Analysis of microbiota associated with dental implant overdenture attachment

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Abstract

This study was to investigate the distribution of pathogenic microorganisms around the attachments and implants of implant overdentures in edentulous patients and to also analyze the correlation between overdenture-related bacteria and clinical indices. Periodontal pathogenic bacteria were detected in most patients, and amounts of bacteria as much as those found in the peri-implant sulcus and around intra-oral attachments were also detected in the attachments of denture bases. These results imply that the increase in the number of periodontal pathogenic bacteria is effected by the periodontal pocket probing depths and the modified sulcular bleeding indices in patients using implant overdentures.

Background and Aim

Implant overdenture plays an important role in treatment option for edentulous patients. Failures on implant restoration by bacteria induced inflammation were frequently reported. Therefore, oral pathogens related with peri-implantitis are actively studied. However, these results are limited to peri-implantitis of fixed restorations. The aim of this study is to investigate and compare the distribution of microbiota around attachment of implant overdenture and implant, and support the information for the treatment protocol for patients who need implant overdenture.

Methods and Materials

The study was performed targeting the patients with implant overdenture. Samples were collected from the oral surface of the abutment (Abutment), the bottom of implant sulcus with the greatest probing depth (Sulcus), attachment housing in denture (Denture) with microbrush. Each patient was evaluated by measuring probing depth, modified sulcus bleeding index. The bacterial DNA was extracted from samples by bacterial DNA extraction kit. Quantification of *Filifactor alocis*, *Fusobacterium nucleatum*, *Streptococcus sanguinis*, *Prevotella intermedia*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* in samples was analyzed by real-time polymerase chain reaction using the extracted DNA. The relationships between quantification of the bacteria and sampling area were investigated with Friedman test. Correlations between quantification of the bacteria and probing depth were analyzed with Spearman rank correlation coefficient. The relationships between DNA quantification of bacteria and modified probing index were investigated with Jonckheere-Terpstra test.

Table I. The primer sequences for real-time PCR.

Name		Primer sequence
Universal	Forward	5'-TGG AGC ATG TGG TTT AAT TCG A-3'
	Reverse	5'-TGC GGG ACT TAA CCC AAC A-3'
<i>F. alocis</i>	Forward	5'-CAA TGA CAG CCT TTT AAT CGA GG-3'
	Reverse	5'-CCT CTA AGT TGT CCT TAG CTG TCT CG-3'
<i>F. nucleatum</i>	Forward	5'-CGC AGA AGG TGA AAG TCC TGT AT-3'
	Reverse	5'-TGG TCC TCA CTG ATT CAC ACA GA-3'
<i>P. endodontalis</i>	Forward	5'-AGC TGC AGC TCA ACT GTA GTC TTG-3'
	Reverse	5'-TCA GTG TCA GAC GGA GCC TGG TAC-3'
<i>P. gingivalis</i>	Forward	5'-TGC AAC TTG CCT TAC AGA GGG-3'
	Reverse	5'-TAC TCG TAT CGC CCG TTA TTC C-3'
<i>P. intermedia</i>	Forward	5'-CGT ATC CAA CCT TCC CTC CAC TC-3'
	Reverse	5'-TCC ACC GAT GAA TCT TTG GTC C-3'
<i>S. sanguinis</i>	Forward	5'-TGG CTC AGG GCA GCC AGT T-3'
	Reverse	5'-GAA CAG TTG CTG GAC TTG CTT GT-3'
<i>T. denticola</i>	Forward	5'-GATAATACC GAATGTGCTCAT TTACAT A-3'
	Reverse	5'-TCA AAG AAG CAT TCC CTC TTC TTC TTA-3'
<i>T. forsythia</i>	Forward	5'-CGA AAG TCG GAC TAA TAC CGC AT-3'
	Reverse	5'-GCC GTT ACC TCA CCA ACT ACC TA-3'

Figure 1. A standard curve was produced using the already-known concentration figure of DNA per number of bacteria and the threshold value.

Results

Table II. The primer sequences for real-time PCR.

Bacteria	Patient count (%)			
	Abutment	Sulcus	Denture	Total
<i>F. alocis</i>	20 (100)	20 (100)	20 (100)	60 (100)
<i>F. nucleatum</i>	20 (100)	20 (100)	20 (100)	60 (100)
<i>P. endodontalis</i>	12 (60)	13 (65)	12 (60)	37 (62)
<i>P. gingivalis</i>	4 (20)	5 (25)	7 (35)	16 (27)
<i>P. intermedia</i>	14 (70)	14 (70)	14 (70)	42 (70)
<i>S. sanguinis</i>	19 (95)	19 (95)	19 (95)	57 (95)
<i>T. denticola</i>	4 (20)	8 (40)	7 (35)	19 (32)
<i>T. forsythia</i>	14 (70)	14 (70)	15 (75)	43 (72)

*Threshold 10³, n=20

Table III. Correlation between bacterial count and probing depth.

Bacteria	Abutment	Sulcus	Denture
<i>F. alocis</i>	0.004*	0.003*	0.013*
<i>F. nucleatum</i>	0.044*	0.048*	0.016*
<i>P. endodontalis</i>	0.001*	0.0001*	0.0002*
<i>P. gingivalis</i>	0.209	0.244	0.469
<i>P. intermedia</i>	0.082	0.046*	0.14
<i>S. sanguinis</i>	0.848	0.521	0.625
<i>T. denticola</i>	0.041*	0.007*	0.02*
<i>T. forsythia</i>	0.031*	0.007*	0.237

(Spearman's rank correlation coefficient test, *p<0.05)

Table IV. Correlation between bacterial count and modified bleeding index.

Bacteria	Abutment	Sulcus	Denture
<i>F. alocis</i>	0.109	0.093	0.023*
<i>F. nucleatum</i>	0.391	0.351	0.015*
<i>P. endodontalis</i>	0.126	0.048*	0.057
<i>P. gingivalis</i>	0.351	0.093	0.167
<i>P. intermedia</i>	0.145	0.314	0.168
<i>S. sanguinis</i>	0.794	0.911	0.911
<i>T. denticola</i>	0.328	0.106	0.232
<i>T. forsythia</i>	0.576	0.434	0.97

(Jonckheere-Terpstra test, *p<0.05)

S. sanguinis, *F. alocis*, *F. nucleatum* *T. forsythia* were detected in most of subject. The detection ratio of *F. alocis*, *F. nucleatum* were 100%. When comparing oral surface of the attachment, the bottom of implant sulcus with the greatest probing depth and attachment housing in denture, no relationships observed between sampling site and the bacterial quantification.

A significant positive correlation between probing depth and the bacterial quantification of *F. alocis*, *F. nucleatum*, *P. endodontalis*, *T. forsythia*, *T. denticola* showed. Also a significant positive correlation between modified bleeding index and bacterial DNA quantification of *F. alocis* in Denture group, *F. nucleatum* in Denture group, and *P. endodontalis* in Implant group exhibited. These results indicate clinical indices related with periodontopathogens.

Conclusions

Quantification of main periodontopathogens as red complex bacteria had increased according to increasing clinical indices. These results indicates oral environment of patient with implant overdenture and attachment housing in denture has possibility of colonization of periodontopathogens. Therefore, it is need to know that implant overdenture may have biological complications similar to peri-implantitis and provide preventive treatment and hygiene education to overdenture patients.

References

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