Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

Streptococcus mutans Colonization on Titanium Implant Abutment with Different Hygiene Instruments

Najahhuddin Shahbudin[1], Mohd Zulkarnain Sinor[2], Suharni Mohamad[3], Akram Hassan[4*]
[1,2,3,4]School of Dental Sciences, UniversitiSains Malaysia 16150 KubangKerian, Kelantan, Malaysia
*drakramhassan@yahoo.com

ABSTRACT

This *in vitro* study was aimed to assess the differences in the number of colony forming units (CFUs) of *Streptococcus mutans(S. mutans)* on the titanium implant abutment surfaces after treatment with an air polishing and rubber cup with pumice powder by using a stereomicroscope. This was an experimental laboratory study which was performed at Craniofacial Science Laboratory, School of Dental Sciences, UniversitiSains Malaysia.
Fifteen DentiumCombi titanium-implant abutments were divided into three groups of treatments; control and treated which were air polishing and rubber cup with pumice powder. *S. mutans* were then cultured on control and treated specimens. The amounts of retained bacteria on the surface were measured by the bacterial culture method. The median amount of *S. mutans*log_{10} CFU was highest in the air polishing group and lowest in the rubber cup group with the value of 5.65(0.25) and 4.75(0.65) respectively. The statistical analysis showed significant differences among these three groups [X^2(df) = 20.39(2), p-value = 0.00].The Mann-Whitney test also revealed the significant differences in median between the control and air polishing (p-value = 0.004), control and rubber cup (p-value = 0.018) as well as air polishing and rubber cup (p-value = 0.00) groups.Rubber cup with pumice powder exhibited lesser amounts of *S. mutans* colonization than air polishing treated surfaces, probably due to surface alteration as the rubber cup created a smoother surface topography.

Keywords
Implant abutment, bacterial colonization

Introduction

Nowadays a dental implant is one of the treatment options for replacing missing teeth. Implant treatment offers superior long-term survival compared to the fixed partial denture treatment option, while the success and survival rate are on par with root canal treatment that retains the natural tooth [1]. Even it is an artificial tooth, it is still affected by diseases just like a natural tooth. It is not exposed to caries, but it is still susceptible to tooth supporting structure diseases known as peri-implant diseases, such as peri-implantitis and peri-implant mucositis. Both diseases of the natural tooth and implant have the same aetiology, which is bacterial biofilm.

The thin biofilm can be removed easily by a simple local measure, such as brushing teeth. However, due to certain problems such as ineffective tooth brushing, some biofilms cannot be cleaned properly, especially at the neck of the tooth or abutment area of dental implant. This leads to further destruction of the tooth or implant supporting structure when it progresses into calculus. Biofilm will mineralise to form calculus and develop towards the apical of the tooth or implant resulting in the destruction of surrounding alveolar bone, as in peri-implantitis[2]. Poor plaque control skills, a history of chronic periodontitis and irregular maintenance care have clearly shown the increase in the risk of developing peri-implantitis[3]. This progression can be

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

halted by removing the plaque and calculus but without any interception, the patient will eventually lose the tooth or implant.

The use of different oral hygiene instruments such as a plastic scaler, air polishing system and rubber cup polish to remove biofilm were reported to cause less damage to the implant abutment surface [4]. Regardless, some studies contraindicate the use of flour of pumice for rubber cup polishing as well as other coarse abrasive polishing pastes [5, 6]. Meanwhile, the use of air polishing for implants remains controversial as it may detach the soft tissue connection from the implant surface due to air pressure leading to emphysema [7, 8].

It is important to ensure that the oral hygiene instrument does not damage the surface of the implant abutment, which can lead to surface alteration and roughness. This would make it more susceptible for further bacterial plaque adhesion [9]. Rough surfaces have a greater contact area between the bacterial cells and the surface that protects it from shear force [10]. In addition, Duarte *et al.* (2009)found no significant difference in the level of bacterial adhesion on smooth titanium surfaces among control and treated groups using Er:YAG laser, plastic curette, metal curette or the air-powder abrasive system; however, on rough titanium surfaces treated with metal curette and air powder abrasion, it showed significantly low levels of bacterial adhesion [11]. Di Salle *et al.* (2018)noticed that there were no significant differences in bacterial colonization among the untreated and treated implant surfaces when using devices such as a metal tip ultrasonic scaler, metal curette, air-polishing device and rubber cup instrumentation but there was a significant reduction in biofilm formation on the rubber cup treated surfaces [12].

