Application of Fluorinated Alkyl Acrylate to Denture Base Resin

Influence of Carbon Chain Length of Fluorinated Alkyl Acrylate on Bacterial Adherence

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Abstract

This study examined the influence of carbon chain length of fluorinated alkyl acrylate on bacterial adhesion to the surface of test specimens. Perfluorobutylethyl acrylate (C4F), perfluorohexylethyl acrylate (C6F), perfluorooctylethyl acrylate (C8F), and perfluorodecylethyl acrylate (C10F) were used as fluorinated alkyl acrylate monomers. All specimens with the fluoro-monomer added exhibited significantly higher water-shedding and oil-shedding surface characteristics when compared to control (MMA/PMMA). Furthermore, contact angle for droplets of water and dodecane on the fluoromonomer-added specimens increased with carbon chain length of the perfluoroalkyl groups. Bacterial adhesion was significantly suppressed on the fluoromonomer-added specimens, both the saliva-uncoated and -coated specimens. In addition, the number of *Candida albicans* adhering to both the saliva-uncoated and -coated fluoromonomer-added specimens decreased with an increase in perfluoroalkyl carbon chain length. The results suggest that the application of fluorinated alkyl acrylate to denture base resin can modify the surface characteristics, and that fluorinated alkyl acrylate with long carbon chains of C8F and C10F successfully inhibit bacterial adherence to the resin surface.

Keywords :

denture base resin, fluorinated alkyl acrylate, hydrophobicity, *Candida albicans*

Introduction

Demographic data indicate an increasing proportion of elderly in the population and a lengthening life expectancy. Elderly adults are retaining their natural teeth longer, and the first removable dentures are being obtained later in life. The use of dentures is beneficial to physical as well as oral function (1). However, plaque adhering to the denture surface can cause regional inflammation in oral mucosa, such as denture stomatitis, and systemic disease, such as bacterial pneumonia and infectious endocarditis (2). Yoneyama *et al.* suggested that oral care might prevent respiration infections in elderly adults because improved oral hygiene in the elderly reduced

the duration of pneumonia-induced fever (3). Denture plaque can be removed with a brush or denture cleaner. However, it can be difficult for ill older adults and people with disabilities to clean their artificial dentures. Inhibiting plaque formation on denture surfaces through the development of a new denture base material that prevents microbial adhesion could solve this problem.

Hayashi *et al* (4). suggested that the surface characteristics of denture base resin could be improved markedly by adding perfluorooctyl ethyl acrylate to the filling polymer. Previously, we examined the influence of carbon chain length of fluorinated alkyl acrylate on mechanical properties (5). In this study,

we examine the influence of carbon chain length of fluorinated alkyl acrylate on the surface properties and surface bacterial adhesion using fluorinated alkyl acrylate monomers such as perfluorobutyl ethyl acrylate (C4F), perfluorohexyl ethyl acrylate (C6F), perfluorooctyl ethyl acrylate (C8F), and perfluorodecyl ethyl acrylate (C10F).

Materials and Methods

Preparation of Polymer beads

Perfluorobutyl ethyl acrylate (C4F), perfluorohexyl ethyl acrylate (C6F), perfluorooctyl ethyl acrylate (C8F), and perfluorodecyl ethyl acrylate (C10F) were purchased from Daikin Chemicals Sales Company, Ltd. and used as the fluoromonomers. Methyl methacrylate (MMA) (Kanto Chemical Company, Ltd.) was used as a control and base monomer. According to the method of Kim *et al* (6), spherical beads were made by suspension polymerization. The results of a preliminary experiment showed that beads with even diameters could be collected using a monomer weight ratio of 25 wt% fluoromonomer and 75 wt% MMA, although spherical beads could not be collected at monomer weight ratios greater than 25 wt% (7). Control beads were made in the same manner using only MMA.

Preparation of Control and Fluoromonomer-added specimen

Test specimens were made using the method described by Hayashi *et al* (5). Briefly, paraffin wax was attached to the four sides of an acryl board (size: 20 mm × 50 mm, thickness: 2 mm) in the shape of a truncated pyramid, and the resulting acryl board was embedded in plaster while being sandwiched by slide glass to prepare a counter-die. The liquid portion was prepared at a monomer weight ratio of 25 wt% fluoromonomer and 75 wt% MMA. The polymer to liquid ratio (wt/wt) was 2:1 for both the control (MMA) and the fluoromonomer-added specimens (C4FA, C6FA, C8FA, and C10FA). Then, 1.5 wt% α - α' -azobisisobutyronitrile (AIBN) was added to initiate polymerization. These ingredients were mixed in a conventional manner, and cast in the

counter-die to allow polymerization to take place at 70 °C for 6 hr and then at 100 °C for 0.8 hr. From the center of the resulting resin plate, four test specimens (10 × 20 mm) were cut. Two of the four specimens were used to prepare polished specimens as follows. The two specimens were polished using #1200, #2000, #4000, and #8000 water-resistant sandpapers (Sumitomo 3M), and the surface of each piece was polished using a lathe and then cleaned ultrasonically with a rouge cleaner. The other two specimens were cleaned ultrasonically in distilled water for testing as unpolished specimens.

