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A three-year study on periodontal microorganisms of short locking-taper implants and adjacent teeth in patients with history of periodontitis

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ABSTRACT

Objective: To analyze the change of six periodontal pathogens around short locking-taper implants and adjacent teeth in patients with different periodontal conditions for three years.
Methods: Sixty implants and 62 adjacent teeth from 24 patients with different periodontal conditions were included: 5 patients with history of aggressive periodontitis (AgP group), 14 patients with history of chronic periodontitis (CP group), and 5 patients with healthy condition or slight gingivitis (H group). Subgingival samples were collected at five timepoints: before implant placement (T1); before second stage operation (T2); one month after restoration (T3); one year after functional loading (T4) and two years after functional loading (T5). *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*, *Fusobacterium nucleatum, Prevotella intermedia,* and *Aggregatibacter actinomycetemcomitans* were detected by polymerase chain reaction (PCR).
Results: Pathogens were hardly found around implants or adjacent teeth until T4. The detection rates of five pathogens other than *A. actinomycetemcomitans* raised up from T3 to T5. *F. nucleatum* and *P. gingivalis* were mostly detected followed by *P. intermedia*, *T. forsythia*, and *T. denticola*. The detection rate of *P. gingivalis* in implants were higher than natural teeth. There was significant correlation between pathogenic bacteria from implants and adjacent teeth. *A. actinomycetemcomitans* were only detected positively in peri-implant sites of AgP group. Peri-implantitis sites showed significantly higher detection rates of *T. denticola*, *F. nucleatum* at T4, and *P. gingivalis*, *F. nucleatum* at T5 than peri-implant mucositis and healthy groups.
Conclusion: This three-year longitudinal study demonstrated that periodontal pathogens accumulate over time around short locking-taper implants and adjacent natural teeth after restoration. Adjacent teeth may become the microbial reservoir for peri-implant bacteria. Therefore, periodontally compromised patients may face higher risk for peri-implant disease.
Clinical significance: Plaque control of implant should be intensified with time instead of diminished. Patients with history of periodontitis need more frequent and individualized implant maintenance. Treatment and maintenance for adjacent teeth is as important as for implants..

1. Introduction

Nowadays, implant restoration has gradually become a common choice for lost teeth caused by periodontitis. But periodontally compromised patients often encounter two common problems when receiving implant therapy. One is severe bone defect or atrophy caused by periodontal lesion. The other is the potential risks for peri-implant diseases [1–4].

Except for bone augmentation such as sinus lift or guided bone regeneration, short implant (implant length ≤ 8 mm) has become an alternative choice in sites with bone defect, and it demonstrated similar survival rate as standard length implants [5–9]. Besides, the locking-

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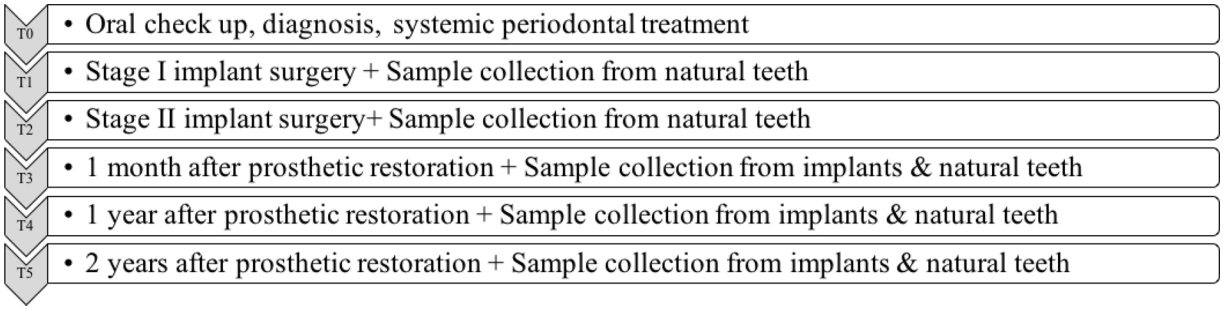

Fig. 1. Flowchart of sample collection.

taper platform shifting design of specific systems were verified to be hermetic to microbial leakage in vitro [10,11]. However, the long-term risk and reliability of short implant and locking-taper platform shifting design for periodontally compromised patients still need further verification.

On the other hand, peri-implant diseases include two clinical varieties: peri-implant mucositis and peri-implantitis. Although they share common pathological condition occurring in tissues around dental implants, the latter one is characterized by progressive loss of supporting bone [12,13]. It is anticipated that peri-implant mucositis is reversible under appropriate treatment but is also able to convert into peri-implantitis [14]. According to the overall analysis of the literatures concerning about the etiology and pathogenesis of periodontitis and peri-implantitis, an impression that these two diseases have more similarities than differences could be drawn. In addition, periodontally compromised patients appear to be more susceptible to peri-implantitis than patients without history of periodontitis [15].

