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RESEARCH ARTICLE

BACTERIAL ADHESION ON DENTAL SURFACES AND ON TITANIUM

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ABSTRACT

Adhesion is a phenomenon of general significance that governs the evolution of microorganisms and their interaction in all the environments in which they occur, that is to say in the whole of the biosphere. The elucidation of the mechanisms at the molecular level of bacterial adhesion to solid surfaces has not been fully accomplished. The oral cavity is part of these environments, the bacterial adhesion is interested in different structures in the mouth: dental structures, mucosal structures and structures of therapeutic interest (composite, brackets, orthodontic wire, ceramic, titanium…). The adhesion phenomenon involves nonspecific factors of different types (ionic, dipolar, hydrophobic, hydrogen bonding) between the macromolecules on the surface of the microorganisms and those of the support and other specific factors. Stereochemical order involves interactive complementary chemical groups. Our study is used to observe the behavior of bacteria in contact with tooth surfaces and titanium, in order to understand the adhesion mechanism. The aim of this work is to study in vitro the behavior of certain bacteria of the oral flora in contact with a dental surface and titanium to:

- Observe the ability of these germs to adhere to tooth surfaces and titanium.
- Evaluate quantitatively the adhesion and proliferation potential of these germs in contact with tooth surfaces and titanium.
- Compare their behavior in contact with these 2 surfaces.

The results of our work have shown that the three germs used have different behaviors and even adhesion capacity on the two surfaces. In contact with the tooth surfaces the three seeds were able to adhere and proliferate, in contrast in contact with the titanium surfaces, an inhibition of this adhesion was observed. Germs need more time in contact with surfaces to adhere and proliferate. The results showed the importance of surface characteristics and more precisely the influence of surface roughness on the adhesion of bacteria.

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INTRODUCTION

Today, orthodontists have multiple therapeutic devices to correct dental malocclusion or skeletal dysmorphosis (Kerner *et al.*). One of the possible classifications of these devices is to distinguish the removable devices (which can be removed from the mouth by the patient) fixed devices, by definition frozen (glued or sealed) on the dental arches throughout the treatment (Kerner *et al.*). Fixed multi-attachment orthodontic devices, long known as multi-rings, allow three-dimensional control of dental displacement, in response to a force applied to the crown of the teeth (Kerner *et al.*). Continuous advances in research and industry over the last 50 years have led to the modernization of tools in terms of fasteners, arches, bonding systems and ligatures, in particular (Kerner *et al.*). However, the arrival of this device in the oral cavity is causing changes in the macrobiological but also microbiological. The various reliefs favor in particular the bacterial colonization and complicate the control of plate. Bacterial plaque constitutes a major risk of occurrence of hard tissue and soft tissue lesions. The scientific literature reports the presence of constant gingival inflammation in patients with orthodontic devices, and up to 97% of initial enamel lesions after removal of the device (Zachrisson, 1972; Boersma *et al.,* 2005). Thus, these modifications of the oral ecosystem, if they are not anticipated and quickly taken care of, can be harmful for the oral environment. They cause consequences on dental tissues and periodontal tissues. Adhesion is a phenomenon of general significance that governs the evolution of microorganisms and their interaction in all the environments in which they occur, that is to say in the whole of the biosphere. The elucidation of the mechanisms at the molecular level of bacterial adhesion to solid surfaces has not been fully accomplished. The oral cavity is part of these environments, the bacterial adhesion is concerned in different structures in the mouth: dental structures, mucosal structures and structures of therapeutic interest (composite, brackets, orthodontic wire, ceramic,

titanium ...). The adhesion phenomenon involves nonspecific factors of different types (ionic, dipolar, hydrophobic, hydrogen bonding) between the macromolecules on the surface of the microorganisms and those of the support and other specific factors. Stereochemical order involves interactive complementary chemical groups.

MATERIALS AND METHODS

Biomaterials: Titanium alloy implants Ti6Al4V (90% Titanium, 6% Aluminum, 4% Vanadium) were machined using a disk placed on a mandrel carried by a handpiece, in different samples of 5 mm in length and 3 mm wide and 2 mm thick. Freshly extracted permanent natural teeth were cut longitudinally in different samples of 5 mm in length and 3 mm in width and 2 mm in thickness consisting essentially of enamel, outer layer of the crown of the tooth. The dental fragments and the titanium fragments were disinfected with (Hexanios G + R, Laboratoire Anios) for 15 minutes and then sterilized with a wet autoclave (Tau Clave 3000, Vacuum) at 120 ° C. for 30 min.

Bacterial strains: Three reference bacterial strains were used in this study:

- Haemophilus influenzae ATCC 49247,
- Streptococcus intermedius ATCC 27335 Staphylococcus Aureus Met ATCC 29213.

The strains were stored as an aliquot in brain-brain broth (BHI) medium supplemented with 10% glycerol and frozen at -20 ° C.