Therefore, the aim of this *in vitro* study was to determine the number of CFUs of *S. mutans* on the titanium implant abutment surface in three different groups, namely the untreated (control), treated with an air flow system and rubber cup with pumice powder by using a stereomicroscope. The knowledge about these hygiene instruments used for implant maintenance could help the dentist in choosing the least damaging method to clean surrounding the implant surfaces and to establish best practice with respect to reducing further bacterial colonization. This will prevent from peri-implant diseases progression and increase the survival rate of the dental implant. Subsequently, the dental implant treatment will be improved.

Material and Methods

Study design

This was an experimental laboratory study which was performed at Craniofacial Science Laboratory, School of Dental Sciences, UniversitiSains Malaysia.

Titanium-implant abutment samples

Fifteen DentiumCombi titanium-implant abutments (CAB 5535L) were randomly divided equally into three groups. Five abutments were selected for untreated/ control (group and the other two groups were treated with rubber cup with pumice powder and air polishing (Air Flow® Master, EMS, Munich, Germany) respectively. The transmucosal part with a surface area of 2mm x 3mm was selected for bacterial colonization. All samples were sterilized with autoclaved.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

Bacterial strain and growth condition

The standard reference strain comprising *S. mutans* (ATCC 25175) (United States) cells were inoculated and incubated under microaerophilic conditions for 24 hours (candle jar; 37°C). Bacterial cells were suspended in a BHI broth (HiMedia Laboratories Pvt Limited, India).

Saliva coating of the samples

Unstimulated saliva was naturally collected from a healthy donor in one visit. The samples were preserved in a cool flask with ice pack before being transported to the laboratory to be stored and frozen at -20°C. The saliva sample was pooled and centrifuged (2500 revolutions per minute (rpm) for 30 minutes. The supernatant was pasteurized for 30 minutes at 60°C inside a water bath to inactivate endogenous enzymes. Then, it was re-centrifuged at 2500 rpm for 30 minutes in a sterile 50 ml centrifuge tube and stored at -20°C. The pasteurization efficacy was evaluated by plating 100µl saliva on a Brain-Heart Infusion (BHI) agar and the absence of bacterial growth was observed after 72 hours The abutments were sterilised using the autoclave (15 minutes at 127°C) and placed into the collected saliva for 4 hours to allow salivary pellicle formation (24-well polystyrene cell culture plate containing 1000µl saliva).

Bacterial colonization

Saliva was aspirated from each well and replaced with 500µl BHI broth and 500µl saliva. Bacterial cells of *S. mutans*were suspended in BHI broth, adjusting the turbidity to Optical Density $(\mathrm{OD})_{630}$ 0.15 (1 x 10^6 colony forming units (CFUs)/ml). Each well was inoculated with 100µl of this inoculum suspension. The plate was incubated for 16 hours under microaerophilic conditions for *S. mutans*.

CFUs counting

Out of fifteen titanium-implant abutments, twelve abutments were randomly selected with four from each group after colonization and incubation of the *S. mutans*. They were washed in sterile saline solution to remove unattached cells. Samples were inserted in microtubes containing 1,000 µl of BHI broth. The microtubes were in vortex for 2 minutes to free bacteria attached on the surface and to disperse the bacterial cells that had undergone serial dilutions (10^{-1} to 10^{-5}). 100 µl of each diluted solution was inoculated onto the BHI agar plates. It was spread on the agar with a glass rod spreader and incubated for 48 hours under microaerophilic conditions (candle jar 37°C). The test was done triplicate and a stereomicroscope was used to determine the CFUs. The total value of one hundred and eighty agar plates was produced for all 12 samples. Confirmation of the microorganism was carried out by visualisation of gram staining under a light microscope at a magnification of x40, which was blue for positive gram stain. The total value of thirty-six CFUs were collected.

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 24 software. CFUs for samples inoculated in triplicate were subjected to logarithmic transformation where total values were thirty-six. Data were statistically analyzed using the non-parametric analysis (Kruskal-Wallis) to compare the amount of bacteria in the three different groups. When two independents group were compared, the Mann-Whitney test (the non-parametric equivalent of independent sample t-test) was used.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

Results

The amount of CFUs of *S. mutans* on the abutment surface from all twelve samples from the respective groups were calculated conventionally for each dilution (10^{-1}to10^{-5}) and triplicated. All thirty-six CFUs were subjected to a logarithmic transformation of log_{10} (Table 1). None of the values were excluded from the study.

