Colonization of *Streptococcus mutans* on Titanium Implant Abutment with Different Hygiene Instruments under Scanning Electron Microscopy

Najahhuddin Shahbudin[1], Suharni Mohamad[1], Akram Hassan[1]*

1. School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Abstract

This study was aimed to describe the differences in amounts of Streptococcus mutans (S. mutans) colonization on the titanium-implant abutment surfaces after treatment with two different hygiene instruments by using Scanning Electron Microscope (SEM).

Fifteen Dentium Combi titanium-implant abutments were randomly divided equally into three groups. Five abutments were selected for untreated/control (C) group and the other two groups which were treated with rubber cup with pumice powder (RC) and air polishing (AP) respectively. S. mutans was then cultured on untreated and treated specimens. One sample from each group was randomly selected and observed for the bacterial colonization on the surface of abutment using SEM.

At x2000 magnification, the machining marks were observed in C and AP groups whereas the surface was smoothened in RC group. Sparse colonies could be observed in both C and RC groups but moderate colonies was noticed in AP group at a magnification of x5000. At x10 000 magnification, multilayers of bacterial chains could be seen in C and AP groups, while a monolayer of bacterial chain was found in RC group.

RC instrumentation revealed lesser amounts of S. mutans colonization compared to instrumentation with AP.

Experimental article (J Int Dent Med Res 2020; 13(4): 1293-1298)

Keywords: Implant abutment, bacterial colonization.

Received date: 13 September 2020 **Accept date:** 22 October 2020

Introduction

Nowadays edentulous patients can now have opportunity to gain back what they had once thought lost with the invention of dental implants which are generally viewed more favourably, in terms of aesthetics, functionality and stability and they are becoming increasingly available[1].

Implant is an artificial tooth, it is also affected with diseases as the natural tooth. It would not expose to caries but it is still susceptible to tooth supporting structure diseases which are peri-implant diseases, i.e peri-implantitis and peri-implant mucositis. Peri-implant mucositis is comparable to gingivitis in the normal tooth, involving the inflammation of the gingiva that is in contact with the implant in the absence of loss of supporting bone[2].

Whereas, peri-implantitis is analogous to periodontitis, which involves the progressive loss of alveolar bone and can cause the implant to be lost[3-5]. Peri-implant mucositis is considered a precursor to peri-implantitis[2]. Both diseases of the natural tooth and implant have the same aetiology, which is bacterial biofilm. The microflora of peri-implantitis looks similar to subgingival biofilm on periodontitis teeth based on culture studies and recent molecular biological studies[6]. However, progression of the inflammatory process in these two diseases is different, in which progression of the peri-implantitis is faster than periodontitis[5,7]. Most of the material of dental implants is titanium. Titanium as a highly polished metal has proven to be fewer plaques retentive than natural teeth[8]. However, it has been reported contradictory that even on relatively smooth implants surfaces e.g. abutments, plaque accumulates faster when compared with natural teeth and they found that up to 23 times more bacteria adhering to rough

Corresponding author:
Associate Professor Dr. Akram Hassan
BDS, MClinDent
School of Dental Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan, Malaysia
E-mail: drakramhassan@yahoo.com

implant surfaces than smooth ones[9]. The main concern about plaque removal from surface of implant is the possibility of damaging to the implant surfaces. Any damage to the implant surface will cause changes in chemical oxide layer and may result corrosion of implant surface. If plaque accumulation occurs and is left for a period of time, inflammation around the implant may spread rapidly and can easily reach the bone[10]. It could cause bone resorption, with subsequent failure of osseointegration.