Reactivation of germs: The reference strains used are cultured, placing them in a liquid enrichment medium (BHI) and then incubated in the oven at 37 ° C for 2 to 4 hours. Then one drop of this broth incubated with a sterile loop, the appropriate media for the culture of these strains:

- Chocolate agar composed of columbia agar with cooked blood supplemented with polyvitaminic supplements allowing the growth of all bacterial strains and especially the deficient bacteria.
- Fresh blood agar supplemented with nalidixic acid and colistin, selective for streptococci.
- Chapman agar (medium hypersaled to 7% Nacl) selective for staphylococci. The inoculated dishes are incubated in the oven at 37 ° C. for 18 to 24 hours under 5% CO 2.

Preparation of media necessary for bacterial adhesion on biomaterials: We used two pairs of dishes from each of the following media: MHS (Mueller Hinton: Mueller-Hinton and Fresh Blood), MH (Mueller Hinton), and Chocolate Agar: The first pair of boxes each contains two dental fragments embedded in the agar so that the enamel surface is on the same plane as the surface of the agar, the enamel surface has been chosen because it is the first surface in contact with the cells. bacteria of the oral cavity. The second pair of dishes each contains 2 titanium fragments embedded in the agar with an accessible surface and on the same plane as the agar.

Inoculation and culture of biomaterials: From a bacterial suspension corresponding to a turbidity of 0.5 McFarland of each bacterium studied (≈ 106 CFU / mL), we have inoculated the surfaces of the 2 pairs of blood agar plates with a sterile swab. (MHS) encrusted with fragments to study. For Haemophilus influenzae is seeded on chocolate boxes. For Staphylococcus aureus is seeded on Mueller Hinton (MH) agar. One box of each pair was incubated for 6 hours at 37 ° C and 5% CO2, the other box of each pair was incubated for 24 hours at 37 ° C and 5% CO2.

Macroscopic and microscopic observations: A macroscopic observation and a binocular magnifying glass (V.M.Z. 1 to 4 Japan, Olympus) of bacterial proliferation was performed regularly after 6h and 24h incubation. An optical microscope observation based on the GRAM staining allowed to visualize the presence or the absence of bacteria and to differentiate them. Confirmation test of bacterial fixation on biomaterials by transplanting on culture media: We scraped a portion of the culture on the surface of each substrate (tooth and titanium) with a calibrated loop of 1 µl and suspended with physiological saline. This suspension was readjusted at a concentration of 0.5 MacFarland and was subjected to different dilutions 1/10, 1/100 and 1 / 1000th. From each suspension prepared, we seeded 1 .mu.l on different media which are specific to them. Interpretative reading of cultures was made after incubation in an oven at 37 ° C for 24 hours.

Bacterial adhesion: Adhesion of Haemophilus influenzae, Streptococcus intermedius and Staphylococcus aureus Meti on dental fragments was compared to that obtained on titanium fragments. For example, the number of bacteria adhered as a function of the number of bacteria inoculated (CFU / ml) was reported for the two pre-incubated samples. The slopes of the lines obtained make it possible to determine the percentages of adhesion. Counting bacteria in culture was carried out by two different methods:

Culture on Congo red medium: The medium was prepared by adding 0.8 g of Congo red and 36 g of sucrose to 1 liter of brain heart agar, then autoclaved at 115 ° C for ten minutes. (50). In each sterile tube containing 0.5 ml of 1x phosphate buffered saline (PBS), introduce the cultured material (tooth or titanium), vortex for 5 minutes to release the bacteria attached to the substrates. We adjusted the bacterial suspension with physiological saline to prepare a suspension of 0.5 Mc Farland. Then we seeded on each box of red Congo medium, 1 µl of a suspension of 0.5 McFarland (a colony in 20 ml of distilled water). The reading was made after 24 hours of incubation at 37 ° C. The adhesive strains gave black colonies with a rough surface and red colonies with a smooth surface for non-adhesive strains.

Bacterial enumeration by optical assay: -Cultivated tooth and titanium fragments are washed with physiological saline to remove any non-stick bacteria.

- In each sterile tube containing 0.5 ml of 1x phosphate buffered saline (PBS), introduce the cultured material (tooth or titan), vortex for 5 minutes to release the bacteria attached to the substrates.
- Following a procedure based on the protocols described by Christensen *et al.* (27). The bacterial suspension was adjusted to an optical density (OD) of 0.5 to 610 nm. A 1: 10, 1: 100 and 1: 1000 dilution were prepared with the adjustment of the bacterial suspension in tryptic soy broth (TBS). The contents of each tube were gently

aspirated for quantification of adhesive bacteria. The OD of the resulting solution was measured at 560 nm.

- Optical dosage: relies on the Beer-Lambert law: The form used is as follows:

A: absorbance of the solution without unit.

ε: molar extinction coefficient in L × mol-1 × cm-1 (sometimes noted with a ξ)

l: length of the tank crossed by the light in cm molar concentration in mol × L-1.

