Original Article

Periodontopathogens around the surface of mini-implants removed from orthodontic patients

André Tortamano[a]; Gladys Cristina Dominguez[b]; Ana Cristina Soares Santos Haddad[c]; Fabio Daumas Nunes[d]; Mônica Nacao[e]; Camillo Morea[f]

ABSTRACT

Objective: To verify if mini-implant mobility is affected by the presence of periodontopathogens, frequently associated with peri-implantitis.

Materials and Methods: The surfaces of 31 mini-implants used for skeletal anchorage in orthodontic patients were evaluated. Polymerase chain reaction was used for identification of the presence of DNA from three different periodontopathogens (*P. intermedia* [*Pi*], *A. actinomycetemcomitans* [*Aa*], and *P. gingivalis* [*Pg*]) in 16 mini-implants without mobility (control group) and 15 mini-implants with mobility (experimental group).

Results: The results showed that *Pi* was present in 100% of the samples, from both groups: *Aa* was found in 31.3% of the control group and in 13.3% of the experimental group. *Pg* was detected in 37.4% of the control group and in 33.3% of the experimental group. The Fisher exact test and the odds ratio (OR) values for *Aa* and *Pg* (OR = 0.34; 95% confidence interval [CI]: 0.05–2.10 and OR = 0.61; 95% CI: 0.13–2.79, respectively) showed no significant association ($P > .05$) between the periodontopathogens studied and the mobility of the mini-implants.

Conclusions: It can be concluded that the presence of *Aa*, *Pi*, and *Pg* around mini-implants is not associated with mobility. (*Angle Orthod.* 2012;82:591–595.)

KEY WORDS: Anchorage control; Microbiology; PCR analysis

INTRODUCTION

In the past decade, the clinical use of mini-implants as temporary anchoring devices has been widespread. This is due, among other factors, to the possibility of absolute orthodontic anchorage and the ease of installation and removal of these devices.[1] Mini-implants are routinely used to anchor retraction of the anterior segment, mesiodistal movement of the posterior teeth, asymmetrical tooth movement, intrusive mechanics, and orthopedic corrections.[2–4] Several studies have demonstrated the stability of mini-implants against orthodontic loads, which may vary between 50 and 250 gF during treatment.[5] However, after installation, some mini-implants show mobility before or during load application, which can lead to their removal or clinical failure as an absolute anchoring device.[6] The failure rate of mini-implants varies from 6.6% to 16.1%, which is higher than that of dental implants (3%) and other temporary anchoring devices, such as mini-plates (2.6 to 7.3%).[2,7–11] The mechanism that leads to mobility, and consequently to the clinical failure of mini-implants, is still unknown.[1,11,12] Some factors have been suggested as causes, such as incorrect positioning and proximity to a dental root.[10] However, Kau et al.[13] have verified, using tomography, the contact of mini-implants with the periodontal ligament in 65.2% of cases. Kim et al.[14] did not suggest that root proximity is an isolated causal factor for the loss of mini-implant stability.

[a] Assistant Professor, Department of Orthodontics, School of Dentistry, University of São Paulo, São Paulo, Brazil.
[b] Associate Professor, Department of Orthodontics, School of Dentistry, University of São Paulo, São Paulo, Brazil.
[c] PhD student, Department of Orthodontics, School of Dentistry, University of São Paulo, São Paulo, Brazil.
[d] Associate Professor, Department of Oral Pathology, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.
[e] Former graduate student, Department of Orthodontics, School of Dentistry, University of São Paulo, São Paulo, Brazil.
[f] Postdoctoral Fellow, Department of Orthodontics, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil.
Corresponding author: Dr Ana Haddad, Faculdade de Odontologia da Universidade de São Paulo, Departamento de Ortodontia, Av. Prof. Lineu Prestes, 2227 CEP: 05508-000, São Paulo, SP Brazil
(e-mail: anacssantos@usp.br)

Accepted: November 2011. Submitted: August 2011.
Published Online: January 17, 2012

