BIOFILM INFLUENCE ON THE PAIR TRIBOLOGICAL WEAR TOOTH _ZIRCONIA

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ABSTRACT

Tooth wear is an inevitable process due to chewing conditions. In the development of new dental restoration materials one of the requirement is exhibiting a low wear and avoid abnormal wear of the antagonist teeth.
Usually the wear tests are carried out in distilled water or artificial saliva without taking into account the existence of microorganisms.
The aim of the present study was to evaluate the effect of bacteria biofilm in the tribological pair wear zirconia/tooth and *Streptococcus salivarius* (*S. salivarius*) was the selected bacteria in the complex oral microbiota.
The wear tests, pin-on-plate type, used cusps of molars and premolars, as pins and zirconia as plates. Three lubricants were used in a comparative study: artificial saliva, artificial saliva with glucose and artificial saliva with glucose and *S. salivarius*, at a temperature of 37 °C and pH 7. Results show that in the presence of *S. salivarius* there is a 70% reduction in the friction coefficient and a 50% reduction in wear. No wear was detected on the zirconia surface.
It was concluded that the existence of biofilm in the zirconia/tooth interface affects the tribological results, leading to a decrease in tooth friction coefficient and wear.

KEY WORDS: Wear, bacteria, biofilm, biotribology

1.- INTRODUCTION

Tooth tissue damage is replaced by using several artificial materials in order to restore the normal mastication and phonetics functions. These materials need to mimic the natural tooth color and translucent and must resist the high stress during mitigation, thermal shook and chemical attach of the environment among others. One of the major requirements in the materials selection for dental proposals it is to be wear resistant and avoid abnormal wear of antagonist natural contacting tooth. Abnormal wear leads to lack on the contacting opposing surfaces resulting in disturbance of the mastication process efficiency. The tribological performance of dental materials is extensively studied, using as lubricant water or artificial saliva, however no attention have been given to the presence of bacteria [1].

The human oral cavity holds one of the most diverse microbiome in the human body. It includes viruses, fungi, protozoa, archaea and bacteria. These microorganisms regulate each other and coexist within the microbial community [2]. They can be found in complex biofilms attached to soft and hard tissues assembled in an ordered process that begins with adherence of microbial colonizers to a surface followed by attachment of different microbial species to initial colonizers [3,4].
In oral microbiota, *Streptococci* are the major group of microorganisms when compared to other genera. The species most frequently isolated from the oral cavity are *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococus mitis*, and *mutans Streptococci* [5-7]. *Streptococcus salivarius* is a lactic acid bacterium and is the first commensal bacterium that appears in the oral cavity of newborns. Is a gram positive bacteria that has adhesion systems that garantee its residence in the oral cavity [8].

The aim of the present study was to evaluate the impact of biofilm on tribological response. For that, it was assessed the effect of *Streptococcus salivarius* (*S. salivarius*) biofilm in the tribological response of the zirconia/tooth pair.

2.- MATERIALS AND METHODS

Reciprocating pin-on-plate wear tests were performed using plates of zirconia and cusps of molar and premolar human teeth as pins in three different lubricants: artificial saliva (AS), artificial saliva with glucose (AS+G) and finally artificial saliva with glucose and *Streptococcus salivarius* (AS+G+Ss). Six tests were performed per lubricant. The tribological tests operational conditions are given in Table 1. The friction force was motorized during the tests.

Table 1. Wear test operational conditions.

Frequency (Hz)	1
Stroke (mm)	5
Load (N)	2
Temperature (ºC)	37
Duration (h)	6

Zirconia plates were obtained from pre-sinter zirconia blocks. These blocks were cut in form of plates and sintered according to the supplier procedure. After, the plates were grinded with 600 mesh sand paper. The plates were characterized by roughness measurement (Mitutoyo Surftest-301).
The pins were prepared using molar and pre molar healthy human teeth and acrylic resin. Each tooth was cut in order to individualise each cusp. The cusps were mounted in acrylic resin. The pins were maintained hydrated in water. For each test condition it was used a cusp belonging to a different tooth. The cup´s radius was evaluated by profilometry (Mitutoyo, Contracer CP. 200).

The lubricating conditions were determined by the growth profile of *Streptococcus salivarius* in artificial saliva (AS) and in artificial saliva with three different supplements: glucose, meat extract and urea respectively. Artificial saliva is a modification of the used by Roger *et al.*, in that porcine stomach type III was not used and urea was used as a supplement [8].
The growth profiles were obtained recording the absorvance at 600 nm over time.

Streptococcus salivarius (MICROBIOLOGICS) was activated in blood agar (Liofilchem), according to supplier information, and upon 24 h incubation at 37 ^{o}C was transferred and precultured twice in AS till absorvance at 600 nm equal 0.6 (corresponding to stationary phase) and then used in tribological tests.

