

# The characteristics of biofilms in peri-implant disease

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#### Abstract

**Aim:** To describe the microbiota associated with peri-implant disease, with a specific emphasis on the differential diagnosis of the condition.

Material and Methods: The potentially relevant literature was preliminarily assessed via scoping searches to find the most appropriate search terms and the most efficient Boolean search algorithm. We identified 29 reports on subjects with osseointegrated implants, with a pathological condition compatible with the definition of "peri-implant disease", and reporting microbiological data from samples taken in affected sites. **Results and Conclusions:** In most studies bacterial samples were obtained by methods that destroy the three-dimensional structure of the biofilm. The samples therefore describe mixtures of bacteria from unspecified districts of biofilm associated with peri-implant diseases. Analyses of such samples with various methods indicate that peri-implant disease maybe viewed as a mixed anaerobic infection. In most cases the composition of the flora is similar to the subgingival flora of chronic periodontitis that is dominated by Gram-negative bacteria. Peri-implant infections may occasionally be linked to a different microbiota, including high numbers of peptostreptococci or staphylococci. Beneficial effects of mechanical and chemical interventions to disrupt the peri-implant biofilm demonstrate that microorganisms are involved in the disease process, even if they may not always be the origin of the condition.

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# The definition of peri-implant diseases

Today the replacement of missing teeth with reconstructions anchored on endosseous implants is a standard treatment option. Dental implants have a high success rate in general, and results may be maintained over many years. Nevertheless, pathological conditions may develop in the peri-implant tissues

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putting implants and reconstructions at risk and potentially affecting the patient's health (Berglundh et al. 2002, Pjetursson et al. 2004). Implant failures may be classified as "early", if they occur before, and "late", if they arise subsequent to functional loading. The causative factors involved in failures at these time points may be unrelated. In the latter case, implant loss may be the consequence of a gradually advancing disease process, or a succession of different events over prolonged periods. The term "Peri-implantitis" (or "Periimplantitis") was introduced more than two decades ago (Levignac 1965, Mombelli et al. 1987) to describe pathological conditions of infectious nature around implants. At the First European Workshop on Periodontology in 1993 it was agreed that this name should be used for destructive inflammatory processes around osseointegrated implants in function, leading to peri-implant pocket formation and loss of supporting bone (Albrektsson & Isidor 1994). The definition implied that initial healing had been uneventful and osseointegration was achieved as anticipated. Pathological conditions associated with implants not designed for osseointegration and problems with no inflammatory component were therefore not included. Hence, bone loss following implant installation due to remodelling had to be distinguished from bone loss due to a subsequent infection.

The typical clinical signs and symptoms of peri-implantitis and periimplant mucositis have been described in reports prepared for previous European Workshops on Periodontology (Mombelli 1994, 1999b, Zitzmann & Berglundh 2008). Clinically, the inflammation of the soft tissues gives rise to bleeding after gentle probing with a blunt instrument, and there may be suppuration from the pocket. Swelling and redness of the marginal tissues may or may not be manifest, and there is usually no pain. As long as the process has not resulted in more bone loss than the one attributable to remodelling, the term "Peri-implant mucositis" may be used. The typical peri-implantitis bone defect is circumferential around the implant, and is well demarcated. Because the bottom part of the implant retains perfect osseointegration, bone destruction may proceed without any notable signs of implant mobility until osseointegration is completely lost.

#### **Biofilms and peri-implant infections**

Classical microbiology has been based to a large extent on the investigation of the properties of pure cultures of microorganisms grown under laboratory conditions that are not representative of how microorganisms are found in nature. In reality, bacteria frequently live in mixed communities, termed biofilms, which are attached to environmental surfaces. This is also true for the oral microbiota that accumulate on implant surfaces to form plaque. Biofilm may be defined as a sessile community of cells that are irreversibly attached to a substratum or interface to each other, embedded in a matrix of extracellular polymeric substances that they have produced. An exhaustive discussion of the research conducted on non-oral biofilms, or using experimental models, is beyond the scope of this article. Comprehensive review papers highlight the importance of this research for the understanding of the aetiology of implant-related infections and therapeutical consequences in a broader perspective (Costerton 2005, Costerton et al. 2005, Marsh 2005, Davey & Costerton 2006). In brief, biofilm-associated infections are notoriously resistant to antimicrobial therapy unless the biofilm is disrupted mechanically. Multiple factors appear to contribute to the overall resistance of biofilm bacteria. These include the protection by extracellular polymeric substances leading to failure of the antimicrobial agent to penetrate the biofilm, and the adoption of a resistant physiological state or phenotype related to the multicellular nature of the biofilm community. Biofilms play an important role in the spread of antibiotic resistance. Within the dense bacterial population, efficient horizontal transfer of resistance and virulence genes takes

place. Biofilm to host tissue interactions are discussed in detail by working group I of this workshop.

It is a common view that oral biofilms are principally noxious. Hence, interfering with biofilm formation is regarded as a universal measure to prevent oral disease. In fact, using the experimental gingivitis model (originally described by Löe et al. 1965), a cause and effect relationship between biofilm formation on teeth and gingivitis, as well as on implants and peri-implant mucositis, can be demonstrated in humans (Pontoriero et al. 1994, Zitzmann et al. 2001). When oral hygiene is abolished to allow undisturbed accumulation of bacterial deposits on teeth or implants, clinical signs of inflammation start to appear in the adjacent soft tissues within a few days. As the deposits are removed, these signs disappear again. The tissue response to plaque formation was studied in a beagle dog model on the histological level (Berglundh et al. 1992). The inflammatory infiltrate emerging as a result of biofilm formation was equal in size adjacent to teeth and implants, indicating that the initial host response in the peri-implant mucosa and in gingiva was alike. The presence of biofilm on implants during 6 months induced an inflammatory lesion in the connective tissue of the peri-implant mucosa that was dominated by plasma cells and lymphocytes (Zitzmann et al. 2002).

