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博 士 論 文

Study of a novel resin cement containing anti-microbial compound CPC-Montmorillonite (CPC モンモリロナイトを含む新規歯科用レジンセメントに関する検討)

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ABSTRACT

Objective: The aim of this study was to evaluate the mechanical property, bonding performance and anti-microbial activity of a novel resin cement containing montmorillonite modified Cetylpyridinium chloride (CPC-Mont), and to determine the optimal particle size and concentration of CPC-Mont to be lorded to the resin cement.

Materials and methods: Montmorillonite filler modified CPC with a median diameter of 30μm and 7μm were prepared, and they lorded to a resin cement at concentrations of 2, 3, 4, 5, 7.5 wt.%. Mechanical property and bonding performance of the resin cements were evaluated by 3 point bending test and micro-tensile bond strength test. The amount of CPC released from the resin disks were quantified using a UV-vis recording spectrophotometer. The anti-biofilm activity was also studied using scanning electron microscope.

Results: Mechanical property and bonding performance of the resin cement decreased with lording 30μm CPC-Mont, but no reduction was observed with lording 7μm CPC-Mont. Although CPC release was decreased as the immersion period passed, 5 and 7.5 wt.% CPC-Mont resin cement inhibited biofilm formation for 30 days.

Conclusions: The lording of CPC-Mont with a median diameter of 7μm at concentrations of 5 to 7.5 wt.% to resin cement was effective in achieving continuous anti-biofilm activity while maintaining the mechanical property and bonding performance.

Keywords: Secondary caries, Biofilm, Resin cement, Montmorillonite, Cetylpyridinium chloride

1. Introduce

In recent years, the increase in awareness of oral care has greatly reduced the incidence of dental caries among young people in Japan. On the other hand, the rapid increase of elderly population and the number of remaining tooth in elderly people have brought concerns about caries and periodontal disease in aged people [1, 2]. Several epidemiological investigations conducted on the causes of tooth extraction suggest that caries is one of the major reasons of the tooth loss as well as periodontal disease [3,4,5]. In the life cycle of restored tooth that repeats the repair treatment for caries and eventually leads to extraction, the prognosis of restoration treatment is significant for preserving the residual tooth and maintaining the oral function.

Generally, it is known that the changes in oral condition, such as decrease in salivary and gingival recession with aging, may increase the caries risk [6]. It is also reported that the bacteria remains on the interface of restored tooth and grows over a long period [7,8]. In addition, the dissolution of the dental cement, the deterioration of its physical properties and the detachment from tooth interface are also considered to be caused of secondary caries from the cement margin [9,10].

An ideal dental cement has sufficient mechanical properties and bonding performance to resist functional forces and degradation in the oral environment [9,10,11]. Furthermore, prevention of biofilm formation on the tooth-cement interface is also important in order to prevent secondary caries and maintain the dental prosthesis function for a long term [10,12]. Resin cements are extensively used in restorative dentistry due to their excellent mechanical and bonding performance. However, resin cement with sustained anti-microbial activity, sufficient mechanical and bond performance has not been developed.

In this study, we studied a novel resin cement with anti-microbial activity containing montmorillonite loaded cetylpyridinium chloride (CPC-Mont) as bactericide releasable compound. CPC is a well-known and effective bactericide, of which its wide use in dental pastes and mouthwashes. The mechanism of anti-bacterial activity of CPC is ascribed to the positive charge of pyridinium group [13,14,15,16]. Montmorillonite is consists two tetrahedral sheets of silica sandwiching a central octahedral sheet of alumina, and has a characteristic retaining the cation between the sheets. It is also known as reinforcing engineering composites [16,17,18,19].

The aim of this study was to evaluate the mechanical property, bonding performance and anti-microbial activity of the experimental cement containing CPC-Mont, and to determine the optimal particle size and concentration of CPC-Mont to be lorded to the resin cement.

2. Materials and Methods

2.1. Preparation of CPC-Mont

CPC-Mont was prepared by exchanging cation of natural sodium montmorillonite clay with cetylpyridinium chloride. Sodium montmorillonite (5g) was dispersed in deionized water (500mL) and stirred for 1 hour. The amount of CPC (10.5g) corresponding to four times the cation exchange capacity of the sodium montmorillonite was dissolved in deionized water (250mL) and preheated to 60°C. The CPC solution was slowly added to the dispersion. Then the dispersion was stirred for 1 day at room temperature. The solid products were separated by centrifugation, washed with deionized water and dried at 60°C for 1 day. The dried solid was ground to powder, and sieved. Two different particle size of CPC-Mont, the median diameter of 30μm (CPC-Mont30) and 7μm (CPC-Mont7) were prepared.

