Peri-implant infections

de Waal, Yvonne Catharina Maria

DIFFERENCES IN PERI-IMPLANT MICROFLORA BETWEEN FULLY AND PARTIALLY EDENTULOUS SUBJECTS: A SYSTEMATIC REVIEW

Y.C.M. de Waal
E.G. Winkel
H.J.A. Meijer
G.M. Raghoebar
A.J. van Winkelhoff

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ABSTRACT

Background

The current evidence suggests that the oral microflora differs between fully edentulous (FES) and partially edentulous subjects (PES). It is unknown whether this leads to differences in peri-implant microflora when implants are installed.

Purpose

The aim of the study was to compare the submucosal peri-implant microflora between FES and PES.

Material and methods

A systematic review was conducted. The MEDLINE, EMBASE and COCHRANE databases were searched for publications up to September 1st 2012. To reduce methodological variations only studies reporting in the same article about the submucosal peri-implant microflora of FES and PES were selected.

Results

Eleven publications describing ten studies were selected. Due to numerous differences among the selected studies no meta-analysis could be performed. Six out of ten studies showed a significant difference in the composition of the submucosal peri-implant microflora in healthy and peri-implant mucositis conditions between FES and PES, with the latter showing a potentially more pathogenic composition. However, microbiological results were not unanimous among the studies.

Conclusion

In healthy and peri-implant mucositis conditions, PES harbor a potentially more pathogenic peri-implant microflora than FES. The currently existing data are insufficient for a clear conclusion regarding peri-implantitis cases. Overall, due to lack of a meta-analysis, the variability in microbiological outcomes and the limited number of studies available, the current evidence seems not to be robust.

INTRODUCTION

The oral cavity is the single site in the human body that provides non-shedding surfaces for microbial colonization. This, and the oral environmental conditions, facilitates growth of numerous micro-organisms and development of dental biofilms (Marsh & Devine 2011). Disturbance of the balance between the oral microflora and the host immune response may result in infection and destructive inflammatory responses in the periodontal tissues. Research on micro-organisms associated with periodontal disease has been extensive. Based on studies using predominately microscopy and cultural techniques a number of species including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* have been associated with periodontitis (Haffajee & Socransky 1994, Van Winkelhoff et al. 2002). These species have also been associated most strongly with periodontal disease progression and unsuccessful periodontal therapy (for review see: Haffajee & Socransky 1994) and have, as such, been designated as periodontal pathogens during the 1996 World Workshop of Periodontology (Zambon 1996). It has been suggested that these periodontal pathogens may also play a role in the development and progression of peri-implant mucositis and peri-implantitis. Several studies have shown distinct differences between the microflora associated with healthy and inflamed peri-implant tissues (Mombelli et al. 1987, Leonhardt et al. 1999, Shibli et al. 2008). In these studies *A. actinomycetemcomitans, Fusobacterium nucleatum, P. gingivalis, Prevotella intermedia and T. forsythia* were detected more frequently around unsuccessful implants than in healthy implant sites. However, the use of new analyzing techniques such as polymerase chain reaction (PCR) and pyrosequencing has revealed that the periodontal and peri-implant microflora are far more diverse than previously thought and may harbor 'uncultivable' species of which the potential pathogenic role in periodontal and peri-implant diseases is unknown (Kumar et al. 2003, Kumar et al. 2012).

It has been suggested that elimination of the subgingival environment by extraction of all teeth reduces the number of periodontal pathogens present in the oral cavity. Extraction of partially erupted third molars has been found to significantly reduce the detection frequency of black pigmenting gram-negative bacteria and *A. actinomycetemcomitans* (Rajasuo et al. 1993). Danser et al. (1994) could no longer culture *A. actinomycetemcomitans* and *P. gingivalis* from oral mucosal sites after extraction of all natural teeth, in subjects initially culture positive for these micro-organisms. This observation suggested that the periodontal sulcus may be the primary habitat of these periodontal species. This was in line with the notion that both pathogens were not detected from peri-implantitis lesions in fully edentulous subjects (FES) (Mombelli et al. 1987). A more recent study, applying real time PCR technology, showed that full-mouth tooth extraction does not result in eradication but merely in a significant reduction of these periodontal pathogens (Van Assche et al. 2009). This finding is supported by various cross-sectional studies showing that *A. actinomycetemcomitans* and *P. gingivalis* can be detected in the oral cavity of FES (Könönen et al. 1991, Socransky & Haffajee 2005, Sachdeo et al. 2008, Cortelli et al. 2008, Fernandes et al. 2010). A comparison between the microflora on removable full dentures in FES and the supragingival plaque from periodontally healthy subjects and chronic periodontitis patients revealed

marked differences between the groups in both the 'hard surface' samples and the soft tissue and saliva samples (Socransky & Haffajee 2005). It was suggested that the nature of the hard tissue surface influences the composition of the biofilm and that gingival sulcus fluid might be essential for the colonization of some bacterial species.

