Pilot Study: In vivo Characterization of the microbiome present on titanium implant surfaces

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Introduction

The vast majority of infections associated with dental implants result from bacterial adhesion to the surface of biomaterials and subsequent formation of biofilm.[1] Peri-implantitis results from a process that comprises bacterial interactions and an excessive inflammatory response from the host.[2] The microbiome in implants with peri-implantitis has been described as similar to that found in patients with chronic periodontitis.[2]
The purpose of the modifications present on implant surfaces is to positively affect the tissues response to implant placement, by reducing bacterial adhesion and increasing osteointegration.[3]

Objectives

Characterization of the formed microbiome on treated and fully machined titanium implant surfaces present in the oral cavity of human volunteers for 48 hours, and to investigate the impact of microorganisms on the implants structure.

Hypothesis 1 - null: Vellox® (treated) surface treatment does not influence the adhesion of bacterial biofilm to titanium implants

Alternative hypothesis 1: Vellox® surface treatment influences the adhesion of bacterial biofilm to titanium implants

Hypothesis 2 – null: Biofilm does not cause changes on implant surfaces

Alternative hypothesis 2: Biofilm causes changes on implant surfaces

Materials and Methods

Twenty-two volunteers used intraoral devices containing, on each side, a machined titanium implant and an implant with a Vellox® surface treatment (double acid etching + aluminum oxide blasting) (SignoVinces®) for a period of 48 hours. After this period, the implants were collected and the adhered bacterial microbiome was characterized due to its composition and diversity using the sequencing of the genes of the 16S portion of the ribosomal RNA. In addition, the studied implants were observed using the Scanning Electron Microscope (Quanta 400FEG ESEM / EDAX Genesis X4M) and their surface wettability was obtained by measuring the contact angle using the deposition of sessile drop.

Figure 1- Occlusal view of the intraoral device in the mouth

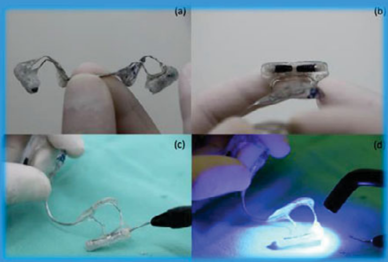

Figure 2- Placement of the implants under study in the intraoral device; (a) Front view of the device with the implants; (b) Side view of the acrylic box of the device with the implants; (c) Placing the composite resin as an anchorage system for the implants in the acrylic boxes; (d) Polymerization of composite resin

Figure 3- Analysis of wettability with deposition of sessile drop in 3 different locations on the implants surface (Vellox®)

Results

Wettability Analysis

Statistical analysis of the contact angles value shows that contact angles are significantly higher on the Vellox surface (135.6 ° ± 2.8), compared to the surface machined (102.6º ± 0.4), based on a 1st order autoregressive structure, with variance and homogeneous correlation ($p < 0.001$). Thus, the Vellox® surface has less wettability than the machined surface.

Microscopic Analysis

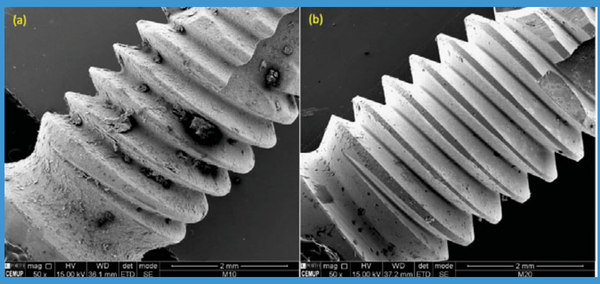

Figure 4-Image obtained due to observation with SEM with 50x magnification of both studied surfaces (without application of the collection protocol); (a) Vellox® surface subject to the environment of the oral cavity (with contamination and without application of biofilm removal protocol), (b) Machined surface subject to the environment of the oral cavity (with contamination and without application of the removal protocol of the biofilm) biofilm). (Image captured by secondary electron receivers)