2.2. Resin cement formulation

A dual-cure adhesive resin cement, SEcure (Parkell Inc., New York, USA), was used in this study. This resin cement is consisted of catalyst past and base past. The composition of each past is shown in Table1. They are contained methacrylate monomers, aromatic amine, inorganic fillers and peroxide. The base past was modified by adding each size of CPC-Mont at concentrations of 2, 3, 4, 5, 7.5 wt.% for the cement’s total weight.

Table 1 - Compositions of Catalyst paste and Base past

Catalyst paste	Methacrylate monomers, Aromatic amine, Inorganic fillers, others
Base paste	Methacrylate monomers, Inorganic fillers, Peroxide, others

2.3. Three point bending test

The mechanical property of the each experimental resin cements were evaluated by three point bending test. The test specimens were prepared using a Teflon mold of 2 mm in width, 2 mm in thickness and 25 mm in length. A slide glass was covered with a polyester film and the Teflon mold was placed on the film.

Catalyst past and Base past modified by CPC-Mont were dispensed onto a mixing pad, manually mixed and filled into the mold. Then another polyester film was pressed against the mold surface with slide glass. After curing at room temperature for 30 minutes, the slide glasses and films were removed and the specimens were stored in water at 37°C for 24 hours.

The three point bending test was performed using a universal tester (AG-IS 500N, SHIMASZU, Japan). The distance between supports was 20 mm and the load was applied to the center of specimen at a crosshead speed of 1mm/min.

The flexural strength, elastic modulus and fracture toughness of each specimen were obtained and the results were statistically analyzed with one-way analysis of variance (ANOVA) and the Bonferroni multiple comparisons test, with the level of significance set at $p < 0.05$.

2.4. Micro-tensile bond strength to dentin

Fifty-five human third molars were randomly divided into each experimental group to measure the micro-tensile bond strength to dentin (5 teeth per experimental group). The teeth were ground to flat dentin with #600 SiC paper under water irrigation.

SEcure Primer (Parkell Inc., New Yoke, USA) was applied for 20 seconds, and dried with gentle air blow. The experimental cement was placed on the dentin surface, and covered with polyester film, pressed it horizontally to the dentin surface. After 30 minutes, an acrylic block of 10 mm in width, 10 mm in thickness and 10 mm in length was mounted on the cement surface with Super-Bond C&B (SunMedical Co.,Ltd, Shiga, Japan).

The specimens were stored in water at 37°C for 24 hours, and sectioned perpendicular to the bonding surface to obtain 1 mm^2 stick-shaped micro-specimens using a low-speed diamond saw (IsoMet, BUEHLER, USA). The micro-specimens were fixed to a jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan) and stressed at a crosshead speed of 1mm /min until failure in a micro-tensile testing device (EZ-test EZ-SX 500N, Shimadzu, Kyoto, Japan). The value of micro-tensile bond strength was calculated in MPa, and the all data were analyzed by one-way analysis of variance (ANOVA) and the Bonferroni multiple comparisons test, with the level of significance set at $p < 0.05$.

2.5. Quantification of released CPC

A slide glass was covered with a polyester film and the Teflon mold of 10 mm in diameter and 2 mm thickness was placed on the film. The experimental cement was filled into the Teflon mold and another polyester film was pressed against the mold surface with slide glass. After curing for 30 minutes at room temperature, the resin disks were immersed in 2 mL distilled water at 37°C for 7 days with changing water every day. The amount of CPC released from the resin disk was quantified with UV-Visible spectroscopy (V-630, JASCO Corporation, Tokyo, Japan) using the peak height from an arbitrary base line at 258.5 nm attributed to the pyridinium ring structure of CPC.

2.6 Biofilm formation

Streptococcus mutans(ATCC25175) were cultivated under aerobic condition in tryptic soy broth, harvested by centrifugation and suspended in brain-heart infusion broth supplemented with 1 wt.% sucrose. The bacteria suspension was adjusted to 1×10^5 CFU/mL.