Placement of dental implants in the edentulous oral cavity establishes a submucosal subgingival-like environment. Several studies have investigated the effect of implant placement on the microflora of the edentulous oral cavity but contradicting results have been reported (Lee et al. 1999, Devides & Franco 2006, Quirynen & Van Assche 2011). Using the checkerboard DNA-DNA hybridization technique, Lee et al. (1999) found no differences in the composition of the tongue microbiota before and after implant placement and between tongue and peri-implant microflora after implant placement. Devides & Franco (2006) evaluated the presence of three periodontal pathogens in the mandibular arch of FES before and after implant placement by analyzing samples obtained from the alveolar ridge and peri-implant sulcus using PCR. Higher detection frequencies of *A. actinomycetemcomitans* and *P. gingivalis* were observed after implant placement and with an increasing detection rate the longer the implants were in function, while no differences were observed for *P. intermedia*. Quirynen & Van Assche (2011) evaluated the oral microflora from full-mouth tooth extraction, over 9 months of full edentulism, up to 1 year after abutment connection. Full-mouth tooth extraction resulted in reduction of the total aerobic and anaerobic bacterial load (culture) and levels remained stable after subsequent implant placement. Using both quantitive polymerase chain reaction (qPCR) and the checkerboard technique, no differences were observed in detection frequency of key periodontal pathogens at the different time intervals. However, significantly higher total numbers of *P. gingivalis*, *T. forsythia* and *P. intermedia*, but not for *A. actinomycetemcomitans*, were observed in teeth, saliva and tongue samples that were taken before full-mouth extraction compared to the edentulous and post-implantation situation.

The proportions of different bacterial species have been found to differ markedly on different intra-oral surfaces (Mager et al. 2003). Using the checkerboard technique to analyze 40 different species, it was concluded that the microflora of the different soft tissue sites differs from the microflora that colonizes the supra- and subgingival locations at teeth. In FES the microorganisms colonizing implants originate primarily from the intra-oral soft tissues, whereas in partially edentulous subjects (PES) the subgingival area of neighboring teeth can also be a principal source. Great similarities in the composition of the subgingival microflora at implants and teeth within the same mouth have been found (Lekholm et al. 1986, Leonhardt et al. 1993, Mombelli et al. 1995, Gouvoussis et al. 1997, Van Winkelhoff et al. 2000). In fact, after implant placement a similar subgingival microflora can be found at implants and teeth after just a few days (Koka et al. 1993, Quirynen et al. 2006, De Boever & De Boever 2006).

A systematic review comparing long-term clinical performance of dental implants in FES and PES showed that FES generally harbor more plaque at their implants than PES (De Waal et al. 2013). However, no differences could be observed regarding probing pocket depth and survival rate, whereas data regarding peri-implant mucosal bleeding were inconsistent. Apparently, the higher plaque levels observed in FES do not lead to impaired peri-implant conditions when comparing to PES. From this,

it might be hypothesized that not the quantity but rather the quality of the plaque, *i.e.* the microbial composition, plays a predominant role in the development of peri-implant infection. Whether a difference exists in the peri-implant microflora of FES and PES in health and/or disease, is unknown at a systematic review level. Therefore, the aim of this study was to review the peri-implant microflora in fully edentulous (FES) and partially edentulous subjects (PES) by means of a systematic review.

MATERIAL AND METHODS

The PRISMA guidelines for reporting a systematic review were followed (Moher et al. 2009).

Focused question

Is the submucosal peri-implant microflora in FES with dental implant supported reconstructions similar to the submucosal peri-implant microflora in PES with dental implant supported reconstructions, both in healthy peri-implant conditions and in peri-implant disease?

Type of studies/patients

Only prospective or cross-sectional clinical studies were considered. No limitations were applied with regard to follow-up period. Studies were excluded if less than five patients per group were evaluated. Studies comparing FES and PES who were treated with implant supported reconstructions were considered. Studies reporting only on FES or PES were excluded, because it was foreseen that major differences in methodology, such as differences in sampling and analytic techniques, exist among studies, making a true comparison between both groups impossible. Studies not clearly describing dental status or not allowing for a breakdown of data corresponding to dental status were not included. Also, studies evaluating implant therapy in a specifically selected subset of patients, such as diabetes patients, were not included.

Type of treatments

Studies describing treatments with titanium endosseous implants were considered. Consequently, studies on ceramic, submucosal, blade, transmandibular, orthodontic and zygoma implants were not included. Also, studies evaluating immediate implant placement in fresh extraction sockets were not included, because the initial microbial colonization, mainly in patients who are fully edentulous apart from the teeth to be extracted, might be influenced by the microflora of the extracted tooth and the extraction socket.

Type of outcomes

Studies reporting on the microbiological composition of the submucosal peri-implant microflora were considered. No microbiological technique was rejected from this study. Qualitative and quantitative data of bacterial species or groups of bacterial types were collected and analyzed.

Table 1. Search strategy

MEDLINE [MeSH terms / all subheadings] Dental Implants/microbiology OR Dental Implants OR Dental Implantation AND [MeSH terms / all subheadings] Microbiology OR [text words] microbi* OR pathogen OR microflora OR flora OR microbe* OR microorganism* OR micro-organism* OR bacteria OR bacterial OR bacterium OR bacteriological OR bacteriology OR bacteriologic OR infection OR peri-implant OR peri-implantitis
EMBASE 'tooth implantation'/exp AND 'microbiology'/exp OR microbial OR microbiological OR 'pathogen'/exp OR 'microflora'/exp OR 'flora'/exp OR 'microbe'/exp OR 'microbes'/exp OR 'microorganism'/exp OR microorganisms OR 'micro-organism' OR 'micro-organisms' OR 'bacteria'/exp OR bacterial OR 'bacterium'/exp OR bacteriological OR 'bacteriology'/exp OR bacteriologic OR 'infection'/exp OR 'peri implant' OR 'peri implantitis'/exp AND [embase]/lim
CENTRAL #1 search [MeSH terms / all subheadings] Dental Implants #2 search [MeSH terms / all subheadings] Dental Implantation #3 search [MeSH terms / all subheadings] Microbiology #4 search microbi* OR pathogen OR microflora OR flora OR microbe* OR microorganism* OR micro-organism* OR bacteria OR bacterial OR bacterium OR bacteriological OR bacteriology OR bacteriologic OR infection OR peri-implant OR peri-implantitis #5 search (#1 OR #2) AND (#3 OR #4)

Search strategy

Studies were identified by searching three electronic databases: MEDLINE (PubMed), EMBASE and CENTRAL (Cochrane Central Register of Controlled Trials). No language restrictions were applied. The three databases were searched for studies published up to the 1st of September 2012. The search strategy is outlined in Table 1.

