Evaluation of the long-term antibiofilm effect of a surface coating with dual functionality of antibacterial and protein-repellent effects

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The provision of antibacterial properties to resinous restorative/reconstructive materials by incorporating polymerizable bactericides such as 12-methacryloyloxydodecylpyridinium bromide (MDPB) has been attempted. Previously, MDPB was combined with 2-methacryloyloxyethyl phosphorylcholine (MPC) to fabricate a copolymer coating to increase antibacterial effectiveness by protein repelling. In this study, we assessed the longevity of the protein-repelling, antibacterial, and antibiofilm effects of the MDPB–MPC copolymer. After 28 days of water immersion, MPC-containing copolymers exhibited lower adsorption of bovine serum albumin and salivary proteins; after 24 h of incubation, MDPB-containing copolymers demonstrated antibacterial effects against *Streptococcus mutans*. The copolymer containing both MDPB and MPC showed thinner biofilm formation with a higher percentage of membrane-compromised bacteria than control. The results were consistent with those before aging, indicating the long-lasting antibacterial, protein-repellent, and antibiofilm effects of this copolymer. The durable copolymer developed in this study can be applied to dental resins to control bacteria in the oral environment.

Keywords: Anti-biofilm effect, Antibacterial activity, Protein, MDPB, MPC

INTRODUCTION

Resin-based materials are generally used in dental restorations and prostheses to restore or replace dental structures in the oral cavity with a challenging bacterial environment. To prevent the secondary diseases caused by bacterial infections, a polymerizable bactericide—a component immobilized with dental resins by polymerization—has been incorporated and investigated¹. Briefly, 12-methacryloyloxydodecylpyridinium bromide (MDPB; Fig. 1a) has been combined with a quaternary ammonium compound (QAC) for antibacterial activity and a methacryloyl group for copolymerization with other methacrylate monomers by Imazato *et al.*².

MDPB has been reported to inhibit bacterial growth *via* immobilization in various resin-based materials including dental adhesives, resin composite restorations, and root canal fillings without releasing the active component from the materials^{3–5}. This antibacterial activity is achieved by contact inhibition between the antibacterial components and the bacteria, which is unavoidably prevented by the coverage of salivary proteins⁶. To solve this problem, 2-methacryloyloxyethyl phosphorylcholine (MPC)^{7,8}—a methacrylate monomer containing a phosphorylcholine group (Fig. 1b)—has been copolymerized with MDPB.

To achieve anti-bacterial adhesion, MPC has been combined in orthodontic cement and adhesive system^{9,10}. Previously, we fabricated a dual-functional surface coating consisting of MDPB, MPC, and n-butyl methacrylate¹¹ (BMA) (Fig. 1c), in which BMA was combined, to form an insoluble copolymer coating for application on the surface of resinous materials through hydrophobic interactions between the hydrophobic units of BMA and resin^{12–14}. MPC has been reported to reduce protein adsorption because of its superhydrophilicity and the existence of a hydration layer of water structures on its surface^{15–18}. The reduction in protein adsorption promoted the exposure of MDPB molecules to enhance the contact inhibition effect. This surface coating could repel proteins and exhibit antibacterial effects against *Streptococcus mutans*, and thereby, effectively inhibit biofilm formation¹¹. As restorations are surrounded by bacteria in the oral cavity for a long period, sustainable effectiveness is required to prevent the occurrence of secondary diseases; however, the longevity of biofunctionalities (*i.e.*, protein-repellent and antibacterial/antibiofilm effects) has not yet been elucidated. It has been reported that a copolymer coating composed of MPC and BMA did not elute significantly into the distilled water after 48 h because of the stability of the hydrophobic interaction between BMA and polymethyl methacrylate (PMMA)¹². The copolymer is expected to exhibit long-lasting biofunctionalities. In

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this study, we aimed to assess the inhibitory effect of a surface coating consisting of MDPB/MPC/BMA on PMMA on protein adsorption and its antibacterial and antibiofilm effects after aging.

