

Supplementary material

Dynamic analysis of *Porphyromonas gingivalis* invasion into blood capillaries during infection process in host tissue using a vascularized three-dimensional human gingival model

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Supplementary Table S1. Primers and probe list

Primer	Sequence
Primers for cell junctions and basement membrane molecules	
<i>ZO-1</i> Fw	CGAGTTGCAATGGTTAACGGA
<i>ZO-1</i> Rv	TCAGGATCAGGACGACTTACTGG
<i>CLAUDIN-1</i> Fw	ATGAGGATGGCTGTCATTGG
<i>CLAUDIN-1</i> Rv	ATTGACTGGGGTCATAGGGT
<i>CLAUDIN-4</i> Fw	TTGTACCTCGCAGACCATC
<i>CLAUDIN-4</i> Rv	CAGCGAGTCGTACACCTTG
<i>E-CADHERIN</i> Fw	CGAGAGCTACACGTTACGG
<i>E-CADHERIN</i> Rv	GGGTGTCGAGGGAAAAATAGG
<i>COLLAGEN IV</i> Fw	ACAGCCAGACCATTTCAGATC
<i>COLLAGEN IV</i> Rv	GCTGTAAGCGTTTGCGTAGT
MVK Fw	AAGGTAGCACTGGCTGTATCC
MVK RV	CCAATGTTGGGTAAGCTGAGG
Primers for <i>P. gingivalis</i>	
16S rRNA Fw	GCGCTCAACGTTTCAGCC
16S rRNA Rv	CACGAATTCCGCCTGC
16S rRNA probe	CACTGAACTCAAGCCCGGCAGTTTCAA

Supplementary Figures

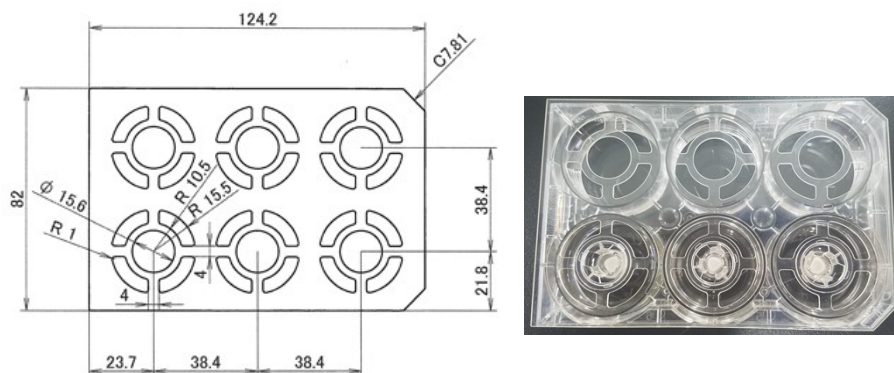


Figure S1. Production the 6 well plate adaptor. (A) Illustration of the home-made 6 well plate adaptor. (B) Image of the insert set in the adaptor.

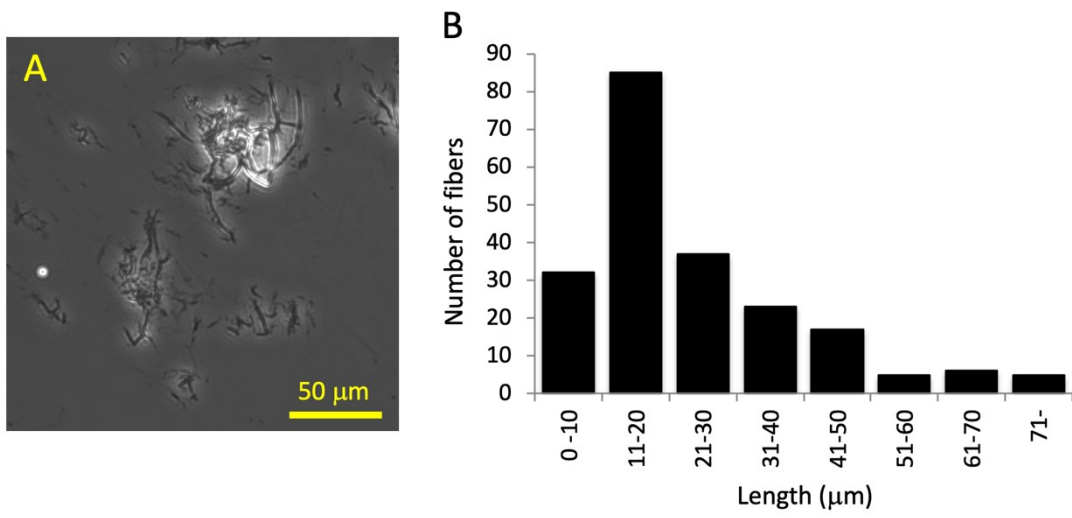


Figure S2. Morphology of collagen microfibers. (A) Photograph of CMFs in medium
(B) Size of CMFs after suspension in medium

