COMPARISON OF DIFFERENT CHEMICAL AND MECHANICAL DECONTAMINATION MODALITIES ON TITANIUM DENTAL IMPLANTS: MICROBIOLOGICAL AND BIOCOMPATIBILITY ANALYSES

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Abstract

The aim of this *in vitro* study was to evaluate the efficacy of chemical and mechanical methods used for decontamination of titanium dental implants previously infected with polymicrobial biofilms in a model simulating a peri-implant defect. Furthermore, the effect of each decontamination protocol on MG-63 cells morphology and adhesion to the treated implants was assessed. A polimicrobic biofilm has been grown on 40 implants. Before treatment the implants were placed into a model simulating a peri-implant defect. Implants were randomly assigned to 5 treatment groups: 1) no treatment, 2) air-abrasion without any powder, 3) air-abrasion with powder of erythritol, amorphous silica and 0.3% chlorhexidine (ESC), 4) a sulfonic/sulfuric acid solution alone (HBX), or 5) a combination of ESC and HBX (ESC+HBX). From 5 implants per group the remaining colonies were counted (as $\log_{10}$CFU/mL) for each bacterial strain and for the total number of colonies. The remaining 3 implants per group and 3 non-contaminated implants were used to assess biocompatibility after treatment. A significant decontaminant effect was achieved using the HBX alone or in combination with ESC while no differences were shown between the groups receiving other treatments. Moreover treatments with HBX were able to reduce the contamination of the implants to a level that didn't interfere with MG-63 regrowth.

Introduction

The main cause of peri-implant diseases is bacteria (1,2). Therefore the treatment of these diseases is targeted on effective removal of the microbial biofilm (3). Peri-implant mucositis and incipient forms of peri-implantitis might be treated by non-surgical debridement. Anyway, more severe forms of peri-implantitis often require additional therapy, since the morphology of the fixture offers macroscopic and microscopic repair to bacterial cells harbored on the surface and the results of non-surgical treatment of such conditions is usually unpredictable.

A variety of chemical and/or mechanical methods have been tested for treatment of implant surface, but none was found to be superior to others (4). A recent systematic review (5) showed that air powder abrasion with glycine powder may result in better clinical outcome than other approaches, even if complete resolution of the disease was still unlikely. Air powder abrasion has shown some advantages in terms of biofilm removal in some *in vitro* experiments when compared to other treatment approaches (6). However, complete surface cleaning is not achievable irrespective of the surgical or non-surgical approach (7,8). For this reason a combination of mechanical and chemical methods has been claimed to provide

better results. In recent years, new approaches have been proposed to treat biofilm-induced diseases. A novel topical sulfonic/sulfuric acid (HBX) solution has been developed. The sulfate components strongly absorb water from vital organic biofilm components. The result is a coagulation of the entire biofilm matrix that i) destroys its attachment mechanisms to the underlying tissues, and ii) kills bacterial cells (12). Up to now, the use of HBX alone or in combination with air powder abrasion has shown promising results for the non-surgical treatment of acute periodontal abscesses and peri-implantitis (13,14,15).
Another important aspect of implant surface decontamination is the effect of treatment modalities on the surface topography and chemical composition that may impair the re-osseointegration. Sodium bicarbonate or a powder composed of erythritol, amorphous silica and 0.3% chlorhexidine (ESC) have been demonstrated effective to remove biofilm from rough implant surfaces without interfering with osteoblast growth over the previously contaminated titanium surface (9-11) whilst chemicals such as chlorhexidine (CHX) and citric acid (CA) has been proven to adversely affect cellular regrowth after treatment.
For these reasons, the aim of this study was the evaluation of the decontamination potential of HBX application followed by air-abrasion with ESC powder on previously biofilm-contaminated implants in terms of residual viable bacterial load measured in $\log_{10}$CFU/ in comparison with the treatment with HBX alone, ESC alone, with air-abrasion without any powder and no treatment. Furthermore, the effect of each decontamination protocol on MG-63 cells morphology and adhesion to the treated implants was assessed.

