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EVALUATION OF OCULAR PROSTHESIS BIOFILM AND ANOPHTHALMIC CAVITY CONTAMINATION AFTER USE OF THREE CLEANSING SOLUTIONS

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ABSTRACT

In addition to an initial socket discomfort, ocular prosthesis (OP) installation may allow the adherence of fungi and/or bacteria due to the superficial characteristics of the prosthesis' material, use of inadequate cleansing solutions and methods, or because the void located between the internal portion of the prosthesis and the anophthalmic cavity (AC) mucosa. Objective: The aim of this study was to evaluate OP biofilm formation and the level of contamination of the internal portion of the OP and the AC in 24 patients. Material and Methods: Material was collected from the AC at the beginning of the study and 15 days after cleansing of the OP with 3 cleansing solutions: a neutral liquid soap, a multiuse solution for contact lens (Complete) and 0.12% chlorhexidine (Periogard). The collected materials were sowed in Petri dishes containing selective media for aerobic and facultative microorganisms, specifically staphylococci (Hipersalt agar with egg yolk), aerobic microorganisms (Brain Heart Infusion Blood Agar), streptococci (Mitis salivarius Agar), gram-negative bacilli (MacConkey Agar) and yeasts (Chromagar Candida™), incubated at 35ºC or 37ºC and the number of colony forming units were counted. Data were analyzed statistically by ANOVA, Friedman's test and Spearman's correlation. Results: Aerobic microorganisms, gram-negative bacilli and S. aureus were found in the OP biofilm and in the AC. There was statistically significant difference ($p<0.05$) between the number of microorganisms before and after the use of the cleansing solutions. Conclusion: There was positive correlation with respect to the microorganisms present in the OP biofilm and AC for the 4 proposed treatments, indicating that the decrease of OP contamination leads to AC contamination as well.

Uniterms: Artificial eye; Eye infections; Prosthesis-related infections.

INTRODUCTION

Ocular prosthesis (OP) is an artificial replacement for the bulb of the eye and has the goal of reestablishing facial esthetics while maintaining the form of the anophthalmic cavity (AC), preserving the palpebral muscle tone, inhibiting palpebral collapse, directing tear drainage, preventing fluid accumulation in the socket and aiding the patient's social contact[7].

OP wearers are preset to infections, inflammations and traumas related to physiological and morphological modifications of the AC; AC colonization by pathogenic microbiota; impaired mobility to inadequate prosthesis installation; neglected prosthesis cleansing without removal from the socket for months or years and no washing; and accumulation of secretion, which may cause giant papillary conjunctivitis and consequent intolerance to prosthesis use[6,9,10,12-14].

The aims of this study were to assess the levels of contamination of OP biofilm and AC in OP wearers by counting the number of colony forming units (cfu) before and after use of different cleansing solutions, and to correlate the contamination levels of the AC with those of the OP.

MATERIAL AND METHODS

Twenty-four OP wearers of both genders with mean age of 44 years were selected from the Rehabilitation Service for Patients with Mutilations of the Face, Head and Neck Regions of the Department of Dental Materials and Prosthodontics (FORP/USP) and followed-up during a 45-day period. Patients attended four visits (0, 15, 30 and 45 days), in which biofilm was collected from the internal surface of the OP as well as from the AC. For collection of material on day 0 (I – Initial), patients did not receive any hygiene instruction. After collection, the patients received cleansing solutions for the OP and were oriented how to clean it 4 times a day, during 15 days. The first solution used, after the initial material collection, was a neutral liquid soap (LS) (Daterra, Ribeirão Preto, São Paulo, Brazil). The patients were instructed to put the LS on their clean hand palms and dab it on the prosthesis for 1 minute, rinsing in running water thereafter. The second solution used was a multiuse solution (MS) for cleansing of contact lenses (Complete, Allergan, Guarulhos, São Paulo, Brazil) and the third was a 0.12% chlorhexidine solution [Periogard (P), Colgate, São Paulo, SP, Brazil]. The cleansing instructions were similar to those given to LS, except for the fact that MS was not rinsed after application, according to the manufacturer's instructions. Material was collected from the OP and AC after 15 days of use of each solution. After the last solution was used for 15 days, the patients attended the fourth appointment and the final collections were performed.

