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研究題目：Development of Periodontal Topical Medication Using Ionic Liquids

目　的：

Eliminating the subgingival pathogen, especially biofilm, is critical in the periodontal treatment. In the previous study, Choline Geranate-Ionic Liquid (CAGE) showed promising effects as a topical ointment against periodontitis with self-penetration into the deep periodontal pockets and gingiva. However, limited efficacy against pathobiont has been studied. In this study, the details of the antibacterial and anti-biofilm activities were assessed.

対象および方法：

CAGE was synthesized by Wakayama Medical University. Two strains of *Porphyromonas gingivalis* (ATCC33277/W83), *Fusobacterium nucleatum* ATCC25586, *Prevotella intermedia* ATCC25611, and *Streptococcus mitis* ATCC903 were chosen to use. Antibacterial efficacy was tested by measuring minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The cell membrane disruption by CAGE was confirmed by transmission electron microscope (TEM) imaging and measuring propidium iodide (PI) uptake. Mature multi-species biofilms were built by 3 days of incubation under anaerobic conditions. The bactericidal effect against the pathobiont in the biofilm was assessed by live/dead BacLight viability staining (Thermo Fisher Scientific Inc., Waltham, MA, USA) and was observed by confocal laser scanning microscopy (CLSM). Change in biofilm biomass was assessed by crystal violet (CV) staining and was observed by scanning electron microscopy (SEM). The penetration of CAGE into the mature multi- species biofilm was confirmed by CLSM imaging. The data are expressed as the means ± standard errors of the mean. Mann-Whitney U test for two groups, Kruskal-Wallis followed by Dunn's test for multiple groups, which were performed with GraphPad Prism 8.3 graphing and statistical software, $^{*}P < 0.05$ was considered statistically significant.

結果および考察：

The antibacterial efficacy against representative planktonic bacteria of CAGE was shown in Table1. The MICs and MBCs of CAGE indicated that it has killing efficacy against the four representative periodontopathic bacteria without any selectivity. Destruction of the cellular membrane on the dead cells after CAGE treatment was observed by TEM (Figure 1A) and the significant PI uptake by CAGE-treated bacteria was observed (Figure 1B). These results

indicated that CAGE has the antimicrobial potential due to the disruption of bacterial membrane.

Table1. The MICs and MBCs values of CAGE

Bacterial strain	CAGE	
	MIC ($\mu g/\mu l$)	MBC ($\mu g/\mu l$)
P. gingivalis W83	1.25	2.5
P. gingivalis ATCC33277	1.25	2.5
F. nucleatum ATCC25586	0.625	2.5
P. intermedia ATCC25611	1.25	2.5

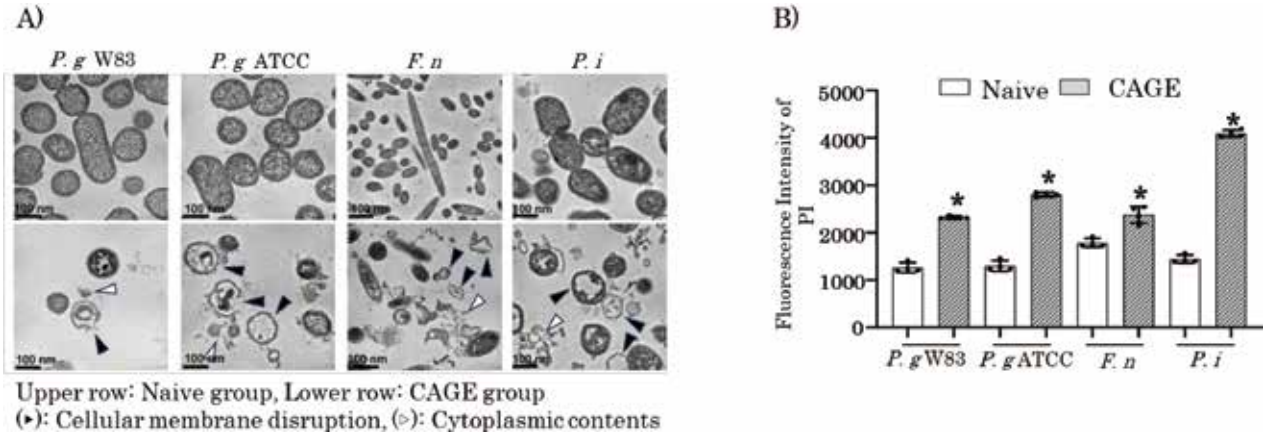

Figure 1. Antibacterial effect on representative planktonic bacteria

The ratio of live to dead cells in the biofilm was significantly decreased after treatment with CAGE (1.25 $\mu g/\mu l$ for 10 min) (Figure 2A). Cell death was obvious in the CLSM images, proving the bactericidal effect of CAGE against the biofilm bacteria (Figure 2B). Biofilm mass reduction after CAGE treatment was demonstrated by CV staining (Figure 2C). The SEM images showed that the biofilm was disrupted and effectively eliminated in the CAGE-treated sample, whereas it was well conserved in the PBS-treated sample (Figure 2D). Thus, the antibiofilm activity of CAGE against the multispecies biofilm was clearly presented.