After wear testing, the worn surfaces were observed by light optical microscopy (LOM) and scan electron microscopy (SEM) and the dental wear was assessed by worn cusp area measurement.

3.- RESULTS AND DISCUSSION

Streptococcus salivarius growth profiles were determined in artificial saliva (AS) and in artificial saliva with three different supplements: glucose (G), meat extract (ME) and ureia (U). Table 2 displays the obtained value for the beginning of the stationary phase. The maximum absorvance value for artificial saliva with glucose (AS+G) shows that the highest bacteria proliferation is obtained in this medium.

Table 2. *Streptococcus salivarius* absorvance in stationary phase for the tested artificial saliva.

Artificial saliva	Absorvance 600 nm
AS+Ss	0.854
AS+G+Ss	1.028
AS+ME+Ss	0.380
AS+U+Ss	0.229

Therefore AS+G was selected as lubricant for the tribological tests.

It was also studied the effect of the material in *Streptococcus salivarius* growth (Table 3) showing that the presence of both, zirconia and tooth, leads to a decrease on plantonic bacteria concentration. The observed reduction is related to the biofilm formation on the tested materials.

Table 3. Material (zirconia/tooth) influence on *Streptococcus salivarius* growth.

Tested condition	Absorvance 600 nm
AS+G+Ss	1.028
AS+G+Ss+zirconia	0.843
AS+G+Ss+tooth	0.759

The zirconia plates roughness obtained was 1.64 ± 0.54 μm. A similar value has been measured in plates whose surface finishing procedure was done according to dental crowds manufacture procedure [9]. According to Bollen *et al.*, the lower limit for bacteria adhesion is Ra= 0.2 μm and increases with Ra value [10]. In fact, biofilm was observed on the zirconia surface after 24 hours of exposition to *Streptococcus salivarius* (Figure 1). Even it is possible to observe that *Streptococcus salivarius* proliferation was higher in plate scratched areas.

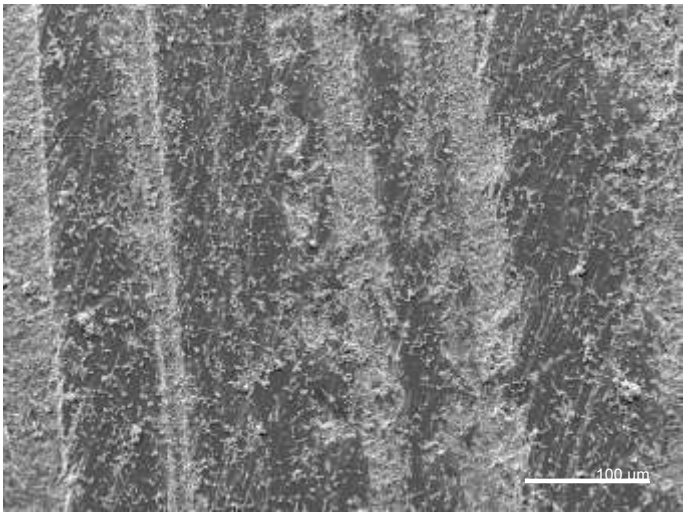


Figure 1. SEM image of the zirconia biofilm obtained by 24 h exposition to AS+G+Ss.

The friction coefficient was quite stable for the three lubricants studied: 0.69 ± 0.05 for AS, 0.50±0.04 for AS+G and 0.16±0.04 for AS+G+Ss. The friction reduction upon glucose addition to AS is probably due to glucose organic molecules adsorption at the sliding surfaces. The reduction observed in the presence of *Streptococcus salivarius* suggests that bacteria works as boundary lubricant reducing the contact between the surfaces during sliding.

No zirconia wear was observed in any of the tests in contrast with dental wear that was always observed. The dental wear was very similar in AS and in AS+G but in the presence of bacteria a reduction of 50% was observed (Figure 2).