In preparation for material collection, the patients were instructed to wash their hands (water and soap) and the antisepsis was made with a 68% alcohol gel. The OP was removed by the patient, placed in a sterile Petri dish (20x100mm) and taken to an aseptic zone (obtained by 2 alcohol lamps). Collection of the AC material and the biofilm from the internal portion of the OP was done by a single operator with a sterile swab (DME – Diagnósticos Microbiológicos Especializados, Araçatuba, São Paulo, Brazil), maintaining the same frequency of movements during 5 minutes. Still in the aseptic zone, the swab was introduced into a test tube containing 2.0 mL of Letheen Broth – Calet (Difco, Detroit, Michigan, USA) and was forwarded to the laboratory packed in ice-filled polystyrene boxes to ensure an adequate conservation.

Thereafter, the OPs were polished with pumice (Vigodent, Rio de Janeiro, RJ, Brazil) and Kaolin (ARJ Chemical do Brazil Ltda, Rio de Janeiro, RJ, Brazil), washed with water and soap, rinsed in running tap water and given back to the patients.

The collected material was agitated for 1 minute in a shaker (Mixtron Toptronix, São Paulo, SP, Brazil) and submitted to decimal dilution up to 10^{-4}. These suspensions were dropped in equidistant points on the Petri dish in a volume of 50 µL, according to Westergren and Krasse[15] (1979) onto MacConkey Agar (Mc; Difco, Detroit, Michigan, USA), Chromagar Candida™ (Cm; CHROMagar, Paris, France), Brain Heart Infusion Blood Agar (As; Difco, Detroit, Michigan, USA), Hipersalt Agar with egg yolk (Ni) according to Ito, et al.[5] (1979) and *Mitis salivarius* Agar (Ms; Difco, Detroit, Michigan, USA).

About 4.0 mL of thioglycollate medium without dextrose or indicator (Tio's; Difco, Detroit, Michigan, USA) were added to the remaining material and incubated at 37ºC for 10 days for detection of less than 20 microorganisms.

Mc, As, and Ni media were incubated at 37ºC for 24 to 48 hours, while Cm medium was incubated at 35ºC for 48 to 72 hours. Ms medium was incubated at 37ºC for 24 to 96 hours under capnophilic conditions by the candle jar system. After incubation, the material was examined with a stereomicroscope (Nikon, Tokyo, Japan) under reflected light and the number of colony forming units (cfu) was counted.

Data were analyzed statistically by analysis of variance, Friedman's test and Spearman's correlation. Significance level was set at 5%.

RESULTS

Aerobic microorganisms, *S. aureus* and gram-negative bacilli were detected in the OP biofilm and in the AC (Table 1 and 2).

The statistical results of ANOVA for aerobic microorganisms are shown on Table 3 while the statistical results of Friedman's test for *S. aureus* and gram-negative bacilli are shown on Table 4. The analysis of the results showed that the initial condition (I) was statistically different from the use of the cleansing solutions (LS, MS, and P) ($p<0.05$). The results of the Spearman's correlation for the microorganisms present in the OP biofilm and AC for the 4 proposed treatments (I, LS, MS, and P) (Table 5) showed a positive correlation, indicating that as the number of microorganisms on OP surface increased, the number of microorganisms in the AC increased accordingly, in any of the tested conditions. The inverse also occurred. A numerical comparison was made and confirmed this correlation (Table 6).

DISCUSSION

In the present study, the varied microbiota observed in the OP biofilm (aerobic microorganisms, gram-negative bacilli and *Staphylococcus aureus*) as well as the presence of these microorganisms in the AC are results consistent with the literature[1-3,6,9,10,12,13].