The measurement of the absorbance is given by a spectrophotometer which measures the optical density. The more a solution is concentrated, the more light is difficult to pass through the medium, which leads to an increase in the absorbance of the solution, the measurements obtained are expressed in OD (optical density), the solutions used in the optical assay are the same dilutions and the mother solution used in the inoculation of the dishes in the first method (bacterial count per culture).

RESULTS

Observations to the naked eye of bacterial cultures on biomaterials after 24 hours of incubation

Staphylococcus Aureus Metis S. ATCC 29213 : The culture dishes inoculated with Staphylococcus aureus, showed the formation of a bacterial carpet that covers the entire box including dental fragments (Figure 1a). Whereas at the level of the second box a decrease in proliferation around the titanium fragments was observed with the appearance of a 1 mm inhibition zone (Figure 1b).

Streptococcus intermedius ATCC 27335: Figure (2a) showed a total invasion of Streptococcus intermedius on both culture dishes and even on the surface of the dental fragments. On the other hand, in the culture dish in the presence of the titanium fragments, we found an inhibition zone of 2 mm around the substrate in the presence of the titanium fragment (Fig 2b).

Haemophilus influenzae ATCC 49247: The plates inoculated with Haemophilus influenzae, showed the formation of a bacterial carpet which covers the whole of the inoculated agar and the surface of the dental fragments (fig: 3a), whereas around the titanium fragments appears a zone of inhibition of 1 mm in diameter (Fig 3b).

Confirmation test of bacterial fixation on biomaterials by transplanting onto culture media:

The culture of Staphylococcus aureus diluted 1/100 on the MH medium showed: After 6 hours of incubation, that whole box has been submerged by bacterial growth of the same surface of the dental fragments. On the other hand, on the agar which contains titanium fragments, there is no bacterial proliferation. After 24 hours of incubation, a growth was observed on the entire agar surface containing dental fragments, but only a few colonies were noted on the agar inlaid with titanium fragments. The results also showed that with the other dilutions there were no bacterial colonies on the agars containing titanium fragments, after 6 h and after 24h incubation, but on the surfaces of the titanium fragments there was no adhesion of these bacteria instead they were inhibited. Therefore Staphylococcus aureus adheres and proliferates more easily on dental fragments on agar with the same dilution. While Staphylococcus aureus does not adhere and therefore does not proliferate in the presence of titanium fragments even with the stock solution. (Fig 4).

The culture of Streptococcus intermedia diluted 1/100 on the MHS medium showed: After 6 hours of incubation, the whole box has been submerged by the bacterial growth of the same surface of the dental fragments. On the other hand, agar containing titanium fragments did not have bacterial growth.

- After 24 hours of incubation, a colony was marked on the entire agar surface containing dental fragments, but the total absence of colony on the agar inlaid with titanium fragments was noted.
- After 24 hours of incubation, a small number of bacterial colonies were grown in the culture dishes with a 1/100 dilution in the presence of the dental fragments and in the presence of agar.
- In the presence of the titanium fragments the results showed the absence of bacterial colonies after 24 hours of incubation with the different dilutions and even with the stock solution.
- At a dilution of 1/1000 we had no culture in all culture-dishes.

Thus Streptococcus intermedius has a weak capacity to adhere and proliferate not only in the presence of dental fragments but also on agar with the same dilutions. While the Streptococcus intermedius did not adhere and therefore did not proliferate in the presence of titanium fragments with all the diluted solutions (Fig 5).

The culture of Haemophilus influenzae diluted 1/100 on the chocolate medium showed:

- After 6 hours of incubation, the same number of colony in the culture dishes are present in the presence of the dental fragments and the agar with the dilutions: 1/10, 1/100, and 1/1000. no colonies in the presence of titanium fragments with different dilutions.
- After 24 hours of incubation, we have a large number of bacterial colonies in the culture dishes in the presence of dental fragments and agar with a dilution of 1/100, on the other hand there are fewer colonies in the presence of titanium fragments and with the same dilution.

Therefore, Haemophilus influenzae needs more incubation time to adhere and proliferate in the presence of titanium fragments. While in the presence of dental fragments and agar this type of bacteria adhered and proliferated much more rapidly (Fig 6).

Evaluation of the adhesion capacity by enumeration by culture on congo red medium: Bacteria grown on the biomaterials are resuspended in distilled water readjusted to a turbidity of 0.5 McFarland, and then we incubate the congo red medium with 1µl of the suspension. After incubation we counted the number of colonies that grew on the medium, which phenotypically marks the colonies in black colonies with rough surface against red colonies, smooth surface for

