Supplementary data – Dingsdag et al.

Table S1: Sample descriptions for 196 samples used herein.

Column labelled ‘PCR Amp.’ indicates whether PCR amplification failed completely (‘×’), and/or was sufficient (‘✓‘) or insufficient (‘~’) for downstream molecular analyses.

Patient	Sample	Site	Description	PCR Amp.	TRFLP	454
1	1A	UL7	Biofilm	✓	✓	
	1B	UR2	Paper point	×		
	1C		Paper point	×		
	1D	UL2	Paper point	×		
	1E		Paper point	×		
2	2A	UL6	Granuloma	✓	✓	✓
3	3A	UL2	Paper point	×		
	3B		Drill bit	×		
	3C		Drill bit	×		
4	4A	UR1	Paper point (7mm)	×		
	4B	UR3	Paper point (12mm)	×		
5	5A	UR5	Paper point (7mm)	×		
	5B	UR7	Paper point (7mm)	×		
6	6A	LR1	Infected tooth	✓	✓	✓
	6B		Drill bit	×		
	6C		Drill bit	×		
7	7A	UR1[a.]	Drill bit	×		
8	8A	UL7	Biofilm	✓	✓	✓
	8B	UR2	Drill bit	×		
	8C		Paper point (13mm)	×		
	8D		Bone fragments	×		
	8E	UL2	Drill bit	×		
	8F		Paper point (13mm)	×		
	8G		Bone fragments	×		
9	9A	UL7	Biofilm	✓		
	9B	UR2	Drill bit	×		
	9C		Paper point (13mm)	×		
	9D		Bone fragments	×		
10	10A	UL7	Biofilm	✓	✓	
	10B	UR1[a.]	Paper point (14mm)	×		
	10C		Drill bit	×		
	10D		Paper point (17mm)	×		
	10E	UL7	Biofilm	✓	✓	
	10F	UR1	Drill bit	×		
	10G		Paper point	×		
	10H		Bone fragments	×		

[a.] Sample was taken 6 months after initial sampling of this patient

Table S1 (cont.)

Patient	Sample	Site	Description	PCR Amp.	TRFLP	454
11	11A	UL7	Biofilm	✓	✓	
	11B	UL3	Drill bit	✗		
	11C		Paper point (15mm)	✗		
	11D		Bone fragments	✗		
	11E	UR1	Paper point (17mm)	✗		
	11F		Drill bit	✗		
	11G		Paper point (20mm)	✗		
	11H	UL7	Biofilm	✓	✓	
	11I	UR1	Paper point	✗		
	11J		Drill bit	✗		
	11K		Bone fragments	✗		
12	12A	UL7	Biofilm	✓	✓	
	12B	UR1	Paper point	✗		
	12C		Drill bit	✗		
	12D		Paper point	✗		
	12E	UL7	Biofilm	✓	✓	
	12F	UR1	Paper point	✗		
	12G		Drill bit	✗		
	12H		Bone fragments	✗		
13	13A	UL7	Biofilm	✓		
	13B	UL3	Drill bit	✗		
	13C		Paper point	✗		
	13D		Bone fragments	✗		
	13E	UL5	Drill bit	✗		
	13F		Paper point	✗		
	13G		Bone fragments	✗		
14	14A	UL7	Biofilm	✓		
	14B	UR1	Drill bit	✗		
	14C		Paper point	✗		
	14D		Bone fragments	✗		
15	15A	UL7	Biofilm	✓		
	15B	LL5	Paper point	✓		
16	16A	UL7	Biofilm	✓		
	16B	LL6	Paper point	✗		
	16C		Drill bit	✗		
17	17A	UL7	Biofilm	✓	✓	
	17B	UL6	Drill bit	~		
	17C		Paper point	✗		
	17D		Paper point	✗		
	17E		Bone fragments	✗		
18	18A	UL7	Biofilm	✓	✓	
	18B	UL2	Paper point	✗		
	18C		Drill bit	✗		
	18D		Paper point	✗		
	18E	UL7	Biofilm	✓	✓	✓
	18F	UL2 [a.]	Paper point	✗		
	18G		Granuloma	✓	✓	✓
	18H		Bone fragments	✗		
	18I		Drill bit	✗		

[a.] Sample was taken 6 months after initial sampling of this patient

Table S1 (cont.)