The hypothesis that bacterial biofilm on implant surfaces causes peri-implantitis, and that the removal of these bacteria is the cure, is an attractive extrapolation of these findings. Beneficial effects of mechanical debridement and systemic antibiotics, demonstrated in nine cases diagnosed with periimplantitis, supported this hypothesis early (Mombelli & Lang 1992). However, based on additional data from subsequent reports, it was concluded at the Sixth European Workshop on Periodontology that the predictability of such treatment was limited and influenced by factors not yet fully understood (Claffey et al. 2008, Lindhe et al. 2008, Renvert et al. 2008). In this context, one needs to consider that bacterial colonization and maturation of biofilms depend on a favourable ecological environment, and lead to shifts in the composition and behaviour of the endogenous microbiota that may become intolerable for host tissues. Thus, changes in local ecological conditions that favour the growth

of bacterial pathogens, or trigger the expression of virulence factors (Pratten et al. 2001), may be viewed as the true origin of peri-implant disease. If such an environment persists, antimicrobial therapy alone unlikely resolves the problem permanently, because re-emergence of a pathogenic microbiota is to be expected. As an example, the fracture of an implant can give rise to a secondary bacterial infection, thus provoke purulent peri-implant disease. The primary origin of the condition is nonbacterial - microorganisms nevertheless cause the infection. Although the disease can be attenuated with antibiotics, the problem is resolved for good only once the fractured implant is removed. Another example is peri-implant infection due to submucosal persistence of luting cement ("cementitis"), where the presence of a foreign body gives rise to a bacterial infection. In a recent study (Thomas 2009) excess dental cement was associated with clinical and/or radiographic signs of peri-implant disease in 81% of 39 cases. Once the excess cement was removed, the clinical signs of disease disappeared in 74%. The differential diagnosis of periimplant disease therefore must include the identification of a possible underlying problem, and this even if suppuration, or the presence of a biofilm points to a bacterial infection. In addition, bone loss due to infection must be discriminated from bone loss due to remodelling, for example, after placement of implants too deep (Hämmerle et al. 1996), or too close to other structures (Tarnow et al. 2000).

The aim of the current review was to describe the microbiota associated with peri-implant disease, with a specific emphasis on the differential diagnosis of the condition.

#### Material and Methods

#### Search strategy

The potentially relevant literature was preliminarily assessed via scoping searches to find the most appropriate search terms and the most efficient Boolean search algorithm. On 1 July 2010 we searched the U.S. National Institutes of Health free digital archive of biomedical and life sciences journal literature (PubMed) to identify all articles that included the following terms in the title: "bacteria" OR "bacterial" OR "biofilm" OR "microbial" OR "microbiological" OR "microbiota" OR "microflora" OR "microorganism(s)"

together with (AND)

"dental implant(s)" OR "oral implant(s)" OR "osseo-integrated" OR "osseointegrated" OR "osteointegrated" OR "osteointegration" OR "peri-implant" OR "peri-implant" OR "peri-implantitis" OR "peri-implantitis".

In addition, we searched previous review articles on the subject as well as the reference lists of the articles already identified for further potentially relevant publications. Although there was no language restriction, the minimum requirement was access to an English version of the title.

#### Study selection criteria

To be eligible for inclusion in the review, cross-sectional or longitudinal studies had to provide microbiological data from samples taken in humans with clinical signs of peri-implant disease. The primary study selection criteria were thus:

- includes human subjects with dental osseointegrated implants,
- describes a pathological condition compatible with the definition of "peri-implant disease",
- microbiological data are available from samples taken in affected sites.

Two independent reviewers screened titles and abstracts of the search results. The full text of all studies of possible relevance was obtained for assessment against the stated inclusion criteria. Any disagreement regarding inclusion was resolved by discussion.

## Data extraction and synthesis of extracted evidence

A preliminary review of the literature revealed considerable heterogeneity of methods and parameters utilized in studies dealing with microbiological aspects of peri-implant diseases. It was therefore decided to tabulate the data where appropriate and report the findings in a narrative manner. The following information was sought: clinical diagnosis, number of cases and implants with the condition, implant type, sampling method, microbiological identification and results.

An attempt was made to stratify the data according to clinical diagnosis. In addition, the extracted data were stratified according to publication date, to provide a historical picture of emergence and evolution of the evidence over time.

#### Results

#### Included studies

The initial search yielded 87 potentially relevant papers. The titles and abstracts of these articles were screened independently by two reviewers (A. M. and F. D.) to determine if they included subjects with osseointegrated implants, with a pathological condition compatible with the definition of "peri-implant disease", and reported microbiological data from samples taken in affected sites.