Materials and Methods

Forty-three sterile dental implants (OSSEOTITE® CERTAIN™ IOS IMPLANT 4.00mm x 11.50 mm; BIOMET 3i LLC, Palm Beach Garden, FL, USA), were included into the study.

Implants contamination

A polymicrobial biofilm has been grown on 40 implants. In order to develop a vial polymicrobial biofilm *in vitro* the following bacteria have been obtained commercially and used:

- *Staphylococcus aureus* (ATCC25923)
- *Staphylococcus epidermidis* (ATCC49461)
- *Streptococcus anginosus* (ATCC33397)
- *Streptococcus salivarius* (ATCC13419)
- *Streptococcus mitis* (ATCC9811)
- *Fusobacterium nucleatum* (ATCC10953)
- *Capnocytophaga ochracea* (ATCC27872)

Whole unstimulated saliva was collected from 10 periodontally healthy volunteers. Subjects who used antibiotics in the previous two weeks have been excluded from the donators. Saliva has then been pooled, aliquoted and stored at -20°C.
Biofilm has been grown on 40 dental implants (BIOMET 3i LLC, Palm Beach Garden, FL, USA) in medium consisting of 60% of whole unstimulated saliva and 40% brain heart infusion (BHI). In brief, each bacterial strain has been separately cultured on CDC ANAEROBE +5% SB plates for 48h at 37°C in CO_2 (S. *aureus*, S. *epidermidis*, S. *anginosus*, S. *salivarius*, S. *mitis* e C. *ochracea*) or in anaerobic conditions (F. *nucleatum*). Then, a bacterial suspension 4 McF (1200 X 106 CFU/ml) in BHI has been prepared. Saliva aliquots were defrosted and the bacteria contained in it were identified by mean of the MALDI-TOF (Matrix Assisted Laser Desorption Ionization – Time of Fly).

Forty dental implants have been then incubated in 3ml of defrosted pooled saliva in anaerobic conditions at room temperature for 4h, in order to promote the formation of the *acquired pellicle* (16). Next, saliva has been substituted with 1.8ml of defrosted pooled saliva, 1.2ml of BHI and 602ml of mixed bacterial suspension (86µl of suspension 4 McF per each strain) that has been removed and renewed 16h after. At 40h the implants have been washed and the culture medium renewed. Incubated dental implants had been repeatedly washed with sterile saline after 16h, 20h, 24h, 40h, 44h, 48h and 64h. Total time of anaerobic incubation was 64h at 37.0 °C.

Model of peri-implantitis defect

A model that simulated a crater-like peri-implant defect was created by the mean of an aluminum hemisphere of 1 cm of diameter inserted into dental impression material (EliteHD+ Putty Normal Set; Zermack, Badia Polesine Italy) contained into a squared plastic box. Implants were then placed into the model that simulated a peri-implant defect with a 5mm deep intrabony, crater-like component and a 5mm deep suprabony component.

Implants decontamination

Fourty contamined implants were randomly assigned to five different groups including 4 decontaminating procedures and 1 control group by the use of a computer generated random sequence of numbers (SPSS 24.0; SPSS Inc., Chicago, IL, USA). :

- Group ESC: air powder abrasion with ESC alone (Air- Flow Master®, E.M.S. Electro Medical Systems GmbH, Munich, Germany; Air-Flow® Plus Sub+Supragingival, E.M.S. Electro Medical Systems GmbH, Munich, Germany);
- Group HBX: HBX alone (EPIEN MEDICAL, Saint Paul, MN, USA);
- Group HBX+ESC: combination of air powder abrasion with ESC and HBX;
- Group AW: using only a spray of air and water coming from the air abrasive device.
- Group C: no treatment

In groups ESC and HBX+ESC, air flow system was used on the dental unit and set at a static water pressure of 4.5 bar and a static air pressure of 6 bar for each specimen. Cleansing time was set at 120 seconds per implant with circumferential movements going all around the implant surface. Efforts were made in order to maintain the spray as perpendicular to the implant long axis as possible.
In groups EBX and HBX+ESC, HBX was applied for 20 seconds to the implant surface proceeding from the most apical part of the defect to the most coronal part of it with circular movements. When the treatment procedure was the combination of ESC and HBX, the latter was applied before the ESC. At the end of the treatment procedures all the implants, including those of group C, were gently rinsed for 60 seconds with sterile saline solution.