There is no denying that implants are a costly investment and should be taken good care to avoid severe complications. Therefore maintenance is crucial to ensure the longevity and lifespan of an implant. Proper implant instrumentation includes removing microbial deposits without altering the surfaces of implant or affecting biocompatibility adversely[11]. During maintenance therapy, cleaning can be done by scaling with a metal instrument to remove the calculus in the case of a natural tooth but it is not advisable to use this hygiene instrument on an implant tooth as cleaning effects can alter the implant surface[12]. The bacterial load and host defence imbalance recognized as the factor in developing of peri-implant diseases[13]. Bacterial colonization on oral implant surfaces starts immediately and rapidly after exposing of implant surface to oral environment[14]. But many studies indicated that bacterial colonization may occur within 30 minutes after placement of the implant with different types of microorganisms[15]. The factor that initiates colonization seems to be influenced by the surface roughness, surface-free energy and chemical composition[16,17].

The long term success of dental implants is dependent upon regular follow up and maintenance regimes and as dental implants become more prevalent, the responsibility for providing this maintenance care will fall increasingly on the general dental practitioner. As the number of patients selecting dental implants as a treatment option continues to grow, the dental team must accept the challenges of maintaining these sometimes complexes restorations. The softness of commercially pure titanium has led to the development of various devices for the cleaning of implant abutments. The efficacy of implant surface cleaning is significantly influenced by the implant surface structures and by the cleaning methods[18]. It is crucial that the instruments used for maintenance be able to remove plaque and calculus from the implant surface effectively and efficiently while causing minimal damage to its circumference. Conventional sonic and ultrasonic scalers cause considerable changes to implant surfaces[19]. Therefore the use of plastic curets, graphite or nylon-type instruments, rubber polishing cups, brushes with abrasive paste and air-powder abrasive systems have been recommended[12]. Studies using scanning electron microscopy showed these implant instruments produced no scratches or gouges on the implant surface.

At the moment, there are no clear protocols for the treatment of peri-implantitis, and there are no data in the literature devoted to the study of the effect of mechanical treatment on the implant surface, depending on its type, and as a result, the prognosis for the treatment of peri-implantitis[20]. There was our previous study and other study before the bacterial colonization comparing the abutment surface after being treated with air polishing and rubber cup with pumice powder since these were among the best-suggested methods for cleaning implants. The abutment surface treated with AP had a significantly rougher surface compared to a surface treated with RC[21,22]. However, Cafiero *et al.* (2017) found that a high-pressure air-powder abrasive system resulted in a smoothened surface compared to a low-pressure setting and rubber cup polishing[23]. Based on previous studies, it theoretically shows that a rough surface has a better colonization of bacterial plaque, which leads to diseases. On the other hand, a statistically significant reduction of bacterial plaque formation was observed for RC in comparison to AP device treatment[22].

Therefore, the aim of this *in vitro* study was to describe the *S. mutans* colonization on the titanium-implant abutment surface topography using SEM in three different groups with two different type of instruments which were rubber cup with pumice powder and an air polishing. 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.

Materials and methods

Study Design

This was an experimental laboratory study which was performed at Craniofacial Science Laboratory, School of Dental Sciences, Universiti Sains Malaysia. *S. mutans* was cultured on a 24-well polystyrene cell-culture plate containing the different treated abutments and was incubated for 16 hours under microaerofilic conditions. The samples of abutments with the bacteria colonies were then scanned with SEM at the Biomaterial Laboratory School of Health Science, Universiti Sains Malaysia.

Titanium-Implant Abutment Samples

Fifteen Dentium Combi titanium-implant abutments (CAB 5535L) were randomly divided equally into three groups. Five abutments were selected for untreated/ control (C) group and the other two groups were treated with rubber cup with pumice powder (RC) and air polishing (AP) (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.

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 (age range, 18 to 35 years) until the amount reached 7.5 ml within 30 minutes in one visit. The saliva samples were collected in a sterile container. 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 $(OD)_{630}$ 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*. All saliva samples were discarded directly into the sluice, which was directly connected to the sewage system.