Study selection

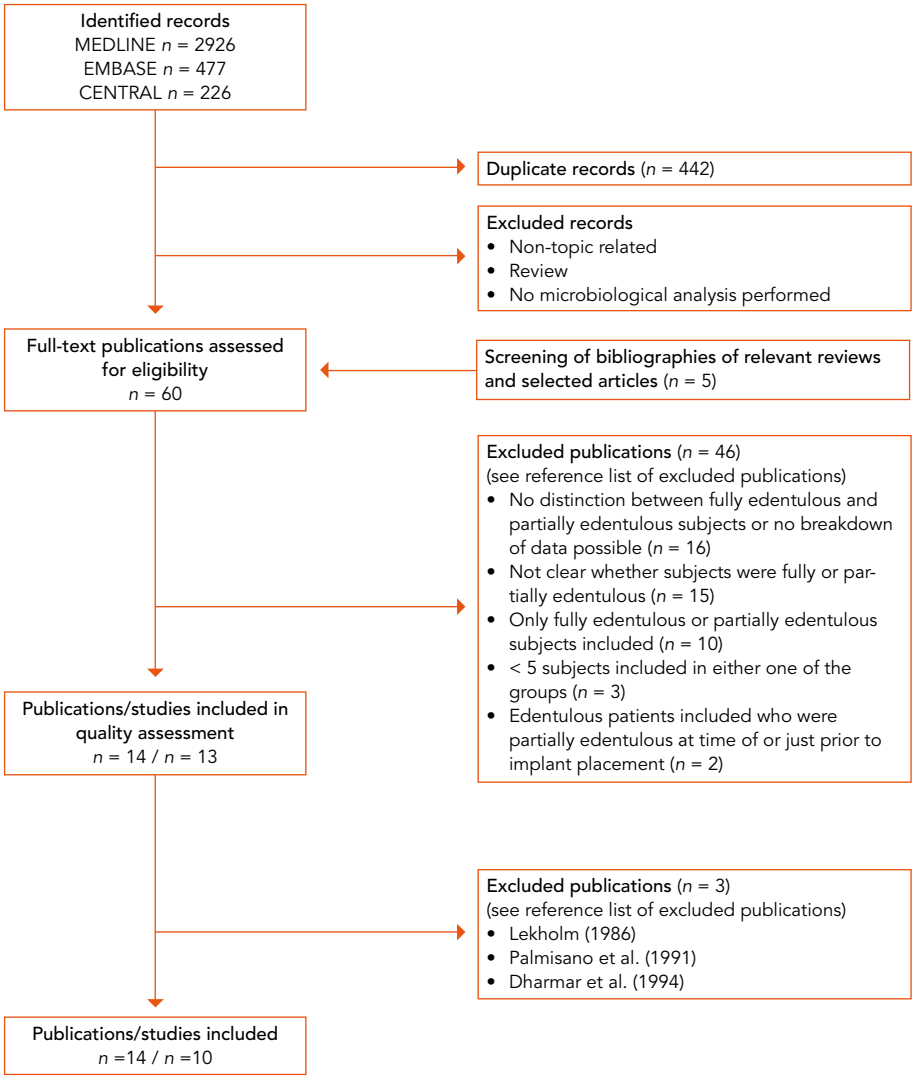
The titles and abstracts of the identified studies were initially screened. Full-text articles were obtained for all potentially relevant studies and eligibility assessment was performed by two independent reviewers (Y.W. and H.S). In addition, bibliographies of the selected publications and previously published reviews relevant to the present review were searched for eligible studies. In case of disagreement between the two reviewers, consensus was reached by discussion.

Data extraction and synthesis

Data were extracted, in duplicate and independent by two reviewers, using a data extraction form containing the following items:

- Number of FES/PES sampled, number of implants sampled, follow-up period/ time since implant placement;
- Method of microbiological sampling and analysis;
- Information regarding the clinical status of the sampled implants (bleeding ten-

Figure 1. Study selection procedure



dency of peri-implant mucosa, presence of plaque, probing pocket depth);
- Information regarding study design, treatment procedure and patient variables (study design, selection criteria, implant system, history of periodontitis);
- Data regarding the following outcome variables:
  - Distribution of bacterial morphotypes;
  - Presence (% of samples/implants/subjects) of five major putative periodontal pathogens (*A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia and F. nucleatum)* and the group of black-pigmenting anaerobic rods (*Porphyromonas* and *Prevotella* species).

Quality assessment

Methodological quality was assessed by two independent reviewers (Y.W. and H.S.) using specific study-design related forms designed by the Dutch Cochrane Collaboration (www.dcc.cochrane.org). Studies scoring five of more 'plusses' were considered methodologically 'acceptable'.

Statistical analysis

Agreement between the two reviewers with regard to the study selection procedure was calculated using Cohen's κ statistics. Due to lack of methodological uniformity of the included studies, no meta-analysis could be performed. Therefore, the outcomes are presented as a narrative review.