MATERIALS AND METHODS

Synthesis of copolymer and preparation of surface coatings

The copolymer was synthesized as reported previously¹¹⁾. MDPB was synthesized by the reaction of 12-bromo-1-dodecanol and methacrylic acid and the subsequent reaction of the product with pyridine. MPC was donated by NOF (Tokyo, Japan) and BMA was purchased from

FUJIFILM Wako Pure Chemical (Osaka, Japan). The copolymer was synthesized by radical polymerization of MDPB, MPC, and BMA at molar ratios of 0/30/70, 15/15/70, and 30/0/70 (weight ratios of 0/47/53, 30/22/48, and 55/0/45, respectively) (Table 1). The initiator, 2,2'-azobisisobutyronitrile (AIBN) (0.5 mol%), was added to the monomer mixture in a reactor. To eliminate oxygen in the reactor, it was filled with argon gas for 15 min before use. The mixture of monomers was stirred at 60°C for 15 h. The mixed monomer was poured into diethyl ether with chloroform to precipitate the copolymer. The copolymer was then dried overnight in a desiccator. Subsequently, all types of coatings were dissolved in ethanol and adjusted to 0.5 wt%.

Preparation of resin disc and coating with copolymer

To evaluate protein adsorption, powder and liquid PMMA (UNIFAST III clear, GC, Tokyo, Japan) at a powder/liquid ratio of 2 g/mL were mixed and prepared as 10 mm diameter and 2 mm thick discs using a silicone mold; the discs were stored at 25°C for 24 h. The cured PMMA discs were polished using silicon carbide paper of grit 120/P120 and grit 320/P400 (CarbiMet, Buehler, Lake Bluff, IL, USA) in wet condition. To evaluate the antibacterial effects, a specimen was prepared using the same method described above. Thereafter, a cylindrical well (7 mm diameter and 1 mm depth) was drilled into the PMMA disc using a cylindrical carbide bur mounted on a low-speed handpiece. For biofilm formation studies, a transparent PMMA disc of 10 mm diameter and 1 mm thickness (Comoglas, Kuraray, Tokyo, Japan) was used. Subsequently, all types of specimens were sterilized with ethylene oxide at 40°C for 24 h.

Each PMMA disc was immersed in 1 mL of each copolymer solution for 10 s, and then the coated disc was air-dried at 25°C for 30 min. The soaking and drying processes were repeated twice. A bare PMMA disc without a coating was used as the control.

The copolymer-coated disc was immersed in 500 μ L of distilled water at 37°C for 28 days and subjected to the following investigations to evaluate the longevity of protein-repellent, antibacterial, and antibiofilm effects of the copolymer coatings.

Evaluation of protein-repellent effect of the copolymer-coated surface

Protein concentrations were measured using bovine serum albumin (BSA) and human salivary proteins.

Fig. 1 Chemical structures of monomers. a: 12-methacryloyloxydodecylpyridinium bromide (MDPB), b: 2-methacryloyloxyethyl phosphorylcholine (MPC), c: n-butyl methacrylate (BMA)

Table 1 Composition of the synthesized copolymer coatings

Coating	MDPB	MPC	BMA
Coating 1	0	30	70
Coating 2	15	15	70
Coating 3	30	0	70

MDPB, 12-methacryloyloxydodecylpyridinium bromide; MPC, 2-methacryloyloxyethyl phosphorylcholine; BMA, n-butyl methacrylate
All values are presented in (mol)%.

BSA (Thermo Fisher Scientific, Kanagawa, Japan) was dissolved in 0.01 mol/L phosphate buffer saline (PBS, pH 7.2–7.4, Wako Pure Chemical) to prepare 4.5 g/L BSA solution. Human saliva was collected unstimulatedly from three donors in healthy condition and passed through a 0.22- μ m syringe filter twice. All experiments involving sample collection from human subjects were approved by the Ethics Review Committee of Osaka University Graduate School of Dentistry and Osaka University Dental Hospital (Approval number: R1-E52).

Each copolymer-coated PMMA was immersed in 1 mL of BSA solution or filtered human saliva at 37°C for 2 h. Irrigation and centrifugation in 1.5 mL PBS at 300 rpm were repeated three times to remove loosely bound proteins from the surface. To collect the adsorbed protein, the specimen was ultrasonicated for 20 min in 1% sodium dodecyl sulfate (SDS) in PBS. A Micro BCA Protein Assay Kit (Thermo Fisher Scientific) was used to determine the concentration of BSA. Briefly, 150 μ L of collected protein solution was mixed with 150 μ L of a solution containing alkaline tartrate–carbonate buffer, bicinchoninic acid, and copper sulfate. The absorbance was measured at 560 nm using a microplate reader (iMark Microplate Absorbance Reader, Bio-Rad, Hercules, CA, USA) after storing for 2 h at 37°C, and the amount of protein was determined using the BSA standard curve (each absorbance value on the y-axis corresponds to a distinct concentration value on the x-axis). The experiment was repeated five times. The amount of protein adsorbed on the copolymer-coated disc was relative to the amount of protein adsorbed on the control specimen.

