 5 years to all the other 10 antiseptics tested (Jarvinen Helina, 1993). Do not forget that long-term use of CHX may have side effects in the oral cavity, such as extrinsic staining of teeth or even burning sensation, according to a 1999 study, a reduction of side effects CHX is possible by combining it with hydrogen peroxide, in order to reduce their side effects, we have tested the hypothesis that a combination of the concentrations of these two agents can act synergistically. We can say that the interactions between hydrogen peroxide (PH) and CHX can reduce side effects such as staining of teeth due to the oxidative properties of PH that can counteract staining caused by CHX. We have found that combinations of certain concentrations of CHX and PH may increase their antibacterial effect relative to their individual antibacterial activity. This synergistic effect at these specific concentrations of CHX and PH inhibited S. faecalis and S. sobrinus at concentrations below the minimum inhibitory concentration of each agent separately (Ben Slama, 2004).

MIC and MBC of Betadine®: The use of the Betadine mouthwash gave very satisfactory results near the S.Mitis bacterial strain at a concentration which is equal to [20 µg / ml], and almost completely eradicated all the colonies visible to the naked eye. This study was conducted to determine the minimum inhibitory concentration of Betadine in order to limit the undesirable side effects associated with this molecule, and to give practitioners and consumers limited ranges to facilitate their control of plaque, and achieve good oral hygiene. Another study was conducted by K Almas, Skaug N, Ahmad I (Almas, 2005) performed an in vitro comparison of the antimicrobial activity of Miswak extracts with other non-alcoholic marketed solutions such as Betadine, and in this comparison, they were able to confirm the effectiveness of the use of this mouthwash on several species of streptococci. According to ADDY and R, WRIGHT (Addy, 1978), in a study carried out to compare the antibacterial properties of two chlorhexidine and polyvidone iodine mouthwashes, which followed the same method of microdilution used in our operating protocol, and were able to find a value of the MIC of PVP-I in the range of [625-2500]. Against Strep.mutans. (NCTC10922), which shows us that S.Mutans need a higher dose of this molecule as necessary to reduce S.mitis to inhibit their multiplication (14). The benefits of using Betadine do not lie in its antibacterial activity per se, but rather in its retention capacity in the oral cavity and its slow release (Addy, 1978). However, the use of Betadine is usually more limited. It is reported to be effective in the preparation of mucosal surfaces preoperatively. Prophylactic topical administration of iodinated compounds has shown very effective results in reducing the number of viable microorganisms in saliva and gingival fluid, which is similar to the results found in our study of S. Mitis strain of oral streptococci (Tanzer, 1977). In addition, surface treatments of teeth infected with S. mutans by Betadine could show in children who have a very high carious index, a reduction of microorganisms present in the oral flora for several weeks. (Tanzer, 1977). In a study conducted by JM TANZER, AM SLEE found **(**Tanzer, 1977**),** they tested three compounds of iodine being anti-plaque agents, the study was conducted in vitro using 4 bacterial strains, one of which is S.Mutans, and the results obtained for polyvidone iodine confirmed that the strain S.Mutans was more resistant than S.Mitis, since the time of contact with the antiseptic was 5 min, a time longer than that used in our study. Other authors have also tested the efficacy of iodine as well as other antiseptic solutions, such as DA Spratt, J. Pratten, M. Wilson & K. Gulabivala, on different bacterial strains responsible for the onset of diseases. periodontal lesions as well as periapical lesions that are: Prevotella intermedia,, Streptococcus intermedius, Fusobacterium nucleatum and Enterococcus faecalis, and they were able to show a reduction in the number of colonies formed by S.Intermedius after the use of Betadine for 15min contact time of 15 min, and after 60 min of contact, there was complete eradication of 100% of colonies formed (Spratt, 2001). This molecule has been studied for several years by being very effective on several bacterial species and giving very remarkable results on oral streptococci especially, which confirms the results found in our research, this study was carried out by Jose L. Zamora, MD, detailing the microbiological and chemical characteristics of solutions based on Polyvidone iodine (Jose, 1996).

Evaluation of antiseptics on enamel and on titanium: The results obtained in our study could demonstrate that the use of antiseptic solutions was as effective on dental fragments as on titanium fragments, even if the contact time of chlorhexidine was greater (5 min) than that of the betadine (2 min), and these two mouthwashes could almost eradicate the formation of bacterial colonies, but let us not forget that bacterial adhesion could change characteristics by changing the substrate of the experiment even if the The conditions of the experiment were similar for both email and titanium. Bacterial adhesion is a phenomenon of general importance that governs the evolution of microorganisms and their interaction in all environments where they occur, that is, in the whole of the biosphere. The elucidation of the mechanisms at the molecular level of bacterial adhesion on solid surfaces has not been fully accomplished (Characklis, 1983). To understand the mechanisms of bacterial adhesion on the dental surface and on the surface of implants, we cultured germs in contact with dental surfaces and titanium surfaces in culture dishes. This allowed us to understand the mechanisms of adhesion and bacterial proliferation through counting methods. The choice of bacteria used was made by their membership of the oral flora and their presence at the time of oral sampling. The evaluation of the adhesion capacity of these germs was carried out by a bacterial count after 24 hours of incubation.

We used a counting method:

- Enumeration by culture on CNA medium of adhering bacteria and bacteria in suspension.
- The results of our work showed that after 24 hours of incubation, the adhesion capacity of bacteria to the surface of dental fragments even with a bacterial

suspension of 0.5McFarland was greater than in the presence of titanium fragments from of the same bacterial solution.