Microbiological tests

Quantification of viable bacterial cells

After decontamination, 5 implants per group were randomly selected and were placed into 15ml Falcon tubes. They were immerged in a 0.1% dithiothreitol solution (DTT), and vortexed for 15 minutes in order to remove the residual biofilm. Then implants were removed and the DTT solution has been centrifuged for 5 minutes at 2500 rpm. The supernatant has been eliminated and the resultant cell suspension was serially diluted (10-fold). 20µl of suspended bacteria were collected and aliquots of 10µl were plated in duplicates on blood agar plates supplemented with 5% defibrinated horse blood. Per each dilution two plates were incubated anaerobically (Gas Pak, Becton, Cockeysville, USA) and the conditions were controlled with affiliated indicator strips. Other two plates were incubated aerobically both at 37°C for 48h. The resulting colonies were counted (as CFU/mL) for each bacterial strain and for the total

number of colonies. *F. nucleatum*, and *Propionibacterium acnes*, which was present in the pooled saliva, were counted on plates in anaerobic conditions. The other bacterial species were counted on the plates in aerobiosis. Only plates containing between 25 to 250 colonies were considered valid (Tomasiewicz, Hotchkiss, Reinbold, Read, & Hartman, 1980). Counts were provided according to bacterial species and total bacterial counts. All counts were then transformed into the $\log_{10}$CFU/ml.

Identification of bacterial cells

The identification of the bacteria grown on culture plates has been performed using the MALDI-TOF.

Biocompatibility test

The remaining 3 implants per treatment group and the 3 non-contaminated/non-treated group (group NC) (total 18 implants) were used for the biocompatibility test.

Osteoblast-like Cells regrowth on treated implants

Immediately after treatment, osteoblast-like cells (osteosarcoma cells; MG-63; ATCC® CRL-1427™; LGC Standards, Wesel, Germany) were seeded onto the top of implants. Before cells were seeded, 1.3 ml of media was placed in each micro-plate well containing the implants. Then, 150 µl of cell suspension, adjusted to 1.5 x 10^5 cells/ml, were pipetted in meandering pattern above prepared specimen. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10% Fetal Bovine Serum (FBS) without phenol red and any antibiotics (to allow concomitant biofilm re-growth) at 37°C in a humidified atmosphere with 5% CO_2 for 5 days, without media changing. Cells were cultivated in tissue culture flasks (Eppendorf Italia Srl, Milan, Italy) and were split at approximately 80% of confluence by trypsin (0.05%)/EDTA (0.02%) solution (Sigma-Aldrich, Milan, Italy), stopped with DMEM containing 20% FBS, to attain an adequate number of cells.

Influence of Decontamination on MG-63 growth

In order to assess the effect of each decontamination protocol on the MG-63 morphology and adhesion to implant surface, after incubation period, samples were fixed with 2.5% glutaraldehyde in buffered saline solution, dehydrated through a graded series of alcohol, dried and observed at Scanning Electron Microscope (SEM) for morphological assessments and Backscattered Electron Microscope (BES) for semi-quantitative analysis (Jeol Nikon JCM-6000P, at 15 kV). All implants were photographed by a blind operator (GP). For each implant, (a) one photo at low magnification (20x) was taken to describe cells distribution on implant surface; (b) 6 photos at a total magnification of 55x were taken in the area where cells had been seeded to perform semi-quantitative analysis; (c) high magnification photos (440x to 1500x) were taken on few randomly selected samples to assess the cellular morphology.

