

Complex Intratissue Microbiota Forms Biofilms in Periodontal Lesions

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Appendix

Appendix Methods

Real-time PCR

Real-time PCR was performed in a 20 µl reaction mix containing 2 µl of bacterial genomic DNA, SYBR *Premix Ex Taq*, ROX Reference Dye II (Takara Bio, Otsu, Japan), and each primer. Universal (forward: 5'-AGTCACTGACGAGTTTGATCMTGGCTCAG-3' and reverse: 5'-CAGTGACTACWTTACCGCGGCTGCTGG-3') and *P. gingivalis*-specific primers (Baek et al. 2013) targeting the bacterial 16S rRNA gene were used. *P. gingivalis* ATCC 33277 genomic DNA was used to generate standard curves.

Appendix Reference

Baek KJ, Choi Y, Ji S. 2013. Gingival fibroblasts from periodontitis patients exhibit inflammatory characteristics in vitro. *Arch Oral Biol.* 58(10):1282–1292

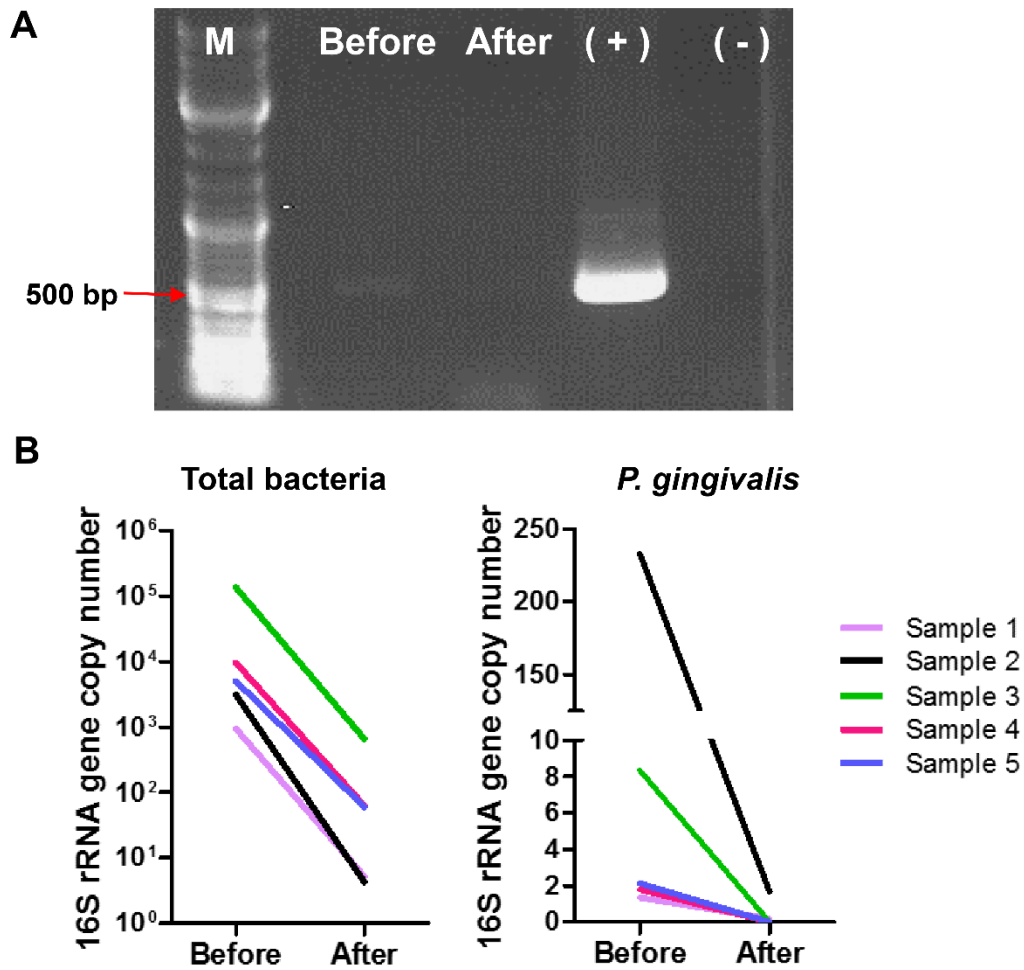
Appendix Table. The demographic and clinical characteristics of subjects