Fifty-seven of the 87 papers did not fulfill all three primary study selection criteria: 10 papers were reviews, commentaries or editorials without original data (none of them satisfied the criteria of a systematic review). Twenty-three articles did not concern human subjects with osseointegrated implants. Of those remaining, 24 did not clearly address a pathological condition compatible with the definition of "peri-implant disease". They concerned issues such as the microbiology of implants in fully opposed to partially edentulous subjects, in subjects with or without a history of periodontal disease, or before and after placement of prostheses, the bacterial colonization of inner spaces of implants or gaps between parts, or of smooth and rough implant surfaces, described bacterial colonization and shifts over time, or compared the microbiota on teeth and implants in clinically successful cases. Even though they did not address a pathological condition clearly recognizable as peri-implant disease, some of these 24 papers were nevertheless of value for this review and will be cited selectively, if appropriate.

Twenty-nine papers fulfilling all the three study selection criteria are listed in Table 1. (Rams & Link 1983, Rams et al. 1984, 1991, Krekeler et al. 1986, Mombelli et al. 1987, 1988, 2001, Becker et al. 1990, Sanz et al. 1990, Alcoforado et al. 1991, Rosenberg et al. 1991, Mombelli & Lang 1992, Kalykakis et al. 1994, Augthun & Conrads 1997, Danser et al. 1997, Salcetti et al. 1997, Muller et al. 1999, Leonhardt et al. 2003, Rutar et al. 2001, Hultin et al. 2002, Botero et al. 2005, Covani et al. 2006, Persson et al. 2006, 2010, Shibli et al. 2008, Emrani et al. 2009, Maximo et al. 2009, Tabanella et al. 2009). One additional paper, published in a local journal in Italian, was disregarded due to unavailability.

In all of these studies, except one (Covani et al. 2006), bacterial samples were obtained by methods that destroy the three-dimensional structure of the biofilm, such as inserting a paper point into the peri-implant sulcus or removing a portion of the microbiota with a curette. Information about the spatial organization of naturally grown biofilm associated with human peri-implant disease is therefore currently unavailable. To study the three-dimensional architecture, discrete samples including the substratum on which the biofilm grows must be obtained with as little structural disturbance as possible. Such samples may be subjected to various analytical methods that have been employed for the study of other biofilms (Mombelli 1999a). In the absence of such data, the following descriptions pertain to samples representing a largely uncontrolled mixture of bacteria from unspecified districts of biofilm associated with peri-implant diseases.

### Clinical diagnosis and microbiological findings

As can be seen in Table 1, authors have used various clinical signs and diverse terms to describe pathological conditions that may fit the definition of periimplant disease. The term "failing implant" was used in five publications. Three of them (Becker et al. 1990, Rosenberg et al. 1991, Covani et al. 2006) reported data from implants with mobility, indicative of complete loss of osseointegration. In one report, however, implants with mobility were not included (Salcetti et al. 1997). One may suppose that the disease had lead to substantial, but not to complete loss of osseointegration. Presence or absence of implant mobility was not reported in the fifth article using the term "failing implant" (Alcoforado et al. 1991). A specific microbial criterion distinguish-

Table I. Repo	rts on subjec	cts with osseointegrated in	nplants, with a pathologic	al condition compat	ible with the delimito	n or peri-impiant uisease, a		ו ממנמ דרטונו פמוווערים נמוצרו ווו
Authors	Year	Clinical diagnosis	N cases	N implants	Sampling	Identification	Principal microbiological finding	Implant type, comments
Alcoforado et al.	1661	''Failing imp'': progressive PPD, marginal BL and/or abscessing after primary healing and osseo- integration	12 (8 m, 4 f), mean age 58.1 (47–70)	8	dd	Culture	6 imp: PM, CR; 5 imp: FU, C. albicans; 4 imp: PI; 3 imp: CA; 2 imp: staphylococci; 1 imp: AA 1; Enteric rods or pseudomonads constituted a significant part of the	10 Branemark, 3 Core-Vent, 3 Integral, 1 Screw-Vent, 1 TPS
Augthun & Conrads	1997	PPD > 5	12 (5 m, 7 f), age 66.5 $\pm$ 10.1	8	Peri-implant tissue removed	Culture	In the second and the second and the second and the second acceae ( <i>Prevotella</i> spp.), AA; 5 imp: EC, staphylococci, entercocci entercocci	IMZ, mean lifetime of imp 74.7 (土 28.7) months. Edentulous pat
Becker et al.	1990	<ul> <li>'Failing imp'': increased mobility, peri- implant radiolucency</li> </ul>	13	28	ЪЪ	DNA-probe analysis	AA detected in 27.8%; PG in 37.5%; PI in 35.4%	5 pat blade-type imp, 1 pat sub-periosteal imp, 9 pat root-form-type imp PPD 6.1, survival time 6 months to 12
Botero et al.	2005	''Peri-implant disease'': PPD>3, BOP, BL	11, mean age 48.7	16	Ч	Culture	12 imp: enterics; 8 imp: FU; 7 imp: PG; 4 imp: PI, EB	Partially edentulous pat, $> 1$ year in function, 14 screw- type. 2 blade-type imp
Covani et al.	2006	"Failed imp": peri-implant radiolucency, mobility	Q	15	Implant and abutment retreived	Histology of abutment/ implant interface	15 imp: Bacteria at the level of imp/abutment interface. Cocci and filaments adherent to imp. Surface, orientation perpendicular to long axis of imp	10 titanium imp, 5 HA- coated imp
Danser et al.	1997	PD>4	Ξ	DN	CT cervical area, PP peri-implant pockets	Culture	11 imp: Peptostreptococci, FU; 8 imp: PN; 6 imp: TF; 5 imp PI; 2 imp CR; AA and PG not detected.	Fully cdentulous pat, history of periodontitis
Emrani et al.	2009	BOP and mucositis	1 f, age 45	4	dd	Culture	PG, PI, TF, Dialister pneumosintes. CR. PM. FU	Case report, 3i Osseotite and TiUnite Nobel Biocare
Kalykakis et al.	1994	Imp in maintenance, PPD 1–7, BOP	24 (9 m, 15 f), age 33–70	98	dd	Latex agglutination assays	Greater PPD, BOP in sites colonized by AA and/or PG, PI	41 imp in 10 partially edentulous, 57 imp in 14 edentulous pat
Hultin et al.	2002	"Peri-implantitis": BL>3 fixture threads (1.8 mm) after 1st year of loading	17 (9 m, 8 f), mean age 62.8	45	dd	DNA-probe analysis	75-100% imp: AA, FN, PN, PI; 50-75% imp: PG, PM, CR, EC	14 pat Branemark, 3 pat ITI solid screw
Krekeler et al.	1986	PPD 1 to 6	10, mean age 63 (49–69)	ND	CT	Culture	High % of Gram-negative anaerobe rods, FU, Selenomonas sp. and black-	Descriptive, preliminary analysis