Previous studies demonstrated that periodontal pathogens have been found in implants of periodontally compromised patients and may play a part in the progress of peri-implant disease [4,15–17]. However, few studies focused on longitudinal microbiology on implants and proximal teeth in Chinese population over a long term. Therefore, the aims of the present study were to explore the long-term microbial change of periodontal pathogens around short locking-taper implants in patients with history of periodontitis at different stages, and to identify the association between bacteria from implants and their adjacent teeth, and to compare the difference of microbial colonization in different periodontal conditions.

2. Materials and methods

This study was conducted under permission of the Ethical Committee (approval no. IRB00001052-10047).

2.1. Sample size calculation

According to the microbiological change at peri-implant sites in pretest, *P. gingivalis* was the primary outcome and effect size was 40 %. Before-after study (paired nonparametric test) was performed with significant level $\alpha = 0.05$ and $\beta = 0.2$, and the minimum size was calculated as 50 using software PASS version 11 (NCSS, USA). Considering a 20% rate of possible loss to follow-up, sample size was set as 60.

2.2. Patients and sampling

24 patients (8 men and 16 women) who received short locking-taper dental implants (Integra-CP, hydroxylapatite surface) from September 2011 to January 2013 were enrolled in this study (age range: 35–62 years; mean age: 47 years). All patients with history of periodontitis had adjacent, occluding, and contralateral teeth to the implant sites. The patients with pregnancy, systemic diseases or smoking history were excluded.

According to initial diagnosis before implant treatment, 5 patients (30 implants / 25 adjacent natural teeth) were categorized as aggressive periodontitis group (AgP Group) while 14 patients (24 implants / 28 adjacent natural teeth) were included into chronic periodontitis group (CP group). The rest 5 patients (6 implants / 9 adjacent natural teeth) were either healthy or gingivitis patients (H group). They all had received systemic periodontal therapy before implant treatment.

After the informed consent was signed by all participants, subgingival plaque samples were collected with Whatman #3 sterile filter papers (Whatman®, UK) trimmed into 2 mm × 10 mm tightly inserted into cervical sulci for 30 s at the mesial buccal site and distal buccal site of the implants and the adjacent teeth. They were collected at 5-time-points: before implant placement (T1); before second stage operation (T2); 1 month after restoration (T3); 1 year after loading (T4) and 2 years after loading (T5). The samples from the implants were collected one month (T3), one year (T4) and two years (T5) after implant prosthetic restoration. The subgingival samples from the adjacent natural teeth were obtained at the time of implant placement (T1) and second-stage surgery (T2) as well as one month (T3), one year (T4) and two years (T5) after implant restoration. The flowchart of sample collecting process is displayed in Fig. 1. The collected samples were suspended in TE buffer (pH 8.0) consisted of 10 mM Tris-HCl (Wako Pure Chemical Industries, Osaka, Japan) and 1 mM EDTA (Wako) to harvest the microorganisms by centrifugation at 13,000 rpm under 4 ˚C for five minutes. The pellets were then stored at −80 ˚C for subsequent detection of periodontal bacteria. There were totally 244 sites added up to 980 samples from five collecting time-points.

Detection of periodontopathic bacteria by the Polymerase Chain Reaction (PCR) using specific primers designed from 16 s rRNA sequences [18]. Genomic DNA of collected samples were isolated with TIANamp Micro DNA Kit (TianGen Biotech, Beijing, China). Detection of *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*, and *Fusobacterium nucleatum* was performed by PCR in a thermal cycler (Gene Amp PCR system 2700, Foster City, CA, USA) using the same primer pairs with Ashimoto's study on detecting periodontal pathogens by PCR [18]. The PCR products were electrophoresed using 2% agarose gel and then examined under 300 nm ultraviolet light (Bio-Rad, USA) after staining with Goldview DNA stain (Takara® Biotechnology, Dalian, PR China). The detail of primers used in this study is displayed in Table 1.

2.3. Statistical analysis

The proportion of the positive sites of the periodontal bacteria was calculated as detection rate. The longitudinal differences of bacterial change was analyzed by McNemar's test. The difference detection between implants with natural teeth was analyzed by McNemar's test. The difference between different periodontal conditions and peri-implant conditions were analyzed by Fisher exact test of Chi-square test. Spearman Correlation Analysis was applied to evaluate the correlation

Table 1
Primers used in PCR analysis.