The actual penetration of CAGE into the multispecies biofilm was validated. A solution of Alexa Fluor 647-labeled BSA was prepared in CAGE and PBS and applied to the multispecies biofilm to visualize penetration. DAPI indicates the nucleus of bacterial cells in the biofilm. In the cross-sectional images, the AF647 signal was only detected in the CAGE-treated sample, indicating CAGE penetration into the deep layers of the biofilm (Figure 3A). This was further confirmed by XZ and YZ cross-sectional images in which the AF647 signal was observed across all biofilm layers after CAGE treatment, but not after PBS treatment (Figure 3B).

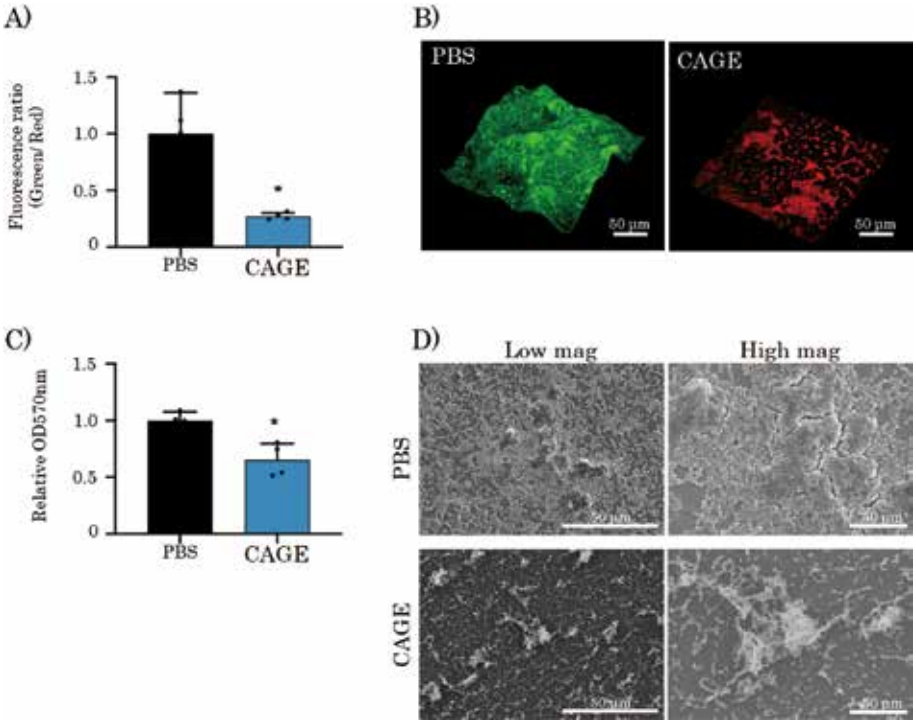

Figure 2. Antibiofilm effects of CAGE on the multispecies biofilm

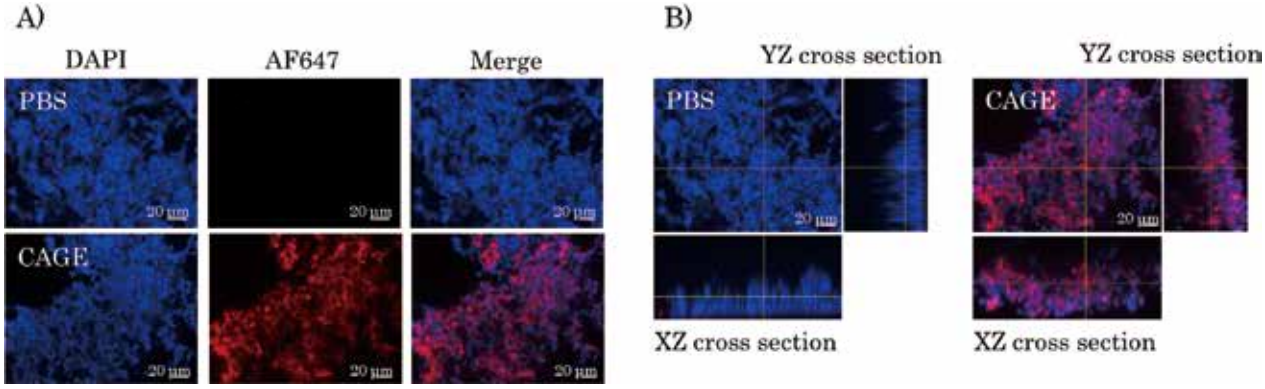

Figure 3. Visualization of the penetration of CAGE into the mature biofilm

This study highlighted the potential of CAGE as a powerful antibiofilm therapeutic. Since topically applied CAGE on the gingival tissue can penetrated deep into the periodontal pockets and periodontium by itself, CAGE has great potential as a new topical periodontal therapeutic.

成果発表：（予定を含めて口頭発表、学術雑誌など）

Poster Presentation

・閻春陽，中島麻由佳，柳川万由子，多部田康一：Anti-bacterial/biofilm activities of Choline Geranate-Ionic Liquid as a novel topical periodontal agent. 第 66 回秋季日本歯周病学会学術大会，長崎，2023 年 10 月 13 日，日本歯周病学会誌 第 65 巻秋季特別号：142 頁，2023.

・Chunyang Yan, Mayuka Nakajima, Mayuko Yanagawa, Koichi Tabeta. Anti-bacterial/biofilm Activities of Choline Geranate-Ionic Liquid for Periodontal Therapy. 102nd General Session & Exhibition of the IADR, New Orleans, USA, Mar. 14, 2024.