The resin disks of 10 mm in diameter and 2 mm in thickness were prepared, and placed in a 24 well plate. 2 mL of bacteria suspension was added to each well and cultivated at 37°C for 7 days and 30 days with changing the suspension every day.

After cultivation, the specimens were rinsed thoroughly with distilled water, fixed with 2.5% glutaraldehyde. The biofilms on the resin disks were sputter-coated with silver and observed under a scanning electron microscope (JSM-5610LV, Tokyo, Japan).

3. Results

3.1. Three point bending test

The flexural strength, elastic modulus and fracture toughness of the resin cements modified with CPC-Mont30 and CPC-Mont 7 were shown in Fig 1 and 2, respectively.

The tendency of reduction in flexural strength was observed with addition of CPC-Mont30. The mean value of 7.5 wt.% CPC-Mon30 was 45.6 MPa, which was significantly lower than control (no CPC-Mont) and 2, 3, 4 wt.% CPC-Mont30. The mean value of elastic modulus ranged from 3.8 to 4.9 GPa and there were no significant differences. The fracture toughness was also decreased with increasing CPC-Mont30 concentration.

Regarding the CPC-Mont7 added groups, 2 wt.% CPC-Mont7 was the highest value in flexural strength and elastic modulus. Although a significant difference were observed between 2 wt.% CPC-Mont7 and 5, 7.5 wt.% CPC-Mont7 in elastic modulus, no reductions were observed in flexural strength and fracture toughness.