	Primer sequence (5′–3′)	Base position (length)	References
Porphyromonas gingivalis			
Forward	AGG CAG CTT GCC ATA CTG CG	729-1,132 (404)	Ashimoto et al.
Reverse	ACT GTT AGC AAC TAC CGA TGT		
Tannerella forsythia			
Forward	GCG TAT GTA ACC TGC CCG CA	120-760 (641)	Ashimoto et al.
Reverse	TGC TTC AGT GTC AGT TAT ACC T		
Treponema denticola			
Forward	TAA TAC CGA ATG TGC TCA TTT ACA T	193-508 (316)	Ashimoto et al.
Reverse	TCA AAG AAG CAT TCC CTC TTC TTC TTA		
Fusobacterium nucleatum			
Forward	AGG GCA TCC TAG AAT TAT G	190-1,006(817)	Baumgartner et al.
Reverse	GGG ACA CTG AAA CAT CTC TGT CTC A		
Prevotella intermedia			
Forward	TTT GTT GGG GAG TAA AGG GGG	458-1, 032 (575)	Ashimoto et al.
Reverse	TCA ACA TCT CTG TAT CCT GCG T		
Aggregatibacter actinomycetemcomitans			
Forward	AAA CCC ATC TCT GAG TTC TTC TTC	478-1, 034 (557)	Ashimoto et al.
Reverse	ATG CCA ACT TGA CGT TAA AT		

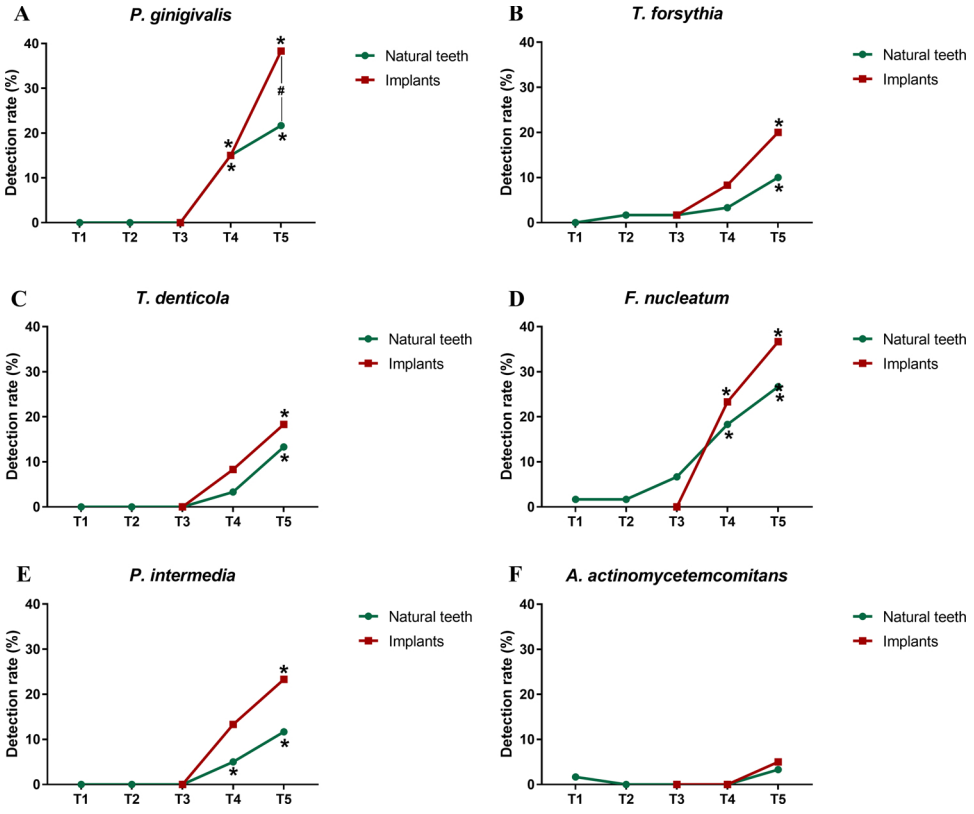

Fig. 2. The longitudinal change of bacteria in implants and adjacent natural teeth. (A) *P. gingivalis*; (B) *T. forsythia*; (C) *T. denticola*; (D) *F. nucleatum*; (E) *P. intermedia*; (F) *A. actinomycetemcomitans*. Analyzed by McNemar's test. * $p < 0.05$, statistically different from baseline (natural teeth, T1; implants, T3). # $p < 0.05$, statistical difference between implants and natural teeth. T1, before implant placement; T2, before second stage operation; T3, one month after functional loading; T4, one year after functional loading; T5, two years after functional loading.

of subgingival plaque between implants and natural teeth. SPSS 23.0 (IBM, NY, USA) was used to for statistical analysis.

3. Results

3.1. Basic information

This research included 24 patients (16 females, 8 males) at an average age of 47 with 60 locking taper implants and 62 adjacent teeth. Although the survival rate for implants was 100 %, 4 implants of 3 patients from CP group were diagnosed as peri-implantitis while 24 implants of 8 patients from CP or AgP groups had peri-implant mucositis after two years. The peri-implant disease diagnosis was in accordance with the standard made by Mombelli and Lang [19]. All implants from H group were healthy. No mechanical failure was observed.

3.2. Longitudinal change of bacteria in implant sites and adjacent periodontal sites

There were only few sporadic sites with positive detection of pathogens in implants in T3 and in natural teeth from T1 to T3. But the detection rates of these pathogens in implants and natural teeth continuously raised from T3 to T5 (Fig. 2). Detection rates of *P. gingivalis* significantly increased from 0% to 38.33 % in peri-implant sites and from 0% to 21.67 % in periodontal sites ($p < 0.05$, Fig. 2A). It's worthy of note that the detection rate of *P. gingivalis* of implants was significantly higher than that of natural teeth ($p < 0.05$, Fig. 2A).

