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A COMPARISON OF MICROBIAL FLORA IN PERI-IMPLANT SULCULAR FLUID OF BALL VERSUS LOCATOR RETAINED MANDIBULAR IMPLANT OVERDENTURES. A RANDOMIZED CROSSOVER STUDY

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ABSTRACT

Purpose: To evaluate the microbial flora in peri-implant sulcular fluid of edentulous patients rehabilitated with ball and socket and locator retained mandibular 2-implant overdentures.

Materials and Methods: 14 edentulous patients were recruited to receive two mandibular implant overdentures and new conventional maxillary complete dentures. Mandibular implant overdentures (MIODs) were retained either by locator or ball attachment systems in random order. After 6 months of function, the attachment systems in the existing dentures were replaced with the other type of attachment. The prevalence of streptococci and staphylococci species (spp.) was analyzedusing blood-agar media at 3, 6 and 9 weeks after pick-up of attachments. The data was then collected, tabulated and statistically analyzed using SPSS.

Results: No significant difference was found in bacterial count of either Streptococcus or Staphylococcus spp. between two attachment systems (Ball and socket, Locator) at any of the evaluated time intervals with *P*-value > 0.05

Conclusions: Within the limitations of this study, it can be concluded that there is no difference in microbial flora between the two attachment systems. It can also be suggested that the locator attachments are valid treatment alternative for ball abutments to retain mandibular 2-implant overdentures from a biological point of view.

INTRODUCTION

Mandibular implant overdentures (MIODS) have been successfully used for the rehabilitation of completely edentulous patients.[1,2] Various attachment systems have been used to retain IODs. Unsplinted ball and locator attachments are frequently used because of their simplicity and satisfactory clinical and prosthodontic outcomes as well as the improved patients' satisfaction reported with both systems.[3,4] Different studies have evaluated retentive force, prosthodontic complications, clinical outcomes

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and the financial aspects of these attachments.[5,6,7,8,9] However, the studies that analyze the peri-implant/abutment microbiological environment and its influence on the clinical outcome with regards to different overdenture attachments are scarce.[10]

Since overdentures are most commonly employed for elderly and geriatric patients, the oral hygiene tends to worsen with time with subsequent plaque accumulation that occur some time after the dentures delivery.[11] Plaque accumulating on the exposed surfaces of the biomaterial at the connection between the implant and the abutment or on the abutment surface may alter the microbiota of the oral cavity and result in soft tissue complications such as peri-implant mucositis, hyperplastic mucositis, and some fistulas originating from the soft tissue compartment.[12-14] The different overdenture attachment designs can also be a contributing factor that influence oral biofilm formation starting with initial bacterial colonization, plaque formation till complete maturation and consequently alter the resultant clinical picture.[12,14]

The locator abutment is designed to have a double aligning, self-retention areas (undercuts) on inner and outer abutment surface. With this pivoting self-aligning design of the locator attachments, there is increased number of undercuts (recesses) that can act as shelter areas for initial colonizer species, such as Streptococcus and Staphyococcus, in locator abutment (patrix) compared to that of the ball attachment. Furthermore, food residues can accumulate in the central depression of the locator patrix that can further complicate the oral hygiene procedures.[15] Whether, this difference in design of the abutment between the two attachment systems has an influence on the microbial flora on peri-implant sulcular fluid has not yet been investigated.

Therefore the aim of this study was to evaluate the prevalence of early colonizers Streptococcus and Staphylococcus species (spp.) in peri-implant sulcular fluid of edentulous patients rehabilitated with ball and locator retained implant overdentures in a crossover study.

MATERIALS AND METHODS

Study Design

Patient population & surgical procedures

Fourteen edentulous patients from Removable Prosthodontic Department, Faculty of Dentistry, Cairo University participated in this randomized crossover trial. All of those patients experienced functional problems with their conventional mandibular dentures and had sufficient interforaminal bone height and width to receive two implants. The implants (SuperLine, Dentium implant system) were placed following a standardized surgical protocol (Fig 1). Participants with a history of radiotherapy in the head and neck region, heavy smoking of more than 10 cigarettes/day or patients with any systematic condition that can preclude surgical implant procedures were excluded from study. All the participants were informed about the treatment options and an informed consent was obtained.