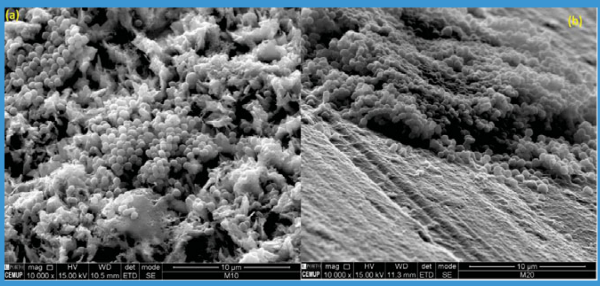

Figure 5-Image obtained due to observation with SEM with 10000x magnification of both studied surfaces (without application of the collection protocol); (a) Vellox® surface subject to the environment of the oral cavity (with contamination and without application of a biofilm removal protocol), (b) Machined surface subject to the environment of the oral cavity (with contamination and without application of the removal protocol of the biofilm) biofilm). (Image captured by secondary electron receivers)

Microbiological Analysis

Readings obtained during the sequencing process were subsequently grouped into taxonomic operational units (OTU's) with 97% similarity, resulting in a total of 2169 OTU's. After identifying the OTU´s, they were added to each taxonomic ranking, obtaining 34 bacterial strains.

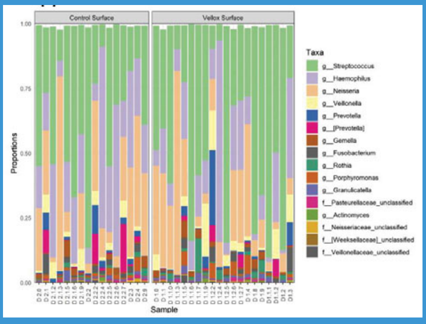

Figure 6-Aggregation rate in each taxonomic ranking (Species)

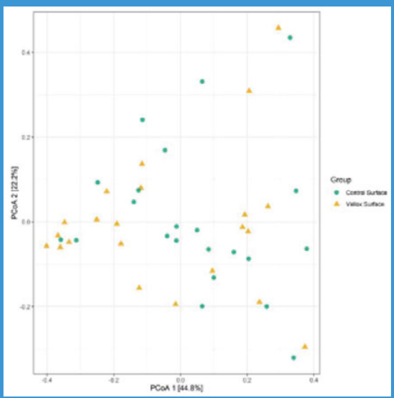

Figure 7-Graphic of the beginning - analysis of the main coordinates of the microbial composition of all samples

Comparing the bacterial adhesion of the two surfaces, it appears that it occurs in a similar way on both surfaces, being marked by the absence of a statistically significant difference in beta diversity.

Discussion

The analysis of this surface using a scanning electron microscope already exists described in the literature. In this investigation it was observed that microscopically the Vellox® surface presents reliefs and cavities with variable dimensions, which are randomly distributed on the previously blasted surface. It was also found that the machined surface has a flat topography with circular grooves resulting from the milling process.[4] The results obtained in this investigation are in agreement with those obtained Giner et al. (2018).[4]
In this study, the characterization of microorganisms adhered to the Vellox® surface *in vivo* was performed for the first time. It was possible to verify that the bacterial composition on both surfaces studied was the same, being this conclusion based on the absence of statistically significant differences in beta diversity and bacterial proportions. These results contrast with what is described in the literature, in which there seems to be a greater bacterial adhesion to irregular implant surfaces than to flat surfaces. Thus, it is possible to state that in vivo, qualitatively, bacterial colonization is not influenced by the topographic characteristics or physicochemical properties of the Vellox® surface. This result corroborates previous studies such as Ferreira Ribeiro et al. (2016) who concluded that the roughness and microtopography of the implant surface did not stimulate the formation of the initial biofilm on titanium surfaces with different surface treatments.[5]

Conclusion

With this investigation, it can be concluded that the application of double acid conditioning in addition to sandblasting with aluminum oxide particles decreases the surfaces wettability, being this decrease represented by the increase in the contact angle. Furthermore, the exposure of the implanted Vellox® surface and the machined surface to the oral cavity for a period of 48 hours during the formation of the initial biofilm does not allow microstructural changes to be observed in them. It is also possible to conclude that bacterial colonization occurred in the same way on the Vellox® surface and on the machined surface (control), having a similar constituent microbiome that microtopography and wettability do not stimulate the formation of the initial biofilm on titanium surfaces with different surface treatments.

Clinical Implications

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This investigation helps, not only to understand the peri-implant disease through its microbiological etiology, as well as the mechanisms that implant manufacturers can adopt to reduce its incidence.

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