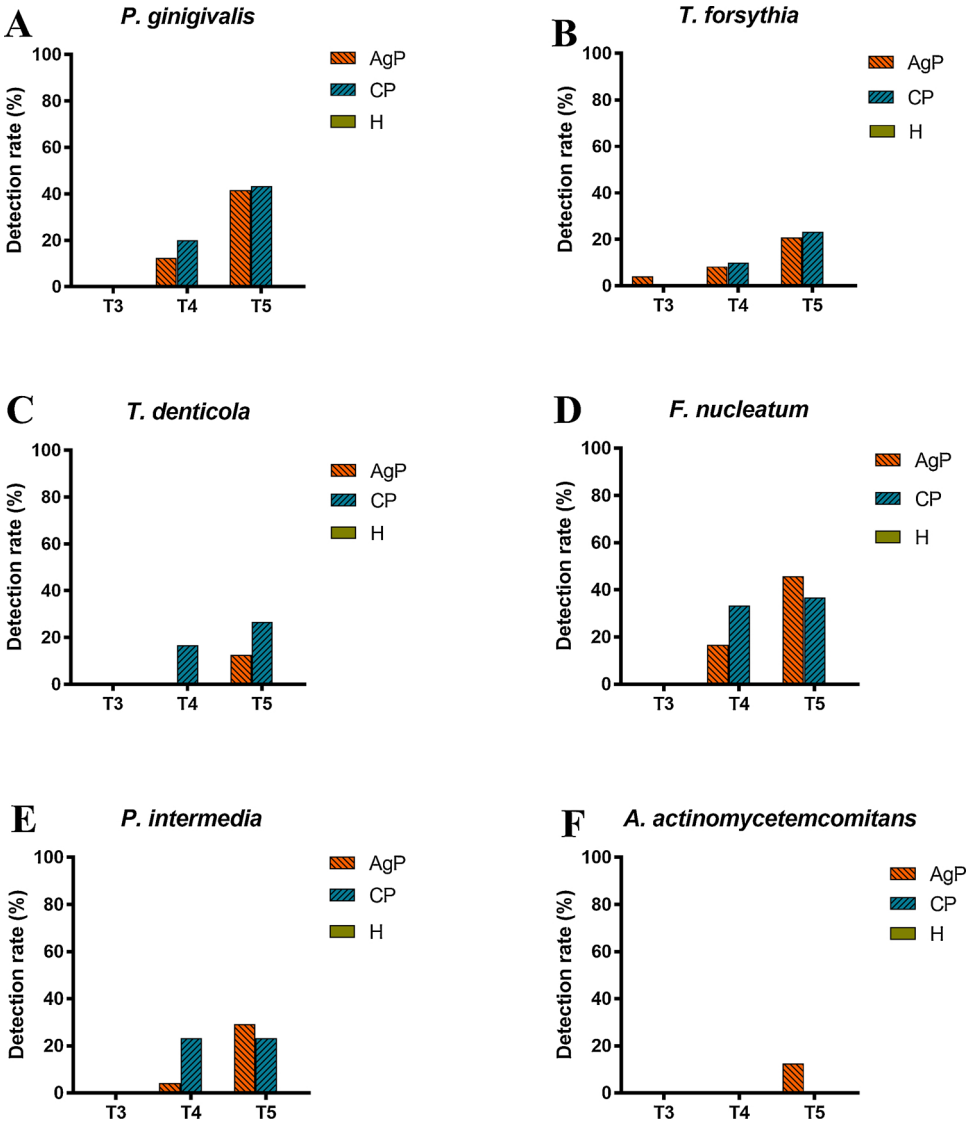

Fig. 3. Comparison of bacteria in peri-implant sites among different periodontal diagnostic groups. (A) *P. gingivalis*; (B) *T. forsythia*; (C) *T. denticola*; (D) *F. nucleatum*; (E) *P. intermedia*; (F) *A. actinomycetemcomitans*. Analyzed by Fisher's exact test of Chi-square test, there was no statistically significant difference of bacterial detection among three diagnostic groups from T3 to T5. T3, one month after functional loading. T4, one year after functional loading. T5, two years after functional loading. AgP, aggressive periodontitis group. CP, chronic periodontitis group. H, healthy group.