OP wearers may present a pathogen microbiota in the AC, mainly those who neglect the cleansing of prosthesis, not removing them for days, months or even years, sometimes leading to an intolerance to prosthesis use[1,3,6,9,10,12-14]. The results of this study agree with those of these authors[1,3,6,9,10,12-14] regarding the fact that, despite the pathogenicity of the microorganisms, their presence does not depend on the type of cleansing solution used given that bacteria persisted the OP and AC, though in a smaller number. This fact is clearly observed when the results of

TABLE 1- Cfu counting for aerobic microorganisms, *S. aureus* and gram negative bacilli on the ocular prosthesis

	Aerobic microorganisms				*S. aureus*				Gram-negative bacilli			
Patient	**I**	**LS**	**MS**	**P**	**I**	**Ls**	**MS**	**P**	**I**	**LS**	**MS**	**P**
1	960	160	250	100	0	0	0	0	0	0	0	0
2	3000	7400	3800	0	1440	4800	3200	0	0	0	0	0
3	54000	21900	1900	17100	0	0	0	0	0	0	0	0
4	12000	850	3300	5900	0	0	0	0	1120	600	760	4260
5	90000	180	840	100	0	0	0	0	0	0	0	0
6	72000	7300	2130	5200	1800	2600	3800	3400	102	60	180	180
7	24000	40	340	80	11800	20	180	40	0	0	0	0
8	44000	10600	22000	980	21000	1600	6000	40	0	0	0	0
9	170	380	20	80	0	0	0	0	0	0	0	0
10	440	220	590	0	430	60	170	0	0	0	0	0
11	28000	58000	74000	3300	15600	3800	800	20	0	0	0	0
12	42000	66000	44000	12000	26000	34400	12000	2800	0	0	0	0
13	38000	790	1840	6000	0	0	0	0	0	0	0	0
14	28000	5000	340	2800	4000	1200	180	1000	0	0	0	0
15	3004000	4658000	402000	80	179000	656400	144200	0	15780	31200	220	0
16	92400	6200	8600	570	1600	1600	980	700	100	960	810	40
17	578000	226000	74000	910000	0	0	0	0	0	0	0	0
18	642000	56000	60000	5300	125000	1400	2660	20	3780	5740	140	760
19	5500	420	80	180	0	0	0	0	0	0	0	0
20	1810000	2132000	2476000	2962000	15700	11000	1800	1800	19940	13580	378000	17020
21	14000	1400	7000	2000	2600	680	1200	80	80	40	1020	60
22	480	790	500	280	0	0	0	0	0	0	0	0
23	14700	720	1110	3000	0	0	0	0	0	0	0	0
24	34000	590	560	330	520	0	0	0	140	80	60	60

I = Initial; LS = Liquid Soap; MS = Complete Multiuse Solution; P = Periogard

TABLE 2- Cfu counting for aerobic microorganisms, *S. aureus* and gram negative bacilli in the anophthalmic cavity