Table 1: Amount of CFU/log_{10} of *S. mutans* in three different groups

Number of samples in each group	Plate 1 (CFUs) X 10^3	Plate 1 (log_{10})	Plate 2 (CFUs) X 10^3	Plate 2 (log_{10})	Plate 3 (CFUs) X 10^3	Plate 3 (log_{10})
Control						
1	94	4.97	93	4.97	93	4.97
2	514	5.71	458	5.66	504	5.7
3	202	5.31	166	5.22	181	5.56
4	80	4.9	42	4.62	63	4.8
Rubber cup with pumice						
1	20	4.3	35	4.54	21	4.32
2	318	5.5	228	5.36	243	5.39
3	54	4.73	74	4.87	59	4.77
4	57	4.76	54	4.73	53	4.72
Air polishing						
1	307	5.49	347	5.49	321	5.51
2	725	5.86	644	5.83	679	5.83
3	551	5.74	528	5.72	520	5.72
4	347	5.54	374	5.57	359	5.56

The median amount of log_{10} CFU was highest in the air polishing group 5.65(0.25) and lowest in the rubber cup group 4.75(0.65). The statistical analysis showed significant differences among these three groups [X^2(df) = 20.39(2),p-value = 0.00] (Table 2).

Table 2: Median amount of CFU/log_{10}*S. mutans* between groups

No	Group	n	Median (IQR) CFU/log_{10}	X^2	df^a	p $value^b$
1	Control	12	5.10(0.72)	20.39	2	0.00
2	Rubber Cup	12	4.75(0.65)	20.39	2	0.00
3	Air Flow	12	5.65(0.25)	20.39	2	0.00

[a]Kruskal-Wallis test [b]p-value significant at <0.05 at 95% confidence interval

The Mann-Whitney test was used to compare the median between two groups. There were significant differences in median between the control and air polishing (p-value = 0.004), control and rubber cup (p-value = 0.018) as well as air polishing and rubber cup (p-value = 0.00) groups (Table 3).

Table 3: Comparison of the median amount of CFU/log_{10}*S. mutans* between groups

No	Comparison of the median amount of CFU/log_{10}*S. mutans* between	n	$Z\ stat^a$	p $value^b$

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

	groups			
1	Control and Air Polishing	12	-2.86	0.004
2	Control and Rubber Cup with pumice	12	-2.37	0.018
3	Air Polishing and Rubber Cup with pumice	12	-4.102	0.000

[a]Mann- Whitney U test [b]p-value significant at <0.05 at 95% confidence interval

Discussion

This study focused on the mechanical effects of surface changes, different modalities of the surface roughness as well as bacterial colonization in the form of *de novo* biofilm formation. This was a critical situation as it directly caused subsequent inflammation. Clinicians require prior recommendation for treating modalities, not only for effectively removing the source of infection but also for preventing further re-colonization of the bacteria.

In this study, the colony was calculated manually using a stereomicroscope. The highest number of *S. mutans* colonies was found in the sample treated with air polishing and followed by the sample without any treatment and the sample treated with rubber cup to have the least number of colonies. This finding was consistent with a previous finding by Di Salle *et al.* (2018) where the sample treated with the rubber cup was statistically significant reduced of *Streptococcus aureus* biofilm formation compared to the air polishing treatment and control groups [12]. This was also supported by findings of significantly higher *S. mutans* adhesion in the control group compared to the rubber cup group, which was measured using atomic force microscopy [13]. In this study, the air polishing treated surface retained the highest *S. mutans* adhesion together with the highest surface roughness among them. This is in conjunction with rougher surfaces having more contact with bacterial cells and providing better protection again shear forces. Conversely, rougher surfaces make it easier for the biofilm to form and stay, which might easily initiate peri-implant diseases instigated by improper oral hygiene practices. Even at below roughness threshold levels, *S. mutans* can still colonize the titanium implant surface [14] but this study proved that the changes in roughness values of above the threshold value of 0.2 μm offers statistically significant differences in biofilm formation. However, the previous studies based on one-time instrumentation found no significant differences in bacterial colonization between the air polishing and control groups [11, 15]. Di Salle *et al*. (2018) found insignificantly similar or higher bacterial amounts in the control group compared to the air polishing group [12]. This could be due to the small sample size as these studies were costly to perform. The environment might not absolutely mimic the clinical situation, which could affect the formation of the biofilm differently. In a clinical situation, biofilm formation does not only involve a single or limited bacterial species that work synergistically for the mutual benefit of the colony's survival.