Patient	Sample	Site	Description	PCR Amp.	TRFLP	454
19	19A	UL7	Biofilm	✓		
	19B	UR4	Drill bit	✖		
	19C		Paper point	✖		
	19D		Bone fragments	✖		
	19E	UR5	Drill bit	✖		
	19F		Paper point	✖		
	19G		Bone fragments	✖		
20	20A	UL7	Biofilm	✓		
	20B	LL6	Paper point	✖		
	20C		Drill bit	✖		
21	21A	UL7	Biofilm	✓	✓	
	21B	LL5	Drill bit	~		
	21C		Paper point	✖		
	21D		Bone fragments	~	✓	
	21E	LL6	Drill bit	~	✓	
	21F		Paper point	✖		
	21G		Bone filter	~		
	21H	LL7	Paper point	✓	✓	
22	22A	UL7	Biofilm	✓	✓	
	22B	LR6	Paper point	✖		
	22C		Paper point	~	✓	
	22D		Paper point	✖		
	22E		Paper point	✖		
23	23A	UL7	Biofilm	✓	✓	
	23B	LL6	Paper point	✓	✓	✓
24	24A	UL7	Biofilm	✓	✓	✓
	24B	UR4	Drill bit	~	✓	
	24C		Paper point	✓	✓	
	24D		Bone fragments	✖		
	24E	UR1	Drill bit	✖		
	24F		Paper point	✖		
25	25A	UL7	Biofilm	✓		
	25B	UL4	Drill bit	✖		
	25C		Paper point	✖		
	25D		Bone fragments	✖		
26	26A	UL7	Biofilm	✓		
	26B	UL1	Drill bit	✖		
	26C		Paper point	✖		
	26D		Bone fragments	✖		
27	27A	UL7	Biofilm	✓		
	27B	UR5	Paper point	✖		
28	28A	UL7	Biofilm	✓		
	28B	LL6	Paper point	✖		
	28C		Paper point	✖		

Table S1 (cont.)

Patient	Sample	Site	Description	PCR Amp.	TRFLP	454
29	29A	UL7	Biofilm	✓	✓	
	29B	UR4	Drill bit	✗		
	29C		Paper point	✗		
	29D		Bone fragments	✗		
	29E	UR2	Drill bit	✗		
	29F		Paper point	✗		
	29G	UL1	Drill bit	✗		
	29H		Paper point	✗		
30	30A	UL7	Biofilm	✓		
	30B	UR4	Drill bit	✗		
	30C		Paper point	✗		
	30D		Bone fragments	✗		
31	31A	UL7	Biofilm	✓	✓	
	31B	LR6	Drill bit	✗		
	31C		Paper point	✗		
	31D		Bone fragments	✗		
	31E	LL6	Drill bit	✗		
	31F		Paper point	✗		
	31G	LL7	Drill bit	✗		
	31H		Paper point	✗		
32	32A	UL7	Biofilm	✓	✓	
	32B	UR2	Drill bit	✗		
	32C		Paper point	✗		
	32D		Bone fragments	✗		
33	33A	UL7	Biofilm	✓		
	33B	UR4	Drill	✗		
	33C		Paper point	✗		
	33D		Bone fragments	✗		
	33E	UL4	Drill bit	✗		
	33F		Paper point	✗		
	33G		Bone fragments	✗		
34	34A	UL7	Biofilm	✓		
	34B	UL2	Paper point	✗		
	34C		Drill bit	✗		
	35A	UL7	Biofilm	✓	✓	✓
35[1]	35B	LL6	Paper point	~	✓	✓
	35C		Failed implant	✓	✓	✓

[1] Patient 35 presented with a long term edentulous site. Following diseased tooth removal, the site was curetted. Radiographs indicated the site had apparently healed. The implant was a two stage procedure with 4 month submergence of a titanium 5x7mm WP Branemark MK 111 TiUnite (non-gradational, 10-15 microns microrough) implant. Patient 35 presented with bone loss 8 months after prosthetic connection.