RESULTS

Study characteristics

The MEDLINE, EMBASE and CENTRAL searches resulted in 2926, 477 and 226 titles and abstracts respectively. After extracting duplicate citations, 3187 remained to be screened (Figure 1). After screening of titles and abstracts, 55 publications were selected for full-text analysis. Screening of bibliographies of relevant reviews and selected publications revealed five additional publications. Of the 60 selected publications, 46 were excluded after full-text analysis (see reference list of excluded publications). Additionally, three publications were excluded after quality assessment. The κ-value for inter-assessor agreement was 0.84. Disagreements were easily resolved in a consensus meeting.

Of the 11 selected publications (Leonhardt et al. 1999, Apse et al. 1989, Quirynen & Listgarten 1990, George et al. 1994, Kalykakis et al. 1998, Papaioannou et al. 1995, Hultin et al. 1998, Hultin et al. 2002, Karbach et al. 2009, Kocar et al. 2010, Quirynen & Van Assche 2012), two were found to present data on the same study (George et al. 1994, Kalykakis et al. 1998). The results of these two publications were grouped and data of 10 studies are presented (see Table 2 for study characteristics). Only one prospective study was included (Quirynen & Van Assche 2012), whereas all remaining studies were cross-sectional.

Techniques
Differential phase-contrast microscopy was used in some studies to classify the microorganisms into morphological categories including coccoid cells, spirochetes, motile rods, fusiforms and rods or filaments (Apse et al. 1989, Quirynen & Listgarten 1990, Papaioannou et al. 1995). For this classification an analysis for dark-field microscopy was used as described by Listgarten & Helldén (1978). With this method the relative distribution of the bacteria was established.
In other studies (Leonhardt et al. 1999, Apse et al. 1989, Kalykakis et al. 1998, Hultin et al. 1998, Hultin et al. 2002, Karbach et al. 2009, Kocar et al. 2010, Quirynen & Van Assche 2012) the prevalence of certain bacteria or groups of bacteria associated with periodontitis was evaluated. In the current systematic review, data on five major periodontal pathogens were considered (*A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia and F. nucleatum*) (Zambon 1996). These bacterial species were identified by culture (Leonhardt et al. 1993, Hultin et al. 1998, Leonhardt et al. 1999), latex agglutination test (Kalykakis et al. 1998), checkerboard DNA-DNA hybridization (Lee et al. 1999, Hultin et al. 2002) or PCR (Karbach et al. 2009, Kocar et al. 2010). One study used both microscopy and culture technique (Apse et al. 1989), whereas another study used three different techniques: culture, checkerboard DNA-DNA hybridization and qPCR (Quirynen & Van Assche 2012).

Outcome variables
The outcomes of the included studies are presented in Table 3. Due to lack of methodological uniformity of the included studies, the outcomes are presented as a narrative review.

Microscopical analysis
Apse et al. (1989) found a significantly higher proportion of motile rods in FES as compared to PES. This finding contrasts the results of Quirynen & Listgarten (1990) who found the opposite. Additionally, Quirynen & Listgarten (1990) found no spirochetes in FES, whereas these forms could be detected, in low numbers, in PES. Papaioannou et al. (1995) demonstrated fewer organisms and higher concentrations of cocci around implants in FES and additionally concluded that the proportion of pathogenic organisms is higher in PES (no significance level provided).

Specific bacterial species
Latex agglutination test
Using the latex agglutination test, Kalykakis et al. (1998) found a significantly higher detection frequency of *P. gingivalis* in PES compared to FES. No statistically significant difference was found for *A. actinomycetemcomitans*.

Culture
By culture technique, Apse et al. (1989) were only able to detect *A. actinomycetemcomitans* in PES, but not in FES. In addition, a significant difference was found in counts of black-pigmenting anaerobes, which appeared higher for PES. The quantitative analysis revealed a greater number of black-pigmenting anaerobic rods in PES

Table 2. Study and patient characteristics

authors	study design	selection criteria	implant surface	sampling method	analysis	dental-status	history of periodontitis	# subjects	# implants	# samples	follow-up	clinical parameters of sampled implants: plaque	BoP	PD (mm)
Apse et al. 1989	CS	consecutive, patients scheduled for recalls, implants ≥ 6 months in function	NR	needle with bent tip, one sample per implant	DPCM / Culture	FES	NR	6	13	13	44.2 m (30-84 m)	1.2 (pi)	55%	3.0
						PES	NR	15	28	28	16.3 m (7-52 m)	0.9 (pi)	36%	3.5
Quirynen & Listgarten 1990	CS	consecutive, patients scheduled for recalls, implants ≥ 6 months in function	turned[§]	curette, one (pooled) sample of one or multiple implants per patient	DPCM	FES	NR	11	NR	11*	6-68 m	1.3 (pi)	0.6 (bi)	2.6
						PES	NR	31	NR	31*	6-68 m	0.6 (pi)	0.1 (bi)	3.0
George et al. 1994, Kalykakis et al. 1998	CS	consecutive, patients scheduled for periodic maintenance, no perio-patients (ppd < 4 mm)	turned[§]	paperpoint, 2 samples per implant (mesial and distal separately)	Latex	FES	NR	14	57	114	≥ 1y (1-4 y)	11%	NR	3.5 (1-7)
						PES	no active periodontal disease, ppd < 4 mm at natural teeth	10	41	82	≥ 1y (1-4 y)	27%	NR	
Papaioannou et al. 1995	CS	consecutive, patients scheduled for recalls, implants succesfully osseointegrated and stable	turned[§]	paperpoint, one sample per implant	DPCM	FES	NR	138	261	169[†]	43.7 m	1.1 (pi)	55%	3.3
						PES	NR	159	300	143[†]	44.1 m	0.8 (pi)	55%	3.6
Hultin et al. 1998	CS	consecutive, patients selected from regular check-up in maintenance program, no patients with loss of implants, no radiographic evidence of marginal bone loss in either teeth or implants	turned[§]	paperpoint, one pooled sample of all implants per patient	Culture	FES	NR	15	4-12 per patient	15	1-11 y	1.0 (pi)	NR	2.5
						PES	no radiographic evidence of marginal bone loss at teeth	16	2-4 per patient	16	1-11 y	1.0 (pi)	NR	2.5
Leonhardt et al. 1999	CS	peri-implant health (without clinical and radiographic signs of peri-implant disease)	turned[§]	paperpoint, one pooled sample of multiple implants per patient	Culture	FES	teeth lost due to periodontitis	15	5.1 per patient	15	≥ 5 y	NR	NR	NR
						PES	teeth lost due to periodontitis, periodontal disease treated before implant installation, no progression of attachment loss during maintenance phase	36	5.1 per patient	36	≥ 5 y	NR	NR	NR