Detection rates of *T. forsythia* significantly increased from 0% to 20.00 % in peri-implant sites and from 0% to 10.00 % in periodontal sites ($p < 0.05$, Fig. 2B). Detection rates of *T. denticola* significantly increased from 0% to 18.33 % in peri-implant sites and from 0% to 13.33 % in periodontal sites ($p < 0.05$, Fig. 2C). Detection rates of *F. nucleatum* significantly increased from 0% to 36.67 % in peri-implant sites and from 1.67 % to 26.67 % in periodontal sites ($p < 0.05$, Fig. 2D). Detection rates of *P. intermedia* significantly increased from 0% to 23.33 % in peri-implant sites and from 0% to 11.67 % in periodontal sites ($p < 0.05$, Fig. 2E). Detection rates of *A. actinomycetemcomitans* significantly increased from 0% to 5.00 % in peri-implant sites and from 1.67 % to 3.33 % in periodontal sites ($p < 0.05$, Fig. 2F). Although the detection rates of *T. forsythia* and *P. intermedia* in implants were almost two folds of that in natural teeth at T5, there were no statistical differences (*T. forsythia*, $p = 0.07$; *P. intermedia*, $p = 0.14$).

3.3. Peri-implant bacteria among different periodontal diagnostic groups

The detection rates of six bacteria increased stepwise from T3 to T4 to T5 for patients with AgP or CP (Fig. 3). It was strikingly found that the detection rates of six periodonto-pathogens in periodontally healthy subjects was 0% (Fig. 3). The detection rates of *P. gingivalis* and *T. forsythia* was nearly equivalent in AgP group and CP group (Fig. 3A–B). *T. denticola* at T4 and T5, *F. nucleatum* and *P. intermedia* at T4 were more prevalent in CP group than AgP group (not less than two folds), although no statistical differences were found (Fig. 3C–E). *A. actinomycetemcomitans* were only detected in peri-implant sites of AgP group (Fig. 3F).

3.4. Peri-implant bacteria among sites with different peri-implant conditions

According to peri-implant condition diagnosed at T5, samples were divided into three groups: peri-implantitis, peri-implant mucositis, and peri-implant health. There was a remarkable result that no bacteria were detected in peri-implant healthy sites (Fig. 4). Five pathogens consisting of *P. gingivalis*, *T. forsythia*, *T. denticola*, *F. nucleatum*, and *P. intermedia*, were more prevalent in sites with peri-implantitis than sites with peri-implant mucositis (Fig. 4A–E). The detection rate of *P. gingivalis* was 75 % in sites with peri-implantitis which was significantly higher than 40 % in peri-implant mucositis at T5 ($p < 0.05$, Fig. 4A). At

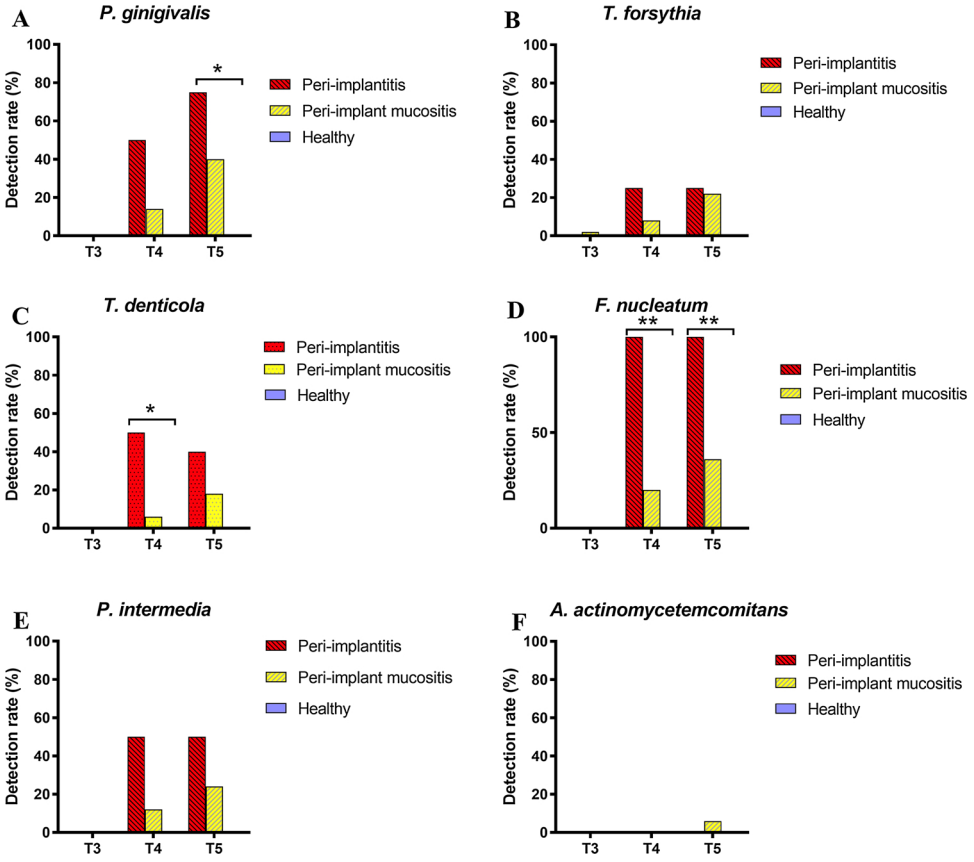

Fig. 4. Comparison of bacteria in peri-implant sites among different peri-implant conditions. (A) *P. gingivalis*; (B) *T. forsythia*; (C) *T. denticola*; (D) *F. nucleatum*; (E) *P. intermedia*; (F) *A. actinomycetemcomitans*. Analyzed by Fisher's exact test of Chi-square test. * $p < 0.05$. ** $p < 0.01$. T3, one month after functional loading. T4, one year after functional loading. T5, two years after functional loading.