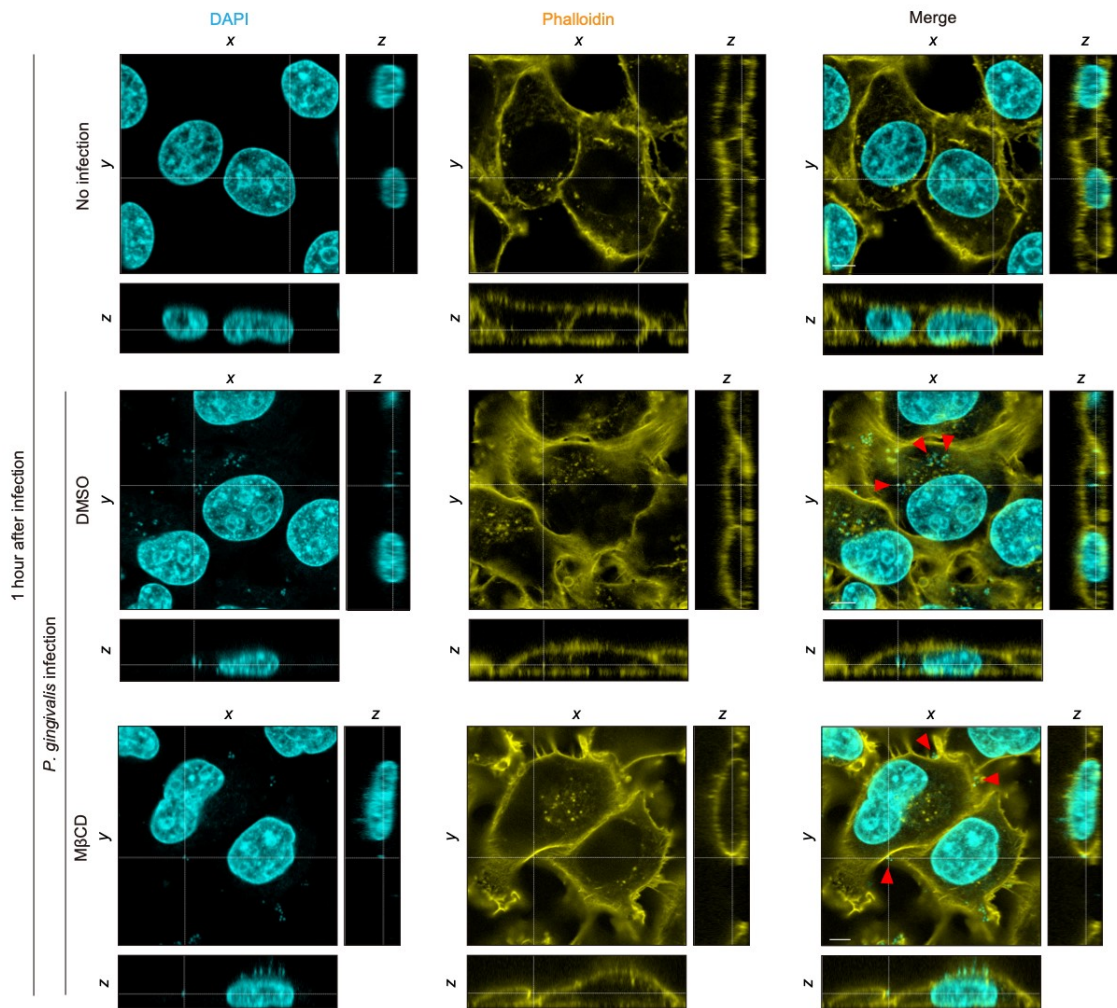


Figure S3. M β CD inhibits *P. gingivalis* internalization by gingival epithelial cells. Immortalized human gingival epithelial (IHGE) cells were treated with DMSO (Mock) or 1 mM M β CD in DMSO. At 30 min after treatment, IHGE cells were infected with *P. gingivalis* ATCC 33277 at an MOI of 100 with DMSO or 1 mM M β CD in DMSO for 1 hr, fixed, stained with DAPI (cyan) and Alexa Fluor 568 Phalloidin (yellow), and analyzed by confocal microscopy. The highlight *P. gingivalis* were indicated by red arrow heads. Scale bars, 5 μ m. See also Figure S4.