The difference that we noticed in our results depends on the composition of the biomaterials used, their nature and the degree of surface roughness, since several studies have shown the influence of these components on cell adhesion and proliferation phenomena (Grizon, 2002). The characteristics and specificities of bacterial adhesion change according to the change in the nature and composition of the substrate on which it occurs, Similar studies (Grizon, 2002) have shown that culturing cells on biomaterials in vitro after 6 hours of incubation is not sufficient for the cells to adhere and proliferate on their substrates. Other studies have shown that seeding density influences the phenomena of adhesion and proliferation of bacteria on the surfaces of the supports used. (Despina, 2001). Membership on enamel: Adhesion is a mechanism that differs from one substrate to another, and the latter would be more important at the level of enamel surfaces since the enamel is a highly mineralized biological structure, which would be covered during the formation of the dental biofilm. the acquired exogenous film, which interacts directly with primary bacteria in their colonization phase (Wim Teughels, 2006). This film is composed of many components including glycoproteins (mucins), proline-rich proteins, phosphoproteins (eg statin), histidine-rich proteins, enzymes (eg, α-amylase) and other molecules can function as adhesion sites for bacteria (receptors). Bacterial adhesion occurs between a film-coated bacterium and a film-coated surface. (Wim Teughels, 2006).

Membership on titanium: Unlike dental surfaces, adhesion to the titanium surface may be different due to its metallic nature and its ability to inhibit the growth and proliferation of bacteria through the presence of ions on its surface. There is indeed a bacterial migration from different mouth niches to peri-implant sites. This microbiology of dental implants is influenced by many factors including the oral state of the patient. These bacterial species, which are normally involved in the etiology of periodontal diseases, are also involved in the irreversible destruction of deep peri-implant structures (. Keller Jean-Francois, 2013). There are different factors that also play a role in conditioning bacterial adhesion and may interfere with the bactericidal action of antiseptics to inhibit bacterial growth:

Roughness: The different biological or metallic materials have on their surface irregularities represented by surface crevices which are considered as bacterial niches which promote the proliferation of bacteria and prevent the effectiveness of mechanical and chemical treatments. We can conclude that bacterial adhesion varies according to surface roughness, Carlos Nelson and al (elson Carlos, 2008) supported this hypothesis by stating that the biological properties of titanium depend on its surface oxide film. Several mechanical and chemical treatments have been used to modify the surface morphology and properties of titanium dental implants. One possible method of improving the biocompatibility of dental implants is to increase the surface roughness and to reduce the contact angle by in vivo and in vitro tests (Nelson Carlos, 2008)

Free surface energy: Surface free energy is one of the initial characteristics that conditions the formation of biofilm on dental and implant surfaces, and is a measurable value relative to the surface of the substrate used and the bacterial strain found, and this value is generally related to average hydrophobicity in the presence of bacterial colonies on dental surfaces (Quirynen, 1995; Umme Salma Durbar1, 2017).

Porosity: Several studies have been conducted (Kjeld, 2009) to determine whether differences in initial bacterial accumulation on dental surfaces could be explained by differences in surface characteristics, in particular the porosity of substrates. A point counting method was used to determine the number and size of porosities in materials. The results showed a great variation between the materials in the number of porosities which could explain the difference of topography of the bacterial colonies (Kjeld, 2009). Some authors have supported this hypothesis, such as Müller and al. (Birte Gröner-Schreiber, 2001), which has confirmed that bacterial adhesion to titanium implant surfaces has a significant influence on the healing and long-term condition of dental implants. Parameters such as surface roughness and the chemical composition of the implant surface have a significant impact on plaque formation.

The purpose of this work was to study in vitro the characteristics of titanium by choosing as bacterial strain, Streptococcus mutans and Streptococcus sanguis, and they also used pure titanium discs that were modified using surface treatments. Polished titanium surfaces served as controls. The surface topography was examined by SEM and the estimation of the surface roughness was performed using a contact profilometer. Contact angle measurements were taken to calculate the surface energy. The titanium disks were incubated in their respective bacterial cell suspension for one hour and single colonies formed by adherent bacteria were counted under a fluorescence microscope. Contact angle measurements showed no significant difference between surface changes. The surface roughness of all examined surfaces was between 0.14 and 1.00 μm. A significant reduction in the number of adherent bacteria was observed on titanium hard materials that had undergone a surface treatment. In conclusion, physical modification of titanium implant surfaces can reduce bacterial adhesion and thus improve clinical outcomes (28). In the same vein, a systematic review (25) was written to show the role of surface condition in the formation of the dental biofilm, and showed that the roughness and grooves of the tooth surface, the more it will be colonized by bacterial niches that will promote their multiplication, in vitro and in vivo studies to examine two essential components in adhesion, surface roughness and surface free energy (Quirynen, 1995). What can be deduced from the results obtained in our study is that the adhesion and proliferation of bacteria depend not only on the type of bacteria, the conditions of the cell culture but also the properties of the biomaterials used. The surface topography of a biomaterial affects not only the adhesion but also the migration and proliferation of bacterial strains in the oral cavity (Shibata, 2015). Let's not forget that biofilm bacteria have a very slow metabolism and are protected by the extracellular matrix which makes them resistant to antiseptics and antibiotics. Indeed, it has been shown that bacteria inside the biofilm have characteristics and properties different from those of planktonic cells (Gilbert, 1997). The minimum inhibitory concentration (MIC) of CHX was found to be 300-fold greater when S. sobrinus is grown as a biofilm than in planktonic form in a study by Shani *et al.* (2000) Another study has shown that repeated exposures of mixed culture of oral biofilms to CHX are effective only at

concentrations 10 times the MIC (Shani, 2000). This may be related to the physical properties of the biological biofilm or the protection by neighboring bacteria (Marsh, 2005). To conclude, the use of mouthwashes with Eludril or Betadine has shown an important role in the fight against the formation of biofilms whatever the surface, as well as an optimal microbial activity against S. mitis strains.

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