	Aerobic microorganisms				*S. aureus*				Gram-negative bacilli			
Patient	**I**	**LS**	**MS**	**P**	**I**	**Ls**	**MS**	**P**	**I**	**LS**	**MS**	**P**
1	460	650	100	120	0	0	0	0	0	0	0	0
2	7800	4100	3100	0	590	810	800	0	0	0	0	0
3	668000	430000	102000	132000	0	0	0	0	0	0	0	0
4	36000	4600	40000	580	0	0	0	0	2800	100	240	100
5	570	430	1600	710	0	0	0	0	0	0	0	0
6	86000	58000	12000	11900	18600	14000	2200	6600	660	220	100	140
7	30000	40	480	700	1700	20	180	490	0	0	0	0
8	76000	46000	44000	22000	22200	6000	13800	140	840	200	140	180
9	440	470	990	330	0	0	0	0	0	0	0	0
10	3400	3400	610	200	860	1600	600	120	0	0	0	0
11	88000	26000	8700	7700	25600	8400	800	660	0	0	0	0
12	40000	70000	24000	5300	16200	26000	5600	1200	0	0	0	0
13	1258000	3200	35900	42000	0	0	0	0	0	0	0	0
14	16500	4500	560	180	6200	600	490	260	0	0	0	0
15	1262000	212000	22000	1800	107800	119800	17200	0	11140	140	620	0
16	102000	2810	9300	360	470	160	280	20	220	60	40	80
17	1742000	1164000	566000	3360000	0	0	0	0	120	80	40	20
18	28000	3700	1600	6000	6200	370	260	20	300	40	100	220
19	10800	40	380	280	0	0	0	0	0	0	0	0
20	3182000	4178000	4024000	3266000	18680	21200	24600	19000	26500	31130	87800	20500
21	5600	3900	44000	18000	31800	2800	25800	340	2040	920	1040	120
22	20000	9800	36000	18000	580	40	20	20	0	0	0	0
23	32000	1800	1890	9700	0	0	0	0	2040	520	250	620
24	42000	610	500	260	850	60	0	0	1180	20	40	40

I = Initial; LS = Liquid Soap; MS = Complete Multiuse Solution; P = Periogard

the cleansing solutions (neutral liquid soap, multiuse solution and Periogard) were compared to the initial condition (no cleansing).

Few studies have addressee OP cleansing methods, the most common being the use of water and soap[11,12]. Removal of the OP, use of solutions indicated for cleansing of contact lenses and periodical examination by a health professional have also been recommended to ensure the proper cleansing and assess the integrity of tissues that cover the AC and the need for changing the prosthesis[4,6,8,13].

The findings of the present study showed that the use of a contact lens multiuse solution for cleansing of the OP yielded a decrease in the number of microorganisms on both the prosthesis and the anophthalmic cavity.

The use 0.12% chlorhexidine is widely widespread for chemical biofilm control because of its bacteriostatic action against gram-positive and gram-negative microorganisms. Periogard was used in this study because it is a readily available product that does not offer risks to patients' health. After use of Periogard, the biofilm presented a smaller number of colony forming units in comparison to the initial condition. These results suggest that, although it does not differ significantly from the other solutions, Periogard had an evident bacteriostatic effect, given that, after its use, bacterial growth in the biofilm or AC was less intense compared to the use of the other solutions. In some cases, no bacterial growth was observed.

There are no studies referring to the correlation between the presence of microorganisms in the OP or AC and cleansing solutions that could serve as a parameter to the

TABLE 3- Results of the analysis of variance for the presence of aerobic microorganisms

(H0) Probability	Anophthalmic Cavity	Ocular Prosthesis
Among Patients	0.0000%	0.0000% *
Among Solutions	0.0009%	0.0009% *

*Statistically significant at 1% level

TABLE 4- Results of the Friedman's test for the presence of *S. aureus* and gram-negative bacilli

Two-by-two comparisons	*S. aureus*		Gram-negative bacilli x	
	AC	OP	AC	OP
I x LS	ns	ns	1%	ns
I x MS	1%	5%	1%	ns
I x P	0.1%	0.1%	1%	ns
LS x MS	ns	ns	ns	ns
LS x P	0.1%	1%	ns	ns
MS x P	5%	5%	ns	ns

Ns = non-significant. I = Initial; LS = Liquid Soap; MS = Complete Multiuse Solution; P = Periogard. AC= Anophthalmic Cavity; OP= Ocular Prosthesis

TABLE 5- Results of Spearman's correlation test for the presence of aerobic microorganisms, *S. aureus* and gram-negative bacilli

Two-by-two comparisons	Ho Probability		
	Aerobic microorganisms	*S. aureus*	Gram-negative bacilli
I AC X OP	0.0000%	0.0000%	0.0000%
LS AC X OP	0.2800%	0.0000%	0.0400%
MS AC X OP	0.0200%	0.0000%	0.0000%
P AC X OP	0.0000%	0.0000%	0.0000%

I = Initial; LS = Liquid Soap; MS = Complete Multiuse Solution; P = Periogard; AC = Anophthalmic cavity; OP= Ocular prosthesis

outcomes of the present study. Portellinha, et al.[13] (1984) correlated the presence of secretion with the time of use and the frequency of prosthesis cleansing and found that the bacterial colonization in the AC and the frequency of OP cleansing had no statistically significant correlation. Campos[2] (1994) did not find a positive correlation between the time of use of OP and the presence of microorganisms.

