***Research Article***

***Porphyromonas gingivalis* gingipains-mediated degradation of plasminogen activator inhibitor-1 leads to delayed wound healing responses in human endothelial cells**

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***Online Figure Legend***

**Online suppl. Fig. 1.** Amidolytic activities of gingipains in lyophilized whole cells or OMVs of *P. gingivalis.* Lyophilized whole cells of *P. gingivalis* W83 (25 mg/mL) were pretreated with 1 mM KYT-1 (Rgps inhibitor) (**c**) or 1 mM KYT-36 (Kgp inhibitor) (**d**) for 15 min at 37°C. **a**-**d** The indicated concentrations (**a**, **b**) or 25 mg/mL (**c**, **d**) of *P. gingivalis* W83 lyophilized whole cells, or the indicated concentrations of *P. gingivalis* W83 OMVs (**e**, **f**) were assayed at 37°C with 0.5-mM BA-pNA (Rgp substrate) (**a**, **c**, **e**) or 0.5-mM HEK-MCA (Kgp substrate) (**b**, **d**, **f**) for 1 h. Results are expressed based on the absorbance of *p*-nitroaniline (**a**, **c**, **e**) or 7-amino-methylcoumarin (**b**, **d**, **f**) released from the substrate, respectively. The data are representative of three independent experiments.

**Online suppl. Fig. 2.** Viability of HUVECs after infection with *P. gingivalis*, HUVECs were infected with the indicated MOI of *P. gingivalis* W83 for 24 h. **b**, **c** HUVECs were stimulated with the indicated MOI of live bacteria (**a**) or with the indicated concentrations of lyophilized whole cells (**b**) or OMVs (**c**) of *P. gingivalis* W83. The luminescence of the NanoLuc substrate was measured using a luminometer. Cell viability was expressed as the percentage of viable cells compared to that of the untreated control. Data are representative of three independent experiments and are shown as the mean ± SD. Statistically, significant differences are indicated as follows: \*\*, *p* < 0.01, compared with the respective untreated control.

**Online suppl. Fig. 3.** Role of mitomycin C in wound healing of endothelial cells **a**, **b** HUVECs in a cell monolayer were scratched, and cell migration after the injury was assessed by measuring the scratched area of the cells. HUVECs were treated with 10 mg/mL mitomycin C for the indicated times (**a**, **b**) or 24 h (**c**). **a** The images show one of the results of three independent experiments. The lines indicate the margins of the scratched area. **b** Percentage of closure of the scratched area. **c** Effect of mitomycin C treatment on HUVEC proliferation. Twenty-four hours after treatment with mitomycin C, HUVEC proliferation was measured using CCK-8. Data are representative of three independent experiments and are shown as the mean ± SD. Statistically significant differences are indicated as follows: \*\*, *p* < 0.01; \*, *p* < 0.05, compared with the respective untreated control.

**Online suppl. Fig. 4.** The role of PAI-1 in wound healing of endothelial cells HUVECs grown in monolayer were scratched, and cell migration after injury was assessed by measuring the scratched area of the cells. A detailed explanation is provided in the legend of Fig. 4. Effect of PAI-1 mutant (**a**, **b**), PAI-039 (**c**, **d**), or PAI-1 mutant plus RAP (**e**) on the closure of the scratch (**a**, **c**, **e**) or the proliferation of HUVECs (**b**, **d**). **a**, **c**, **e** The images show one of the results of three independent experiments. The lines indicate the margins of the scratched areas. **b**, **d** Twenty-four hours after the treatment, the proliferation of HUVECs was measured using CCK-8. Data are representative of three independent experiments and are shown as the mean ± SD.

**Online suppl. Fig. 5.** *P. gingivalis* induces a delay in the wound healing of endothelial cells in a PAI-1-dependent manner. HUVECs in a cell monolayer were scratched, and cell migration after injury was assessed by measuring the scratched area of the cells. A detailed explanation is provided in the legend of Fig. 5. Effect of stimulation with live bacteria or lyophilized whole cells of *P. gingivalis* W83 (**a**, **b**), or lyophilized whole cells of *P. gingivalis* W83 or ATCC 33277 ± rhPAI-1 mutant on the closure of the scratch (**a**, **c**) or the proliferation of HUVECs (**b**). **a**, **c** Images show the results of one of the three independent experiments. The lines indicate the margins of the scratched areas. **b** Twenty-four hours after treatment, HUVEC proliferation was measured using CCK-8. Data are representative of three independent experiments and are shown as the mean ± SD. Statistically significant differences are indicated as follows: \*\*, *p* < 0.01 compared with the respective untreated control.

**Online suppl. Fig. 6.** Kgp preferentially induces a delay in wound healing in endothelial cells. HUVECs in a cell monolayer were scratched, and cell migration after injury was assessed by measuring the scratched area of the cells. A detailed explanation is provided in the legend of Fig. 6. The effect of the stimulation of lyophilized whole cells of *P. gingivalis* ATCC 33277, KDP136, or heat-pretreated *P. gingivalis* ATCC 33277 (**a**, **b**), purified Kgp (**c**), purified RgpA (**d**) on the closure of the scratch (**a**, **c**, **d**) or the proliferation of HUVECs (**b**). **a**, **c**, **d** The images show the results of one of the three independent experiments. The lines indicate the margins of the scratched areas. **b** Twenty-four hours after treatment, HUVEC proliferation was measured using CCK-8. Data are representative of three independent experiments and are shown as the mean ± SD.