		peri-implantitis (bone loss ≥ 3 threads compared to 1-year intra-oral radiographs + presence of bleeding and/or pus, no implant mobility)	turned§	paperpoint, one pooled sample of multiple implants per patient	Culture	FES	teeth lost due to periodontitis	6	4.7 per patient	6	≥ 5 y	NR	NR	NR
						PES	teeth lost due to periodontitis, periodontal disease treated before implant installation, no progression of attachment loss during maintenance phase	31	4.7 per patient	31	≥ 5 y	NR	NR	NR
Hultin et al. 2002	CS	selected from regular maintenance program, no clinical or radiographic signs of marginal tissue destruction around implants	turned§	paperpoint, one sample of implant with greatest amount of radiographic bone loss per patient	Check	FES	NR	6	NR	6	≥ 1 y	38%	NR	2.2
						PES	NR	13	NR	13	≥ 1 y			
Karbach et al. 2009	CS	screened at routine implant recall, removable restoration in situ for ≥ 6 months, no active periodontitis at residual teeth, and more than 6 residual teeth present in partially edentulous subjects	minimally rough or rough (not specified)	paperpoint, one sample of implant with deepest pocket per patient	PCR	FES	NR	47	NR	47	1-19 y	67%	55%	3.0‡ (1-11)
						PES	NR	53	NR	53	1-19 y			
Kocar et al. 2010	CS	regular clinical assessment, stable implants without bleeding, peri-implant ppd ≤ 4 mm, no clinical or radiographic signs of peri-implantitis, at least 12 months of loading	grit-blasted acid-etched ll	paperpoint, one sample of implant with deepest pocket per patient	PCR	FES	NR	19	NR	19	30 m	NR	0	≤ 4 mm
						PES (ppd teeth ≤ 4 mm)	periodontal pockets 1-4 mm	19	NR	19	18 m	NR	0	≤ 4 mm
						PES (ppd teeth > 4 mm)	deepest periodontal pocket > 4mm (range 5-6)	15	NR	15	18 m	NR	0	≤ 4 mm
Quirynen & Van Assche 2012	P (RCT)	selected before implant placement	turned§ or porous anodized surface¶ (split-mouth)	paperpoint, 2 pooled samples of multiple implants per patient	Culture qPCR Check	FES	6-12 months edentulous, history of severe periodontitis	10	4-6 per patient	20	1 y	20%	15%	2.7 (1-5)
						PES	history of moderate/severe periodontitis, non-surgically treated, only a few pockets 4-6 mm remaining	8	4-6 per patient	16	1 y	20%	40%	3.8 (2-7)

CS = cross-sectional; P = prospective; ppd = probing pocket depth; mm = millimeters; NR = not reported; NB= Nobel Biocare/Brånemark; DPCM = Differential phase-contrast microscopic analysis for dark-field microscopy; Latex = Latex agglutination test; Check = Checkerboard DNA-DNA hybridization method; qPCR = quantitative polymerase chain reaction; * = Patients with unstable implants (> 0.1 mm bone loss per year after the first year) or samples with less than 25 countable cells were excluded; † = samples included in statistical analyses; Follow up = time from implant placement to follow-up in months (m) or years (y); pi = modified plaque index (Mombelli et al. 1987); bop = bleeding on probing; bi = modified bleeding index (Mombelli et al. 1987); ‡ = 36% of implants showed 'inflammation' (plaque, BOP, PPD ≥ 5mm), 64% of implants had healthy peri-implant conditions; § = Brånemark, Nobel Biocare; ll = Friadent plus surface, Ankylos, Denstply-Friadent; ¶ = TiUnite, Nobel Biocare