Assessment of Bacterial Colonization

Out of fifteen titanium-implant abutments, three abutments were randomly selected with one of each group and observed under the SEM after colonization and incubation of *S. mutans*. Samples were primarily fixed in McDowell's and Trump's fixation, at 4°C for 24 hours. Subsequently, samples were washed with phosphate-buffered saline buffer, secondarily fixed with 1% osmium tetroxide for 10 minutes at 4°C, dehydrated in ascending acetone concentrations up to 100% before air dried overnight. The flat surface of the prepared portion of specimens faced the stub and mounted to the stub. Meanwhile, the 2mm x 3mm convex surface at the transmucosal portion of the abutment was standardized for observation and randomly scanned at three points after all abutments were sputtered gold in an SC005 Leica sputter machine. Representative areas of samples after bacterial incubation were photographed at a magnification of x2000, x5000 and x10, 000.

Ethical consideration

Ethical clearance was obtained from the Research Ethics and Committee (Human), Universiti Sains Malaysia with the reference number was USM/JEPeM/16090313.

Results

At a magnification of x2000, the machining marks that ran in uniform oblique lines

could be seen in C group but not in RC group where the surface was smoothened by the rubber cup and pumice powder. Similarly in AP group, the machining marks were observed and intact as in C group. However, the surface looked rougher with a presence of generalized micro pores created by high velocity of air flow and amino acid-glycine powder (Figure 1).

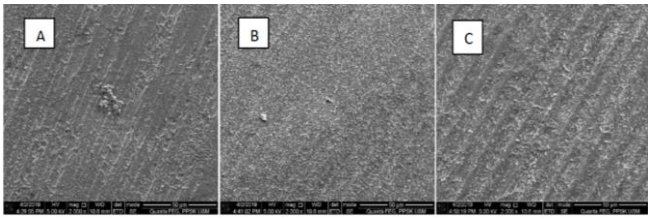

Figure 1. SEM images of *S. mutans* adhesion on the titanium–implant abutment surface topography in three different groups at x2000 magnification. (A) Control, (B) Rubber Cup with Pumice Powder, and (C) Air Polishing.

Sparse *S. mutans* colonies could be observed in both C and RC groups at a magnification of x5000. Moderate *S. mutans* colonies in AP group reflected higher amounts of bacterial colonization compared to others (Figure 2).

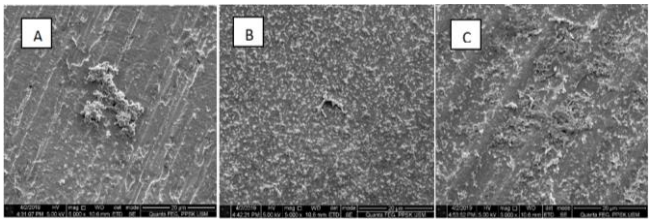

Figure 2. SEM images of *S. mutans* adhesion on the titanium–implant abutment surface topography in three different groups at x5000 magnification. (A) Control, (B) Rubber Cup with Pumice Powder, and (C) Air Polishing.

Multilayers of bacterial chains could be seen in the C and AP groups, while a monolayer of bacterial chain was found in RC group at a magnification of x10 000. At this magnification level, the biofilm clearly showed bacteria cell shape, which was rounded or rod-like while making a long chain. Debris from the sample processing could be seen in RC and AP groups. However, some of them exhibited branched bacteria rather than in chain form (Figure 3).

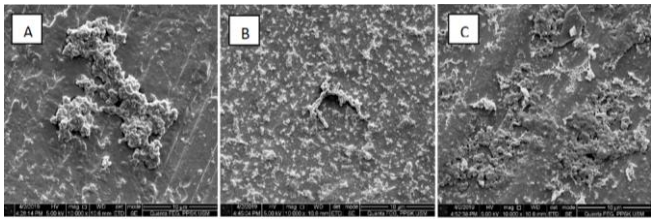

Figure 3. SEM images of *S. mutans* adhesion on the titanium–implant abutment surface topography in three different groups at x10 000 magnification. (A) Control, (B) Rubber Cup with Pumice Powder, (C) Air Polishing.