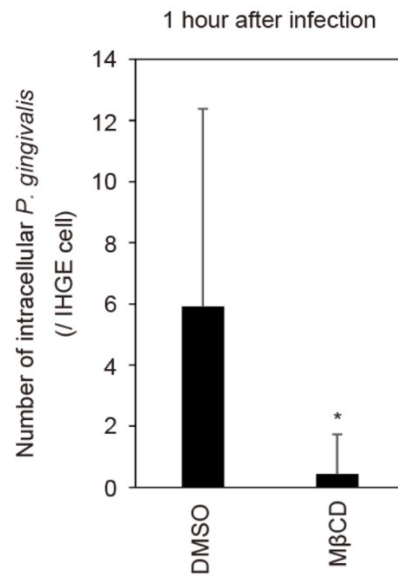


Figure S4. Quantification of *P. gingivalis* internalization by IHGE cells in Figure S3. Each bar represents the number of intracellular *P. gingivalis* organisms in a single IHGE cell, which was analysed using projection images. Bacteria located on the inside at a distance of 1 μm from the outermost phalloidin-stained actin filament were manually counted. DMSO: n = 57; M β CD: n = 61. * $p < 0.05$, two-tailed t -test.

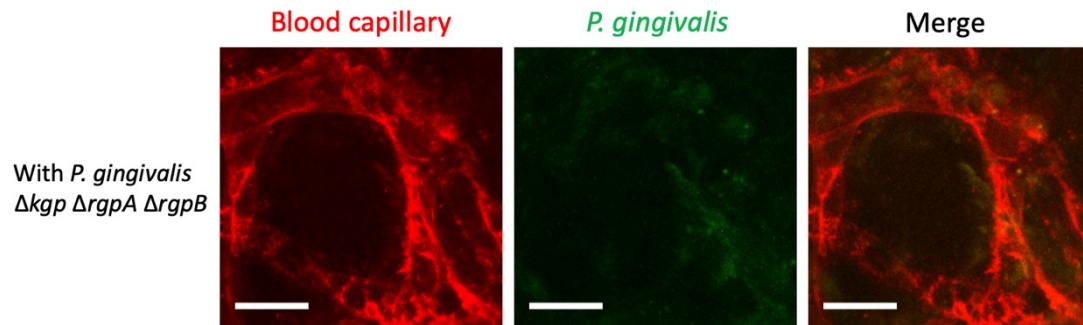


Figure S5. Invasion of *P. gingivalis* $\Delta kgp \Delta rgpA \Delta rgpB$ into the 3D gingival model. The degree of invasion of *P. gingivalis* $\Delta kgp \Delta rgpA \Delta rgpB$ into the blood capillaries less than WT was observed by confocal laser microscopy. Scale bars are 20 μm . See also Fig. 4C.

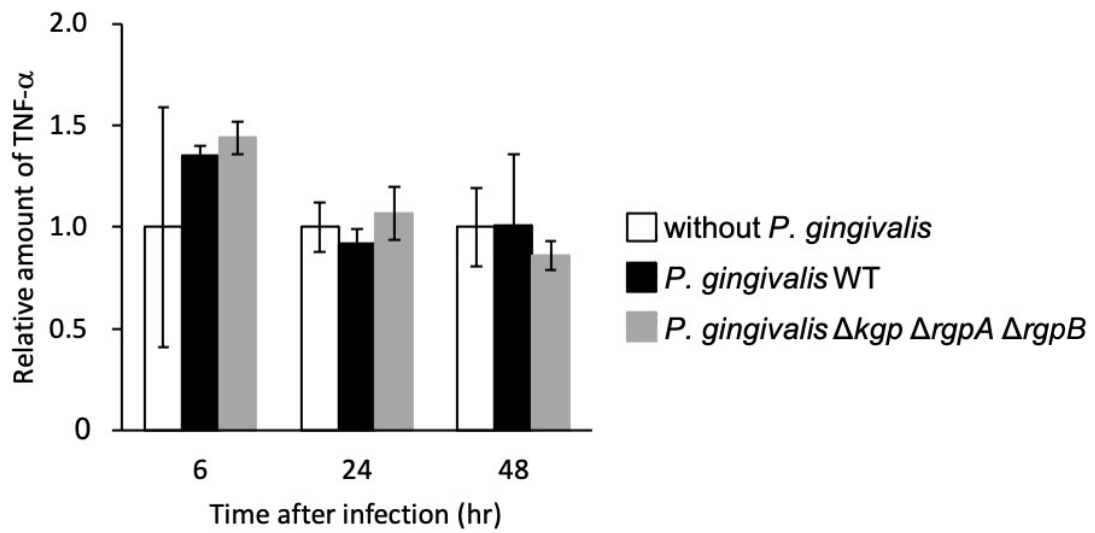


Figure S6. Effect of *P. gingivalis* on TNF- α of 3D gingival models.

TNF- α from the 3D gingival models with or without wild and deficient *P. gingivalis* after 6, 24 and 48 hr treatment were detected by ELISA. Each column represents the mean value for three independent tissues, and the error bar represents its standard deviation.