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Short communication

Antibacterial activity of povidone–iodine against an artificial biofilm of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*

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ABSTRACT

Objective: To investigate the antibacterial activity of povidone–iodine (PVP–I) on an artificial dual species biofilm of periodontal pathogens.
Design: *Porphyromonas gingivalis* or *Fusobacterium nucleatum* grown in broth culture was inoculated on polycarbonate membrane (PCM) tissue culture inserts. After incubation for 72 h, PVP–I solutions were applied to the biofilm for the time period ranging from 0.5 to 5 min. After addition of a deactivator, each PCM was removed and the biofilm on the PCM was serially diluted and plated on blood agar plates and cultured anaerobically for 7 days. Then viable bacteria were enumerated.
Results: In the dual species biofilm model, *F. nucleatum* showed an approximately 200-fold increase in viable counts when compared with mono-microbial biofilm. In dual species biofilm, PVP–I with concentration equal to or greater than 2% was required to significantly reduce *P. gingivalis* and *F. nucleatum*. When the contact time of PVP–I was increased to 1 min or greater, no difference in antibacterial activity of PVP–I was observed in any concentration.
Conclusion: These results suggest that 30 s application of 2% PVP–I would be effective in suppressing both *P. gingivalis* and *F. nucleatum* in dual-species biofilm, and this provides clinical implication for the control of subgingival biofilm.

1. Introduction

More than 400 bacterial species have been recorded in samples taken from the human gingival crevices.[1] Dental plaque is a biofilm consisting of poly-species of oral bacteria and their metabolic products. Periodontal disease is a plaque biofilm-induced inflammatory disease of the supporting tissue of the tooth and includes both gingivitis and periodontitis,[2] and can be described as one of the predominant polymicrobial infections of humans.[3] Periodontal pathogens such as *Fusobacterium nucleatum* initially adhere to 'early colonizers' including gram-positive cocci, and enhance the adherence of 'late colonizers' such as *Porphyromonas gingivalis* and

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Treponema denticola.[4,5] Mechanical methods of oral hygiene, brushing and flossing, are considered by clinicians as the gold standard methods of plaque (biofilm) control. For the treatment of periodontitis, mechanical intervention such as scaling and root planing is necessary in order to effectively disrupt biofilm in the subgingival milieu. In the meanwhile, there is a growing interest in using antimicrobial agents as an adjunct therapy.[6]

In a previous study, we tested the antibacterial activity of several antibiotics against periodontopathic bacteria, and found that a wide variation existed in the susceptibility of monomicrobial cultures to a given antibiotic.[7] In order to be effective against biofilm, the concentration of a typical antibiotic has to be approximately 100 times higher than that needed for planktonic state,[8,9] supporting the need for mechanical intervention in periodontal therapy. Given the recent growing importance for a provision of periodontal care to systemically compromised patients, it is sometimes difficult to implement a meticulous subgingival debridement. Therefore, there is a need for the better use of periodontal chemotherapy.

Povidone–iodine (PVP–I) has been used as an oral rinse in patient home care.[10] PVP–I, which possesses an efficient and broad-spectrum microbicidal property, has also demonstrated an anti-virus effect.[11] Despite long-term use, development of PVP–I resistance in microorganisms has not been reported.[12,13] Although the effect of PVP–I on biofilms other than those in the oral cavity has been reported,[14,15] information regarding its effect on biofilms organized with periodontal pathogens is limited.

This study is part of our ongoing effort to develop an effective antimicrobial regiment against periodontopathic biofilm. A prototype model of dual-species biofilm of *P. gingivalis* and *F. nucleatum* was used to evaluate the antibacterial activity of PVP–I and to propose an optimal condition for its use in periodontal treatment.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The following bacterial strains were used; *P. gingivalis* ATCC 33277 (American Type Culture Collection, Rockville, MD, USA), *F. nucleatum* #20 (a clinical isolate and working strain in our laboratory). The *P. gingivalis and F. nucleatum* were grown in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) supplemented with hemin (5 μg/ml) and menadione (0.5 μg/ml). The bacterial cultures were grown to mid-log phase (range at OD 660 nm of 0.8–1.0) at 37 °C under anaerobic conditions.

2.2. Evaluation of biofilm forming activity

A 150 μl aliquot of TSB was added to each well of a 24-well tissue culture-treated polystyrene plate. For the evaluation of biofilm forming activity, a polycarbonate membrane (PCM) insert (Transwell, No. 3413, Corning Life Sciences, Acton, MA, USA) was placed into each well. For the mono-microbial biofilm formation, 50 μl of *P. gingivalis* or *F. nucleatum* was inoculated onto the insert. For the establishment of dual-species biofilm, *P. gingivalis* and *F. nucleatum* (25 μl each) were added onto the insert. The plates were incubated anaerobically for 72 h at 37 °C. *P. gingivalis* and *F. nucleatum* were in stationary phase at 72 h.

2.3. Effect of PVP–I on the mono- and poly-bacterial biofilm

Biofilms were formed as described above. After incubation, the inserts were washed twice in sterile phosphate-buffered saline (PBS, pH 7.4) to remove nonadherent cells. Then PVP–I (Meiji Seika Kaisha, Tokyo, Japan) solutions with varied concentration (0.23–7%, 50 μl each) were applied to the biofilm for the time period ranging from 0.5 to 5 min. A negative control (PBS) only was included in each assay. After addition of a deactivator containing 10% Tween 80, 3% lecithin and 0.5% sodium thiosulfate[16] (50 μl), wells were washed once with PBS. Each PCM was then excised using a surgical blade (No. 15), placed into a tube containing 1 ml of sterile PBS, and mixed thoroughly at the highest setting on a Vortex mixer for 60 s. The mixtures were serially diluted and plated on blood agar plates supplemented with hemin and menadione, and incubated anaerobically at 37 °C for 7 days. Colony-forming units of recovered organisms were then enumerated.

2.4. Statistical analysis

All experiments were performed in duplicate or triplicate for each condition and repeated at least three times. Kruskal–

Table 1 – Effect of PVP–I contact time and concentration on *P. gingivalis* mono-biofilm.

Contact time	PVP–I concentration							
	Control	0.23%	0.47%	1%	2%	3.5%	5%	7%
30 s	3487.1 (1956.0)	1063.3 (1069.3)	2359.5 (2428.3)	125.9** (108.0)	430.5* (383.1)	67.7** (38.6)	6.4** (7.7)	2.3** (4.4)
1 min	2516.1 (1469.2)	450.0* (369.7)	2408.8 (512.7)	675.0 (512.7)	325.7** (296.1)	260.3* (210.0)	28.4** (38.9)	3.8** (7.5)
3 min	2480.9 (1447.2)	509.0* (268.5)	972.2 (85.0)	224.8** (106.2)	140.0** (23.1)	25.2** (29.1)	0.03** (0.1)	0.0** (0.0)
5 min	2268.4 (1737.7)	370.8 (95.4)	589.2 (874.5)	169.6* (43.8)	53.3** (48.5)	43.0** (54.0)	33.3** (57.7)	0.0** (0.0)

Values are the mean CFU (standard deviations) $\times 10^4$ of triplicate independent determinations from a typical experiment.
Significantly different from control, $^*p < 0.05$, $^{**}p < 0.01$, Kruskal–Wallis test with Dunn post test.