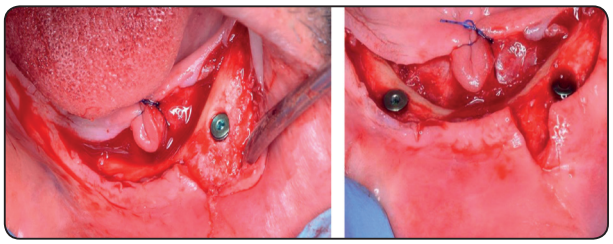

Fig. (1) Implants placement following standard surgical procedures

Prosthodontic procedures:

Following the inclusion in the study, all the participants received new well-fitted maxillary and mandibular complete dentures according to standard prosthodontic procedures[16] using balanced occlusal scheme. The dentures were used for 3 months to ensure full adaptation to the newly constructed prostheses.

Three months following the first stage surgery, the healing abutments were removed and impression copings were installed at implant level. A master cast was then poured and used for the relining of the diagnostic dentures. An experienced laboratory technician performed all laboratory procedures. The patients were randomly selected to initially receive either ball or locator attachment system (Implantium, Implantium II, Superline) and the attachments were changed after 6 months of function. The attachment system was picked up chair side in the fitting surface of the relined denture. Then a wash out period of 1 month was allowed where patients wore the dentures with no attachments in the fitting surface. Following wash out period the other type of attachment system was picked and the patients wore it for further six months so that all the patients received both the locator and ball attachments in an alternating sequence. In such a way, the same denture base was used throughout the whole trial to ensure the reliability of the results. At the end of the trial, patients' wishes regarding their preferred attachment system was fulfilled.

Locator attachment system consisted of double aligning self-retention abutment with retention areas (undercuts) on inner and outer abutment surface and white nylon inserts in the fitting surface of the denture. The ball attachments comprised the conventional ball abutment, female metal housing and plastic O-ring.

Outcome Measures

Microbiologic Analysis:

For microbiological sampling, one implant per patient was selected, and the samples were taken on 3 separate occasions from all the subjects at 3, 6 and 9 weeks after the pick-up of attachments. A requisite for site selection was healthy peri-implant sulcus with a sulcus depth of less than 3mm. The samples were collected at noon (around 12 pm) giving chance for the patients to use the dentures several hours before taking the swabs. No history of antibiotic administration or use of bacterial disinfectants was recorded within two months before taking the microbiological specimens and patients were instructed not to eat any food before taking the swabs.

The samples were taken by careful air-drying and isolating the gingiva around the implants from moisture contamination with a saliva ejector and cotton pellets placed in buccal and lingual vestibule. Sulcular samples were then taken using a sterile endodontic paper points (Densply Dental,Tianjin) inserted into the peri-implant sulcus on buccal, lingual, mesial and distal for 10 seconds (Fig. 2).

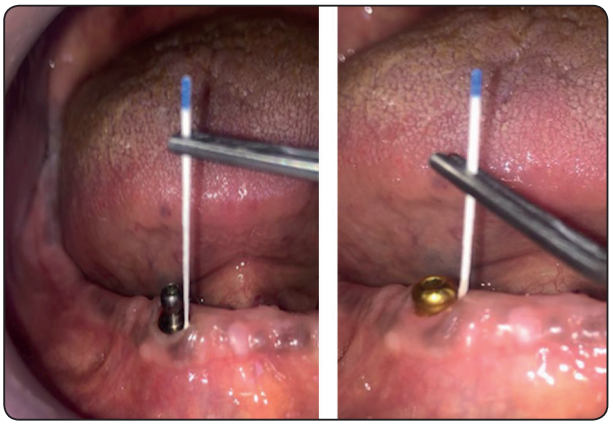

Fig. (2): Microbiological Sampling around ball and socket, and locator attachment

Each sample was then immediately placed in a sterile tube containing 1 ml sterile saline. For each sample, three sterile dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were used. Then 50 micron liter from each dilution were plated onto blood agar plate using a micro pipette, and the samples was then spread on each dilution using a sterile glass rod and incubated thereafter at 37°c for 24 hours.