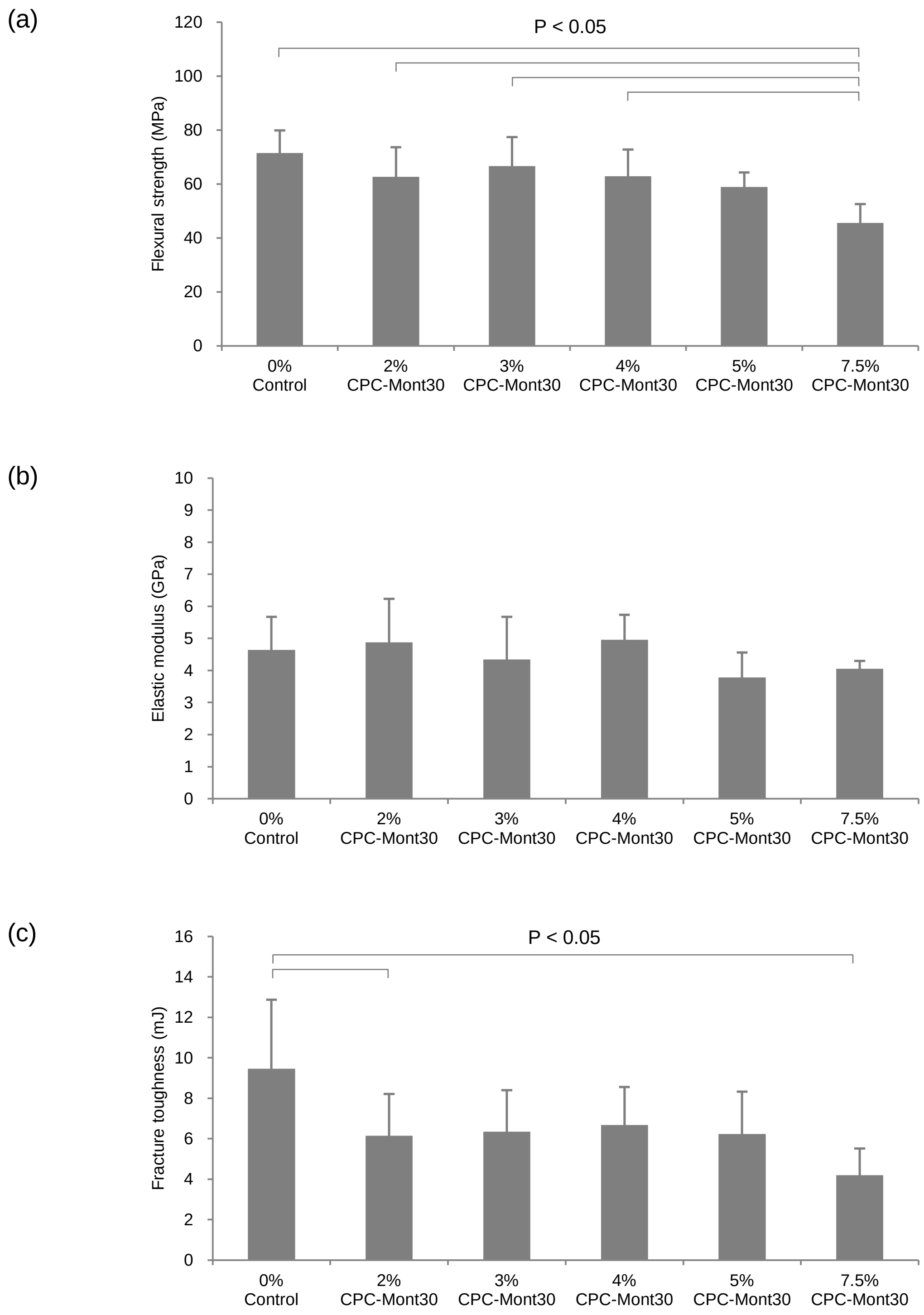


Fig.1 - Three point bending test results of CPC-Mont30 resin cement formulations, (a) Flexural strength, (b) Elastic modulus, (c) Fracture toughness.