Evaluation of the antibacterial effects of the copolymer-coated surface

Streptococcus mutans NCTC10449 (National Collection of Type Cultures, London, England) was used to evaluate the antibacterial activity as reported previously¹¹. Bacterial cells were grown in brain heart infusion (BHI; Becton Dickinson, Sparks, MD, USA) broth with 1% sucrose at 37°C for 24 h and adjusted to approximately 1×10^6 colony-forming units (CFU)/mL. Bacterial growth was evaluated by inoculating 20 μ L of *S. mutans* suspension into the wells of each copolymer-coated disc and the control. After incubation at 37°C for 24 h, the inoculated discs were transferred to BHI broth to collect the bacterial cells from the surface by vigorous shaking. After serial dilution, an aliquot of the diluent was spread on a BHI agar plate (Becton Dickinson). After 48 h of anaerobic incubation at 37°C, the colonies were counted. The experiment had five replications.

Evaluation of the antibiofilm effects of copolymer-coated surface

Each copolymer-coated PMMA disc was placed in 1.5 mL of suspension of *S. mutans* NCTC 10449 ($\sim 1 \times 10^6$ CFU/mL) in BHI with 1% sucrose in a 24-well microplate. After shaking at 100 rpm for 6 h anaerobically at 37°C, the specimen was transferred to a new well with 1.5 mL

of newly prepared bacterial suspension ($\sim 1 \times 10^6$ CFU/mL) with 1% sucrose and was anaerobically incubated. After 18 h, the specimen was moved to a new well with a newly prepared bacterial suspension containing 1% sucrose and further incubated for 24 h.

To evaluate the percentage of membrane-compromised bacteria in the biofilm and the thickness of biofilm, the specimens were irrigated by gentle shaking in 1 mL of PBS twice, and the biofilm on the surface was stained using LIVE/DEAD BacLight bacterial viability kits (L7007, Molecular Probes, Eugene, OR, USA). The staining mixture was prepared as reported previously¹¹. The specimen was stained with 50 mL of the mixed component and incubated at 37°C for 15 min in the dark. After gentle irrigation with distilled water, the biofilm was visualized using a confocal laser scanning microscope (CLSM; LSM 700, Carl Zeiss, Oberkochen, Germany) (the excitation wavelengths, 488 and 553 nm and the emission wavelengths, 520 and 568 nm). The images were analyzed using ZEN (Carl Zeiss) and Imaris software (Bitplane, Zurich, Switzerland) to detect the biofilm thickness and the percentage of membrane-compromised bacteria, respectively.

Statistical analysis

Statistical analysis of the data of protein adsorption, bacterial growth, thickness of biofilm, and percentage of membrane-compromised bacteria was carried out with IBM SPSS Statistics 25 (SPSS, Chicago, IL, USA), using the one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests, with a significance level of $p < 0.05$. The data within a group before and after aging were analyzed using Student's *t*-test with a significance level of $p < 0.05$.

RESULTS

Protein-repellent effect of the copolymer-coated surface

Figure 2a shows the relative amount of adsorbed BSA on each specimen relative to the amount on the control specimen. The protein adsorption ability of the copolymer coating containing MPC (*i.e.*, coating 1 and coating 2) after 2 h of immersion was significantly lower than that of the control specimen and the copolymer coating without MPC (*i.e.*, coating 3) ($p < 0.05$, ANOVA, Tukey's HSD test). Figure 2b shows the adsorption of the human salivary proteins. The salivary proteins adsorbed on the surfaces exhibited the same tendency with BSA adsorption for the copolymer containing MPC (*i.e.*, coatings 1 and 2), which displayed a significantly lower amount of adsorbed proteins, whereas adsorption on coating 3 was not significantly different from that of the control ($p > 0.05$, ANOVA, Tukey's HSD test).

The relative amounts of both BSA and human salivary proteins adsorbed on the surface of each specimen before and after aging were not significantly different among the groups ($p > 0.05$, Student's *t*-test).