Table 3. Microbiological outcomes of selected studies

authors	dental status	bacterial morphotypes (%)[M]				detection freq. specific bacteria (%)						authors conclusions
		cocci	others	motile rods	spiros	Aa	Pg	Pi	Tf	Fn	BPA	
Apse et al. 1989	FES	85.1	5.8	5.9 *	4.5	0					9.1 *	Proportion of motile forms significantly higher in fully edentulous group, counts of BPAs significantly higher in partially edentulous group.
	PES	87.1	7.9	2.7*	4.3	3.6					50.0*	
Quirynen & Listgarten 1990	FES	71.3	28.4	0.4 *	0.0 *							A higher % of cocci and a significant lower % of motile flora around implants in fully edentulous patients.
	PES	68.6	28.0	1.8*	1.6*							
George et al. 1994, Kalykakis et al. 1998	FES					12.3	19.3*†					Significantly greater occurrence of *P. gingivalis*°/*P. intermedia* in the partially edentulous patients.
	PES					17.0	39.0*†					
Papaioannou et al. 1995	FES	60.0	38.1	1.4	0.5							Fewer organisms and higher concentrations of cocci counted around implants in fully edentulous patients. In the partially edentulous patients, the proportion of pathogenic organisms was higher (3.8 vs 1.9%, no significance level provided)
	PES	55.0	43.7	2.7	1.1							
Hultin et al. 1998	FES					0	0				13.3	No statistical differences in the microbiota among the partially and fully edentulous patients.
	PES					0	0				37.5	
Leonhardt et al. 1999	FES (health)					0	0	0*‡				None of the healthy edentulous patients harbored the analyzed microorganisms (13 species) whereas among the healthy dentate patients at least one or more of the analyzed species were found in 40% ($p < 0.001$). In diseased fully edentulous and partially edentulous patients the recovery rates were 62% and 90% respectively.
	PES (health)					3	3	26‡				
	FES (peri-implantitis)					13	25	38‡				
	PES (peri-implantitis)					31	3	66‡				
Hultin et al. 2002	FES					100	83	50	17	67		< 10^6 of target bacterial cells in each sample. Edentulous patients harbored a microflora similar to that in partially edentulous subjects.
	PES					69	53	62	15	100		
Karbach et al. 2009	FES					15* (= Aa, Pg, Pi, Tf or Td)						The % of implants with periodontal pathogens was significantly lower in fully edentulous patients than in partially edentulous patients ($p = 0.037$)
	PES					34* (= Aa, Pg, Pi, Tf or Td)						

Kocar et al. 2010	FES	0	5.3	0	0	The presence of four periodontopathogenic bacteria (Aa, Pg, Tf, Td) in healthy peri-implant sulci is common in partially edentulous patients. These bacteria were absent from the peri-implant sulci of completely edentulous patients.
	PES (pd teeth ≤ 4 mm)	21.1	36.8	5.3	57.9	
	PES (pd teeth > 4 mm)	13.3	73.3	0	33.3	
Quirynen & Van Assche 2012	FES (culture)					The number of CFU/ml were comparable at day 3 after implant placement, but with time, the partially edentulous group harbored higher counts for aerobes (0.5-0.6 log10 difference) and anaerobes (0.2-1 log10 difference) (not significant, p > 0.05)
	PES (culture)					
	FES (check)	80.0	50.0	100	100	An ongoing maturation is seen in the partially edentulous group, especially for the orange and red complex.
	PES (check)	18.8	68.8	62.5	87.5	
	FES (qPCR)	45.0	90.0	60.0	35.0	The concentration of pathogens was similar at day 3, but changed after 1 year, with higher numbers in de partially edentulous group (especially Pg (> 2log10 difference, $p = 0.01$) and Pi (1 log10 difference). In the partially edentulous group Aa was detected less frequently but a higher concentrations (> 1 log10 difference), whereas Tf was detected more frequently at comparable concentrations.
	PES (qPCR)	18.8	68.8	62.5	87.5	

pd = probing pocket depth; mm = millimeters; [M] = Differential phase-contrast microscopic analysis for dark-field microscopy; cocci = coccoid cells; others = non-motile rods, fusiforms and/or filaments; motile rods = motile rods other than spirochetes; * = statistically significant difference; spiros = spirochetes; Aa = *Aggregatibacter actinomycetemcomitans*; Pg = *Porphyromonas gingivalis*; † = Pg and Pi taken together; Pi = *Prevotella intermedia*; ‡ = Pi and *Prevotella nigrescens* taken together; Tf = *Tannerella forsythia;* Fn = *Fusobacterium nucleatum*; BPA = black pigmenting anaerobes; Td = *Treponema denticola*; CFU/ml = colony forming units per milliliter

compared to FES (mean log 1.44 versus 0.21, $p < 0.04$), whereas no significant difference was found in total anaerobic colony forming units (mean log 4.24 versus 4.07). Hultin et al. (1998) were not able to detect *A. actinomycetemcomitans* and *P. gingivalis* around any implant by culture, but some black- pigmenting anaerobic rods were detected in both FES and PES. Although not reaching the level of significance the frequency of detection of black-pigmenting anaerobic rods appeared lower in FES than in PES. In that study (Hultin et al. 1998), gram-positive facultative cocci were the most predominant cultivable bacteria at all examined sites: 55% of the cultivable bacteria at implants in FES and 30 to 40% at implants in PES. Black-pigmenting anaerobic rods accounted for 0.12% and 2.5% of the cultivable microflora in FES and PES respectively (no significant differences). In the long-term follow-up culture study by Leonhardt et al. (1999) healthy peri-implant sites and implants affected by peri-implantitis in FES and PES were compared. Although *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia/nigrescens*, amongst 10 other micro-organisms, could not be detected in healthy sites in FES these micro-organisms were present in healthy sites in PES (at least one of the tested micro-organisms in 40% of the patients, $p < 0.001$) and in peri-implantitis sites in both FES and PES (62% and 90% respectively). In that study (Leonhardt et al. 1999), the group of *P. intermedia/nigrescens* was most commonly detected in all cases. Furthermore, the non-recovery rates, *i.e.* the proportion of subjects at which non of the tested micro-organisms could be detected, of healthy subjects and peri-implantitis patients were significantly different, both for FES and PES. At implants affected by peri-implantitis *P. gingivalis* was more frequently detected in FES (2 out of 8 (25%) versus 1 out of 29 (3%)) whereas *A. actinomycetemcomitans* and *P. intermedia/nigrescens* were more frequently detected in PES (9 out of 29 (31%) versus 1 out of 8 (13%) and 19 out of 29 (66%) versus 3 out of 8 (38%) respectively). Quirynen & Van Assche (2012) showed that three days after implant placement the total number of colony forming units (CFU) (aerobe and anaerobe) at implants in both FES and PES was comparable. However, as time proceeded, the total number of CFU tended to increase in PES whereas the levels in FES remained fairly stable. However, the differences at one year after implant placement were not significant.