The percentage of implant surface covered by adherent cells was calculated by the same blind operator (GP) on 55x photos using an image analysis system (adobe, Photoshop CS5).

Statistical analysis

In order to assess the decontaminant effect of the different treatment methods the viable CFU/ml was determined per each identified bacterial species and per the total bacteria grown on culture plates from duplicate experiments to provide stringent estimates of reliable results of CFU/ml drawn from this specific methodology. Thereafter, CFU/ml was calculated and the results were converted in the log scale to obtain a normal distribution. Inter-group log reduction was provided. The results were statistically analyzed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA). Kologorov-Smirnof test revealed normal distribution of data ($p > 0.005$) for all groups except for group ESC ($p < 0.005$). Levene's test revealed heterogeneity of variances ($p < 0.005$). Since the variance was not homogenous, Kruskal-Wallis test was run. In order to

investigate inter-group differences pair-wise comparisons were performed. P-value was set at 0.05. The influence of decontamination treatment on cellular adhesion was analyzed by descriptive statistics. The percentage of implant surface covered by MG-63 cells was computed for all samples (n=3) of each group, then mean and standard deviation were calculated for each group.

Results

Microbiological test - Quantification of viable bacterial cells

The effect of the five different decontamination methods on the viability of the implant-associated biofilm (log10 CFU/ml) is shown in **Figure 1**.
The presentation of the results is according to the type of treatments and viable log_{10}CFU/ml. Means, medians, standard deviations, minimum, and maximum of log_{10}CFU/ml per the total bacterial counts are presented in **Table 1**. The Kruskal-Wallis test revealed that at least one group was different from the others (p = 0.001). Pair-wise comparisons showed that the use of HBX and the combination of HBX + ESC were superior to group C (p = 0.012 and p = 0.037 respectively) in reducing total bacterial counts. HBX performed also better than AW (p = 0.018). The differences between the HBX + ESC and AW were on the threshold of statistical significance (p = 0.056).
The percentage reduction of total viable log_{10}CFU/ml is reported in **Table 2**.
The MALDI-TOF identified the following bacteria in the pooled defrosted saliva: *Pseudomonas aeruginosa, Serratia marcescens, Streptococcus parasanguinis, Streptococcus faecalis, Klebsiella Oxytoka, Granulicatella adiacens, P. acnes, and Micrococcus luteus*. Limit of detection (LOD) was < 1.0×10^2 CFU/ml. Limit of quantification (LOQ) was set at < 2.5×10^3 CFU/ml. Means, medians, standard deviations, minimum, and maximum of log_{10}CFU/ml per single species are presented in **Table 3.**
The bacteria detected on all implants of group C were *S. epidermidis*, *S.anginosus*, *S.mitis*, *S.salivarius*, *P. aeruginosa*, *S. marcescens*, and *S. parasanguinis.* Changes in terms of log_{10} CFU/ml are presented in **Figure 2**. Treatment HBX and HBX + ESC resulted in a shift of viable CFU/ml under the LOD on all the treated implant for *S. epidermidis*, *S. anginosus*, *S. salivarius*. *S. parasanguinis* was undetectable in all the implants receiving HBX or HBX + ESC either, except for 1 implant in the group HBX + ESC, however it was under the LOQ [estimated 2.40 log_{10}(CFU/ml)]. *S. mitis*, *P. aeruginosa*, and *S. marcescens* were significantly reduced, but were still detectable on the majority of the implants despite always being below the LOQ. Estimated values have been reported anyways.

Biocompatibility test - Influence of Decontamination on MG-63 morphology and adhesion

At morphological analysis at SEM, in all groups cells appeared housed on the implant surface, with clear cytoplasmic extensions that allow the between cells connection as well as the adhesion to the rough surface. No functional orientation was observed in any group.
At analysis at BES, differences between groups were found on cellular distribution. In group C and AW pictures showed spread cells distributed mainly among implant threads. In group C bacterial aggregates were visible. In group ESC cells covered homogenously the implant surface but were not densely packed. In one specimen no cell was visible. In groups HBX, HBX+ESC and NC cells were more densely packed on the implant surface. However in one specimen of group HBX, few cells covered the implant surface (**Figure 3**). Semi-quantitative analysis revealed a trend toward an increasing percentage of implant surface covered by adherent cells from group C to group HBX+ESC and NC (**Table 4**).