206

Mombelli & Décaillet

	Nobel Biocare, partially edentulous	Branemark imp, in function	>1 year, non-smokers			ITI hollow cylinder imn				ITI imp, prospective data on	imp developing into failure		ITI hollow cylinder, at least	6 months following	installation		ITI hollow cylinder or full	body screws	Case report	ITI imn	4 	Predominantly Branemark	imp				2 ceramic blades, 1 ceramic			1 ramus frame assembly, 1 ceramic post. 2 blade	QN
pigmenting bacteroides (PI/PG)	7 pat: PI/PN; 6 pat: AA; 3	PG, TF, PI, FU. S. sanguinis,	S. gordonii, V. parvula and Actinomycetes at elevated	levels in peri-implantitis. N. mucosa, PG, PN, FU and	Actinomycetes at elevated	revers in mucosius Complex microhiota 40%	Gram-negative anaerobic	rods, prominently FU and	Pl. Spirocnetes, Justforms, motile and curved rods	Successive rise of AO, FU,	spirochetes with	development of peri- implantitis	Gram-negative anaerobic	bacilli > 40% of mean total	cultivable count. 7 imp: FU;	5 imp: PI; 3 imp: AO, CR, 1 imp: PG	>50%: CR, FU, PI/PN; TF	36%	Presence of AA, BF, PG, PI, CA. EC. FN. CR	Present at $> 10F5$ in $> 50\%$	imp: PN, FN; > 30% imp: PG, PM, PI, EC, <i>Neisseria</i> , <i>Lontrichia</i>	Present at $> 10E4$ in $> 50\%$	imp: AA, FN, F.	CA, CR, E. saburreum, N.	mucosa, PI, T. socranskii, V.	parvula, H. influenzae, H. pylori, SA	Spirochetes (small and intermediate_cized) rod-	shaped microorganisms with	negative	32% spirochetes (versus 2.3% in healthy imp)	11 imp (in 9 pat) positive for staphylococci: 1/3 SA, 2/3 S.
	Culture	DCH				Culture DFM				Culture, DFM			Culture				Culture, DFM		Indirect immunofluorescence	DCH		Expanded DCH assay					Transmission EM			PCM	Culture
	dd	ст				dd	:			ΡP			ΡΡ				РР		ЪР	CT	5	Ы					CT			CT	dd
	26	20: peri-implantitis 16:	mucositis			×	)			1			6				30		7	25	l	31					ŝ			4	20
	9 (4 m, 5 f)	2 mucositis (4 m, 8 f)	nd 13 peri-implantitis (7 m, 6 f)			L				1 f			6				25		1 m, age 65	2.1	i	31					3 (1 m, 2 f)			ND	13
	BL $\geq 3$ threads, BOP and/or SUP	"Mucositis": BOP and 1	marginal bleeding MB, a absence of BL or SUP;	''peri-implantitis'': PPD > 4, BOP and/or	SUP and BL $\geq 3$	unreaus ''Peri-implantitis'''	PPD>5, SUP, BL			SUP, PPD 6			PPD>4, BL				<pre>', peri-implantitis'':</pre>	circumferential BL, PPD>4	PPD>6, BOP, SUP, BL	", 'Peri-imnlantitis'' -	$BL \ge 2$ , $PPD \ge 5$	". "Peri-implantitis":	$BL > 2.5$ , $PPD \ge 4$ with BOD or SUD				BOP, PPD>10, montressitive RI			PPD>5	''Peri-implantitis''
	2003	2009				1987				1988			1992				2001		1999	2006		2010					1983			1984	1990
	Leonhardt et al	Maximo	et al.			Momhelli	et al.			Mombelli	et al.		Mombelli	& Lang			Mombelli	et al.	Muller et al.	Persson	et al.	Persson	et al.				Rams & I ink			Rams et al.	Rams et al.