T4, the detection rate *T. forsythia* in peri-implantitis group was more than 3 folds of that in peri-implant mucositis group (Fig. 4B). The detection rate *T. denticola* in peri-implantitis group was 8.33 folds at T4 ($p < 0.05$) and 2.22 folds at T5 of that in peri-implant mucositis group (Fig. 4C). It was striking that *F. nucleatum* harbored a 100 % detection in peri-implantitis group at T4 and T5. The detection rate *F. nucleatum* were significantly higher in peri-implantitis group than peri-implant mucositis group and health group ($p < 0.05$, Fig. 4D). The detection rate of *P. intermedia* in peri-implantitis group was 50 % at T4 and T5, which was 4.17 folds and 2.08 folds of that in peri-implant mucositis group, respectively (Fig. 4E).

3.5. The differences of peri-implant bacteria associated with gender

All bacteria, except *T. forsythia* at T3 and T4, were more prevalent in males than that in females, although no significant differences were found (Fig. 5). The detection rates of *T. forsythia* at T5 and *P. gingivalis*, *T. denticola*, *F. nucleatum* at T4, T5 in males were almost two folds of that in females. The detection rates of *P. intermedia* and *A. actinomycetemcomitans* in males were also slightly higher than that in females.

3.6. Correlation of peri-pathogens between implants and adjacent teeth

Spearman rank correlation analysis revealed significant correlation of bacteria detected between implants and natural teeth (Table 2). *P. gingivalis* showed a moderate correlation between implants and natural teeth at both T4 and T5 ($p < 0.05$). *T. forsythia* showed a weak correlation at T4 with r = 0.28, and a strong correlation at T5 with r = 0.53 ($p < 0.05$). *T. denticola* showed strong correlation between implants and natural teeth with r = 0.62 at T4 and r = 0.57 at T5 ($p < 0.05$). *F. nucleatum* harbored a strong correlation at T4 with r = 0.55, however, weak correlation at T5 with r = 0.29 ($p < 0.05$). *P. intermedia* at T4 and *A. actinomycetemcomitans* at T5 showed moderate correlation between peri-implant sites and periodontal sites with r = 0.36 and 0.38, respectively. Six bacteria at T3 and *A. actinomycetemcomitans* at T4 failed to calculate correlation due to low detection rate.

4. Discussion

Our study is the first longitudinal microbiological study on implants and proximal teeth in Chinese population of three years. The periodontal pathogenic bacteria were not extensively detected either in implants or adjacent teeth until one year after functional loading. One of the possible reasons leading to low microbiological detection from T1 to T3 could be systemic periodontal therapy before implant placement and restoration. It was routine to prescribe amoxycillin (500 mg/per time, Tid, p.o.) and to rinse chlorhexidine (10 ml/per time, Bid) for two weeks after implant placement which may contribute to reduction of these pathogens. Oral hygiene and periodontal non-surgical therapy were so emphasised that all dental plaque, calculus, and tartar were removed before surgery and restoration through hand-by-hand oral hygiene instruction under plaque staining and necessary professional scaling. The benign effect of non-surgical periodontal treatment to reduce pathogens of natural teeth and implant had already been proved by previous researches from our group and other teams [20–23]. It was such effort on periodontal health by multiple methods from mechanical cleaning to medication that intensively control the bacteria in the lowest level from T1 to T3.

Whereas, an apparent rising trend of periodontal pathogens appeared one year after functional loading (T4 & T5) in patients with history of periodontitis. Besides, all the periodontal bacteria other than *A. actinomycetemcomitans* were found around implants as well as adjacent natural teeth. In peri-implantitis, the detection rates of *P. gingivalis*, *T. denticola*, *F. nucleatum*, *P. intermedia* at T4, T5 and *T. forsythia* at T4 were more than two times of that in peri-implant mucositis. The detection rate *P. gingivalis* in peri-implant sites was also higher than that