Conclusions

In the present *in vitro* model treatment with HBX either alone or in combination with ESC provided a significant decontaminant effect on previously contaminated implants while no differences were shown between the groups receiving other treatments. Moreover, it was observed that the percentage of implant surface covered by adherent MG-63 cells after 5 days of incubation was influenced by the treatment method. In particular the percentage of surface covered by adherent cells showed progressive increase through groups C, AW, ESC, HBX, HBX+ESC, and NC.

In recent years, the most used antimicrobial agents have been CA, CHX and hydrogen peroxide (H_2O_2). A systematic review identified CA as the most effective agent against single-species or multi-species biofilms killing up to 99.9% of bacteria (17) . CA also demonstrated some potentiality in the removal of single-species biofilm from titanium surfaces (17,18). However it often does not achieve complete removal with effectiveness equivalent to those of water and saline rinses. CHX has shown good and limited bactericidal effect against early and mature biofilms, respectively , but no cleaning properties *per se* (18,19,20). H_2O_2 has a moderate to good bactericidal effect, but no obvious cleaning properties (18,19,21). Interestingly in our research, we demonstrated that HBX is able to produce a significantly greater reduction of viable bacteria compared to group C (99.99% greater bacterial load reduction). In a previous paper it was reported that 40% CA followed by PBS rinses was unable to inactivate 12-hours old bacterial biofilms formed on smooth titanium discs intraorally in humans after submerging the discs in it for 1 minute (19), probably due to the glycocalix which protects the bacteria. On the other hand, we demonstrated that HBX followed by saline rinses was able to completely suppress *S. epidermidis*, *S. anginosus*, *S. salivarius,* and *S. parasanguinis* and reduce significantly *S. mitis*, *P. aeruginosa*, *S. marcescens* and the total viable CFU/ml also when not combined with ESC. This could be explained by the anti-biofilm properties of HBX (12).

If we take into consideration that mechanical debridement with air abrasive devices has been proven to leave consistent amount of untouched implant surface in conditions simulating a surgical access (8) we can assume that disinfection of infected titanium surfaces by mechanical means only, might not be adequate. This is in agreement with previous studies (22,23), which concluded that mechanical debridement alone was insufficient for biofilm disruption or elimination due to the complex implant surface topographies, and claimed for a combination of mechanical and chemical modalities of implant surface decontamination. In our study implant surface decontamination with ESC alone didn't differ in terms of residual viable $\log_{10}$CFU/ml from groups receiving no treatment. This is in contrast with the conclusions of a systematic review which found the *in vitro* cleaning efficacy of air-powder abrasive devices consistent (24). In general studies using sodium bicarbonate, glycine or ESC *in vitro* reported more than 84% removal of bacteria or bacterial products irrespective of the surface type (10,25,26). Conversely, in the present research ESC failed to reduce significantly the viable counts of bacteria on implant surfaces (72.44%), probably because the model of the peri-implant defect and the screw-shaped implants impeded the direct abrasion of the biofilm from the majority of the implants surface. In fact the studies reporting promising results for air-powder abrasive were in general performed either on titanium discs (10,11,25,27) or implants outside of peri-implantitis defect model (26) where the air abrasive could easily reach the whole titanium surface. However this is not the case during clinical practice, where accessibility is a major issue.

Interestingly, we found no differences in terms of residual viable $\log_{10}$CFU/ml between AW and ESC. This is in contrast with the findings of two in vitro studies performed on titanium discs (27,28), which demonstrated that the use of an air abrasive device without

powder (only water) resulted in significantly less biofilm removal compared with the use of the same device with different powders. A possible explanation for this difference resides again in the limited accessibility for the powder to the implant surface due to the peri-implant defect model and/or the implant macrostructure.