For the quantitative assessment, the visible colonies of each organism were counted in every plate, and the number of colonies/plate was multiplied by the corresponding dilution factor and by 10 to determine the total colony forming units per ml of suspension (Fig. 3).

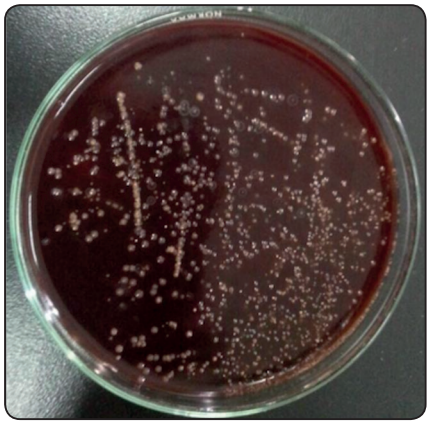

Fig. (3): Bacterial culture on blood agar

Statistical Analysis

The mean and standard deviation values were calculated for each group. Viable counts of antibacterial activity were transformed to their log10 values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Will tests and showed non-parametric normal distribution.

Independent sample t-test was used to compare between two groups in non-related samples. Repeated measure ANOVA was used compare between more than two groups in related samples. Paired sample t-test was used to compare between two groups in related samples.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

No significant difference was found in bacterial count of either Streptococcus or Sstaphylococcus spp. between the two attachment systems or at any of the time intervals (Fig. 4).

I. Streptococcus Spp.

No significant difference was found in bacterial count of Streptococcus Spp. between either of the two attachment systems (Table 1).

At 3 weeks interval:

No statistically significant difference was found between Ball and Socket and Locator attachments (P=0.353).

The highest mean count was found in Locator while the lowest mean count was found in Ball and Socket.

At 6 weeks interval:

No statistically significant difference was found between Ball and Socket and Locator (P=0.580).

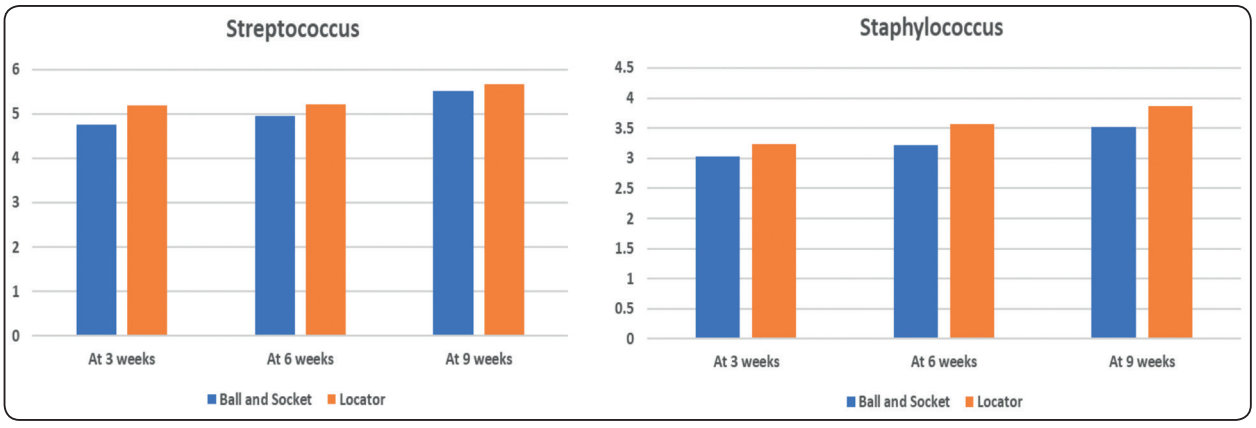

Fig. (4): Bar chart representing Streptococcus & Staphylococcus count for two different attachments at different time intervals

The highest mean count was found in Locator group while the lowest mean count was found in Ball and Socket attachment.

