Abstract of Thesis

Name (THONGTHAI PASIREE)	
Title	Development of novel surface coating material with dual functionality of antibacterial activity and protein repellency （抗菌性およびタンパク吸着阻害作用を備えた新規表面コート材の開発）

[Objective]

One of the effective approaches for preventing infectious diseases caused by bacterial biofilm formation on restorative and prosthodontic materials is to provide these materials with antibacterial activities. 12-methacryloyloxydodecylpyrimidinium bromide (MDPB) is a polymerizable bactericide, developed to immobilize the antibacterial component on resinous materials. Hence, it is beneficial to use MDPB as a surface coating for resinous restorative/prosthetic materials. However, the effectiveness of immobilized MDPB is reduced by coverage with salivary protein. The addition of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, which possesses protein repellency, is considered to be a useful strategy to address this limitation.

The purpose of this study was to fabricate a novel, dual-functional surface coating material composed of MDPB and MPC and to evaluate its protein-repellency and antibacterial/anti-biofilm effects.

[Materials and Methods]

1. Synthesis of copolymers and coating of dental resins

1.1. Synthesis of copolymers and preparation of surface coatings: The copolymer was synthesized by radical polymerization of MDPB, MPC, and n-butyl methacrylate (BMA) in five different molar ratios: 0/30/70 (D0/C30), 5/25/70 (D5/C25), 15/15/70 (D15/C15), 25/5/70 (D25/C5), and 30/0/70 (D30/C0). Fluorescein *O*-methacrylate was introduced into copolymers to obtain fluorescein-conjugated copolymer (f-copolymer). Five types of each coating were prepared by dissolving each copolymer in ethanol and adjusting their concentrations to 0.5 wt%.

1.2. Evaluation of surface coating ability: Polymethyl methacrylate (PMMA) resin and resin composite disc (10 mm diameter and 2 mm thickness) were prepared and coated with each f-copolymer. The uncoated PMMA disc was used as a control. The coated surface was visualized using a fluorescence microscope. The fluorescence intensity was analyzed.

1.3. Contact angle measurement: Dynamic contact angles were recorded on the coated PMMA surface.

2. Evaluation of protein adsorption on the copolymer-coated resin

2.1. Adsorption of bovine serum albumin (BSA): The PMMA disc coated with each copolymer was immersed in BSA for 2 h. The amount of BSA adsorbed was determined by using a micro BCA protein assay kit. Additionally, FITC-conjugated BSA was used to visualize adsorbed protein by fluorescence microscopy on the disc coated with D0/30, D15/C15, or D30/C0.

2.2. Adsorption of human saliva protein: Quantitative measurement of human salivary protein adsorption was determined as described in section 2.1.

3. Evaluation of antibacterial effects of the copolymer-coated resin

3.1. On-disc culture assay: The PMMA disc with a cylindrical cavity well (7 mm diameter and 1 mm depth) was prepared. Twenty μL of *Streptococcus mutans* NTCT10449 suspension adjusted to 1×10^6 colony-forming units (CFU)/mL was inoculated into the well of the specimen coated with each copolymer. After anaerobic incubation at 37°C for 24 h, the number of viable

bacteria was counted.

3.2. Evaluation of longevity of antibacterial effects: The disc coated with D0/30, D15/C15, or D30/C0 was aged by immersion in sterilized distilled water at 37˚C for 28 days, and the same experiment as described above was performed.

4. Evaluation of anti-biofilm effects of the copolymer-coated resin

4.1. Biofilm formation on copolymer-coated resin: Biofilm formation on the PMMA disc coated with D0/30, D15/C15, or D30/C0 was evaluated before and after 28-day ageing. The coated specimen was immersed in human saliva for 2 h and placed in a suspension of *S. mutans* adjusted to approximately 1×10^6 CFU/mL, and cultured for 48 h at 37˚C. Then, the specimen was transferred to a fresh bacterial suspension at 6 and 24 h.

4.2. Evaluation of biofilms formed on the copolymer-coated resin: The adherence of the biofilm was visualized with a scanning electron microscope (SEM). The thickness of the biofilm formed on the surface and the viability of bacteria in the biofilm were observed using a confocal laser scanning microscope (CLSM) after LIVE/DEAD staining.

[Results and Discussion]

1. Using fluorescence microscopy, all five f-copolymers prepared were confirmed to be coated on the PMMA or resin composite by exhibiting brighter fluorescence than bare resins. The advancing and receding contact angles were significantly smaller on the PMMA surface coated with MPC ($p < .05$, Tukey's HSD test), indicating an increase in the hydrophilicity upon incorporating the MPC polymer.
2. The copolymer containing ≥15% MPC (D0/C30, D5/C25, and D15/C15) significantly reduced the adsorption of BSA and salivary protein ($p < .05$, Tukey's HSD test). On the contrary, the coating without MPC (D30/C0) and the control exhibited bright fluorescence of FICT-BSA, indicating greater BSA adsorption compared with MPC-containing coatings.
3. Although *S. mutans* demonstrated growth on the disc coated with D0/C30 and D5/C25, bactericidal effects were obtained when incubated on the specimens coated with copolymer containing ≥15% MDPB (D15/C15, D25/C5, and D30/C0). No significant difference in the number of viable *S. mutans* after 24-hour incubation was found between the specimens before and after 28-day ageing for D0/30, D15/C15, and D30/C0 groups ($p < .05$, Student's *t*-test).
4. CLSM and SEM images of D0/C30 and D15/C15 showed sparser biofilm formation than the control, and the biofilm thickness of these groups were significantly smaller than for other groups ($p < .05$, Tukey's HSD test). Additionally, D15/C15 demonstrated a greater percentage of dead bacteria ($p < .05$, Tukey's HSD test). After aging, the biofilm thickness formed on the coated specimens was consistent, as in before aging ($p > .05$, Student's *t*-test). The percentage of dead bacteria was also found to be similar before and after aging ($p > .05$, Student's *t*-test).

[Conclusion]

Due to the ability of MPC polymer to inhibit protein adsorption, the coating composed of ≥15% MPC inhibited the adsorption of protein, which is required for the initial step of biofilm formation. Additionally, ≥15% MDPB in the copolymer exhibited bactericidal effects due to its killing of *S. mutans* upon contact. Accordingly, the copolymer composed of 15% MDPB/15% MPC demonstrated both protein adsorption inhibition and an antibacterial effect on *S. mutans* biofilm formation; these functions were effective even after aging. This novel, dual-functional surface coating can be applied to a variety of dental resins for controlling the growth of bacteria in an oral environment.

様式 7

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、抗菌性モノマー12-methacryloyloxydodecylpyridinium bromide (MDPB) とタンパク付着阻害作用を有する 2-methacryloyloxyethyl phosphorylcholine (MPC) を組み合わせた二元系ポリマーからなる表面コート材を試作し、それを用いてコートした歯科用レジン表面でのタンパク吸着阻害効果、および抗菌・抗バイオフィルム効果を *in vitro* で評価したものである。

その結果、MDPB および MPC をともに 15(mol)%の割合で混合して作製したコポリマーからなるコート材は、レジン表面への唾液タンパクの吸着を阻害し、唾液の存在下でも接触型の抗菌・抗バイオフィルム効果を発現することが明らかとなった。

以上の研究成果は、口腔内において抗菌効果を発現できる新規の表面コート材の開発に成功したことを示すものであり、本研究は博士（歯学）の学位授与に値するものと認める。