Within the limitation of this study it has been demonstrated that different treatment modalities have different impacts on the MG-63 cells proliferation. Semi-quantitative analysis showed HBX alone or in combination with ESC may reduce the bacterial load to an extent, which may render the previously contaminated implant surfaces as biocompatible as the non-contaminated controls. Conversely, treatment with AW or ESC showed a percentage of covered implant surface which was lower. This is in line with the results obtained in the first part of the experiment, where it was demonstrated that neither AW or ESC were able to significantly reduce the bacterial load on contaminated implants. Schwarz et al. (29) previously observed that the plaque removal efficacy of various mechanical methods used for the treatment of peri-implantitis failed to predict the biologic response of decontaminated titanium surfaces and did not restore their biocompatibility. This may be partially in contrast with the findings of the present study in which it may be observed that the detoxification potential of the treatment modalities is directly related to the cellular growth close to the implants.

We observed that HBX didn't prevent osteoblast-like cells to recolonize the implant surface. This is different from previous reports on other chemical decontaminants such as CHX or CA. Kotsakis et al. (30) showed that CA and CHX has cytotoxic activity, and cellular growth was inhibited in the CHX group compared to non contaminated controls. CA has been demonstrated to possess a transient inhibitory effect of on osteoblastic cell proliferation that last for approximately 5 days (31). In the present study it was observed that in the groups that received HBX cellular morphology was not altered and may be related to no or limited cytotoxic activity by HBX. This is further confirmed by the tests for cytotoxicity using the ISO Agarose Overlay with L-929 Mouse Fibroblast Cells method performed with HBX when it was considered to be non-toxic under the conditions of that test.

In conclusion, within the specific conditions and limitations of this *in vitro* study, it has been demonstrated that, despite the limited accessibility due to the model simulating the peri-implant Class Ie defect, a significant decontaminant effect on the moderately rough implants involved in this study was achieved using the sulfonic/sulfuric acid solution in gel while no differences were shown between the groups receiving other treatments. Moreover treatment with HBX and the combination treatment with HBX and ESC were able to reduce the contamination of the implants to a level that didn't interfere with MG-63 cells growth on the decontaminated implants. These findings prompt further investigations on dental implants decontamination using chemical decontamination. Combination of physical and chemical therapy may provide more predictable results in the future.

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Figure 1. Total viable log_{10}(CFU/ml) in the five treatment groups.

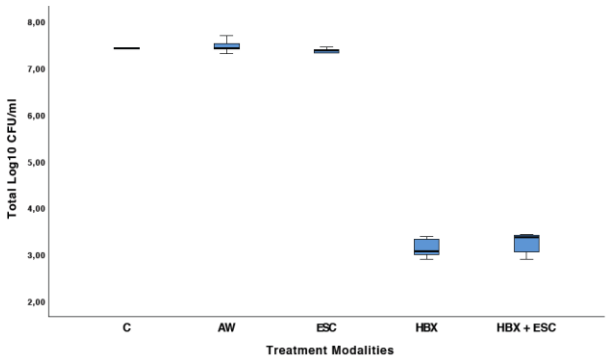

Figure 2. Changes in terms of log_{10} CFU/ml of *S. epidermidis*, *S.anginosus*, *S.mitis*, *S.salivarius*, *P. aeruginosa*, *S. marcescens*, and *S. parasanguinis* in the five treatment groups.