At 9 weeks interval:

No statistically significant difference was found between Ball and Socket and Locator (*P*=0.413).

The highest mean count was found in Locator while the lowest mean count was found in Ball and Socket.

For both attachment types, no significant difference was found between 3 weeks, 6 weeks and 9 weeks with *P Values*=0.26 and 0.51 respectively for Ball and socket and Locator attachments.

Data in table (2) shows the results of two-way ANOVA analysis for the interaction of different variables. The results showed that different attachments had no statistically significant effect at *P*-value of 0.248. Also, time period had no significant effect at *P*-value 0.105. The interaction between the two variables also had no statistically significant effect at *P*-value 0.886.

II. Staphylococcus Spp.

At 3 weeks interval:

No significant difference was found between Ball and Socket and Locator attachments with *P* value =0.481. The highest mean count was found in Locator group while the lowest mean count was found in Ball and Socket group (Table 3).

TABLE (1) The mean, & standard deviation (SD) of Streptococcus spp for each attachment at different time interval

Attachment	Streptococcus						
	3 weeks		6 weeks		9 weeks		P-value
	Mean	SD	Mean	SD	Mean	SD	
Ball and Socket	4.75	0.67	4.94	0.81	5.52	0.53	0.263 (ns)
Locator	5.19	0.39	5.21	0.83	5.67	0.68	0.511(ns)
P-value	0.353 (ns)		0.580 (ns)		0.413 (ns)		

****Significant P<0.05, ns; non-significant P>0.05***

TABLE (2): Results of Two-way ANOVA for the effect of different variables on Streptococcus count.

Source	***Type III Sum of Squares***	***df***	***Mean Square***	***F***	***Sig.***
Corrected Model	2.959[a]	5	.592	1.320	.289
Intercept	815.574	1	815.574	1818.948	.000
Attachment type	.628	1	.628	1.400	.248
Time period	2.222	2	1.111	2.478	.105
Attachment type * Time period	.109	2	.055	.122	.886
Error	10.761	24	.448		
Total	829.294	30			
Corrected Total	13.721	29			

df: degrees of freedom = (n-1), * Significant at P ≤ 0.05

At 6 weeks interval:

No significant difference was found between Ball and Socket and Locator (*P*=0.374).

The highest mean count was found in Locator while the lowest mean count was found in Ball and Socket.

At 9 weeks interval:

No significant difference was found between Ball and Socket and Locator (*P*=0.472).

The highest mean count was found in Locator while the lowest mean count was found in Ball and Socket.

Similarly, like in Streptococcus spp. no significant difference was found between 3 weeks, 6 weeks and 9 weeks with *P Values*=0.23 and 0.183 respectively for Ball and socket and Locator attachments.

Data in table (4) shows the results of two-way ANOVA analysis for the interaction of different variables. The results showed that different attachments had no significant effect at *P*-value of 0.113. Also, time period had no statistically significant effect at *P*-value of 0.062. The interaction between the two variables also had no statistically significant effect at *P*-value of 0.933.