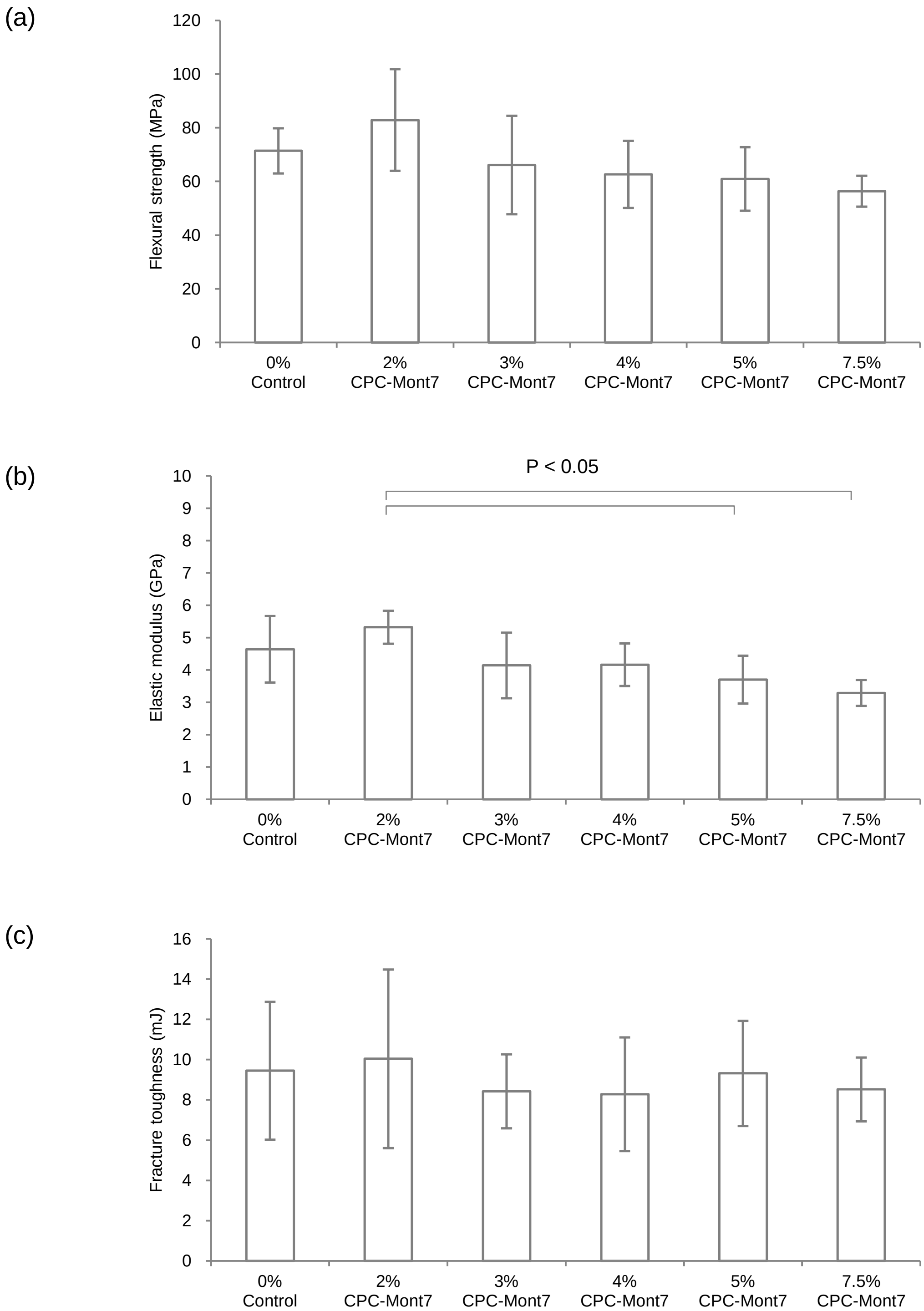


Fig.2 - Three point bending test results of CPC-Mont7 resin cement formulations, (a) Flexural strength, (b) Elastic modulus, (c) Fracture toughness.

3.2. Micro-tensile bond strength test

The micro-tensile bond strengths to dentin were shown in Fig 3. The bond strength of the experimental resin cement formulations decreased with increasing CPC-Mont30 concentration. 7.5 wt.% CPC-Mont30 presented the lowest bond strength of all other concentration groups, and the mean value was 6.4 MPa. The statistically differences were observed between 3, 4, 5 wt.% CPC-Mont30 and control, also observed between 7.5wt.% CPC-Mont30 and all other groups.

In CPC-Mont 7 added groups, these were statistically differences between 7.5 wt.% CPC-Mont7 and control, however no obvious decrease tendency was observed.

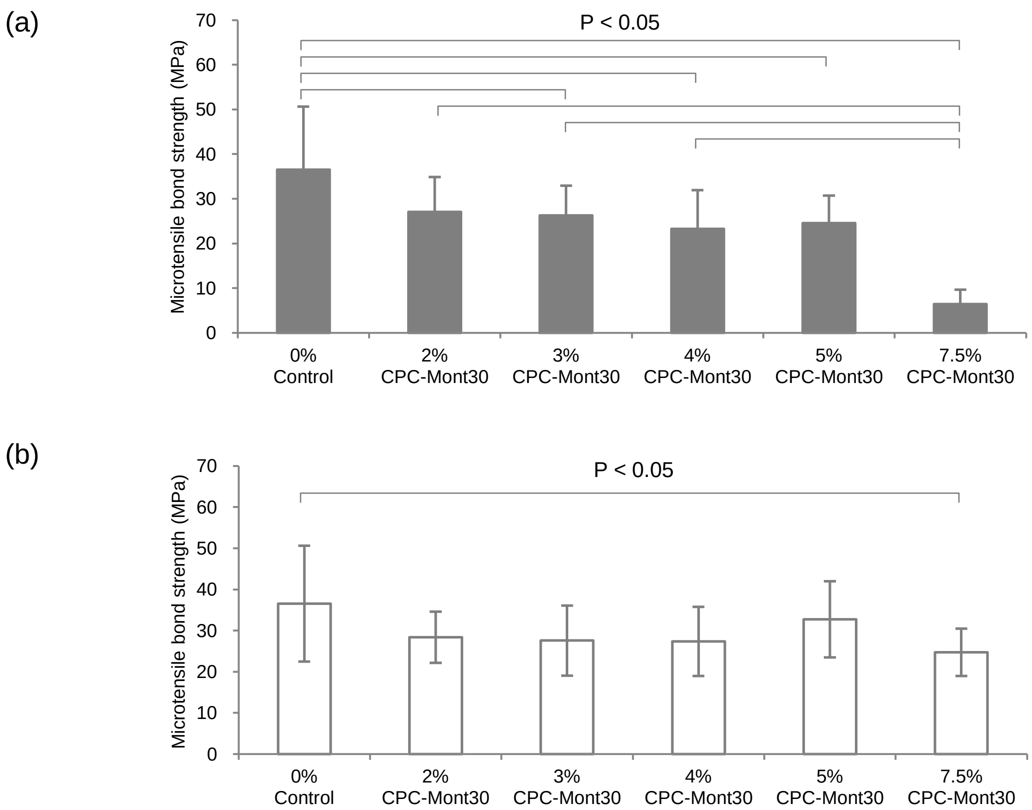
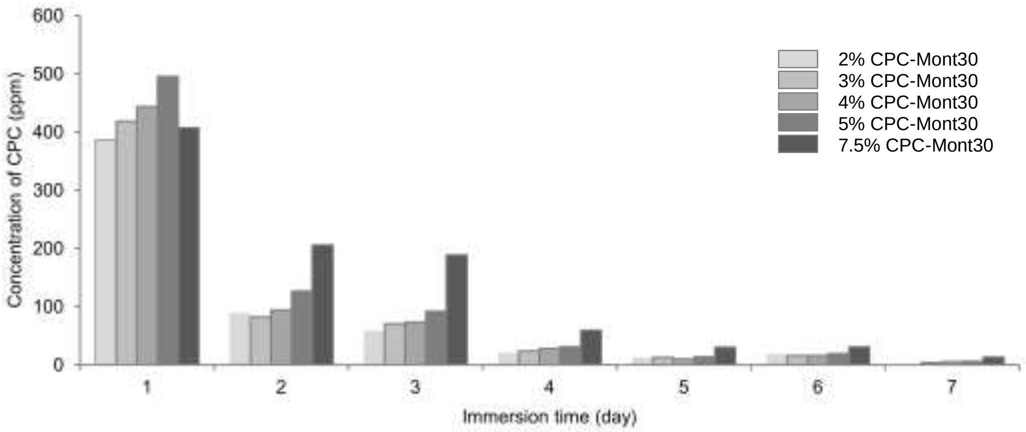