Antibacterial effects of copolymer-coated surface

Figure 3 shows the number of viable *S. mutans* after

24 h of incubation on copolymer-coated and control specimens. On the control specimen, the number of viable bacteria increased during the 24 h of incubation

Fig. 2 Protein adsorption on the surfaces after 2-h immersion in protein solution relative to control. a: Adsorbed BSA, b: Adsorbed salivary protein. The hatched bar represents protein adsorption on the coated discs after 28 days of immersion. Error bars represent the standard deviation. A, B, C, a, b, c: No significant difference between the bars is indicated with identical letters (ANOVA, Tukey's HSD test, $p > 0.05$, $n = 5$). n.s. denotes no significant difference between groups (Student's t -test, $p > 0.05$, $n = 5$).

from the initial bacterial number ($\sim 2 \times 10^4$ CFU; $4.30 \log_{10}$ CFU to $7.00 \log_{10}$ CFU). The number of bacterial cells also increased to $6.01 \log_{10}$ CFU on coating 1. In contrast, the copolymer coatings containing MDPB (i.e., coatings 2 and 3) reduced the number of bacteria to $3.25 \log_{10}$ CFU and $3.39 \log_{10}$ CFU, respectively.

The antibacterial effect of the copolymers containing MDPB was consistent after 28 days of immersion in water. The number of viable cells on the specimen with coating 1 increased to $6.88 \log_{10}$ CFU, whereas the number of bacterial cells on the specimens with coating 2 and coating 3 reduced from the initial number of $4.30 \log_{10}$ CFU to $3.19 \log_{10}$ CFU and $3.11 \log_{10}$ CFU, respectively.

The number of bacterial cells on the specimens with

Fig. 3 Number of *Streptococcus mutans* on the copolymer-coated disc after 24 h of incubation.

The hatched bar represents the number of *S. mutans* on the coated discs after 28 days of immersion. Error bars represent the standard deviation. A, B, a, b: No significant difference between the bars is indicated with identical letters (ANOVA, Tukey's HSD test, $p > 0.05$, $n = 5$). n.s. denotes no significant difference between groups (Student's t -test, $p > 0.05$, $n = 5$). Note: The data on the number of *S. mutans* before 28 days of immersion are from our previous study¹¹.

Fig. 4 CLSM images of *Streptococcus mutans* biofilm formation after 48 h of incubation on the surface of the coated specimen after 28 days of immersion in water.

copolymers containing MDPB (*i.e.*, coating 2 and coating 3) was significantly lower than that on the control and specimens with copolymers without MDPB (*i.e.*, coating 1) ($p < 0.05$, ANOVA, Tukey's HSD test). There was no significant difference in the number of viable bacterial cells in all groups before and after aging ($p > 0.05$, Student's *t*-test).

Antibiofilm effects of copolymer-coated surface

The CLSM images of specimens coated with each copolymer after 48 h of incubation are shown in Fig. 4. After 28 days of immersion in water, the biofilm formed on the MPC-containing copolymer coatings (*i.e.*, coatings 1 and 2) was thinner and more dispersed than that on coating 3 and the control, whereas the biofilm on the MDPB-containing copolymer (*i.e.*, coatings 2 and 3) demonstrated a stronger red color, which indicated the presence of membrane-compromised bacteria.

The image analysis using ZEN software showed that the copolymers containing MPC (*i.e.*, coatings 1 and 2) exhibited significantly thinner biofilm than coating 3 and control ($p < 0.05$, Tukey's HSD test) (Fig. 5). A comparison of the biofilm thicknesses before and after immersion in water for 28 days showed no significant difference among all groups ($p > 0.05$, Student's *t*-test). An image analysis using Imaris software indicated the percentage of membrane-compromised bacteria, shown in red in the CLSM images in Fig. 6. The results revealed that coatings 2 and 3 provided a greater percentage of membrane-compromised bacteria in the biofilm than control and coating 1 ($p < 0.05$, Tukey's HSD test).

Fig. 5 Thickness of biofilm analyzed from CLSM images of *Streptococcus mutans* biofilm formation after 48 h of incubation on the surface of the coated specimen.

The hatched bar represents the thickness of the biofilm formed on the coated disc after 28 days of immersion. Error bars represent the standard deviation. A, B, a, b: No significant difference between the bars indicated with identical letters (ANOVA, Tukey's HSD test, $p > 0.05$, $n = 5$). n.s. denotes no significant difference between groups (Student's *t*-test, $p > 0.05$, $n = 5$). Note: The data of biofilm thickness before 28 days of immersion are from our previous study¹¹.