Table S1 (cont.)

Patient	Sample	Site	Description	PCR Amp.	TRFLP	454
36	36A	UL7	Biofilm	✓	✓	
	36B	LR6	Drill bit	×		
	36C		Paper point	×		
	36D	LR4	Drill bit	×		
	36E		Paper point	×		
37	37A	UL7	Biofilm	✓		
	37B	UR2	Drill bit	×		
	37C		Paper point	×		
	37D		Bone fragments	×		
	37E	UR1	Drill bit	×		
	37F		Paper point	×		
	37G	UL1	Drill bit	×		
	37H		Paper point	×		
	37I	UL4	Drill bit	×		
	37J		Paper point	×		
	37K	UL5	Drill bit	×		
	37L		Paper point	×		
	37M	UR5	Drill bit	×		
	37N		Paper point	×		
38	38A	UL7	Biofilm	✓		
	38B	UL1	Drill bit	×		
	38C		Paper point	×		
	38D		Bone fragments	×		
39	39A	UL7	Biofilm	✓	✓	
	39B	LL7	Paper point	✓	✓	
	39C		Paper point	×		
40	40A	UL7	Biofilm	✓	✓	
	40B	UR3	Paper point	✓	✓	
	40C	UL1	Paper point	~	✓	
	40D	UL3	Paper point	~	✓	

Table S2: 16S rRNA pyrosequencing quality control filters. Each column indicates the sequences remaining after applying filtering criteria.

Sample	Raw Sequences	Quality Trimming	Alignment	Chimera Removal
2A	4052	2398	2260	2258
6A	7212	4137	3724	3660
18E	7524	3959	3458	3404
18G	2297	1391	1210	1189
23B	684	444	428	428
24A	8072	4213	3582	3546
35A	8087	4799	4333	4298
35C	6283	3848	3573	3562
Total	44211	25189	22568	22345
Sequences Removed		19022 (43%)	2621 (10%)	221 (1%)

Table S3: Summary of samples, sequences and OTUs

Sample	Description	Sequences	OTUs (97% similarity)
2A	Granuloma	2258	72
6A	Infected tooth	3660	173
18E	Biofilm	3404	129
18G	Granuloma	1189	51
23B	Paper point	428	17
24A	Biofilm	3546	88
35A	Biofilm	4298	115
35C	Failed implant	3562	85
Total	**Unique**	2464	373
	Health-associated	11248	216 (±12)
	Disease-associated	11097	278 (±26)

Table S4: Diversity (sequences and OTUs) of reference biofilm (**18E**) and diseased granulomatous tissue (**18G**) from the same patient. Percentages next to samples in brackets represent sequences or OTUs that are shared.

Threshold	**Metric**	**18E (n = 3404)**	**Shared**	**18G (n = 1189)**	**Total OTUs**
97%	**Sequences**	1535 (45.1%)	2995 (65.2%)	63 (5.3%)	
	OTUs	171 (75.4%)	42 (30.4%)	93 (54.8%)	138

Table S5: Diversity (sequences and OTUs) of reference biofilm (**35A**) and disease associated (failed implant, **35C**) samples from the same patient. Percentages next to samples in brackets represent sequences or OTUs that are shared.