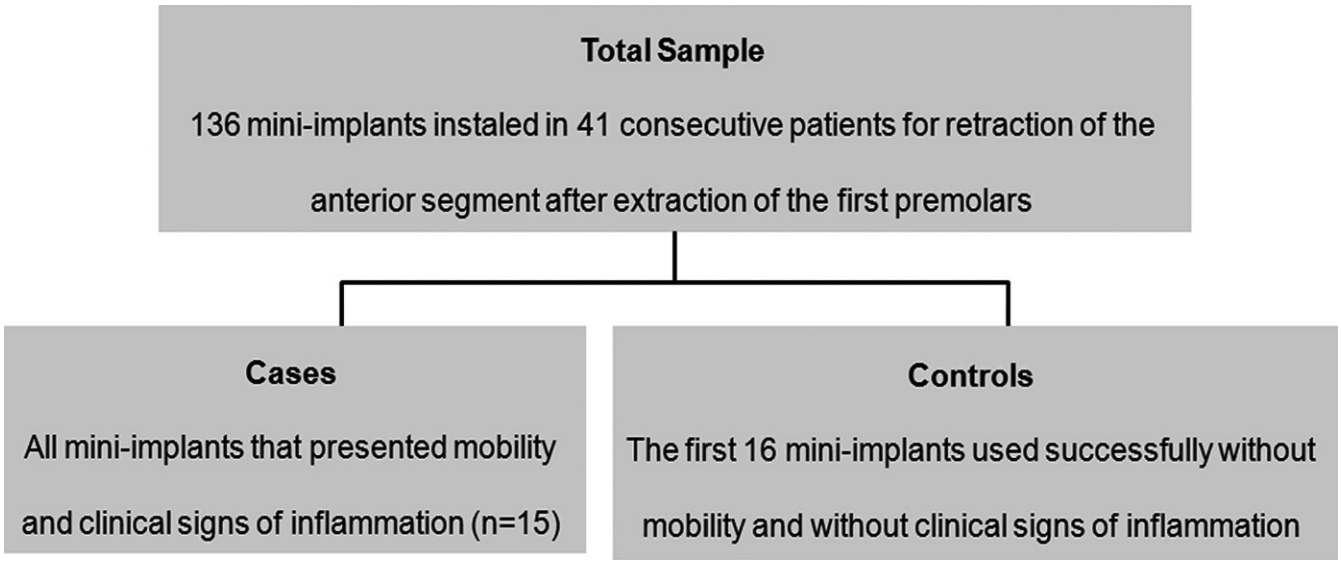

Figure 1. Flow diagram showing distribution of cases and controls.

Some authors, in retrospective studies, have identified co-factors that could increase the rate of success or failure of mini-implants.[7,8] Among the contributing factors for mini-implant failure is the colonization of the surfaces by pathogenic bacteria, which still needs to be investigated.[15] Apel et al.[16] conducted an analysis of the microflora collected around the head of orthodontic mini-implants with and without mobility and did not observe a cause-effect relationship between the observed bacteria and clinical failure of the implants.

However, several studies have observed that the microflora of peri-implantitis, which leads to implant mobility and loss, is similar to that found in periodontitis. This microflora is characterized predominantly by bacilli colonies, such as *P. intermedia* (*Pi*), *P. gingivalis* (*Pg*), and *A. actinomycetemcomitans* (*Aa*).[15,17–20] In vitro studies using scanning electron microscopy have also showed adhesion of these bacteria to titanium surfaces.[21,22] Therefore, this study verifies whether mini-implant mobility is affected by the presence of periodontopathogens, frequently associated with peri-implantitis.

MATERIALS AND METHODS

The sample consisted of 41 consecutive patients (11 men and 30 women) aged 16 to 40 years (average, 20.0 years). All subjects met the following inclusion criteria: (1) diagnosis of dentoalveolar biprotusion with convex facial profile, (2) treated with orthodontic braces (Victory-MBT, 3M Unitek, Monrovia, Calif) for retraction of the anterior segment after extraction of the first premolars, (3) nonsmoker, and (4) nondiabetic. Patients were followed monthly during the orthodontic treatment. This study was approved by the ethics committee of the School of Dentistry, University of São Paulo (project number 112/70). Patients signed an informed consent agreeing to participate in this research.