Table I. (Conti	d.)							
Authors	Year	Clinical diagnosis	N cases	N implants	Sampling	Identification	Principal microbiological finding	Implant type, comments
							epidermidis; 2 pat 60.9% and 100% staphylococci	
Rams	1661	PPD>7, BOP, marked	1 f	1	Ы	Culture, PCM	26.9% FU, 7.7%	Tri-stage, HA-coated root-
et al. Rosenberg	1991	ыс "Infectious failure":	11	32	ЬР	Culture. PCM	Peptostreptococcus prevotu 21% spirochetes. 21%	rorm 1mp Versus traumatic etiology.
et al.		BOP, SUP,	:	1			motile rods; $> 10\%$ of	Branemark, Core Vent,
		granulomatous tissue upon imp removal					cultivable flora: PM, FU, enterics. <i>Candida</i> : >50%	Screw Vent, Swede Vent
							imp: PG, PI, CR, PM, FU, <i>Candida</i>	
Rutar	2001	History of "peri-	ND	15	ЪР	Culture, DFM	Imp with history of	ITI implants, retrospective
et al.		BOP and/or SUP BI )					perumplantitis more frequently nositive for small	study
		(177, 100, 100, 100, 100, 100, 100, 100,					and medium-sized	
							spirochetes; 4 imp: PG; 2	
							imp: AA	
Salcetti et al.	1997	"Failing imp": peri- implant radiolucency	21 (7 m, 14 f), age 33–70	21	CT	DCH	Of 40 taxa only 4 positively associated with failing	Versus 8 patients with only healthy imp
		and/or vertical BL>2					implant: PN, PM, FN ss	Jury Common
		after 1st vear					vincentii. FN. 97.5% failing	
							imp harboured PN or PM;	
							AA not detected	
Sanz	1990	'diseased gingiva'':	7	7	ЪР	Culture	High % of Gram-negative	Sapphire ceramic imp
et al.		GI > I, $PPD > 3$ (4 mp with PPD 6)					anaerobic rods, incl. <i>Prevotella</i> and	
		~					Prophyromonas	
Shibli et al.	2008	", 'Peri-implantitis'':	22 peri-implantitis	22: peri-implantitis	CT	DCH	Higher counts of PG, TD,	Branemark-like imp
		BL>3, BOP and/or	(3 m, 19 f) and 22	22: healthy			TF in peri-implantitis.	
		SUP	healthy (8 m, 14 f)				Supra- and submarginal	
							profiles not substantially different	
Tabanella	2009	'Ailing implant'':	15 (6 m. 9 f). age	15	Ър	Culture	9 imp: FU. TF: 7 imp: CR.	11 Branemark. 4 3i imn
et al.		BL > 3 threads	31–72 (mean 56)				PM; 5 imp: PG, PI	-
<sup>II</sup> Clinical param Sampling: CT, c	eters: pat, p surette; PP,	atient; imp, implant; m, 1 paper points.	male; f, female; BL, bone	: loss; PPD, peri-implan	it probing depth; BC	)P, bleeding on probing; S	sUP, suppuration.	

Microbiological methods: DCH, DNA-DNA checkerboard hybridization; DFM, darkfield microscopy; PCM, phase-contrast microscopy.

Bacteria: AA, Aggregatibacter actinomycetemcomitans; AO, Actinomyces odontolyticus; CA, Capnocytophaga sp.; CR, Campylobacter rectus; EB, Eubacterium sp.; EC, Eikenella corrodens; FN, Fusobacterium num actinomycetem sp.; PA, Pseudomonas aeruginosa; PG, Porphyromonas gingivalis; PI, Prevotella intermedia; PM, Parvimonas micra; PN, Prevotella nigrescens; SA, Staphylococcus aureus; TF, Tannerella forsythia. ND, no data.

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ing "failing implants" with or without mobility could not be identified.

Two papers clearly identified subjects with peri-implant mucositis. The first (Emrani et al. 2009) was a report of one single case, the second (Maximo et al. 2009) included 12 patients with mucositis, 13 with peri-implantitis and 10 healthy controls. Of 40 species, quantified with checkerboard DNA-DNA hybridization, only three were found at significantly different levels among the three groups: Actinomyces gerencseriae was found in lower counts while Tannerella forsythia was found at higher levels in the peri-implantitis group, when compared with the healthy and mucositis group. Capnocytophaga ochracea was increased in the mucositis group compared with the other two groups. The remainder of the publications concerned conditions compatible with the definition of "peri-implantitis" at various stages. Although specific findings were reported in several papers with regards to certain microbiota, no clearly visible trend emerged justifying a subdivision of the material with regards to clinical diagnosis or implant system.

## The microbiology of peri-implant diseases in a historical perspective

The first documented microbiological investigations on human peri-implant disease were carried out using transmission electron microscopy (Rams & Link 1983) and phase-contrast microscopy (Rams et al. 1984). "Intact plaque" was collected with a curette from the most apical portion of the peri-implant space from 17 implants with variable peri-implant tissue conditions and of various designs (ramus frame assembly, blade implants, carbon and ceramic posts). Samples from implants considered "relatively healthy", with stabilized pockets not exceeding 5 mm, contained a predominantly coccoid microbiota. Samples from implants with deeper probing depths showed a significantly lower proportion of coccoid cells and a higher proportion of spirochaetes. In the same year a paper was published showing, in saliva samples, a marked colonization with potentially pathogenic microorganisms such as Staphylococcus aureus. Pseudomonas sp. and enterobacteria after abutment operation, which was attributed to the use of a surgical dressing (Heimdahl et al. 1983).