Threshold	**Metric**	**35A (n = 4298)**	**Shared**	**35C (n = 3562)**	**Total OTUs**
97%	**Sequences**	2202 (51.2%)	2550 (32.4%)	3108 (87.3%)	
	OTUs	157 (73.2%)	42 (30.4%)	127 (66.9%)	186

Table S6: Bacterial taxa (>0.5% relative abundance) in (diseased) granulomas (**2A**, **18G**) from two different patients[1,2]

	Abundance (%)	
Taxonomic Assignment	**2A**	**18G**
Fusobacteria Fusobacteriaceae *Fusobacterium*	69.4	33.4
Firmicutes Veillonellaceae *Veillonella*	2.5	20.7
Bacteroidetes Prevotellaceae *Prevotella*	3.8	12.6
Firmicutes Veillonellaceae *Selenomonas*	1.0	8.8
Actinobacteria Actinomycetaceae *Actinomyces*	1.7	8.2
Synergistes Dethiosulfovibrionaceae *TG5*	5.4	0.0
Firmicutes Streptococcaceae *Streptococcus*	3.2	3.4
Actinobacteria Coriobacteriaceae *Atopobium*	0.0	2.9
Firmicutes Peptostreptococcaceae *Filifactor*	2.5	0.0
Spirochaetes Spirochaetaceae *Treponema*	2.5	0.0
Firmicutes Lactobacillaceae *Lactobacillus*	1.9	0.0
Proteobacteria Campylobacteraceae *Campylobacter*	1.7	6.0
Bacteroidetes Flavobacteriaceae *Capnocytophaga*	0.0	0.7
Firmicutes Clostridiaceae *Mogibacterium*	0.7	0.0
TM7 unclassified	0.1	0.8
Tenericutes Erysipelotrichaceae *Bulleidia*	0.0	0.5
Unclassified	1.6	0.4

[1] Only OTUs of >0.5% relative abundance in at least one of the two sample groups are shown.

[2] Sequences are arranged from highest to lowest abundance in the combined data set.

Table S7: Bacterial taxa (>0.5% relative abundance) from an infected tooth (**6A**)[1].

	Abundance (%)
Taxonomic Assignment	**6A**
Actinobacteria Actinomycetaceae *Actinomyces*	5.2
Actinobacteria Corynebacteriaceae *Corynebacterium*	6.7
Actinobacteria Micrococcaceae *Rothia*	0.7
Bacteroidetes Porphyromonadaceae	0.6
Bacteroidetes Prevotellaceae *Prevotella*	0.5
Bacteroidetes Flavobacteriaceae *Bergeyella*	0.6
Bacteroidetes Flavobacteriaceae *Capnocytophaga*	11.0
Firmicutes Streptococcaceae *Streptococcus*	3.7
Firmicutes Veillonellaceae	0.5
Fusobacteria Fusobacteriaceae *Fusobacterium*	13.6
Fusobacteria Fusobacteriaceae *Leptotrichia*	2.0
Proteobacteria Burkholderiaceae *Lautropia*	21.1
Proteobacteria Comamonadaceae *Hylemonella*	1.4
Proteobacteria Neisseriaceae *Bergeriella*	1.4
Proteobacteria Neisseriaceae *Eikenella*	1.4
Proteobacteria Neisseriaceae *Kingella*	0.6
Proteobacteria Neisseriaceae *Neisseria*	12.0
Proteobacteria Campylobacteraceae *Campylobacter*	2.2
Proteobacteria Cardiobacteriaceae *Cardiobacterium*	1.7
Proteobacteria Pasteurellaceae *Aggregatibacter*	3.8
Proteobacteria Pasteurellaceae *Haemophilus*	3.9
Proteobacteria Rhodocyclaceae *Rhodocyclus*	0.6
SR1 unclassified	0.7
TM7 EW055 unclassified	1.1

[1] Only OTUs of >0.5% relative abundance of sample 6A are shown.

Table S8: Bacterial taxa (>0.5% relative abundance) from a paper point sample of a tooth extraction socket (**23B**)[1].