Mini-implants were installed by the same surgical dentist in all patients, in the superior arch and/or inferior to the region between the second premolars and first molars, using a surgical guide made of acrylic resin.[23] The mini-implants were placed near the mucogingival line. After insertion, patients were instructed to rinse, by swishing, with 0.12% chlorhexidine gluconate (PerioGard, Colgate-Palmolive, São Paulo, SP, Brazil) three times daily for a week and advised to avoid trauma to the mini-implant during tooth brushing. A total of 136 mini-implants were inserted. After a healing period of 3 weeks, a distal ligature attached to an elastic hook activated for arch retraction was attached to the mini-implant, in all cases by the same orthodontist so that the elastic was doubled in size. The mini-implants used were Tomas (Dentaurum, Inspringen, Germany), 8 mm in length and 1.6 mm in diameter.

In this prospective case-control study, 31 removed mini-implants were sent for analysis (Figure 1). All mini-implants that failed (n = 15) constituted the experimental group, whereas the first consecutive 16 mini-implants used successfully were removed and used as controls. The matching was carried out considering the success or failure of the mini-implants' integration to the tissues. The observed parameters were presence or absence of mobility and clinical presence or absence of inflammation (exudate, swelling, redness, pain):

- Cases: Mini-implants with mobility and with clinical signs of inflammation

Table 1. Polymerase Chain Reaction Primer Sequences[a]

Target Gene	Primers (5′–3′)	Tm, °C	Amplicon, bp
Aa	F: AAACCCATCTCTGAGTTCTTCTTC R: ATGCCAACTTGACGTTAAAT	56.9	556
Pg	F: AGGCAGCTTGCCATACTGCG R: ACTGTTAGCAACTACCGATGT	60	404
Pi	F: CCGCATACGTTGCGTGCACTAAG R: CGTGCCAGCAGCCGCGGTATTAGG	59	163

[a] Tm indicates melting temperature.

- Controls: Mini-implants without mobility and without clinical signs of inflammation

The mini-implants of the experimental group were removed because of mobility after periods that varied between 7 and 731 days. The control mini-implants (successfully used) were removed after use for skeletal anchorage without mobility, without inflammation of peri-implant tissues, and without pain after a period ranging from 169 to 1023 days.

Each mini-implant removed was placed in a separate microcentrifuge tube with 500 μL of buffer (1 M NaCl, 1 M Tris-HCl pH 8.0, 0.5 M EDTA pH 8.0, 10% SDS, H_2O up to 100 mL) and the samples stored at −20°C until DNA extraction. Polymerase chain reaction (PCR) analysis was performed at the Laboratory of Molecular Pathology, Department of Stomatology, School of Dentistry, University of São Paulo.

After centrifugation at 14,000 g for 10 min, DNA was extracted and purified using the ChargeSwitch Forensic DNA Purification kit (CS11200, Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions. After eluting DNA from the beads, supernatant containing the DNA was stored at −20°C until use.

Positive controls of the bacteria *Aa*, *Pi*, and *Pg* were supplied by the Anaerobic Laboratory in the Department of Microbiology at the Institute of Biomedical Sciences of the University of São Paulo. Each bacterial sample was mixed with 500 μL of sterile water and washed twice at 12,000 g for 10 minutes. Pellets were resuspended in 500 μL of sterile water and boiled for 10 minutes. After centrifugation (14,000 g, 10 minutes), the supernatant was transferred to a new microcentrifuge tube and used as a control.[24]

Primer sequences were obtained according to Ashimoto et al.[25] (Table 1), from Invitrogen. Primers were resuspended in TE buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA pH 8.0) and stored at −20°C. The Platinum Taq DNA polymerase kit (10966-030, Invitrogen) was used for PCR.

PCR amplification of the samples and positive controls was performed in a 25 μL volume, containing 2.5 μL 10× de Buffer PCR Solution (200 mM Tris-HCl, pH 8.4; 500 mM KCl), 0.5 μL 10 mM dNTPs (2.5 mM dATP, 2.5 mM dTTP, 2.5 mM dCTP, 2.5 mM dGTP; 10297-018, Invitrogen), 1.5 μL $MgCl_2$ (50 mM), 2 μL (10 μM) of each primer, 0.25 μL of Taq DNA polymerase 5 U/μL, 2.0 μL of DNA template, and Milli-Q water to 25 μL.

PCR amplification was performed in a DNA thermal cycler (PTC-100, MJ Research Inc, Watertown, Mass) with the following cycles and temperature parameters: (1) initiation at 94°C for 3 minutes, (2) denaturation at 94°C for 1 minutes 30 seconds, (3) melting temperature (Tm) as per Table 1, (4) extension at 72°C for 2 minutes, and (5) final extension at 72°C for 10 minutes. Cycles 2 to 4 were repeated 40 times.