Discussion

The pathogenicity of *S. mutans* is associated with the ability to produce extracellular polysaccharides and lactic acid. The acidity causes the release of metal ions that can lead to antimicrobial effects. This ion release can inhibit biofilm formation, particularly during the early stage of biofilm growth [24]. This explained the lower amounts of bacterial composition in this study compared to previous studies that utilised *Streptococcus sanguinis*, which was not acidogenic when observed under a SEM[25]. In contrast, Olmedo *et al*. (2013) found high concentrations of metal-like particles in patients suffering from implantitis[26]. However, Schwarz *et al*. (2018) differed and concluded that the available evidence is not enough to associate the role of titanium or metal particles in the pathogenesis of peri-implantitis[5].

Abutment surfaces reflected light under SEM. At large magnifications, apart from the bacterial composition, it was evident that untreated abutment surfaces were not smooth. Definite machined parallel grooves could be seen circumferentially, which were horizontally lined and perpendicular to the abutment axis. The distance between each machining mark was about 5-6 µm[27]. Similarly, the C group in this study exhibited some pits and scratches. The machining marks could be seen in the C group, which ran in uniform oblique lines at x2000 magnification.

SEM images in this study also revealed different surface topographies according to different treatments. The machining mark with irregular scratches and small pits resulting from machining could be clearly seen on the C abutment; however, it was reduced on the RC treated surfaces suggesting a smoothening effect.

Studies had found that RC resulted in lesser roughness, obliterated surface asperities and scratches[12] and reports also suggested that the use of RC polishing did not increase surface roughness[23].

Inversely, they were enhanced on AP treated surfaces, suggesting a roughening effect. It was concluded that abutments treated with AP created the roughest surface, while the RC created a smoother surface by obliterating the original milling marks [12]. This was similar to Duarte *et al.* (2009), who found that AP gave some sand-blasting effects and irregular crater-like defects[25]. In addition, AP treatment significantly increased the surface roughness of the titanium surface though it was still considered safe[28].

The surface alteration still occurred even though the amino-acid-glycine powder used in AP was the softest available powder for subgingival use that should not affect the root surface and soft tissue even more in harder surfaces such as titanium [12]. Furthermore, it was found that AP that used three different abrasive materials, such as glycine based powder, soft powder and erythritol powder, gave an insignificantly rougher surface compared to non-treated surfaces viewed under SEM [27]. The roughness effect could be obvious on laser profilometry as its measurements are more sensitive compared to SEM[5].

This study was consistent with Di Salle *et al.* (2018), who found that AP increased roughness values, while RC polishing significantly reduced roughness values compared to the C group. Therefore, both samples, C group and RC group showed sparse distribution of *S. mutans* colonies compared to the sample treated with AP. However, the sample treated with the RC had a monolayer *S. mutans* cell chains, while the sample without any treatment had multilayer *S. mutans* cell chains. Whereas the sample treated with air flow had moderate distribution of colonies and multilayered *S. mutans* cell chains [22]. Hence, it was found that the control titanium abutment supported denser biofilm growth of *S. mutans* compared to the RC treated titanium abutment [29]. Somehow, Duarte *et al.* (2009) found similar and moderate number of *S. sanguinis* colonies in C and AP treated surfaces, respectively, with a predominantly monolayer present[25]. The spatial arrangement of *S. mutans* cells resembling long chain beads, as its morphological characteristic, was observed at a high magnification of x10 000[30]. Some bacteria exhibited in the branch might suggest that it was not *S. mutans*. However, efficacy of the culture plate and gram staining was carried out to detect any contamination.