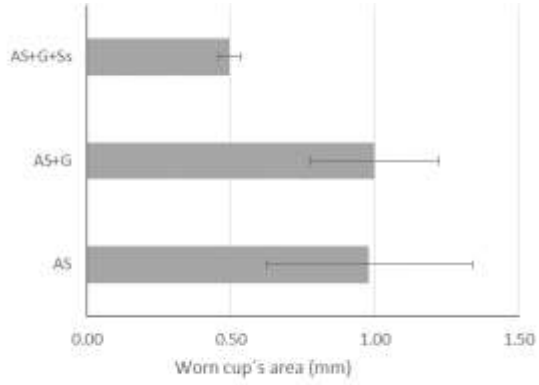
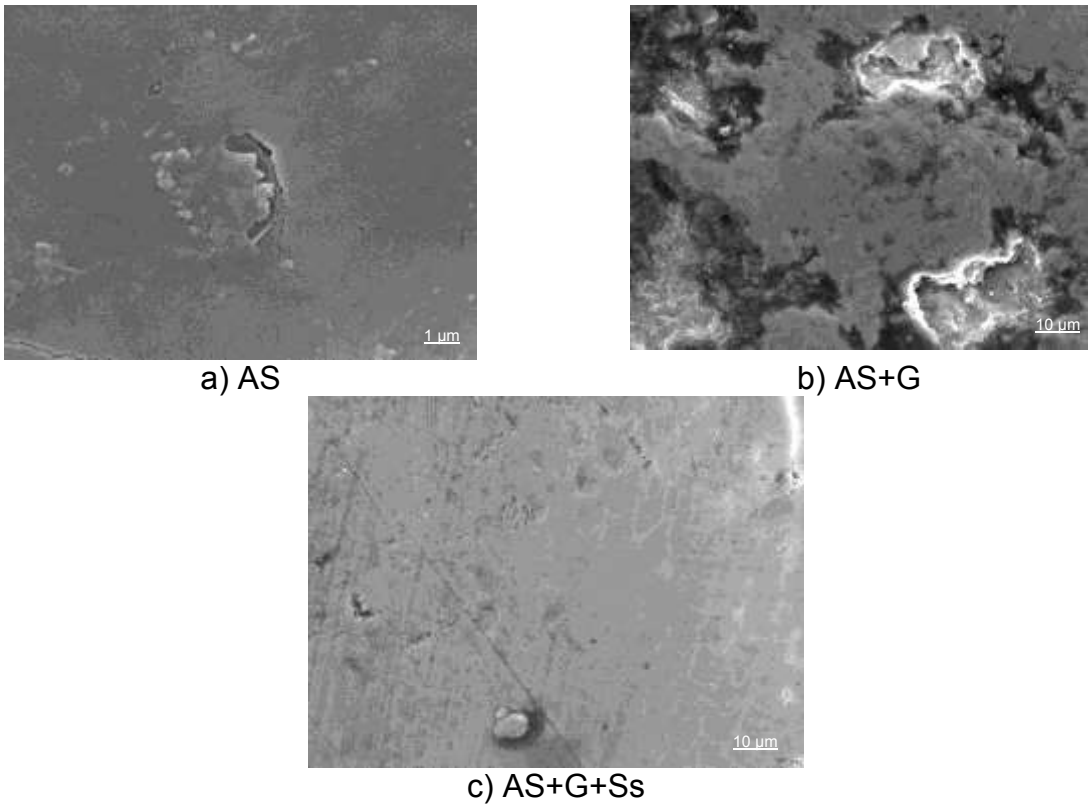


Figure 2. Cusps worn area in the tested lubricants.

A granulose tribolayer on the worn surface of the cusps tested in artificial saliva (AS) and artificial saliva with glucose (AS+G) was observed by SEM (Figure 3). In contrast, the cusps tested with the lubricant containing bacteria (AS+G+Ss) shown a very tiny layer allowing the observation of the prismatic enamel structure. The layer with a granular structure suggests that it was obtained by wear particles compaction. The results suggest that glucose may play a role in this compaction process. In fact the layers obtained for AS+G are thicker (Figure 3 b). According to Eisenburger and Addy the proteins (enamelins and amelogenins), that covers the hibroxapatite

crystals, function as binders leading to particles agglomeration on the worn tooth surface [11].
The present study also suggests that glucose works was binder. Probably glucose is absorbed to the enamel debris increasing the compaction process. Additionally, in all tested surfaces, it was found features associated to delamination. In presence of bacteria this layer was much thinner (Figure 3 c) and the wear was lower (Figure 2).



a) AS b) AS+G

c) AS+G+Ss

Figure 3. SEM images of the cusp surfaces worn in different lubricants: a) AS; b) AS+G and c) AS+G+Ss.

Overall the results showed that the tribological response of the tribosystem is influenced by the presence of *Streptococcus salivarius.*

4.- CONCLUSIONS

The present study had the objective to study the influence of the presence of bacteria on the tribological response. For that pin-on-plate reciprocating wear tests were performed using pins of human cusps and zirconia plates. *Streptococcus salivarius* was the selected bacteria and the growth conditions were studied. The results showed:

a) The best *Streptococcus salivarius* growth conditions were achieved in artificial saliva with glucose as supplement;
b) The presence of tooth and zirconia reduced the concentration of *Streptococcus salivarius* in the planktonic form, demonstrating the ability to form biofilm on the material;
c) The presence of bacteria reduces the friction coefficient in ~70% and the dental wear in ~50%.

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