TABLE (3): The mean, standard deviation (SD) of Staphylococcus spp. for each attachment at different time interval

Variables	Staphylococcus						
	At 3 weeks		At 6 weeks		At 9 weeks		p-value
	Mean	SD	Mean	SD	Mean	SD	
Ball and Socket	3.03	0.38	3.22	0.32	3.52	0.52	0.230 (ns)
Locator	3.23	0.36	3.57	0.68	3.87	0.64	0.183 (ns)
p-value	0.481 (ns)		0.374 (ns)		0.472 (ns)		

*Significant P<0.05, ns; non-significant P>0.05

TABLE (4): Results of Two-way ANOVA for the effect of different variables on Staphylococcus count.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.309[a]	5	.462	1.822	.146
Intercept	348.298	1	348.298	1374.805	.000
Attachment type	.687	1	.687	2.712	.113
Time period	1.586	2	.793	3.131	.062
Attachment type * Time period	.035	2	.018	.069	.933
Error	6.080	24	.253		
Total	356.686	30			
Corrected Total	8.389	29			

*df: degrees of freedom = (n-1), * Significant at P ≤ 0.05*

DISCUSSION

The aim of this randomized crossover trial was to evaluate the microbial flora namely initial colonizer Strpetococcus and Staphylococcus spp. in peri-implant sulcular fluid samples of edentulous patients rehabilitated with ball and locator-retained implant overdentures. No significant difference was found in bacterial count between either of the two attachment systems at any of the evaluated time intervals.

Peri-implant bacterial biofilm is one of the important factors that influence the long-term prognosis of osseointegration. The formation of oral biofilm is initiated by the generation of an acquired pellicle and subsequent adherence of early colonizing species (spp). This initial species create the optimal environment for the accumulation of gram-negative anaerobic late colonizing microorganisms. [17] The microbial diversity of oral biofilms in the peri-implant sulcuar fluid depends on individual host factors such as oral hygiene, salivary composition and nutrition of each subject.[18, 19] On the other hand, important implant related factors include implant/abutment materials and surface roughness as well as plaque-retentive sites of implant attachments or implant supra-structures.[20]

All subjects maintained oral hygiene measures and microbiological swaps were collected at 3,6, and 9 weeks to detect any changes in the count of the initial colonizers between the two attachment systems. Previous studies reported that at a period of 3-5 months of undisturbed plaque formation, the microbiota in peri-implant mucosa was similar as around the teeth but extended further apically.[21, 22]

Locator attachments are commonly used for mandibular implant overdenture retention because of the ease of replacement of components as well as the unique attachment design resulting from the low profile and dual alignment and retentive features of such systems.[23] Nevertheless it can be speculated that the inner and outer retentive areas imparting those unique qualities to locator abutments may enhance the abutment colonization with early colonizing spp. However, based on the findings of the current study and the lack of significant difference in the bacterial count between the locator and ball attachment systems, the later assumption can be refuted. This finding can be attributed to the ease of cleaning which is permitted by both attachment systems. Further, It is acknowledged that strict oral hygiene measures and follow-up protocol that was followed might have influenced the results. Whereas in a dwelling with old frail patients and in a non-trial setting different outcomes could have been observed. Furthermore, the surface roughness of implant and abutment surfaces may play more of a role than the abutment design in the process of initial bacterial colonization and supra-gingival plaque formation. Several studies reported faster supra-gingival plaque formation on abutment surface with increased surface roughness compared to those with smoother surfaces.[24-26] However, the different rate of bacterial colonization and plaque formation between smooth and rough surface was less obvious when oral hygiene measures were optimal. [14]

The Presence of streptococcus spp. in peri-implant sulcus in this study corroborate with findings of other studies [27,28] where high proportions of coccoid cells, a low number of Gram anaerobic species and periodontopathogens were isolated from healthy peri-implant pockets.

Further research, should focus on recruiting subjects with ball or locator retained overdentures who suffer from perimucositis or peri-implantitis and further assessment of oral microbiota in the peri-implant sulcus to provide broader understanding of the whole biological picture and its potential influence on long-term clinical outcome. The influence of surface roughness on the microbial film formation in peri-implant sulcular fluid should also be evaluated.

CONCLUSIONS

Within the limitations of this study, it can be concluded that there is no difference between the two attachment systems with regards to microbiological environment in the peri-implant/abutment sulcular fluid. It can also be suggested that the locator attachments are valid treatment alternative for ball abutments to retain mandibular 2-implant overdentures from a microbiological point of view.

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