This finding indicated a low bacterial adhesion rate on the sample treated with the RC compared to the sample without any treatment and treated with AP. However, previous studies found that smooth surfaces treated with dental hygiene instruments, including AP and RC demonstrated the same level of *S. sanguinis* adhesion as the untreated control surface regardless in the differences of the surface texture between groups[25,31]. These observations were too subjective and carried out under a SEM, which could contain inaccurate data and impediments when studying the *S. mutans* biofilm. The extracellular polymeric substance was devastated, removing supporting structures and causing cracks on the biofilm surface during the intensive dehydration process when using SEM. The variable pressure scanning electron microscopy (VPSEM) offers the most comprehensive representation of bacterial biofilm morphology by avoiding the dehydration process and high chamber vacuum[32].

In this study, particles and debris noted on the samples treated with the RC and AP instruments, whereas surfaces without treatment were clear. These could be due to the pumice residue, rubber cup remnant or the amino acid glycine powder residue. Similarly, presence of sodium carbonate deposits on the treated titanium surface after AP compared to the control group, might be related to a low bacterial adhesion rate[25]. All these residues might interact with the chemical during sample processing and influence bacterial[33]. They might also have other unknown biologic implications on periodontal tissues, such as cytotoxicity, impairment of the fibroblast and osteoblast adhesion.

Conclusions

In conclusion, under SEM, the topography of titanium surfaces after treatment with RC polishing showed lower *S. mutans* colonization compared to AP samples due to the smoothening effect of the instruments. *S. mutans* colonies were sparse or thinly scattered at a higher magnification compared to surfaces treated with

AP, which was moderately scattered due to the roughening effect of the instruments.

Acknowledgments

The study was supported by Bridging Incentive Grant, No. 304/PPSG/6316452 of Universiti Sains Malaysia and School of Dental Sciences, USM.

Declaration of Interest

The authors report no conflict of interest.