Amplification products were verified by bromophenol blue (10× Blue Juice gel loading Buffer, 10826-015, Invitrogen; 65% w/v sucrose, 10 mM Tris-HCl pH 7.5, 10 mM EDTA, and 0.3% w/v bromophenol blue) by horizontal electrophoresis (90 V and 100 mA; Sub-Cell, Bio-Rad Laboratories Inc, Hercules, Calif) in a 2% agarose gel (15510-027, UltraPure Agarose, Invitrogen) stained with ethidium bromide 10 mg/mL (15585-011, Invitrogen) in 1× TAE (2M Tris-acetate pH 8.3; 50 mM EDTA; 24710-030, Invitrogen). Gels were photographed using an Olympus SP-500U2 (Olympus Imaging America Inc, Center Valley, Penn), in a UV transilluminator (model no. 3-3500, Foto/Prep, Fotodyne Incorporated, Hartland, Wis). Product size was compared using the Low DNA mass ladder (10068-013, Low DNA mass ladder, Invitrogen).

The association between the presence of studied periodontopathogens and the failure of mini-implants was determined using the Fisher exact test (*P* values, $\alpha < .05$), complemented by odds ratio (OR) values with 95% confidence intervals (CIs). Statistical analysis was performed using STATA software (Stata 9, StataCorp, College Station, Tex).

RESULTS

Pi DNA was amplified in all samples of both groups. *Pg* was detected in four of 15 samples of the experimental group (33.33%) and in six of 16 control samples (37.4%). *Aa* was present in two of the 15 experimental samples (13.33%) and five of the 16 control group samples (31.25%).

Table 2. Statistical Analysis of the Frequency of *Aa* in the Studied Groups[a]

Group	*Aa* (Expected Frequency) Presence	Absence	Total	*P* Value	OR (95% CI)
Experimental	2 (3.4)	13 (11.6)	15 (15.0)	.39	0.34
Control	5 (3.6)	11 (12.4)	16 (16.0)		(0.05–2.10)
Total	7 (7.0)	24 (24.0)	31 (31.0)		

[a] $\alpha = .05$, Fisher exact test. OR indicates odds ratio; CI, confidence interval.

Table 3. Statistical Analysis of the Frequency of *Pg* in the Studied Groups

Group	*Pg* (Expected Frequency) Presence	Absence	Total	*P* Value	OR (95% CI)
Experimental	4 (4.8)	11 (10.2)	15 (15.0)	.70	0.61
Control	6 (5.2)	10 (10.8)	16 (16.0)		(0.13–2.79)
Total	10 (10.0)	21 (21.0)	31 (31.0)		

[a] $\alpha = .05$, Fisher exact test. OR indicates odds ratio; CI, confidence interval.

Statistical analysis showed no association between the presence or absence of *Aa* ($P = .39$) and *Pg* ($P = .70$) and the success or failure of the mini-implants. The OR values with their respective 95% CI values also showed no association between the presence of *Aa* (OR = 0.34; 95% CI: 0.05–2.10) and *Pg* (OR = 0.61; 95% CI: 0.13–2.79) and the clinical performance of the mini-implants (Tables 2 and 3).

DISCUSSION

This prospective study evaluated the presence of bacteria around mini-implants removed after being used, successfully or not, as orthodontic anchorage. No association between mini-implant mobility and infection by the bacteria was found.

The sample size may be considered low for certain comparisons. However, it was higher than that of the previous study.[16] In addition, the total number of mini-implants that were placed was high (136), but fortunately, the failure rate was low (11%), and it has determined the sample size.

Regarding the nature of the failure, the only mobile mini-implant that we suspect to know the etiology of the failure was the case that failed after 731 days, which suffered a trauma. However, we believe it was relevant to include this case in the sample since the presence of bacteria could be added to the trauma. The remaining cases have unknown etiology, and this was the reason to perform this research. Besides that, this study points out that the loss of stability may be observed at any time during orthodontic treatment, and it seems to be associated with the lack of primary stability (trauma or root proximity).