cross-sectional examination of 20 fully edentulous patients with implants in function for a period of between 6 months to 15 years were published (Lekholm et al. 1986). Submucosal plaque samples were obtained from the sites showing the deepest and shallowest pockets and analysed with regards to the percent distribution of bacterial morphotypes. Although 15% of probing depths were deeper than 6 mm, this was not perceived as a pathological condition (the reason why the paper is not listed in Table 1). After a combined evaluation with the results of a 3-year prospective trial of the same authors (Adell et al. 1986) they stated that "the presence of gingivitis and the occurrence of filiforms and small spirochaetes" were correlated and that "deeper pockets were found significantly correlated with increasing presence of small spirochaetes". In the same year, preliminary results from bacterial culture of peri-implant plaque, collected in 10 patients with titanium implants, were published in a German journal (Krekeler et al. 1986). A predominance of Gramnegative anaerobes with increasing periimplant probing depth was suggested. In 1987, a study compared the peri-implant microbiota of successful and unsuccessful osseointegrated titanium implants using continuous anaerobic culture and darkfield microscopy (Mombelli et al. 1987). Forty-one per cent of the cultivated organisms from implants with probing depths  $\geq 6 \text{ mm}$ , suppuration and radiographic evidence of bone loss were Gram-negative anaerobic rods. Fusobacterium sp. and Prevotella intermedia (then referred to as Bacteroides intermedius) were regularly detected among these organisms, and were often found at high levels. Samples from successful implants yielded very low cultivable counts and consisted predominately of Gram-positive cocci. In the darkfield microscope, samples from failing implants showed abundant motile rods, fusiform bacteria and spirochaetes, while samples from successful implants contained only a small number of coccoid cells and very few rods.

In 1986 microbiological data from a

The longitudinal clinical and microbiological development of a periimplant infection was documented for the first time in one subject participating in a study on the colonization of newly set implants, where samples were taken in weekly intervals from the periimplant sulcus (Mombelli et al. 1988). High anaerobic cultivable counts were noted in this person already 2 weeks after implantation. *Fusobacterium* sp. was isolated for the first time 42 days after implantation. Increasing numbers were noted in the subsequent samples. From day 21 on, a steady decrease of coccoid cells and a simultaneous increase of rods were observed. At day 120 small spirochaetes were found for the first time, pus formation was noted clinically and a pocket probing depth of 6 mm was recorded.

Towards the end of the decennium several other groups had started to investigate the peri-implant microflora as well, and by 1990 the publication rate increased. Sanz et al. (1990) investigated endosteal sapphire ceramic implants. Diseased sites harboured a microbiota with a large segment of Gram-negative anaerobic rods, including black-pigmented organisms and surface translocators. Healthy sites in the same patients vielded small amounts of mainly facultative, Gram-positive bacteria. Becker et al. (1990) used commercially available DNA probes to test for the presence of the three periodontal marker organisms Aggregatibacter actinomycetemcomitans (then referred to as Actinobacillus actinomycetemcomitans), P. intermedia and Porphyromonas gingivalis (then referred to as Bacteroides gingivalis) in 36 failing implant sites of 13 patients with different types of implants (blade-type, subperiosteal and root-form type). They reported high levels of P. gingivalis in one patient with a failing blade implant and high levels of P. intermedia in two additional patients with unsuccessful blades. In the other cases, some weak signals were obtained for one or several of the three tested organisms. Rams et al. (1990) found a limited number of patients demonstrating particularly high counts of Staphylococcus sp., implying these organisms in the development of pathology in some cases.

The differential diagnosis of periimplant diseases was addressed 1991 for the first time by Rosenberg et al. (1991). Thirty-two failing implants in 11 patients were subdivided into two groups: an "infection group" (including implants exhibiting one or more of the following signs: bleeding, suppuration, pain, high plaque and gingival indices, presence of granulomatous tissue upon surgical removal) and a "trauma group" (implants showing mobility and a peri-implant translucency in the absence of the signs listed for the first group). Direct phase-contrast microscopy and culture analysis exhibited distinct bacterial profiles in samples from the two groups. Implants in the first group showed high proportions of spirochaetes and motile rods and culture revealed the frequent presence and high numbers of periodontal marker organisms such as P. gingivalis, P. intermedia, Campylobacter rectus (then referred to as Wolinella recta), Fusobacterium sp. In addition A. actinomycetemcomitans, Parvimonas micra (then referred to as Peptostreptococcus micros) were only detected in samples from this group. S. aureus, Staphylococcus epidermidis and Candida sp. were detected more frequently in the "infection group" as well.

Alcoforado et al. (1991) examined the submucosal microflora of 18 failing osseointegrated implants of various designs (Brånemark, Core-Vent, Integral, Screw-Vent and TPS) for potentially pathogenic oral bacteria. P. micra was recovered from six failing implants. C. rectus from six. Fusobacterium sp. from five, and P. intermedia from four. The authors reported significant numbers of enteric rods or pseudomonads in the microflora of five failing implants. A. actinomycetemcomitans, non-pigmented Bacteroides species, Capnocytophaga sp. and staphylococci were also detected in some implant failures. In addition, five cases were positive for Candida albicans.