Clinical characteristics	P1	P2	P3	P4	P5	P6	P7	Mean
Age (years)	53	60	55	49	73	75	53	59.0±10.3
Gender	M	F	F	M	M	M	M	
Smoking	C	N	N	C	N	N	C	
No. of total teeth	26	27	26	25	25	28	28	26.4 ± 1.3
Marginal bone loss (%)^{a, b}	21.6 ± 18.4	28.5 ± 11.5	35.5 ± 17.6	22 ± 11.3	42.9 ± 16.2	27 ± 15.6	28.8 ± 13.6	29.5 ± 16.4
Bacteria-sampled site								
PD (mm)	7	5	8	5	8	8	7	6.9±1.0
CAL (mm)	9	6	11	7	9	10	8	8.6±1.7
Tissue-sampled sites for pyrosequencing								
PD (mm) ^a	5.0 ± 1.2	6.7 ± 0.9	8.0 ± 0.6	5.0 ± 0.0	7.7 ± 0.3	9.3 ± 0.7	5.3 ± 0.9	6.8 ± 0.4
CAL (mm) ^a	7.0 ± 1.2	7.7 ± 0.9	11.0 ± 0.6	7.0 ± 0.0	8.7 ± 0.3	11.0 ± 0.7	6.3 ± 0.9	8.5 ± 0.5
Tissue-sampled sites for histology								
PD (mm) ^a	4.0 ± 0.6	5.3 ± 0.3	No sample	5.0 ± 0.0	7.7 ± 0.3	6.3 ± 0.7	8.3 ± 1.7	6.4 ± 0.4
CAL (mm) ^a	5.0 ± 0.6	6.3 ± 0.3	No sample	6.0 ± 0.0	8.7 ± 0.3	7.3 ± 0.7	9.3 ± 1.7	7.7 ± 0.5

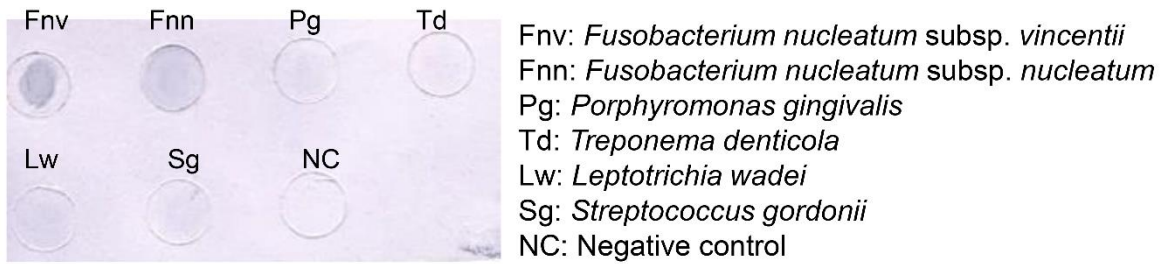
C: current smoker; N: never smoker

^aExpressed as mean ± SD

^bRepresents full-mouth data obtained from panoramic radiographs taken before scaling and root debridement.