Studies evaluating the microflora associated with mini-implants are scarce. Apel et al.[16] evaluated the presence of 20 different bacterial species present in the peri-implant sulcus surrounding eight mini-implants with mobility and four control mini-implants, whereas the present study aimed to detect a lower number of bacterial species present in the mini-screw region, which is in contact with bone tissue. Different findings were observed by Apel et al.[16] for the same bacteria studied here: *Aa* and *Pg* were not observed in any of the samples, and *Pi* was observed in one experimental case (12.5%) and in one control case (25%).

The difference in findings between the two studies may be that Apel et al.[16] used paper cones for sample collection, while in this study, the mini-implants were removed and immediately immersed in buffer. The collection method used here provides greater sensitivity since the paper cones may not have come into contact with all the bacteria present in the sulcus and is an additional step before DNA extraction. Their conclusions that the microflora present in mini-implants with mobility showed no specific aggressive characteristics coincide with the findings of this study. However, additional information was presented by Apel et al.[16] regarding the absence of *A. viscosus* and *C. gracilis* in seven of the eight failed cases, while being almost always present in the controls was remarkable because as both species are more prominent markers for periodontal health than disease, their absence in the sulcus surrounding failed screws could be interpreted as a first symptom of a changing microflora.

This study presents a great advantage: the selection of 41 individuals with similar initial conditions, mini-implant installation by the same surgeon in the same location (mucogingival line between the second premolars and first molars with the use of a guide[23]), and activation of the distal ligatures by the same orthodontist in all cases. These procedures minimize differences and possible confounding factors.

PCR is the method of choice for detecting DNA of micro-organisms, since oral bacteria, including *Aa*, *Pi*, and *Pg*, are difficult to cultivate.[16] However, PCR is unable to detect whether the bacteria were active or inactive at the time of mini-implant removal. PCR, therefore, cannot determine whether the patient's immune system was capable of controlling bacterial growth, keeping bacterial levels static, or if the bacteria were still active at the time of mini-implant removal. Another limitation of this study refers to the numbers of bacterial species studied. This study focused on the analysis of bacterial microfilms, often associated with peri-implantitis, but the role of the presence of other bacterial species around mini-implants as protectors or perpetrators remains to be investigated.

The results presented here showed that the presence of *Aa*, *Pg*, and *Pi* was not the primary causal factor for the mobility of the mini-implants. Bacterial infection responsible for peri-implantitis begins in soft tissues due to poor oral hygiene and slowly extends over the implants, causing mobility and consequently clinical failure.[26] Since progression of peri-implantitis as well as chronic periodontitis is usually slow and often takes several years, peri-implant inflammation may not be so important from a practical point of view in determining the clinical effectiveness of a temporary anchorage device, considering the short function time of the mini-implants. Since the lack of primary stability can be responsible for early failures also for implants, this seems to be more implicated to mini-implant mobility than bacterial colonization.

CONCLUSIONS

- Statistical analysis showed no association between the presence of the studied periodontopathogens (*Pi*, *Pg*, or *Aa*) and mini-implant mobility. The presence of these particular bacteria was not related to the mobility that caused failure of the mini-implants.
- Peri-implant bacterial colonization may not be so important in determining the clinical effectiveness of a temporary anchorage device.

ACKNOWLEDGMENTS

The authors would like to thank Katiúcia Batista Silva Paiva for PCR analysis support and Toni Ricardo Eugênio dos Santos for statistical analysis.