Nine subjects with peri-implantitis were included in a study testing an antimicrobial treatment regimen (Mombelli & Lang 1992). Gram-negative anaerobic bacilli contributed with almost 40% to the total cultivable count in mean, with a maximum of 71% in one patient. P. intermedia and Fusobacterium sp. were frequently found, and reached considerable proportions, when present. There was one partially edentulous patient, who was positive for P. gingivalis. Seven patients harboured motile, eight fusiform rods in the diseased sites. Four patients were positive for small- and medium-sized spirochaetes; two of the four were also positive for large spirochaetes.

Ten edentulous and 14 partially edentulous patients with Brånemark implants were evaluated using a latex agglutination test (Kalykakis et al. 1994). *A. actinomycetemcomitans* was found in 12% of the edentulous and in 17% of the partially edentulous patients. Signals indicative for presence of bacteria of the group *P. intermedia*/*P. gingivalis* (referred to as indicators for black pigmenting bacteria) were obtained in 39% of the partially edentulous and 19% fully edentulous subjects. Implants harbouring one of the three microorganisms had significantly greater probing depths, a higher gingival bleeding tendency and a higher crevicular fluid flow rate.

Peri-implant tissue removed in the context of a surgical intervention to treat a peri-implantitis in 12 patients was analysed by culture techniques for the presence of periodontal microorganisms (Augthun & Conrads 1997). Bacteroi-daceae and A. actinomycetemcomitans were frequently found (16/18). Capnocytophaga, Fusobacterium nucleatum, Eikenella corrodens and other taxa were detected less frequently.

The peri-implant microbiota of 22 patients with failing implant sites were examined using DNA oligonucleotide probes for 40 different microbes (Salcetti et al. 1997). This study found greater detection frequencies of *P. nigrescens, P. micros, F. nucleatum* ss *vincentii*, and *F. nucleatum* ss *nucleatum* in mouths with failing implant sites as compared with mouths with healthy control implants. From a clinical and microbial perspective, risk appeared to be primarily at a patient level and secondarily at a site or implant level.

The presence of periodontal bacteria on oral mucosa and in peri-implant sulci was studied in 20 edentulous subjects with a history of periodontal disease (Danser et al. 1997). P. intermedia was detected only in those 11 subjects with peri-implant probing depths 5 mm or deeper. A. actinomycetemcomitans and P. gingivalis were never detected. All subjects harboured Peptostreptococcus spp., Fusobacterium spp., and other Prevotella species. Actinomyces odontolyticus, T. forsythia (then referred to as Bacteroides forsythus), C. rectus, Pseudomonas spp., and enterobacteria were detected infrequently.

Eleven papers have provided additional microbiological data from periimplantitis since the year 2000. In most cases these are baseline data from interventional studies to treat peri-implant disease.

Thirty peri-implantitis affected implants in 25 patients showed high culture frequencies of *C. rectus*, *T. forsythia*, *Fusobacterium* spp., *P. intermedia/nigrescens* (Mombelli et al. 2001). Frequencies of A. actinomycetemcomitans, P. gingivalis, and E. corrodens were low.

When 64 implants in 45 partially edentulous subjects were examined 5–10 years after implant installation, 15 of them showed a probing pocket depth exceeding 4 mm (Rutar et al. 2001). A statistically significant relationship was established between peri-implant probing depth and the total anaerobic cultivable microbiota as well as the frequency of detection of *P. gingivalis*.

Seventeen partly edentulous patients with a total of 98 implants, of which 45 showed marginal bone loss of more than three fixture threads after the first year of loading harboured high levels of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia* and *Treponema denticola* (Hultin et al. 2002).

Out of nine partially dentate individuals with 26 titanium implants with peri-implantitis six were positive for *A*. *actinomycetemcomitans*, seven for *P*. *intermedia*/*P*. *nigrescens*, one for *P*. *gingivalis*, one for *S*. *aureus* and three for enterics (*Escherichia coli* and *Enterobacter cloace*) (Leonhardt et al. 2003).

Significant differences were noted in microbial samples from 16 implants with signs of pocketing and stable controls (Botero et al. 2005). *P. gingivalis* was detected in peri-implant lesions but not in stable implants. The frequency of detection of Gram-negative enteric rods (75%) and *P. intermedialnigrescens* (25%) was significantly higher in peri-implant lesions.

In 25 cases with peri-implantitis the DNA-DNA checkerboard hybridization method was used to detect bacterial presence (Persson et al. 2006). The majority of the microorganisms in the panel were found in >20% of the samples; however, the distribution of 40 bacteria varied considerably from implant to implant. P. nigrescens and fusobacteria were the most prevalent organisms, followed by P. micras and A. actinomycetemcomitans. An expanded checkerboard DNA-DNA hybridization assay encompassing 79 different microorganisms was used to study bacterial counts in 34 cases with peri-implantitis (Persson et al. 2010). The most prevalent bacteria were: F. nucleatum sp., Staphylococcus sp., A. actinomycetemcomitans, Helicobacter pylori, and T. forsythia.

Studying 22 subjects with periimplantitis, and 22 subjects without, Shibli et al. (2008) did not find substantial differences in microbial profiles of supra- and submucosal samples from the same implant sites determined by DNA–DNA hybridization. Targeting 36 microorganisms, they noted higher mean counts of *P. gingivalis*, *T. denticola* and *T. forsythia* in the peri-implantitis group, both supra- and submucosally.