Checkerboard DNA-DNA hybridization

The checkerboard DNA-DNA hybridization technique was used in two studies (Hultin et al. 2002, Quirynen & Van Assche 2012). *A. actinomycetemcomitans, P. gingivalis, P. intermedia* and *T. forsythia* were detected at high frequencies in both FES and PES. However, in the study by Hultin et al. (2002) none of the species reached the level of 10^6 target bacterial cells. In both studies some periodontal pathogens were detected more frequently in FES and some more in PES, but large and conflicting differences existed between both studies. Although the differences in detection frequencies of bacterial species between FES and PES may not have been clear, Quirynen & Van Assche (2012) showed a distinct difference in maturation pattern between FES and PES. As the submucosal peri-implant microflora remained fairly stable in FES between three days and one year after implant placement, the microflora in PES showed an ongoing maturation, especially in the orange and red complex micro-organisms (Socransky et al. 1998).

Polymerase chain reaction

Using the PCR-analyzing technique Karbach et al. (2009) found a significantly lower percentage of implants with periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia or T. denticola*) in FES compared to PES (p = 0.037). This is consistent with Kocar et al. (2010) who used the same analyzing technique and found that *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* were commonly present in healthy peri-implant sulci of PES, but virtually absent in the peri-implant sulci of FES. Quirynen & Van Assche (2012) used a qPCR technique, which, in contrast to PCR, allows for a quantitative analysis (number of bacteria) in addition to a qualitative analysis (frequency of detection of bacterial species). At three days after implant placement the concentration of periodontal pathogens was quite similar between FES and PES, but after one year significantly higher numbers were observed in PES compared to FES, especially for *P. gingivalis* (> 2 log10 difference, p = 0.01) and *P. intermedia* (nearly 1 log10 difference). *A. actinomycetemcomitans* was detected more frequently in FES, but at lower concentrations (> 1 log10 difference) compared to PES, whereas *T. forsythia* was detected less frequently at comparable concentrations.

DISCUSSION

To our knowledge, this is the first systematic review evaluating the composition of the submucosal peri-implant microflora in FES and PES treated with dental implant supported reconstructions. To avoid methodological variances, only studies reporting on the microbiology of FES and PES in the same article were selected for this study.

The majority of the selected studies (6 out of the 10, Leonhardt et al. 1999, Quirynen & Listgarten 1990, Kalykakis et al. 1998, Papaioannou et al. 1995, Karbach et al. 2009, Kocar et al. 2010) tended to reveal higher proportions of putative peri-implant pathogens in PES than in FES. However, microbiological results were not unanimous among all studies selected. One study (Hultin et al. 1998) showed no statistically significant differences between FES and PES, whereas three studies (Apse et al. 1989, Hultin et al. 2002, Quirynen & Van Assche 2012) demonstrated, to some extent, conflicting microbiological outcomes (Table 3). The latter study revealed a potentially more pathogenic microflora in PES compared to FES based on the quantitative analysis, but showed conflicting results based on the qualitative analysis.

Nevertheless, on the basis of the available data, it can be stated that, in healthy and peri-implant mucositis conditions, PES harbor a potentially more pathogenic peri-implant microflora compared to FES, that is, harbor higher proportions of bacteria that have been associated with periodontal and peri-implant disease (Mombelli et al. 1987, Zambon 1996, Leonhardt et al. 1999, Shibli et al. 2008). This might possibly explain the observation that implants in FES seem to perform at least as well as implants in PES with regard to long-term survival, despite higher plaque levels generally observed in FES (De Waal et al. 2013). Based on the observation that lack of accessibility/capability for appropriate oral hygiene measures and, consequently, local plaque build-up is associated with a diagnosis of peri-implantitis (Serino & Ström 2009), it

might be hypothesized that the higher plaque levels observed in FES are counterbalanced by the lower pathogenicity of the plaque, resulting in implant survival rates that are comparable to PES.

Unfortunately, the currently existing data are quantitatively insufficient for a clear conclusion with respect to peri-implantitis cases. The only study reporting on this condition in both FES and PES showed conflicting results (Table 2) (Leonhardt et al. 1999). However, in both FES and PES, *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia/nigrescens* were significantly more frequently detected around implants affected by peri-implantitis than around implants with healthy peri-implant conditions. Although several periodontal pathogens have been associated with peri-implant disease, it is unclear whether the composition of the microflora is the cause or the result of peri-implant disease. In the natural dentition, the significance of the mere presence of periodontitis-associated bacteria as predictors for future periodontal attachment loss is limited (Wennström et al. 1987, Listgarten 1988, Maiden et al. 1990). Only the subgingival presence of *A. actinomycetemcomitans* has been identified as a risk factor for the onset of periodontitis (Van der Velden et al. 2006). No such relationships have been established for peri-implantitis, which may be explained by the lack of long-term prospective studies evaluating both clinical and microbiological parameters of implant treatment in a sufficiently large group of patients. However, it is evident that the presence of putative periodontal pathogens does not necessarily lead to a destructive process (Leonhardt et al. 1993, Quirynen et al. 2005). The initiation of the disease is suggested to be the result of a multifactorial process, involving iatrogenic, anatomical, genetic, environmental and microbiological factors.