Fig. 6 Percentage of membrane-compromised bacteria analyzed from CLSM images of *Streptococcus mutans* biofilm formation after 48 h of incubation on the surface of coated specimen.

The hatched bar represents the percentage of membrane-compromised bacteria on the coated discs after 28 days of immersion. Error bars represent the standard deviation. A, B, C, a, b, c: No significant difference between the bars is indicated with identical letters (ANOVA, Tukey's HSD test, $p > 0.05$, $n = 5$). n.s. denotes no significant difference between groups (Student's *t*-test, $p > 0.05$, $n = 5$).

Moreover, no significant difference in the percentage of membrane-compromised bacteria was observed before and after immersion in all groups ($p > 0.05$, Student's *t*-test).

Among these four groups, the coating containing both MDPB and MPC (*i.e.*, coating 2) exhibited a sparser and thinner biofilm, in addition to a greater percentage of membrane-compromised bacteria, indicating that this group was the most effective in terms of its antibiofilm effect, even after aging.

DISCUSSION

Most oral diseases are caused by bacterial infections, and it is crucial to control bacterial growth to maintain and promote oral health. Restorative and prosthodontic materials with antimicrobial properties are promising for preventing secondary diseases that occur in the oral cavity, abundant in bacteria. To develop useful antibacterial materials such as polymerizable bactericides, a novel coating material that combines the functions of MDPB and MPC was developed in the present study. Since the antibacterial effects of immobilized MDPB increased from contact inhibition of bacteria, which was retarded by protein coverage^{6,19}, MPC was introduced to facilitate the antibacterial effect of MDPB by repelling the proteins.

Previously, we fabricated novel coatings composed of MDPB, MPC, and BMA. BMA at 70 mol% was required to strengthen the binding interactions of the coatings with PMMA, which could be observed using fluorescein-conjugated copolymers¹¹. When water-soluble MPC was combined with BMA, the copolymer became water-

insoluble and stably attached to the PMMA by the hydrophobic interactions between the BMA moieties and PMMA¹²). These hydrophobic interactions were achieved by a simple dipping method, which is easy and repeatable, and the desired properties of the polymers can be reproduced¹³). However, the ratio of the combination of MDPB and MPC was within 30 mol%. According to our preliminary experiment (data not shown), MDPB and MPC at 15 mol% were essential to achieve effective antibacterial and protein-repellent effects, respectively.

MPC is a polymer that hinders protein adsorption due to the superhydrophilicity of its phospholipid layer^{15,17,20}). Previously, it was found that the surface coated with the MPC-containing copolymer provided more hydrophilicity by showing less advancing and receding contact angles. This hydrophilicity is related to a greater ability to reduce protein adsorption of MPC-containing copolymers¹¹), as reported by several studies, that is, increasing the hydrophilicity of the biomaterial surface reduces the protein adsorption^{21,22}). MPC can form a hydration layer in the surrounding aqueous media, that is almost the same as that of pure liquid water with high mobility^{23,24}). Thus, the proteins surrounded by bound water cannot change their conformation to interact with material surfaces, and float in the hydration layer. This phenomenon facilitates the detachment of proteins from the surface^{25,26}).

For the protein adsorption test, BSA was used as a model protein for evaluation in the first step before using human saliva. BSA has been widely used in the study of protein-surface interactions due to its stability, availability, low cost, and high solubility in water²⁷). The results indicated that BSA adsorption on specimens coated with copolymers containing MPC (*i.e.*, coatings 1 and 2) was relatively lower than that on the control and coating 3. Protein adsorption was also assessed using human saliva, which provided a more complex protein solution. Human saliva is a mixture of oral fluids, including secretions of major and minor salivary glands and constituents of non-salivary origin derived from gingival crevicular fluid, serum, and blood cells from oral wounds, oral bacteria, and desquamated epithelial cells. More than 2,000 proteins with different molecular weights and isoelectric points have been identified in the saliva collected from healthy donors^{28,29}). The results of salivary protein adsorption revealed that the specimens coated with copolymers containing MPC (*i.e.*, coatings 1 and 2) showed greater protein repellency than those with the control and coating 3, similar to the case of BSA. These results from both proteins demonstrate consistency in protein adsorption before and after treatment. However, coating 2 showed higher protein repellency than coating 1 only for BSA. This can be explained by the isoelectric point of BSA at around pH 4.5–5. With increasing pH, the amount of negatively charged groups in BSA increases³⁰). In this study, BSA was dissolved in PBS of pH 7.2–7.4, resulting in a negatively charged surface, which was attracted by the positive charge of the cationic portion of MDPB through electrostatic affinity³¹).