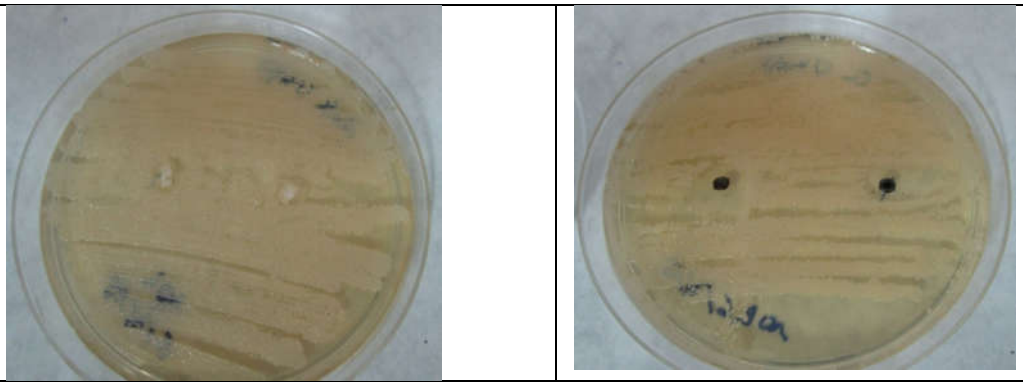


Figure 1. Culture of Staphylococcus aureus on MHS agar encrusted with dental fragments and titanium fragments after 24 hours of incubation

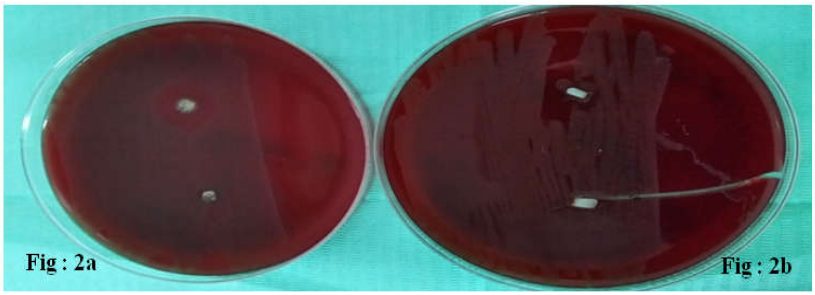


Figure 2. Culture of Streptococcus intermedius on MHS agar encrusted with dental fragments and titanium fragments after 24 hours of incubation

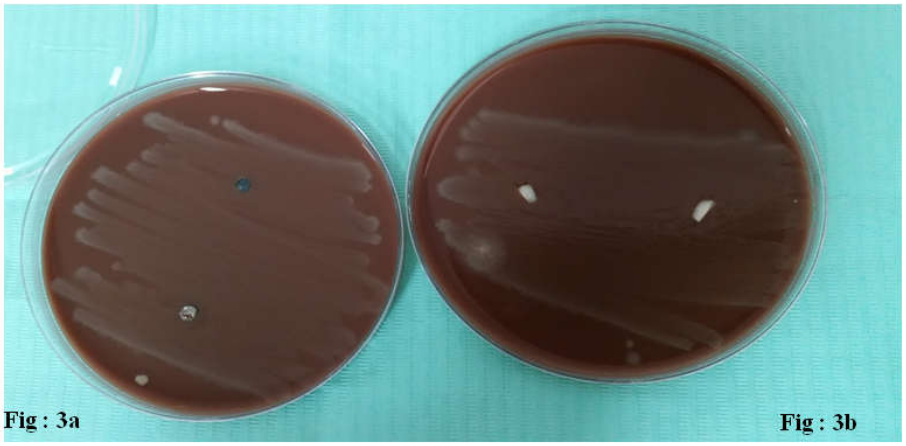


Figure 3. Culture of Haemophilus influenzae on chocolate agar encrusted with dental fragments and titanium fragments after 24 hours of incubation.

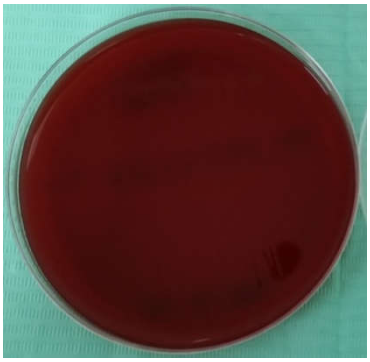
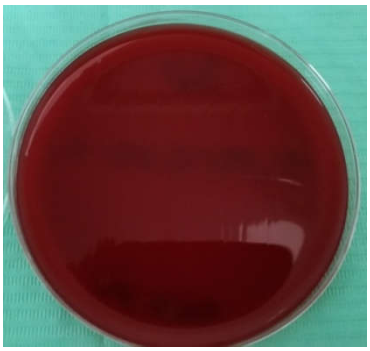
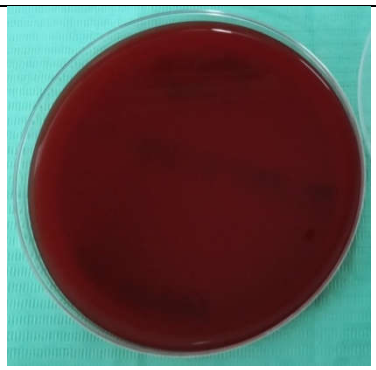
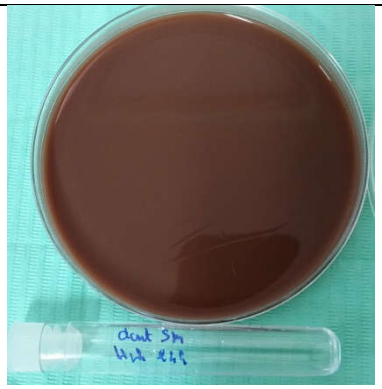
Table 1: Bacterial enumeration (colonies / 1µl) by culture on red Congo medium, stock solutions after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar