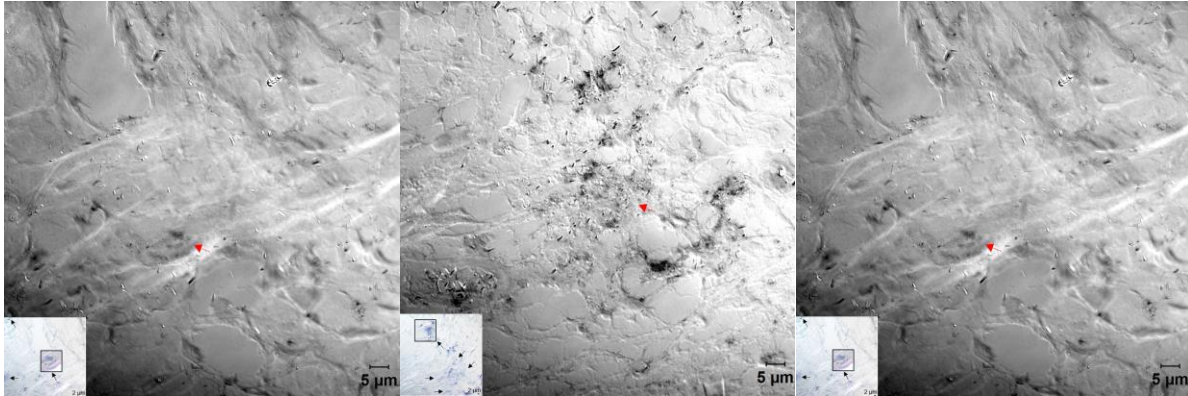


Appendix Figure 1. Efficiency of treatment with lysozyme and DNase I. (A) Bacterial genomic DNA was extracted from wash solutions of before and after DNase I treatment, and the bacterial 16S rRNA gene was amplified by PCR. Genomic DNA from *P. gingivalis* was used as a positive control. (B) The 16S rRNA gene copies of total bacteria and *P. gingivalis* were estimated by Real-time PCR using additional plaque samples, treatment with lysozyme and DNase I.

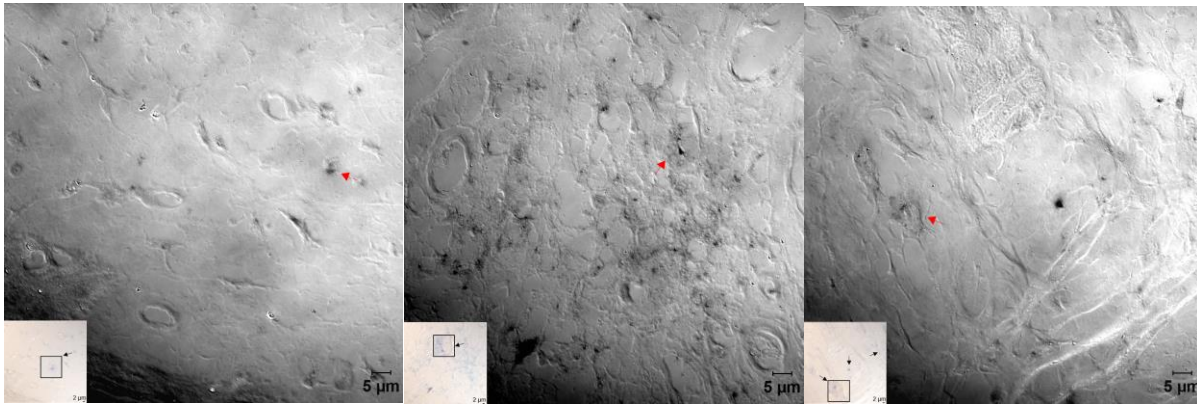


Appendix Figure 2. Specificity test of *F. nucleatum*-specific probe. The specificity of digoxigenin-labeled probe for *F.uncleatum* was confirmed by dot blotting using six bacterial genomic DNA.

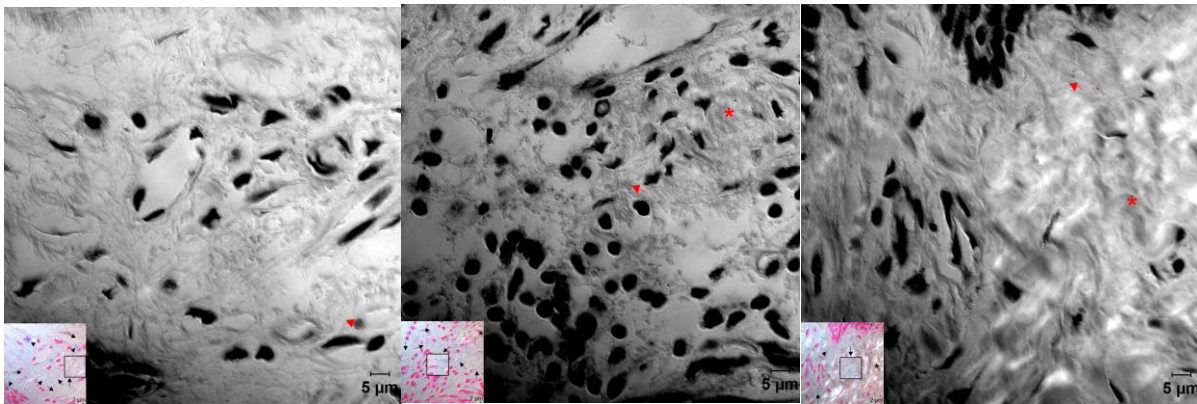
A



B



C



Appendix Figure 3. Images with z-stack. The areas a, b, and c shown in Figure 3B – D were examined by confocal microscopy with z-stack, then z-stack animation has been produced in GIF format.