Cochis*et al*. (2012) found that the air polishing treated surface was not significantly different amounts of bacterial colonization compared to the control group but significantly lower amounts when using glycine instead of bicarbonate powder in the air polishing group [16]. The study concluded that the lower amounts of bacteria were due to the bacterial inhibition effect of glycine in excess amounts. Furthermore, different pressures might have different effects on the surface, and this could change the amount of bacterial colonization. Multiple factors should be considered, such as different devices that work and act differently. Standardization of air polishing devices are difficult to implement as the operator needs to follow the manufacturer's

instructions. There were limited studies that focused on these topics. Most of the literature focused on the efficacy of different instruments used for removing biofilm [17]. One systemic review concluded that the single use of rubber cup with pumice on titanium surfaces did not clean effectively, regardless of rough or smooth surfaces. Whereas, air-powder abrasives with either sodium bicarbonate or amino acid glycine powder appeared to clean all types of titanium surfaces effectively [18]. The use of a brush was more effective in cleaning than the cup type, as more surface area was covered [19] but Schmage*et al.* (2014) found that both methods had similar effectiveness [20]. This should be considered as an *in vitro* study, which is not similar to an oral cavity where the presence of gingival tissue covers the surface and might provide a different result. Furthermore, Wenneberg*et al.* (2003) found that there was no association between the inflammatory response *in vivo* and the surface roughness even though statistically it was significantly difference to biofilm accumulation [21]. The effects of different instruments used on surfaces in these studies were measured based on the alteration of the surfaces by studying roughness under a laser profilometer or Scanning Electron Microscopy (SEM). In addition, there was a lack of studies on the effects of biofilm formation after using different instruments. Studies on the amount of biofilm in CFU/ml were limited compared to studies that subjectively described the formation of the biofilm on SEM.

The trend now focuses on studies on the effectiveness of new technology, such as the Vector scaler, lasers and photodynamic therapy (PDT). One study found that new technology, such as the Vector™ scaler with a carbon tip, is effective in removing bacterial plaque and significantly decreased initial biofilm formation [18]. The laser does not damage the titanium surface or influence periodontal cell attachment compared to the Vector scaler that has negative effects [22]. Photodynamic therapy recommends that new emerging technology be used, especially in the maintenance phase, as it prevents further removal of tooth surface and targets the bacteria itself [23]. However, the use of these new technologies seems clinically impractical as it might not be feasible to implement it in all dental clinics. Therefore, the study on rubber cup and air polishing devices are still relevant as they are readily available in almost every facility as part of the professional hygiene care for implant maintenance [8].

Conclusion

In conclusion rubber cup with pumice powder exhibited lesser amounts of *S. mutans* colonization than air polishing treated surfaces and untreated samples, probably due to surface alteration as the rubber cup created a smoother surface topography.

Acknowledgements

This work was supported by a UniversitiSains Malaysia Research University Grant (Grant Number: 1001/PPSG/8012365) and School of Dental Sciences, UniversitiSains Malaysia.

Declaration of Interest

The authors report no conflict of interest.

References

[1] Torabinejad M., Anderson P., Bader J., Brown J., Chen L. H., Goodacre C. J., Kattadiyil M. T., Kutsenko D., Lozada J., Patel R., Petersen F., Puterman I. and White S. N. (2007). Outcomes of root canal treatment and restoration, implant-supported single crowns, fixed partial dentures, and extraction without replacement: A systemic review. *The Journal of Prosthetic Dentistry,* 98(4),287-311.

[2] Rompen E., Domken O., Degidi M., Pontes A. E. F. and Piattclli A. (2006). The effect of marginal characteristics, surface topography, implant components and connections on soft tissue integration: Literature review. *Clinical Oral Implants Research,* 17 (Suppl. 2),55-67.

[3] Schwarz F., Derks J., Monje A. and Wang H. L. (2018). Peri-implantitis. *Journal of Clinical Periodontology,* 45(Suppl 20),S246-S266.

[4] Louropoulou A., Slot D. E. and Weijden F. A. (2012). Titanium surface alterations following the use of different mechanical instruments: A systematic review. *Clinical Oral Implants Research,* 23,643-658.

[5] Bergendal T., Forsgren L., Kvint S. and Lowstedt E. (1990). The effect of an air abrasive instrument on soft and hard tissues around osseointegrated implants. A case report. *Swedish Dental Journal,* 14(5),219-223.