	Tooth Number of colonies				Titanium Number of colonies				Agar alone Number of colonies			
	6h		24h		6h		24h		6h		24h	
	CB	CR	CB	CR	CB	CR	CB	CR	CB	CR	CB	CR
S. aureus	80	110	300	450	O	0	25	60	200	440	600	1400
S. intermédius	10	30	150	400	0	0	0	15	20	60	300	750
H. influenzae	40	80	100	220	0	0	10	36	100	321	300	410

CB: Black colony CR: Red colony

Table 2. Optical determination of stock solutions after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar

	Tooth		Titanium		Agar	
	6h	24h	6h	24h	6h	24h
S.aureus	0,011	0,053	0	0	0,012	0 ,179
S.intermeduis	0	0,028	0	0	0	0,178
H.influenzae	0,026	0,048	0	0,050	0,008	0,11

<table>
<tr><td></td><td colspan="2">TOOTH</td></tr>
<tr><td rowspan="6">Streptococcus intermédius</td><td>6h</td><td>24h</td></tr>
<tr><td> 
11a : Dilution : 1/1OO</td><td> 
11b : Dilution : 1/1OO</td></tr>
<tr><td>TITANIUM
6h</td><td>24h</td></tr>
<tr><td> 
11c : Dilution : 1/1OO</td><td> 
11d : Dilution : 1/1OO</td></tr>
<tr><td>MHS Agar
6h</td><td>24h</td></tr>
<tr><td> 
11e : Dilution : 1/1OO</td><td> 
11f : Dilution : 1/1000</td></tr>
<tr><td></td><td colspan="2">TOOTH</td></tr>
<tr><td rowspan="2">Haemophilus influenzae</td><td>6h</td><td>24h</td></tr>
<tr><td> 
12a : Dilution : 1/100</td><td> 
12b : Solution mère</td></tr>
</table>

Continue …..

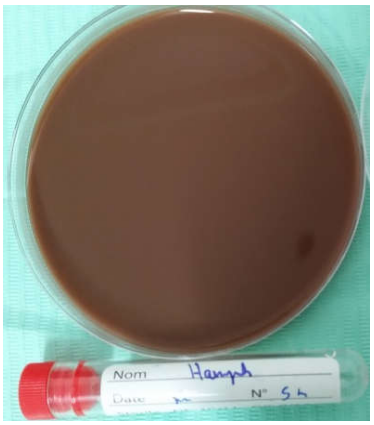
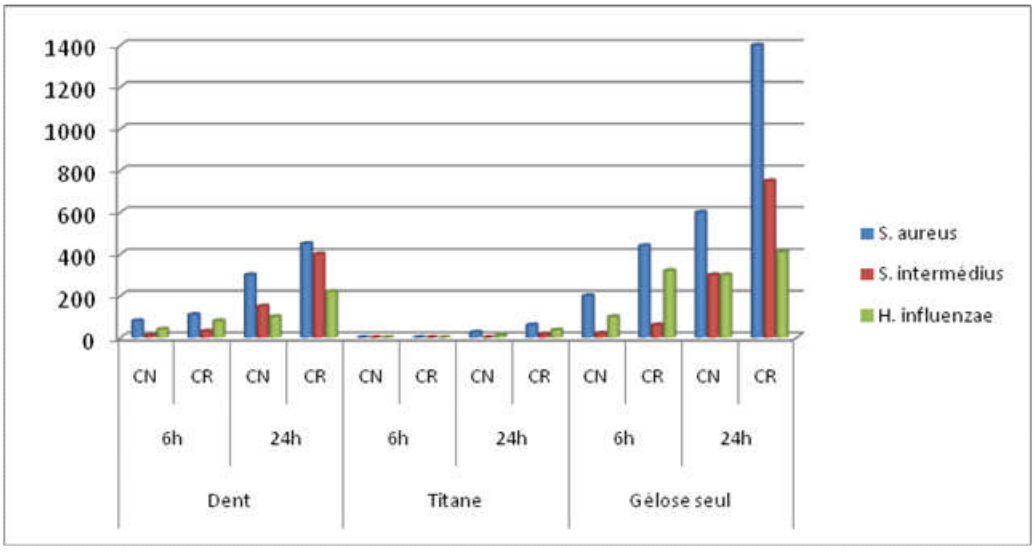
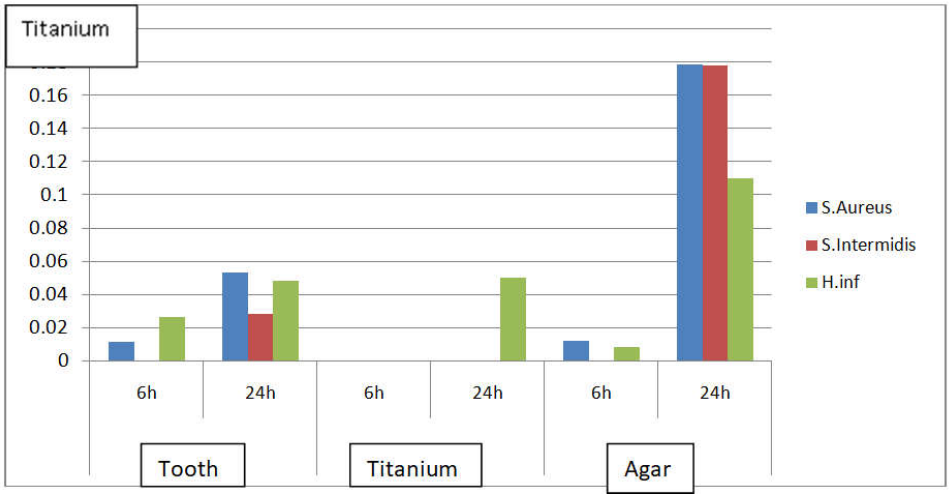
Haemophilus influenzae	**TITANIUM** 6h	24h
	 12c : Solution mère	 12d : Dilution :1/10
	MHS Agar 6h	24h
	 12e : Dilution : 1/100	 12f : Dilution : 1/1000