Measurement of Contact angle

Contact angles were measured by the sessile drop method using a contact angle analyzer (KYOWA Contact Angle Mater CA-D, Kyowa Science Co., Ltd.) on the polished specimen surfaces. Droplets of distilled water (D.W., 6 μ l) or dodecane (Sigma-Aldrich, 6 μ l) were placed at two different locations on test specimens to measure the contact angles (8).

Analysis of Microbial Adhesion

Candida albicans strain ATCC90029 was used to examine microbial adhesion. Microbial adhesion to saliva-uncoated and -coated specimens was analyzed according to the methods of Otake *et al* (9), and Kohinata (10), respectively. Briefly, unstimulated whole saliva samples were collected from a healthy male donor to eliminate sample variation. The saliva was clarified by centrifugation at 10,000 × g for 10 min. Saliva samples were stored at -80 °C prior to use. A hole was made at the edge of the polished and unpolished specimen surfaces, which were then immersed in distilled water for a week (saliva-uncoated specimen). The polished surface specimens, which were immersed in distilled water for one week, were incubated in 3 ml saliva with agitation at 37 °C for 1 hr (saliva-coated specimen).

A test solution was prepared by culturing *C. albicans* in liquid culture medium according to conventional methods. *C. albicans* was subcultured in liquid BHI (Difco®) culture medium and incubated with agitation at 37 °C for 24 hr to achieve the middle of

the logarithmic growth phase. The resulting culture was centrifuged at 3,000 rpm for 10 min. After washing the centrifuged *C. albicans* three times with phosphate-buffered saline (PBS), *C. albicans* was incubated in 2 ml saliva with agitation at 120 shakes per minute for 1 hr and centrifuged with PBS. A sonic disrupter (ultrasonic cell disrupter ; Ohtake Seisakusho ; sound output : 20 kHz, 50 W, 30 sec) was used to disperse *C. albicans*, and PBS was added to adjust microbial concentration to approximately 1×10^8 cells/ml. Test specimens were suspended by stainless steel wires into 3 ml of the test solutions, followed by incubation with agitation for 1 hr at 120 shakes per minute. After removing excess test solution by washing, the resin specimens were stained using 0.01% crystal violet for one minute and rinsed with D.W. After drying the specimens, the numbers of adhered *C. albicans* were counted using a digital high fidelity microscope (VH-8000, Keyence Cooperation) at $\times 400$ magnification.

Statistical Analysis

The data were analyzed for statistical differences between control and experimental groups using one-way ANOVA, two-way ANOVA, and Tukey's test for multiple comparisons (11). Statistical calculations were performed using STATISTICA (Stat Soft®). The correlation between the contact angle for water and the number of adhered *C. albicans* was determined using Pearson's correlation coefficient (12).

Results

Contact Angle

Tables 1 and 2 show the contact angles for water and dodecane, respectively. Contact angles for water on C4FA, C6FA, C8FA, and C10FA were 88.5°, 90.0°, 90.5°, and 91.2°, respectively ; contact angle for the control was 71.9°. Contact angles of oil on C4FA, C6FA, C8FA, and C10FA were 22.1°, 28.6°, 31.9°, and 30.8°, respectively ; contact angle for the control was 10.1°. All fluoromonomer-added specimens possessed significantly greater water-shedding and oil-shedding abilities. The contact angle of C4FA for water

was lower than those of C8FA and C10FA. Furthermore, the contact angle of C4FA for dodecane was lower than those of C6FA, C8FA, and C10FA.

Table 1. Contact angle for water

Specimen	Contact angle (°)
Control	71.9 ± 1.4
C4FA	88.5 ± 1.5
C6FA	90.0 ± 2.1
C8FA	90.5 ± 2.2
C10FA	91.2 ± 1.9

(n=8, mean±S.D.)

** : p<0.01

Table 2. Contact angle for dodecane

Specimen	Contact angle (°)
Control	10.1 ± 5.9
C4FA	22.1 ± 1.5
C6FA	28.6 ± 2.2
C8FA	31.9 ± 5.0
C10FA	30.8 ± 3.3

(n=8, mean±S.D.)