In the present investigation, a positive correlation was found between the microorganisms on the prosthesis/ cavities and the four types of treatment (no cleansing and three cleansing solutions), which indicates that the decrease of OP contamination would lead to a decrease of AC contamination. Therefore, OP cleansing is essential to reduce contamination of AC, improving the comfort of OP wearers and consequently their life quality.

CONCLUSIONS

1. Aerobic microorganisms and gram-negative bacilli were found in OP biofilm as well as in the AC before and after the use of the studied cleansing solutions.
2. All solutions were similarly effective in decreasing the number of aerobic microorganisms in the OP and AC compared to the initial condition;
3. Periogard and MS decreased of the number of *S.*

TABLE 6- Comparison of the number of cfu of aerobic microorganisms, *S. aureus* and gram-negative bacilli in the OP biofilm and AC before and after use of the cleansing solutions

Two-by-two comparisons	Aerobic microorganisms		*S. aureus*		Gram-negative bacilli	
	OP	AC	OP	AC	OP	AC
I	24 +	24 +	14 + 10 -	15 + 9 -	8 + 16	11 + 13 -
I X LS	17 ↓ 7 ↑	19 ↓ 4 ↑ 1 =	8 ↓ 4 ↑ 1 = 11 -	10 ↓ 5 ↑ 9 -	5 ↓ 3 ↑ 16 -	10 ↓ 1 ↑ 13 -
I X MS	17 ↓ 7 ↑	19 ↓ 5 ↑	11 ↓ 2 ↑ 11-	12 ↓ 2 ↑ 10 -	4 ↓ 4 ↑ 16 -	10 ↓ 1 ↑ 13 -
LS X MS	11 ↓ 13 ↑	14 ↓ 10 ↑	7 ↓ 6 ↑ 11 -	9 ↓ 5 ↑ 10 -	4 ↓ 4 ↑ 16 -	5 ↓ 6 ↑ 13 -
P X I	20 ↓ 3 ↑ 1 -	20 ↓ 2 ↑ 2 -	9 ↓ 1 ↑ 14 -	11 ↓ 1 ↑ 12 -	5 ↓ 2 ↑ 17 -	10 ↓ 14 –
P X LS	16 ↓ 6 ↑ 2 - 14 -	14 ↓ 9 ↑ 1 -	8 ↓ 2 ↑ 14 -	12 ↓ 12 –	3 ↓ 4 ↑ 17 -	5 ↓ 4 ↑ 1 =
P X MS	14 ↓ 8 ↑ 2 -	16 ↓ 7 ↑ 1 -	8 ↓ 1 ↑ 1 = 14 -	9 ↓ 2 ↑ 1 = 12 -	4 ↓ 2 ↑ 1 = 17	4 ↓ 5 ↑ 5 ↑ 14 -

I = Initial; LS = Liquid Soap; MS = Complete Multiuse Solution; P = Periogard. AC= Anophthalmic Cavity; OP= Ocular Prosthesis. (?) decrease in the number of microorganisms / (?) Increase in the number of microorganisms. (+) positive culture; (-) negative culture; (=) unchanged number of microorganisms

aureus in the OP and AC compared to the initial condition;

4. There was no significant difference between the initial condition and the studied solutions regarding the presence of gram-negative bacilli in the OP biofilm; for AC, the three solutions were yielded better outcomes than the initial condition.

5. Under the tested conditions, there was a positive correlation for the presence of aerobic microorganisms, *S. aureus* and gram-negative bacilli.

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