Figure 6. Results of cultures of different dilutions after 6h and 24h incubation of Haemophilus influenzae in the presence of dental fragments, titanium fragments and agar



Graph 1. Bacterial enumeration (colonies/1µl) by culture on Congo red medium, stock solutions after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar



Graph 2. Optical determination of stock solutions after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar

non-adhesive strains. In order to determine the time required for the bacteria to be in the exponential phase, a proliferation kinetics of S. aureus, S. intermedius and H. influenzae in suspension was carried out for 24 hours. The results expressed in CFU / ml for two culture times were reported in tab.3. They showed that the culture time required for bacteria to be in the exponential phase is 24 hours. This period was chosen to study adhesion on titanium and dental surfaces. Indeed, it is during this phase of growth that Bacterial Adhesins are more expressed but the concentration differs from one strain to another and from one medium to another.

The results showed:

Staphylococcus aureus:

In the presence of dental fragments:

+After 6 hours of incubation, 80 colonies were obtained.
+After 24 hours of incubation, 300 colonies were obtained.
-In the presence of titanium fragments:
+After 6 hours of incubation, the number of colonies is zero.
+After 24 hours of incubation, 25 colonies were obtained.
-In the presence of agar:
+After 6 hours of incubation, 200 colonies were obtained.
+After 24 hours of incubation, 600 colonies were obtained.

Streptococcus intermedius

In the presence of dental fragments:
+After 6 hours of incubation, 10 colonies were obtained.
+After incubation, 150 colonies were obtained.

In the presence of titanium fragments:
+After 6 hours of incubation, the number of colonies is zero.
+After 24 hours of incubation, the number of colonies is zero.

In the presence of agar:
+After 6 hours of incubation, 20 colonies were obtained.
+After 24 hours of incubation, 300 colonies were obtained.

Haemophilus influenza:

In the presence of dental fragments:
+After 6 hours of incubation, 40 colonies were obtained.
+After 24 hours of incubation, 100 colonies were obtained.

In the presence of the titanium fragment:
+After 6 hours of incubation, there is no colony
+After 24 hours of incubation, 10 colonies were obtained.

In the presence of agar:
+After 6 hours of incubation, 100 colonies were obtained.
+After 24 hours of incubation, 300 colonies were obtained.

Evaluation of adhesion potential by optical dosing (tab 4)

Staphylococcus aureus

In the presence of dental fragments:
+After 6 hours of incubation, an OD of 0.011 was obtained.
+After 24 hours of incubation an OD of 0.053 was obtained.

In the presence of titanium fragments:
+After 6 hours of incubation, an OD of 0 was obtained.
+After 24 hours of incubation an OD of 0 was obtained.

In the presence of agar:
+After 6 hours of incubation, an OD of 0.012 was obtained.
+After 24 hours of incubation, an OD of 0.179 was obtained.

Streptococcus intermedia

In the presence of dental fragments:
+After 6 hours of incubation, the optical density is zero.
+After 24 hours of incubation, an OD of 0.028 was obtained.

In the presence of titanium fragments:
+After 6 hours of incubation, an OD of 0 was obtained.
+After 24 hours of incubation, an OD of 0 was obtained.

In the presence of agar:
+After 6 hours of incubation, the optical density is zero.
+After 24 hours of incubation, an OD of 0.178 was obtained.

Haemophilus influenza:

In the presence of dental fragments:
+After 6 hours of incubation, an OD of 0.026 was obtained.
+After 24 hours of incubation, an OD of 0.048 was obtained.