* : p<0.05 ** : p<0.01

Adherence of *C. albicans* organisms to saliva-uncoated specimen surfaces

Fig. 1. shows the numbers of *C. albicans* organisms adhered to saliva-uncoated specimens. The numbers

Fig. 1. Adherence of *C. albicans* to saliva-uncoated specimen

adhered to unpolished surfaces of C4FA, C6FA, C8FA, and C10FA were 60.9, 58.8, 29.4, and 17.5 cells/mm², respectively, while the number for the control was 171.2 cells/mm². In contrast, the numbers of *C. albicans* organisms adhered to polished C4FA, C6FA, C8FA, and C10FA were 25.6, 25.4, 17.6, and 9.0 cells/mm², respectively, while that of the control was 126.5 cells/mm². The numbers of *C. albicans* organisms adhered to unpolished C4FA and C6FA were greater than those found for polished C4FA and C6FA. The numbers adhered to specimens containing fluorinated alkyl acrylate were significantly less than that of the control. Furthermore, the numbers of *C. albicans* organisms adhered to fluoromonomer-added specimens tended to decreased with the carbon chain length of the fluorinated alkyl acrylate used as comonomer, although no statistical difference was obtained for the difference between C8FA and C10FA. In addition, Pearson's correlation coefficients between the contact angle for water and the number of *C. albicans* adhered to unpolished and polished specimens were similar -0.977 ($p < 0.01$).

Adherence of *C. albicans* to saliva-coated specimen surfaces

Fig. 2. shows the numbers of *C. albicans* organisms adhered to saliva-coated specimens. The numbers found on saliva-coated C4FA, C6FA, C8FA, and C10FA were 19.4, 16.2, 12.4, and 10.3 cells/mm², respectively, while that of the control was 53.2 cells/mm². The numbers of *C. albicans* organisms adhered to the specimens made using fluorinated alkyl

Fig. 2. Adherence of *C. albicans* to saliva-coated specimen

acrylate were significantly lower than that found for the control. Furthermore, the number of *C. albicans* organisms adhered to C10FA was significantly lower than those found for C4FA and C6FA. Pearson's correlation coefficient between contact angle for water and the number of adhered *C. albicans* was -0.996 ($p < 0.01$).

Discussion

Fluorine has a relatively small Van der Waals force and relatively large electronegativity. In addition, the C-F bond possesses greater stability and strength than the C-H bond (13). These properties give fluoro-materials heat resistance, low surface tension, high physiological activity, and superior heat, chemical, and weather resistance. The poly-fluoroalkyl or polyfluoroalkylene group (RF group), particularly, is less likely to bend due to repulsion between the fluorine atoms, and its intermolecular interactions with other polar molecules are suppressed significantly because the C-F bond distance is slightly smaller than the C-H bond distance, allowing the fluorine atoms to completely surround the carbon chain (14). Therefore, rod-shaped RF groups aggregate in an orderly fashion. Because the surface energy of this aggregate is extremely low, the fluoroalkyl side chain orients toward the surface exposed to air of the resulting fluoropolymer.

In this study, we examined the influence of carbon chain length of fluorinated alkyl acrylate on the surface properties and bacterial adhesion to copolymers. All specimens with fluoromonomers exhibited significantly higher water- and oil-shedding surface characteristics compared to the control, as shown in Tables 1 and 2. This surface modification produces superior resistance to discoloration and plaque formation on the denture base in the oral cavity (15, 16). Furthermore, the contact angle for water and dodecane on the fluoromonomer-added specimens increased with carbon chain length of the perfluoroalkyl groups, although no statistical difference existed among the contact angles of C6FA, C8FA, and C10FA. A previous study (17) described the relation between hydrophobicity and co-poly-

mers such as acrylate and fluorinated alkyl acrylate. When the carbon chain length of the fluorinated alkyl acrylate was less than eight, the static contact angles of the co-polymers were relatively low. The crystallization of the fluoroalkyl carbon chain affects the contact angles for water and dodecane.

The number of *C. albicans* that adhered to saliva-uncoated and -coated fluoromonomer-added specimens was significantly lower compared to the control (Figs. 1. and 2.). Furthermore, the numbers of *C. albicans* that adhered to fluoromonomer-added specimens decreased significantly with the carbon chain length of the perfluoroalkyl group, although no statistically significant difference existed between C8FA and C10FA. In contrast, the number of *C. albicans* that adhered to polished specimens were approximately 75% of that found for non-polished specimens. Surface characteristics of the mold affect the orientation of the fluoroalkyl side chain in the polymer during processing. Therefore, the surface of non-polished fluoromonomer-added specimens is relatively hydrophilic compared to the polished specimens.

Since the electronegativity of fluorine is high, the surface energy of an object decreases upon addition of fluorine atoms over the surface. Therefore, the surface of polymers containing fluorine is less likely to become wetted by a liquid with a high degree of intermolecular cohesion, is hydrophobic, and has a high contact angle for water. Ichimaru *et al* (18). reported that a greater contact angle reduced plaque adhesion and increased the ease of plaque removal. It has been reported that plaque is less likely to adhere to materials with low surface energy (16), and the number of *C. albicans* organisms adhered to fluoromonomer-added specimens decreased with carbon chain length of the perfluoroalkyl groups.

This study suggests that the addition of fluorinated alkyl acrylate to denture base resin increases the hydrophobicity of the surface and that fluorinated alkyl acrylates with relatively long carbon chains, such as C8F and C10F, inhibit bacterial adherence to the resin surface.

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