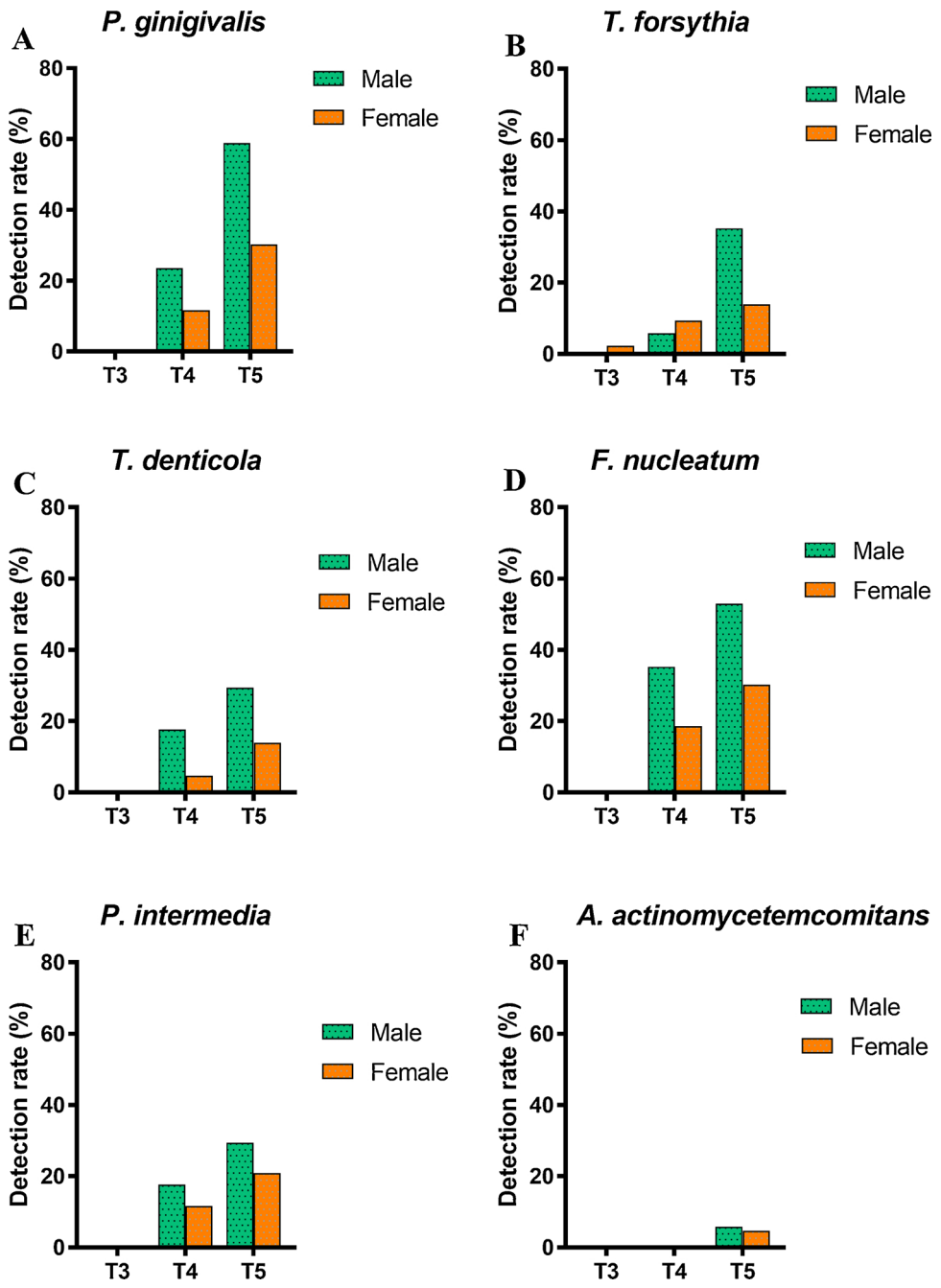

Fig. 5. Comparison of bacteria in peri-implant sites between males and females. (A) *P. gingivalis*; (B) *T. forsythia*; (C) *T. denticola*; (D) *F. nucleatum*; (E) *P. intermedia*; (F) *A. actinomycetemcomitans*. Analyzed by Fisher's exact test of Chi-square test, there was no statistically significant difference of bacterial detection between males and females from T3 to T5. T3, one month after functional loading. T4, one year after functional loading. T5, two years after functional loading.

Table 2
The correlation of bacteria in peri-implant sites and adjacent periodontal sites.

Bacteria	T3		T4		T5	
	r	P-value	r	P-value	r	P-value
P. gingivalis	–	–	0.48*	< 0.001	0.50*	< 0.001
T. forsythia	–	–	0.28*	0.03	0.53*	< 0.001
T. denticola	–	–	0.62*	< 0.001	0.57*	< 0.001
F. nucleatum	–	–	0.55*	< 0.001	0.29*	0.02
P. intermedia	–	–	0.36*	0.01	0.05	0.73
A. actinomycetemcomitans	–	–	–	–	0.38*	0.002

of adjacent periodontal sites. Although the detection rates of *T. forsythia* and *P. intermedia* in implants were almost two folds of that in natural teeth at T5, there were no statistical differences detected. It could be explained by the relatively low detection rates. A correlation between implants and natural teeth was confirmed as well, which indicated the adjacent teeth might be a reservoir of pathogenic bacteria transferred to proximal implants. Another study by our team found the residual pockets and implants position were identified as predictors for the peri-implantitis [24]. These presumption ulteriorly advocate the requisite for implant health should contain plaque control of implant adjacent teeth. A lower microbial detection in natural teeth could be caused by the resistance of natural teeth or by the susceptibility of implants to microbial invasion.