	Abundance (%)
Taxonomic Assignment	**23B**
Actinobacteria Propionibacteriaceae *Propionibacterium*	1.2
Bacteroidetes Flavobacteriaceae *Capnocytophaga*	1.6
Firmicutes Streptococcaceae *Streptococcus*	12.9
Fusobacteria Fusobacteriaceae *Fusobacterium*	65.9
Proteobacteria Campylobacteraceae *Campylobacter*	1.9
Spirochaetes Spirochaetaceae *Treponema*	3.0
Unclassified	2.3

[1.] Only OTUs of >0.5% relative abundance of sample 23B are shown.

Table S9: Bacterial taxa (>0.5% relative abundance) in a biofilm sample from a reference biofilm tooth (**24A**).

	Abundance (%)
Taxonomic Assignment	**24A**
Actinobacteria Actinomycetaceae *Actinomyces*	54.7
Actinobacteria Corynebacteriaceae Corynebacterium	21.5
Actinobacteria Micrococcaceae *Rothia*	0.5
Bacteroidetes Flavobacteriaceae *Capnocytophaga*	3.6
Firmicutes Streptococcaceae *Streptococcus*	2.3
Firmicutes Veillonellaceae *Selenomonas*	0.6
Firmicutes Veillonellaceae *Veillonella*	4.0
Fusobacteria Fusobacteriaceae *Fusobacterium*	0.6
Fusobacteria Fusobacteriaceae *Leptotrichia*	3.2
Proteobacteria Burkholderiaceae *Lautropia*	2.8
Proteobacteria Neisseriaceae *Kingella*	0.9
Proteobacteria Campylobacteraceae *Campylobacter*	2.3
Proteobacteria Pasteurellaceae *Haemophilus*	1.9

[1.] Only OTUs of >0.5% relative abundance of sample 24A are shown.

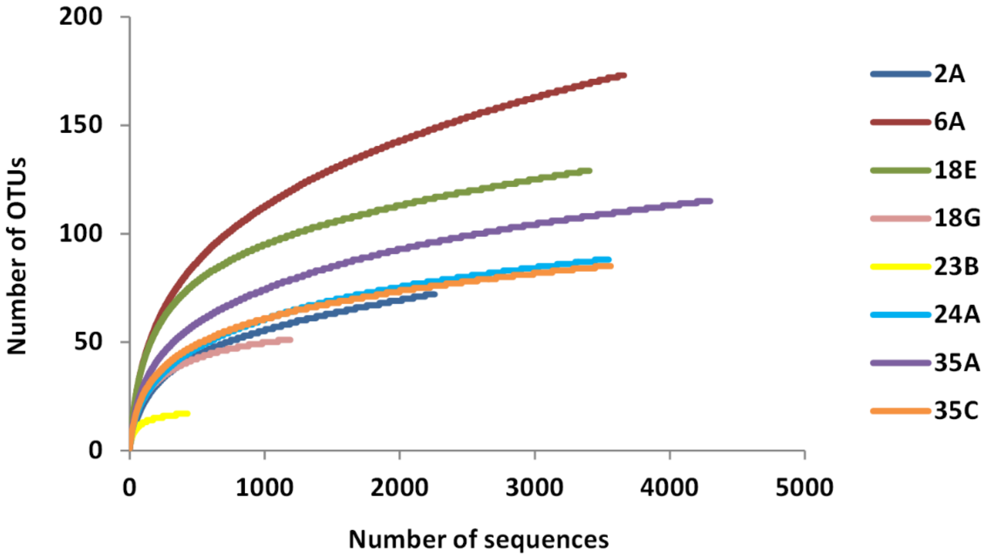

Figure S1: Rarefaction plots of reference biofilm and disease-associated 16S rRNA pyrosequences in different samples (at 97% sequence similarity).
See Table S1 for sample descriptions.