MDPB has been studied in various types of dental

materials because of its antibacterial effect against various oral microbes^{4,5}); however, the effect was reduced after curing, which limited the molecular movement caused by immobilization³²). Thus, various resin-based materials incorporating MDPB, such as dental adhesives and resin composites, have been investigated for bacteriostatic effects rather than bactericidal effects³). In our previous study, the copolymer coating consisting of MDPB demonstrated bactericidal effect; it was attributed to the comparatively higher ratio of MDPB than that in past materials¹¹). Here, the number of bacteria on the specimens coated with copolymers containing MDPB was smaller than that on the specimens coated with the control and non-MDPB copolymers. The log reduction of *S. mutans* suspension inoculated on these MDPB-coated specimens was more than 3.61–3.89, compared with that on the control specimen both before and after aging. In addition, they reduced the bacterial number from its initial value of 4.30 log₁₀CFU to 3.11–3.39 log₁₀CFU (*i.e.*, 0.91–1.19 log reduction), indicating the bactericidal activity both before and after aging.

The copolymer coatings were also assessed for their antibiofilm effect when MDPB and MPC were combined¹¹). MPC reportedly inhibits oral biofilm formation by suppressing bacterial adherence *via* inhibition of protein adsorption; subsequently, the initial interaction between microorganisms and material surfaces is retarded⁹). However, MPC in the absence of MDPB did not inhibit the proliferation of bacteria. Thus, the biofilm formed on the specimen with coating 1 was sparser and thinner than that on the control specimen, but it did not increase the percentage of membrane-compromised bacteria; in other words, the bacterial membrane was not affected by the coating and was still intact compared with that by the control. Conversely, MDPB was found to have an antibiofilm effect, but it was diminished by salivary protein adsorption³³). This was supported by the biofilm formed on the specimen coated with coating 3, which provided a higher percentage of membrane-compromised bacteria but was not reduced in thickness. The antibiofilm effect was enhanced when MDPB was combined with MPC, where bacteria were revealed on MDPB due to the absence of proteins on the surface and finally killed in a surrounding of salivary proteins. The biofilm formed on the specimen coated with coating 2 was thinner and sparser than that on the specimen coated with the control and coating 3, additionally demonstrating the highest percentage of membrane-compromised bacteria among all groups.

This study revealed that after specimen aging in distilled water, the protein-repellent, antibacterial, and antibiofilm effects were similar to those before aging. Even after aging, MDPB and MPC were assumed to be held on the surface, as demonstrated by the antibacterial effect and reduction in protein adsorption. These results indicate that the hydrophobic interactions in the coating were sufficiently stable, even after water immersion and aging. However, stronger solvents, such as ethanol, may affect the stability of coating because this copolymer is prepared in ethanol, and therefore, may solubilize in it.

Another study also reported that a copolymer composed of BMA and MPC coating on polyvinylidene fluoride membrane was durable after ultrasonic cleaning with SDS solution¹³; thus, it is possible to clean a coated restoration by ultrasonication. This dipping method is simple, repeatable, and provide durable antibacterial activity, beneficial for improving oral cavity restoration. However, the oral environment is indeed challenging, and multiple factors need to be considered, such as mechanical stress from mastication and cleaning.

CONCLUSIONS

The MPC-MDPB copolymer coating is promising for achieving long-term biofunctionalities of protein-repellent ability and antibacterial/antibiofilm effects. After 28 days of immersion in distilled water, the coating composed of MPC inhibited the adsorption of protein required for the initial step of biofilm formation, owing to the presence of the MPC polymer. Additionally, MDPB in the copolymer exhibited bactericidal effects owing to the contact killing of bacteria. Eventually, the copolymer composed of both MDPB and MPC effectively inhibited biofilm formation even after aging. This durable dual-functional surface coating can be applied to various dental resins to control bacteria in the oral environment. However, oral microbes consist of diverse microorganisms that produce more complex and harsher oral biofilms. Therefore, an assessment of the benefits of this coating in *in situ* experiments is required to determine its potential for clinical applications in the future.

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