The result was coherent with the study by other scholars [25–33]. These studies carried out different methods such as cultivation, PCR, Real-time PCR, check board DNA-DNA hybridization or Sanger sequencing to detect subgingival bacteria. *F. nucleatum*, *P. gingivalis*, *P. intermedia*, and *T. forsythia* were usually the most prevalent pathogens. Additionally, the consistency of microbial detection of implants with proximal natural teeth from their results also indicated the possible microbial transportation from proximal or residual periodontal pockets to implant colonization.

Given the limited samples, there was no significant difference among different periodontal conditions. However, *A. actinomycetemcomitans* was only detected positive in peri-implant sites of AgP group. It has been established that *A. actinomycetemcomitans* was a peculiar bacterium of aggressive periodontitis for many years [34,35]. In addition, pathogenic bacteria of peri-implant sites in AgP group or CP group were more prevalent than H group, which indicated that periodontal condition had important implication on subsequent peri-implant bacteria. It could be inferred that *P. gingivalis*, *T. denticola*, *T. forsythia*, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans* may play a part in peri-implant disease development and their high-level existence may become latent risk for peri-implant disease. Therefore, history of periodontitis could increase the susceptibility of peri-implant diseases by increasing pathogenic bacteria. It is of great necessity to schedule close monitor for patients with history of periodontitis.

The result displayed higher detection rates of *P. gingivalis*, *T. denticola*, *F. nucleatum* of periodontal bacteria in peri-implantitis samples and peri-implant mucositis. Healthy implants almost detected no bacteria. Maybe this was influenced by our active treatment in accordance with Mombelli and Lang's peri-implantitis therapy decision tree [19] to interrupt disease development. The morbidity of peri-implant disease of our subjects disappointedly exceeded the average epidemiological statistics [36,37], but it could be attributed that all subjects from AgP and CP groups had severe periodontitis and tremendous bone defect in implant placement area. Another study by our team showed that the use of 6-mm-long implants is still a predictable treatment in situations with limited bone height in posterior regions for patients with history of periodontitis [38]. Our subjects strictly followed the annual follow-up maintenance or more frequent procedure from T3 to T5, but it seemed to be insufficient for periodontally compromised patients to keep pathogenic microbe at low level. Rokn et al. found irregular maintenance facilitated peri-implantitis to 20 % after 5 years [39]. Monje et al. concluded in a meta-analysis that individualized risk assessment and maintenance plan are necessary while follow-up interval should behold under 5–6 months to effectively prevent from implantitis [40]. All these researches indicated the significance of maintenance based on individualized assessment for implant and teeth health.

The academic understanding toward microbial difference between history of periodontitis and peri-implantitis has been changing with the development of detecting technique [41–44]. More and more recent researches with gene sequencing displayed a more distinctive diversity of microbiome around implant tissue. Thus, the influence of the six periodontal pathogens on implant may be just a piece of the puzzle and further randomized controlled trials (RCT) with lager sample amounts and more advanced detecting technique are required. On the other hand, the design of internal conical connection, smaller gap and rigid implant-abutment interface are presumed to prevent from the permeability of bacteria spreading. However, the annually continual ascending level of periodontal pathogenic bacteria even after disciplinary maintenance should still raise the caution of practitioner for the risk of peri-implant disease in patients with history of periodontitis. Thus more *in vivo* data are demanded for demonstrating the efficiency of this connection's effect of stopping microbial infiltration for long terms.

5. Conclusion

The long-term dynamic microbiological study displayed that six kinds of periodontal pathogens (*F. nucleatum*, *P. gingivalis*, *P. intermedia*, *T. denticola*, *T. forsythia*, and *A. actinomycetemcomitans*) in peri-implant sites accumulated with time especially in patients with history of periodontitis under annual maintenance. The microbial detection in adjacent natural teeth revealed correlation with implant's detection, which indicated natural teeth could be a microbial reservoir and suggested the essentiality of plaque control of adjacent teeth for peri-implant health. It highlighted that the detection rate of *P. gingivalis* in implants were higher than natural teeth. *A. actinomycetemcomitans* was only detected positively in patients with history of aggressive periodontitis, and patients with history of periodontitis showed higher pathogenic bacterial detection level than healthy subjects. Therefore, it can be concluded that patients with history of periodontitis may face higher potential risk for peri-implant disease and individualized maintenance is imperative for implants of periodontally compromised patients.

Author contributions

Xia Yan: Sample collection, Laboratory work, Writing.
Hongye Lu: Data analysis, Visualization, Writing.
Li Zhang: Supervision, Editing.
Bin Zhu: Laboratory work.
Muzi Piao: Sample collection.
Baoxin Huang: Design.
Haidong Zhang: Sample collection.
Huanxin Meng: Patient recruitment, Conceptualization, Supervision.

Data availability

The datasets analyzed during the current study are available in the Figshare
https://figshare.com/s/2910ed779e4ff0cb0137.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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