In the presence of titanium fragments:
+After 6 hours of incubation, the optical density is zero.
+After 24 hours of incubation, an OD of 0.050 was obtained.

In the presence of agar:
+After 6 hours of incubation, an OD of 0.008 was obtained.
+After 24 hours of incubation, an OD of 0.11 was obtained.

Comparison between enumeration results from cultures and optical assay: Both counting methods showed similar and correlated results for all three germs after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar. (Graph 1, Graph 2). Enumeration by both methods showed that the number of the three bacteria increased with incubation time in the presence of dental fragments, titanium fragments and agar. The adhesion and proliferation of Streptococcus intermedia are lower than those of Staphylococcus aureus and Haemophilus influenzae after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar. The results also showed that the count of the three bacteria after 6 hours of incubation and in the presence of titanium is zero, which confirms the primary results that we obtained. After 24h incubation we found that the number of bacteria increases in the presence of dental fragments and agar. On the other hand, the number of bacteria increases only with Haemophilus influenzae in the presence of the titanium fragments, whereas the count is zero with Streptococcus intermedia and Staphylococcus aureus.

DISCUSSION

The use of titanium dental implants in oral rehabilitation is becoming more common. One of the main causes of failure of their osseointegration is represented by dental and peri-implant infections. In order to understand the mechanisms of bacterial adhesion on the dental surface and implants, we have cultured germs in contact with dental surfaces and titanium surfaces. This allowed us to understand bacterial adhesion and proliferation mechanisms through enumeration methods (culture and optical assay). The choice of bacteria used was made by their presence in the oral flora and their availability at the bacteriology laboratory at the University Hospital Center of Casablanca. The evaluation of the adhesion capacity of these germs was carried out by a bacterial count after 6h and 24h incubation. We used two counting methods:

- Enumeration by culture on a special medium by pigmentation of adhering bacteria and non-adhering bacteria,
- Enumeration by optical determination of the bacteria adhering to the substrates; for this count we used decreasing dilution solutions of the stock solution prepared at a dilution of 1/1000.
- The results of our work showed that after 6 hours of incubation, there is a total absence of adhesion of Streptococcus intermedius on the surface of dental fragments, titanium fragments and on agar. But after 24 hours of incubation, our results showed the adhesion capacity of these bacteria to the surface of the dental fragments and the agar even with a dilution of 1/100. On the other hand, there was no bacterial adhesion after 24 hours of incubation in the presence of the titanium fragments with 1/10 dilution and with the stock solution.
- Similar studies (Campoccia *et al.,* 2013) have shown that cells culturing 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 inoculation density influences adhesion and proliferation phenomena (Badihi Hauslich, 2013).
- Our results also showed that the culture of Haemophilus influenzae after 6h incubation, there is the formation of bacterial colonies in the presence of dental fragments and agar with a 1/100 dilution and absence of colonies in the presence of titanium fragments. There was an increase in the number of colonies after 24 hours of incubation in the presence of the dental fragments and agar with a 1/100 dilution and the formation of bacterial colonies in the presence of the titanium fragments only with the stock solution.
- Also, the results showed that the culture of Staphylococcus aureus after 6h incubation, there is a bacterial adhesion ability in the presence of dental fragments with 1/100 dilution and absence of colonies with titanium fragments and agar with dilution 1 / 100 and with the stock solution.
- On the other hand, after 24 hours of incubation and with a dilution of 1/100, we observed an increase of the bacterial colonies in the presence of the dental fragments and the formation of bacterial colonies in the presence of the agar but total absence of colonies in the presence of the fragments Titanium with 1/100 dilution and with the stock solution.
- This disparity in the results depends on the composition of the substrates and the nature of its surface state since several studies have shown the influence of these components on cellular adhesion phenomena and proliferation (Faia Torres, 2014; Schneider *et al.,* 2018).
- The optical microscopy examination of the bacteria showed from the first hours of culture that the presence of the dental fragments and the agar do not alter the cell adhesion and spreading capacities. Numerous studies have shown that the porous or fibrillar structure of a material (Teixeira *et al.,* 2007; Palmer *et al.* 2016, Bohner M. et al 2012), its topography or roughness (Faia Torres *et al.,* 2014; Schneider *et al.,* 2018; Bohner M. 2012) and its physicochemical properties (Gargi and Malard, 2007; Li *et al.,* 2012) play an important role. Determining role in the migration, adhesion and synthesis phenomena of the extracellular matrix.
- The results obtained with the cultures of the germs in the presence of the dental fragments can be explained by the roughness of its surface state resembling that of the trabecular bone. Indeed, many in vivo studies have shown that rough surfaces allow better bone integration than smooth surfaces. (Badihi Hauslich, 2013; Aifang *et al.,* 2016; Duske *et al.,* 2015).
- The substrates used in our study have rough surfaces, which could explain the proliferation of bacteria around tooth fragments and titanium fragments. Contrary to the results obtained with steel with a smooth surface, a significant inhibition of the proliferation of Staphylococcus aureus has been observed (Marzak *et al.,* 2010).
- In general, plaque buildup is much greater on rough surfaces than on smooth surfaces, such as metal alloys (Bertrand Anne-Lise, 2004; Derks *et al.,* 2015)
- Plaque adheres not only in larger amounts, but is also more difficult to remove when the surface of the material is irregular (Noda *et al.,* 2015; Costa *et al.,* 2011). Indeed, the grooves and other surface defects cause an increase in the potential surface to be colonized, and are favorable places for the creation of microbial niches (Bertrand Anne-Lise, 2004).