Unfortunately, data were insufficient to allow for an analysis separately for PES who were periodontally healthy and PES who were (treated) periodontitis patients. Many studies did not report on, or were not clear about the criteria used to define the periodontal condition of the remaining teeth, although this factor may be important for the establishment of the submucosal peri-implant microflora. Specifically because it has been shown that microflora at implants and teeth shows great similarities, already within a few days after implant placement (Lekholm et al. 1986, Leonhardt et al. 1993, Mombelli et al. 1995, Gouvoussis et al. 1997, Van Winkelhoff et al. 2000). In addition, most studies did not report on smoking habits. Only Karbach et al. (2009) evaluated the influence of smoking habits on the peri-implant microflora. Smoking, in contrast to dental status, could not be identified as potential explanatory variable for the presence of periodontal pathogens. In addition to the influence of dental status, Quirynen & Van Assche (2012) evaluated the influence of implant surface roughness on the peri-implant microflora. No statistically significant differences were found in the subgingival microbiota between minimally and moderately rough surfaces. This is consistent with Karbach et al. (2009) who could also not identify implant surface roughness as explanatory variable for the presence of periodontal pathogens.

The microbiological techniques used to detect and quantify bacterial species may have also had a major impact on the results of the selected studies. The sensitivity and specificity to detect microorganisms in clinical samples differs among the various methods. Microorganisms must be present in sufficiently large numbers to be detectable by microscopy (10^4 cells/ml) and it is not possible to distinguish species based

on morphology (Fredricks & Relman 1999). The sensitivity of the culture technique varies among bacterial species as some species may be more difficult to grow in the laboratory than others. Generally, the sensitivity of culture is 10^4-10^5 cells/ml using non-selective media and ≥ 100 cells/ml using selective media, which is somewhat less than the checkerboard DNA-DNA hybridization method (10^2-10^4 cells/ml). The sensitivity of PCR-based techniques is usually between 1-100 bacterial cells (Zambon & Haraszthy 1995). The high sensitivity of PCR and the ability to detect non-viable bacterial cells, which is also possible with the checkerboard DNA-DNA hybridization, raises the question what a clinically relevant level of detection actually is. Most PCR techniques (except qPCR) do not allow for quantification of bacterial species. Generally, specificity is high using culture or PCR, but may be much lower using checkerboard DNA-DNA hybridization. Low specificity is caused by false-positive outcomes due to cross-reactions with related species, which may particularly be the case when whole genomic probes are used (Socransky & Haffajee 2005). In plaque samples the proportion of target bacterial DNA is relatively low to non-target DNA. The specificity of the checkerboard technique might also be further impaired due to binding of the DNA probe to proteins or other substances in the plaque sample (Leonhardt et al. 2003, Socransky et al. 2004). The detection frequencies of the specific bacteria investigated, as reported in the two studies using the checkerboard DNA-DNA hybridization method (Lee et al. 1999, Hultin et al. 2002), were found to be much higher than reported in studies using other techniques. In both studies whole genomic DNA probes were used.

Conclusion and clinical relevance

Due to numerous differences among the selected studies, *i.e.* study design, patient selection, clinical implant condition, sampling method and microbiological analysis, no meta-analysis could be performed. This, combined with the variability in the microbiological outcomes and the limited number of studies available for this systematic review, are the main reasons why the currently available evidence is not robust. However, despite these limitations, the majority of the studies selected in the present systematic review point toward the presence of a difference in submucosal peri-implant microflora in healthy and peri-implant mucositis conditions between FES and PES, with the former showing a potentially less pathogenic composition. Due to insufficient data no clear conclusion can be drawn with respect to peri-implantitis cases.

Although several periodontal pathogens have been associated with peri-implant disease, it is unclear whether the composition of the microflora is the cause or the result of peri-implant disease. Long-term prospective studies evaluating clinical and microbiological parameters of implant treatment in FES and PES are needed to establish the role of teeth and specific bacterial species as potential risk factors for the onset and progression of peri-implant infection. From the available literature it is known however, that a history of periodontitis is a major risk indicator for peri-implant disease (Heitz-Mayfield 2008). Amongst other factors such as social-behavioral aspects and genetic susceptibility, this association may, in part, be explained by the composition of the oral microflora. It has been shown that the micro-organisms most strongly associated with periodontal disease, *i.e. A. actinomycetemcomitans*, *P. gingivalis* and

T. forsythia (Zambon 1996), may also be associated with peri-implant disease (Mombelli et al. 1987, Leonhardt et al. 1999, Shibli et al. 2008). Therefore, it is appropriate to recommend proper periodontal infection control and concomitant reduction of putative periodontal pathogens prior to implant placement and continued supportive periodontal care therapy thereafter to reduce the risk of infection of peri-implant tissues. As it has been shown form the current systematic review that, in healthy and peri-implant mucositis conditions, PES show a potentially more pathogenic microflora than FES, it may be expected that PES are at higher risk for development of peri-implantitis than FES. Precautions may be taken accordingly.

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