REFERENCES

1. Reynders R, Ronchi L, Bipat S. Mini-implants in orthodontics: a systematic review of the literature. *Am J Orthod Dentofacial Orthop*. 2009;135:564.e1–564.e19.
2. Park HS, Lee SK, Kwon OK. Group distal movement of teeth using miniscrew implant anchorage. *Angle Orthod*. 2005;75: 602–609.
3. Prabhu J, Cousley RRJ. Current products and practice: bone anchorage devices in orthodontics. *J Orthod*. 2006;33: 288–307.
4. Leung MT, Lee TCL, Rabie ABM, Wong RW. Use of miniscrews and miniplates in orthodontics. *J Oral Maxillofac Surg*. 2008;66:1461–1466.
5. Liou EJ, Pai BCJ, Lin JCY. Do miniscrews remain stationary under orthodontic forces? *Am J Orthod Dentofacial Orthop*. 2004;126:42–47.
6. Luzi C, Verna C, Melsen B. Immediate loading of orthodontic mini-implants: a histomorphometric evaluation of tissue reaction. *Eur J Orthod*. 2009;31:21–29.
7. Antoszewska J, Papadopoulos MA, Park HS, Ludwig B. Five-year experience with orthodontic miniscrew implants: a retrospective investigation of factors influencing success rates. *Am J Orthod Dentofacial Orthop*. 2009;136:158.e1–158.e10.
8. Chen YJ, Chang HH, Lin HY, Lai EH, Hung HC, Yao CC. Stability of miniplates and miniscrews used for orthodontic anchorage: experience with 492 temporary anchorage devices. *Clin Oral Implants Res*. 2008;19:1188–1196.
9. Miyawaki S, Koyama I, Inoue M, Mishima K, Sugahara T, Takano-Yamamoto T. Factors associated with the stability of titanium screws placed in the posterior region for orthodontic anchorage. *Am J Orthod Dentofacial Orthop*. 2003;124:373–378.
10. Morea C, Domínguez GC, Tortamano A, Vigorito JW. Frequency and cause of failure of miniscrews for orthodontic absolute anchorage. *Eur J Orthod*. 2007;29:e27–e28.
11. Schätzle M, Roland M, Zwahlen M, Lang N. Survival and failure rates of orthodontic temporary anchorage devices: a systematic review. *Clin Oral Implants Res*. 2009;20: 1351–1359.
12. Feldmann I, Bondemark L. Orthodontic anchorage: a systematic review. *Angle Orthod*. 2006;76:493–501.
13. Kau CH, English JD, Muller-Delgardo MG, Hamid H, Ellis RK, Winklemann S. Retrospective cone-beam computed tomography evaluation of temporary anchorage devices. *Am J Orthod Dentofacial Orthop*. 2010;137:166.e1–166e5.
14. Kim SH, Kang SM, Choi YS, Kook YA, Chung KR, Huang JC. Cone-beam computed tomography evaluation of mini-implants after placement: is root proximity a major risk factor for failure? *Am J Orthod Dentofac Orthop*. 2010;138: 264–276.
15. Pye AD, Lockhart DE, Dawson MP, Murray CA, Smith AJ. A review of dental implants and infection. *J Hosp Infect*. 2009; 72:104–110.
16. Apel S, Apel C, Morea C, Tortamano A, Dominguez GC, Conrads G. Microflora associated with successful and failed orthodontic mini-implants. *Clin Oral Implants Res*. 2009;20: 1186–1190.
17. Becker W, Becker BE, Newman MG, Nyman S. Clinical and microbiologic findings that may contribute to dental implant failure. *Int J Oral Maxillofac Implants*. 1990;5:31–38.
18. Mombelli A, Van Oosten MAC, Schürch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol*. 1987;2: 145–151.
19. Rams TE, Link CC Jr. Microbiology of failing dental implants in humans: electron microscopic observations. *J Oral Implantol*. 1983;11:93–101.
20. Rosenberg ES, Torosian JP, Slots J. Microbial differences in 2 clinically distinct types of failure of osseointegrated implants. *Clin Oral Implants Res*. 1991;2:135–144.
21. Kuula H, Könönen E, Lounatmaa K, Konttinen YT, Könönen M. Attachment of oral gram-negative anaerobic rods to a smooth titanium surface: an electron microscopy study. *Int J Oral Maxillofac Implants*. 2004;19:803–806.
22. Otke E, Sultan N, Doğan B, Asikainen S. Bacterial adhesion of *Actinobacillus actinomycetemcomitans* serotypes to titanium implants: SEM evaluation. A preliminary report. *J Periodontol*. 1999;70:1376–1382.
23. Morea C, Domínguez GD, Wuo AV, Tortamano A. Surgical guide for optimal positioning of mini-implants. *J Clin Orthod*. 2005;39:317–321.
24. Ávila-Campos MJ. PCR detection of four periodontopathogens from subgingival clinical samples. *Braz J Microbiol*. 2003;34:81–84.
25. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol*. 1996;11:266–273.
26. Lindhe J, Meyle J. Peri-implant diseases: consensus report of the sixth European workshop on Periodontology. *J Clin Periodontol*. 2008;35:282–285.