Checkerboard DNA-DNA hybridization for 40 bacterial species was also used to analyse samples from 13 subjects with peri-implantitis and 12 with mucositis (Maximo et al. 2009). P. gingivalis, T. forsytha, P. intermedia, Fusobacterium ssp., S. sanguinis, S. gordonii, V. parvula and actinomycetes were detected at elevated levels in periimplantitis. C. ochracea, Neisseria mucosa, P. gingivalis, P. nigrescens, Fusobacterium ssp. and actinomycetes were detected at elevated levels in mucositis. As mentioned above, only three species were found at significantly different levels in samples from mucositis or peri-implantitis: T. forsythia (higher levels in the peri-implantitis group), A. gerencseriae and C. ochracea (lower counts in the peri-implantitis group).

One case report (Emrani et al. 2009) described the submucosal microbiota of a 45-year-old female with advanced periodontitis before and after complete edentulation. Microbiological culture of three inflamed peri-implant sites showed a spectrum of pathogens, including *P. gingivalis*, *T. forsythia*, and other major pathogenic bacteria characteristic of aggressive periodontitis.

Anaerobic culture techniques were used to investigate the microbiota associated with peri-implantitis (Tabanella et al. 2009). Peri-implantitis was associated with the presence of *T. forsythia*, *Campylobacter* species, and *P. micra*. Pain was associated with *P. micra*, *Fusobacterium* and *Eubacterium* species.

#### **Discussion and Conclusions**

By looking at the chronological evolution of the knowledge on the microbiology of peri-implant disease, it can be concluded that this process was rather continuous over time and cumulative in nature. Early reports pointed to a microbiological similarity between peri-implant disease and chronic periodontitis. Over time, additional reports pointed to the possibility that a limited number of cases may harbour a different microbiota, which would rather be similar to the microbiota generally associated with infections of implanted medical devices.

## Peri-implant disease as a mixed anaerobic infection

The analysis with various methods has shown that the microbiota associated with peri-implant disease is (i) mixed, (ii) rather variable, and (iii) in most cases dominated by diverse Gram-negative anaerobic bacteria. Many investigators have employed methods adapted for the study of the subgingival microbiota in periodontal pockets of natural teeth, and have searched for so-called putative periodontal pathogens in the first place. Table 1 clearly indicates that ubiquitous organisms in chronic periodontitis, such as Fusobacterium spp. and P. intermedia, are also regularly detected in specimens from peri-implantitis. Microorganisms that are less frequently found in chronic periodontitis, for example A. actinomycetemcomitans (Mombelli et al. 2002), are also less frequently associated with peri-implant diseases. Several studies listed in Table 1 have shown that there is a difference in the composition of the peri-implant microflora in deep and shallow pockets, reflecting differences in ecological conditions also known from the situation around natural teeth. Pockets 5 mm deep or more can be viewed as protected habitats for putative pathogens and may be an indicator of a risk for periimplant disease. As mentioned above, information about the spatial organization of naturally grown biofilm from human peri-implant disease is lacking because the available data derive from specimens obtained by methods disrupting the biofilm.

#### Microbial status and differential diagnosis of peri-implant diseases

Surprisingly little is thus far known about microbiological differences that may be characteristic for certain forms of peri-implant disease. The article by Rosenberg et al. (1991), comparing two groups of implant failures microbiologically, and the one by Maximo et al. (2009), comparing mucositis and periimplantitis, are lone examples for studies of this kind. The lack of marked microbiological differences between mucositis and peri-implantitis, or moderate and severe peri-implantitis, may signify that in most cases the disease evolves gradually from mucositis to peri-implantitis.

Although there is no evidence for the existence of one or a limited number of specific pathogens for peri-implantitis in general, reports have repeatedly indicated that peri-implant infections may occasionally be linked to a microflora with a different profile than in chronic periodontitis. This concerns in particular reports of sporadic high numbers of peptostreptococci (i.e. P. micra), or staphylococci (i.e. S. aureus and S. epidermidis). Peptostreptococci are commensal organisms in humans that can cause abscesses and necrotizing soft tissue infections. S. aureus and S. epidermidis are well-established pathogens implicated in infections of implanted medical devices crossing the epidermal barrier (Christensen et al. 1989). Longitudinal observations have shown that S. aureus may colonize implants early after placement (Fürst et al. 2007), and may persist long term (Salvi et al. 2008).

As developed in the introduction, beneficial effects of mechanical and chemical interventions to disrupt the peri-implant biofilm demonstrate convincingly that microorganisms are involved in the disease process. However, this is not a proof that they are always the origin of the condition.

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#### **Clinical Relevance**

Scientific rationale for the study: Beneficial effects of antimicrobial interventions suggest that bacteria are involved in the pathogenesis of peri-implant diseases. For optimal targeting of prevention and therapy the microbiological features of the disease need to be elucidated. face topography. An experimental study in dogs. *Journal of Clinical Periodontology* **29**, 456–461.

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*Principal findings:* Peri-implant disease maybe viewed as a mixed anaerobic infection where Gram-negative microorganisms, also implicated in chronic periodontitis, seem to play an important part. Peri-implant infections may however occasionally be linked to another microbiota, involAddress: Andrea Mombelli School of Dental Medicine Division of Periodontology and Oral Pathophysiology University of Geneva Rue Barthelemy-Menn 19 CH-1205 Geneva, Switzerland E-mail: andrea.mombelli@unige.ch

ving peptostreptococci or staphylococci.

*Practical implications:* Strategies for prophylaxis and therapy should be aiming at a mixed anaerobic microbiota. The issue of differential diagnosis of peri-implant infections needs to be approached in clinical trials.

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