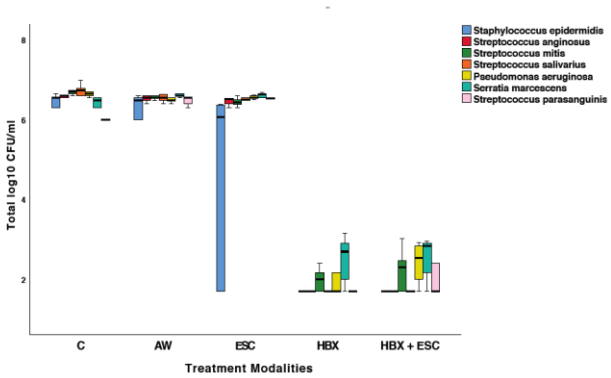

Figure 3. 55x microphotographs of the implants from different experimental groups

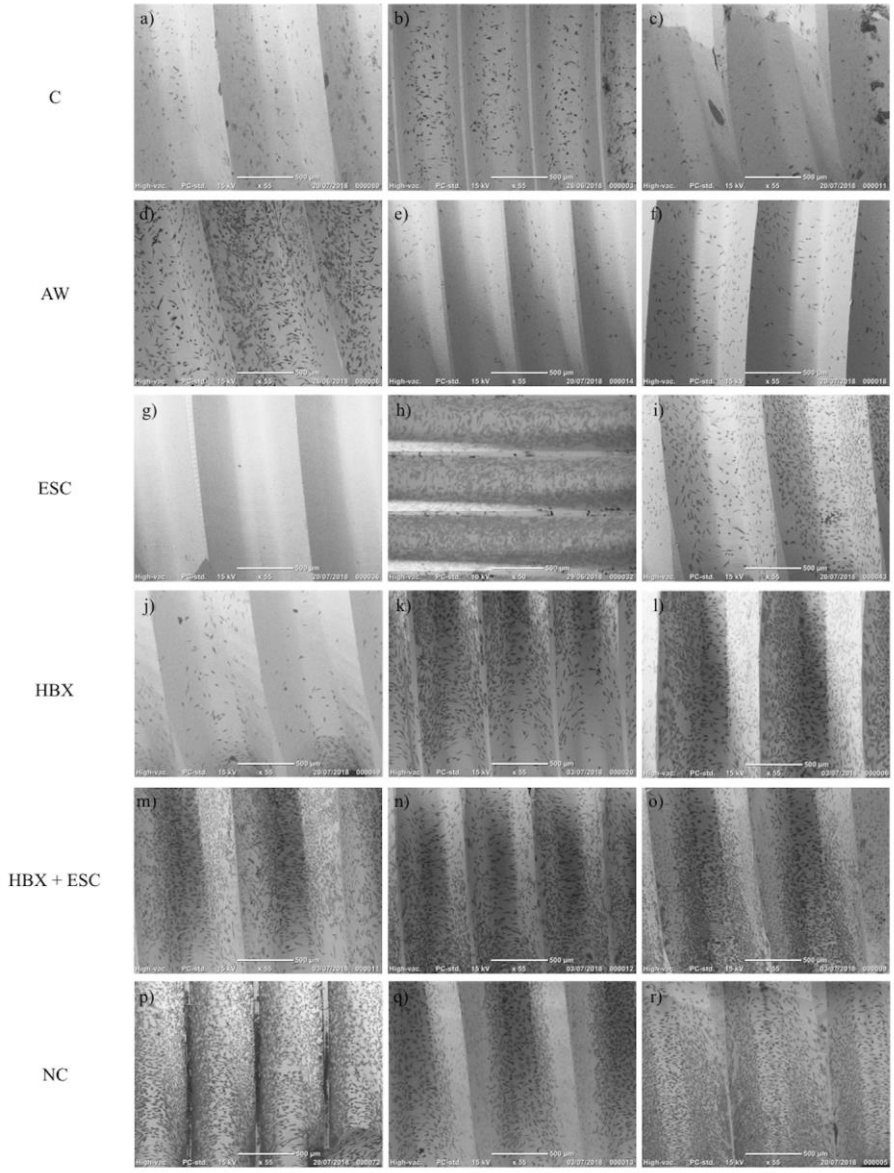

Table 1 - Mean, median, standard deviation, minimum, and maximum of total viable log_{10}(CFU/ml) in the five treatment modalities