References

1. Rustemeyer J, Bremerich A. Patients' knowledge and expectations regarding dental implants: Assessment by questionnaire. Int J Oral Maxillofac Surg. 2007; 36: 814–17.
2. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. Journal of Periodontology 2018; 89(Suppl 1): S257-66.
3. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. Journal of Clinical Periodontology 2008; 35: 286-91.
4. Berechet CA, Ionaseu AM, Sirbu V, Sirbu I. Peri-implantitis versus periodontitis-Similarities and differences. Literature Review. Romanian Journal of Stomatology 2013; 59(1): 32-7.
5. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. Journal of Clinical Periodontology 2018; 45(Suppl 20): S246-66.
6. Larsen T, Fiehn NE. Dental biofilm infection-An update. APMIS 2017; 125: 376-84.
7. Laosuwan K, Epasinghe DJ, Wu Z, Leung WK, Jung HS. Comparison of biofilm formation and migration of Streptocoocus mutans on tooth roots and titanium mini screws. Clinical Experimental Dental Research 2018; 1-8.
8. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. International Journal of Oral Surgery 1981; 10: 387-416.
9. Quirynen M, Bollen CM. The influence of surface roughness and surfaces-free energy on supra and subgingival plaque formation in man. A review of the literature. Journal of Clinical Periodontology 1995; 22: 1-14.
10. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J. Long-standing plaque and gingivitis at implants and teeth in the dog. Clinical Oral Implants Research 1992; 3(3): 99-103.
11. Meschenmoser A, et al. Effects of various hygiene procedures on the surface characteristics of titanium abutments. Journal Periodontology 1996; 67: 229-235.
12. Schmage P, Thielemann J, Nergiz I, Scorziello TM, Pfeiffer P. Effects of 10 cleaning instruments on four different implant surfaces. The International Journal of Oral and Maxillofacial Implants 2012; 27(2): 308-17.
13. Tonetti MS, Schmid J. Pathogenesis of implant failures. Periodontology 2000 1994; 4: 127–38.
14. Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation of oral titanium implants. Clinical Oral Implants Research 2007; 4: 501–8.
15. Wifaq MAA, Warkaa MAA, Afya SDA. Microbiological and cytological response to dental implant healing abutment. Journal of International Dental and Medical Research 2017; 10(3): 891-8.
16. Quirynen M, van der Mei H, Bollen C, et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra-and subgingival plaque. Journal of Dental Research 1993; 72(9): 1304-09.
17. Rimondini L, Fare S, Brambilla E, et al. The effect of surface roughness on early in vivo plaque colonization on titanium. Journal of Periodontology 1997; 68: 556–62.
18. Renvert S, Polyzois I, Claffey N. How do implant surface characteristics influence peri-implant disease? Journal of Clinical Periodontology 2008; 38(11): 214-22.
19. Sato S, Kishida M. Ito K. The comparative effect of ultrasonic scalers on titanium surfaces: An in vitro study. Journal of Periodontology 2004; 75(9): 1269-73.
20. Furtsev TV, Zeer GM. Efficiency of cleaning the various types of dental implants' surfaces (Tiu-Nite, Sla, Rbm) using the air-flow erythritol method. Journal of International Dental and Medical Research 2020; 13(2): 448-52.
21. Hassan A, Taib H, Abas N, Zhen DPL, Hanafi NN. Evaluation of different hygiene instruments on titanium implant abutment. International Medical Journal 2016; 24(3): 275.
22. Di Salle A, Spagnuolo G, Conte R, Procino A, Peluso G, Rengo C. Effects of various prophylactic procedures on titanium surfaces and biofilm formation. Journal of Periodontal & Implant Science 2018; 48(6): 373-82.
23. Cafiero C, Aglieta M, Iorio-Siciliano V, Salvi GE, Blasi A, Matarasso S. Implant surface roughness alterations induced by different prophylactic procedures: An in vitro study. Clinical Oral Implant Research 2017; 28: 16-20.
24. Mabilleau G, Bourdon S, Joly-Guillou ML, Filmon R, Basle MF, Chappard D. Influence of Fluoride, Hydrogen Peroxide and Lactic Acid on the Corrosion Resistance of Commercially Pure Titanium. Acta Biomaterialia 2006; 2(1): 121-9.
25. Duarte PM, Reis AF, de Freitas, PM, Ota-Tsuzuki C. Bacterial adhesion on smooth and rough titanium surfaces after treatment with different instruments. Journal of Periodontology 2009; 80(11): 1824-32.
26. Olmedo DG, Nalli G, Paparella ML, Cabrini RL. Exfoliative cytology and titanium dental implants: a pilot study. Journal of Periodontology 2013; 84(1): 78-83.
27. Schmidt KE, Auschill TM, Heumann C, et al. Influence of different instrumentation modalities on the surface characteristics and biofilm formation on dental implant neck, in vitro. Clinical Oral Implants Research 2017; 28: 483-90.
28. Bennani V, Hwang L, Tawse-Smith A, Dias GJ, Cannon RD. Effect of air-polishing on titanium surfaces, biofilm removal, and biocompatibility: A pilot study. BioMed Research International 2015; 1-8.
29. Barbour ME, O'Sullivan D, Jenkinsson HF, Jagger DC. The effects of polishing methods on surface morphology, roughness and bacterial colonisation of titanium abutments. Journal of Materials Science: Materials in Medicine 2007; 18: 1439-47.
30. Krzysciak W, Koscielniak D, Papiez M, Jurczak A, Vyhouskaya P. Methods of biotyping of streptococcus mutans species with the routine test as a prognostic value in early childhood caries. Evidence-Based Complementary and Alternative Medicine 2017; 1-15.
31. Pita PPC, Rodrigues JA, Ota-Tsuzuki C, et al. Oral streptococci biofilm formation on different implant surface topographies. BioMed Research International, 2015; 1-6.
32. Weber K, Delben J, Bromage TG, Duarte S. Comparison of SEM and VPSEM imaging techniques with respect to Streptococcus mutans biofilm topography. Federation of European Microbiological Societies 2013; 175-9.
33. Cochis A, Fini M, Carrasi A, Migliario M, Visai L, Rimondini L. Effect of air polishing with glycine powder on titanium abutment surfaces. Clinical Oral Implants Research 2012; 1-6.