- Numerous studies have shown that surface roughness is an important factor in oral formation and retention (Derks *et al.,* 2015; Costa *et al.,* 2011;Extremina *et al.,* 2010). The influence of surface characteristics on bacterial adhesion (Staphylococus aureus) has been demonstrated (Duske *et al.,* 2015; Thomas *et al.,* 2006), a significant increase in the number of bacteria has been observed on modified titanium surfaces compared to the relatively smooth surface of a pure titanium implant. It has been shown that this increased adhesion of bacteria may be the cause of the high rate of peri-implant infection. DRAKE *et al.* (Arciola, 2012) showed in their study that colonization of blood streptococci is greater on rough surfaces.
- In contrast, for Thomas A. Schildhauer (Thomas *et al.,* 2006), there was no statistically significant correlation between bacterial adhesion and the roughness of the implant surface. The chemical composition of the implant is a factor that influences proliferation and bacterial adhesion.
- The titanium alloy is composed of 90% Titanium, 6% Aluminum, 4% Vanadium. These different materials have a sensitivity to adhesion, showing values not significantly different from those agar plates inoculared by bacteria.
- Thus, the chemical composition of the material can influence the adhesion and colonization of bacteria on the implant surface. Indeed, several studies are currently focused on the chemical and topographic surface modifications of implantology materials in order to develop surfaces with bacterial adhesion inhibiting properties (Noda *et al.,* 2015; Ahimou *et al.,* 2001; Rosa *et al.,* 2003).
- Our results also showed that the three bacterial strains formed a cellular mat covering the entire box including the dental fragments. On the other hand, at the level of the second box, a decrease in proliferation around the titanium fragments has been observed with the appearance of a zone of inhibition of 1-2 mm, with a total invasion of the box in culture with agar. Similar studies Elagli A. (Sgolastra *et al.,* 2015) have worked on the effects of titanium powder on seven bacteria commonly found in dental plaque or in the gingival sulcus, they showed that the titanium alloy has no inhibitory activity or stimulator on bacterial adhesion.
- The adhesion of the bacteria to the implant depends on the physicochemical and topographical properties of the surface thereof. Other studies have shown (Campoccia *et al.,* 2013; Goodman, 2013) that the modification of the surface condition of titanium renders it antibacterial, in another study a surface treatment which converts amorphous titanium oxide into a crystalline layer enriched with could provide titanium with antibacterial properties (Duske *et al.,* 2015). To the naked eye we observed on the culture dishes in the presence of titanium fragments, an inhibition zone all around these fragments, these areas are identical to those observed in the presence of antibiotic discs. Other studies have shown that titanium could release toxic ions and cause inhibition of adhesion and hence proliferation (Singh, 2012).
- Both counting methods showed similar and correlated results for the three bacterial strains after 6h and 24h incubation in the presence of dental fragments, titanium fragments and agar. Enumeration by both methods also showed that the number of the three bacteria increased with incubation time in the presence of dental fragments, titanium fragments and agar.
- The adhesion and proliferation of Streptococcus intermedia is lower than Staphylococcus aureus and Haemophilus influenzae after 6 hours of incubation in the presence of dental fragments, titanium fragments and agar.
- The results also showed that the count of the three bacteria after 6 hours of incubation and in the presence of the three substrates is very low to zero, which confirms the primary results that we obtained. After 24h incubation we found that the number of bacteria increases in the presence of dental fragments and agar.
- On the other hand, the number of bacteria increases only with Haemophilus influenzae in the presence of the titanium fragments whereas the count is nil with the Streptococcus intermedius and the Staphylococcus aureus. The adhesion and proliferation of bacteria depend on the types 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 (Anselme, 2010; Shibata, 2015)

Conclusion

Bacterial adhesion to tooth surfaces and titanium surfaces is a prerequisite for the formation and maturation of the biofilm responsible for pathological processes such as tooth decay and periodontal disease. Therefore, a great interest has been shown for many years in the study of adhesion mechanisms on dental surfaces and on titanium surfaces and in particular the study of changes in the surface state of these structures. Indeed, this bacterial adhesion on these surfaces has been approached by several methods, such as the chemical or immunological blocking of adhesion sites and the mechanical removal of bacteria and the surface treatment of titanium. Many studies on the mechanisms governing adhesion of oral bacteria have been carried out in powder form with hydroxyapatite as a substrate, but only a few have been made on solid substrates. In our study we used solid surfaces to try to understand the mechanisms of bacterial adhesion in conditions similar to the oral cavity.

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