Treatment modality	Mean	Median	St. Dev.	Min	Max
C[a;c]	7.48	7.43	.12	7.41	7.69
AW[b]	7.48	7.43	.15	7.31	7.70
ESC	7.34	7.38	.10	7.18	7.45
HBX[a;b]	3.14	3.08	.21	2,90	3.39
HBX + ESC[c]	3.23	3.38	.24	2.90	3.43

[a], p = 0.012; [b], p = 0.018; [c], p = 0.037

Table 2 – Logaritmic reduction and percentage reduction of total viable log_{10}(CFU/ml) in the treatment modalities compared to group C

Treatment modality	Log reduction	Percentage (%)
C	-	-
AW	0	0
ESC	0.14	72.44
HBX	4.34	99.99
HBX + ESC	4.25	99.99

Table 3

Table 3 – Mean ± standard deviation viable log_{10}(CFU/ml) in the five treatment modalities for each bacterial species.

Bacterial Species	C	AW	ESC	HBX	HBX + ESC
S. aureus	4.28 ± 2.37	3.54 ± 2.52	2.62 ± 2.06	< 2.00	< 2.00
S. epidermidis	6.47 ± 0.16[a]	5.46 ± 2.12	4.44 ± 2.50	< 2.00[a]	< 2.00[a]
S.anginosus	6.61 ± 1.00[b]	6.53 ± 0.08[c]	6.46 ± 0.11	< 2.00[b;c]	< 2.00[b;c]
S. mitis/oralis	6.72 ± 0.13[b;e]	6.63 ± 0.20[d]	6.44 ± 0.11	1.99 ± 0.30[b;d]	2.24 ± 0.56[e]
S. salivarius	6.74 ± 0.15[f]	6.60 ± 0.22	6.47 ± 0.19	< 2.00[f]	< 2.00[f]
F. nucleatum	2.59 ± 2.00	3.55 ± 2.54	2.64 ± 2.10	< 2.00	< 2.00
C. ochracea	2.62 ± 2.06	2.62 ± 2.06	< 2.00	< 2.00	< 2.00
P.aeruginosa	6.71 ± 0.20[b;h]	6.56 ± 0.20	6.56 ± 0.05[g]	2.03 ± 0.53[b;g]	2.40 ± 0.54[h]
S. marcescens	6.62 ± 0.27	6.66 ± 0.14[j;k]	6.57 ± 0.12[i]	2.49 ± 0.62[i;j]	2.52 ± 0.56[k]
S. parasanguinis	6.08 ± 0.18	6.55 ± 0.25[l;m]	6.59 ± 0.14[f;n]	< 2.00[f;l]	1.98 ± 0.38[m;n]
S. faecalis	2.56 ± 1.92	3.48 ± 2.44	< 2.00	< 2.00	<2.00
K. oxytoka	2.56 ± 1.92	3.48 ± 2.44	< 2.00	< 2.00	<2.00
G. adiacens	< 2.00	3.45 ± 2.40	< 2.00	<2.00	<2.00
P. acnes	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
M. luteus	< 2.00	2.56 ± 1.92	< 2.00	< 2.00	< 2.00

[a], p = 0.019; [b], p = 0.003; [c], p = 0.047; [d], p = 0.039; [e], p = 0.008; [f], p = 0.004; [g], p = 0.044; [h], p = 0.017, [i], p = 0.045; j, p = 0.019; [k], p = 0.022, [l], p = 0.006; [m], p = 0.029; [n], p = 0.020.

Table 4

Table 4 – Mean and standard deviation of the percentage of implant surface covered by MG-63 cells in the 5 different treatment groups and in the non-contaminated implants

Treatment Modality	Mean	St.Dev
C	7,41%	4,75%
AW	12,41%	4,38%
ESC	24,11%	6,72%
HBX	33,55%	11,33%
HBX + ESC	51,69%	9,55%
NC	60,13%	9,34%