[6] Rapley J. W., Swan R. H., Hallmon W. W. and Millls M. P. (1990). The surface characteristics produced by various oral hygiene instruments and materials on titanium implant abutment. *The International Journal of Oral and Maxillofacial Implants,* 5(1),47-52.

[7] Kurtzman G. M. and Silverstein L. H. (2007). Dental implants: Oral hygiene and maintenance. *Dentistry Today,* 1(3),48-53.

[8] Gulati M., Govila V., Anand V. and Anand B. (2014). Implant maintenance: A clinical update. *International Scholarly Research Notices*, 1-8.

[9] Yeo I. S., Kim H. Y., Lim K. S. and Han J. S. (2012). Implant surface factors and bacterial adhesion: A review of the literature. *International Journal Artificial Organs,*35 (10),762-772.

[10] Song F., Koo H. and Ren D. (2015). Effects of material properties on bacterial adhesion and biofilm formation. *Journal of Dental Research,* 94,1027-1034.

[11] Duarte P. M., Reis A. F., de Freitas P. M. and Ota-Tsuzuki C. (2009). Bacterial adhesion on smooth and rough titanium surfaces after treatment with different instruments. *Journal of Periodontology,* 80(11),1824-1832.

[12] Di Salle A., Spagnuolo G., Conte R., Procino A., Peluso G. and Rengo C. (2018). Effects of various prophylactic procedures on titanium surfaces and biofilm formation. *Journal of Periodontal & Implant Science,* 48(6),373-382.

[13] Barbour M. E., O'Sullivan D., Jenkinsson H. F. and Jagger D. C. (2007). The effects of polishing methods on surface morphology, roughness and bacterial colonization of titanium abutments. *Journal of Materials Science: Materials in Medicine,* 18,1439-1447.

[14] da Rocha S. S., Bernardi A. C. A., Pizzolitto A. C., Adabo G. L. and Pizzolitto E. L. (2009). *Streptococcus mutans* attachment on a cast titanium surface. *Materials Research,* 12(1),41-44.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13559 - 13566
Received 05 March 2021; Accepted 01 April 2021.

[15] Schmidt K. E., Auschill T. M., Heumann C., Frankenberger R., Eick S., Sculean A. and Arweiler N. B. (2017). Influence of different instrumentation modalities on the surface characteristics and biofilm formation on dental implant neck, *in vitro*. *Clinical Oral Implants Research,* 28,483-490.

[16] Cochis A., Fini M., Carrasi A., Migliario M., Visai L. and Rimondini L. (2012). Effect of air polishing with glycine powder on titanium abutment surfaces. *Clinical Oral Implants Research***,** 1-6.

[17] Curylofo F. D. A., Barbosa L. A., Roselino A. L., Fais L. M. G. and Vaz L. G. (2013). Instrumentation of dental implants: A literature review. *RevistaSul-Brasileira de Odontologia,* 10(1),82-88.

[18] Louropoulou A., Slot D. E. and Weijden F. (2014). The effects of mechanical instruments on contaminated titanium dental implant surfaces: A systematic review. *Clinical Oral Implants Research,* 25(10),1149-1160.

[19] Matarasso S., Quaremba G., Coraggio F., Vaia E., Cafiero C. and Lang N. P. (1996). Maintenance of implants: An *in vitro* study of titanium implant surface modifications subsequent to the application of different prophylaxis procedures. *Clinical Oral Implants Research,* 7,64-72.

[20] Schmage P., Kahili F., Nergiz I., Scorziello T. M., Platzer U. and Pfeiffer P. (2014). Cleaning effectiveness of implant prophylaxis instruments. *The International Journal of Oral and Maxillofacial Implants,* 29(2),331-337.

[21] Wennerberg A., Sennerby L., Kultje C. and Lekholm U. (2003). Some soft tissue characteristics at implant abutments with different surface topography. A study in humans. *Journal of Clinical Periodontology,* 30,88-94.

[22] Schwarz F., Rothamel D., Sculean A., Georg T., Scherbaum W. and Becker J. (2003). Effects of an Er:YAG laser and the Vector ultrasonic system on the biocompatibility of titanium implants in cultures of human osteoblast-like cells. *Clinical Oral Implants Research,* 14(6),784-792.

[23] Takasaki A. A., Aoki A., Mizutani K., Schwarz F., Sculean A., Wang C. Y., Koshy G., Romanos G., Ishikawa I. and Izumi Y. (2009). Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. *Periodontology 2000,* 51,109-140.