Fig.3 - Micro-tensile bond strength of (a)CPC-Mont30 and (b)CPC-Mont7 resin cement formulations

3.3. Quantification of released CPC

The concentration of CPC released from each resin disk was shown in Fig 4. Both CPC-Mont30 and CPC-Mont7 experimental resin cement formulations presented similar tendency in CPC release behavior. The amount of CPC released from each resin disk was the highest at 1 day, the concentrations ranged from 280 to 495 ppm. As the immersion period passed, the concentrations decreased, but CPC was still released after 7 days. CPC release tended to increase with increasing the CPC-Mont additive rate.

(a)



(b)

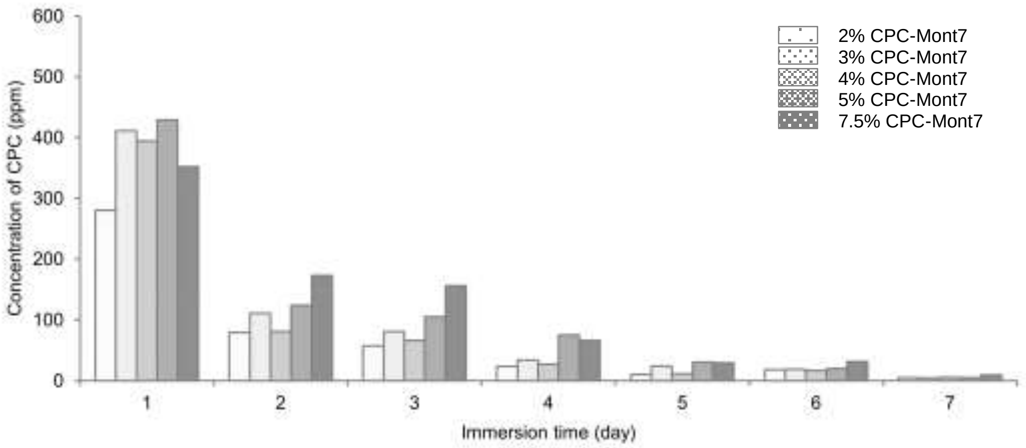


Fig.4 - The amount of CPC released from (a) CPC-Mont30 and (b) CPC-Mont7 modified resin cement formulations for 7 days as measured using UV spectroscopy.

3.4. Biofilm formation

The surface of 2 wt.% CPC-Mont 30 and 2, 3 wt.% CPC-Mont7 resin disks were almost covered with a biofilm, but that of other specimens were not covered after 7 days. (Fig 5)

On 30 days cultivation using CPC-Mont7 resin disks, the surface of 2 and 3 wt.% CPC-Mont7 resin disks were totally covered with a biofilm, and that of 4 wt.% CPC-Mont7 resin disk was partially covered. The biofilm formation was not observed on the surface of 5 and 7.5 wt.% CPC-Mont7 disks after 30 days. (Fig. 6)

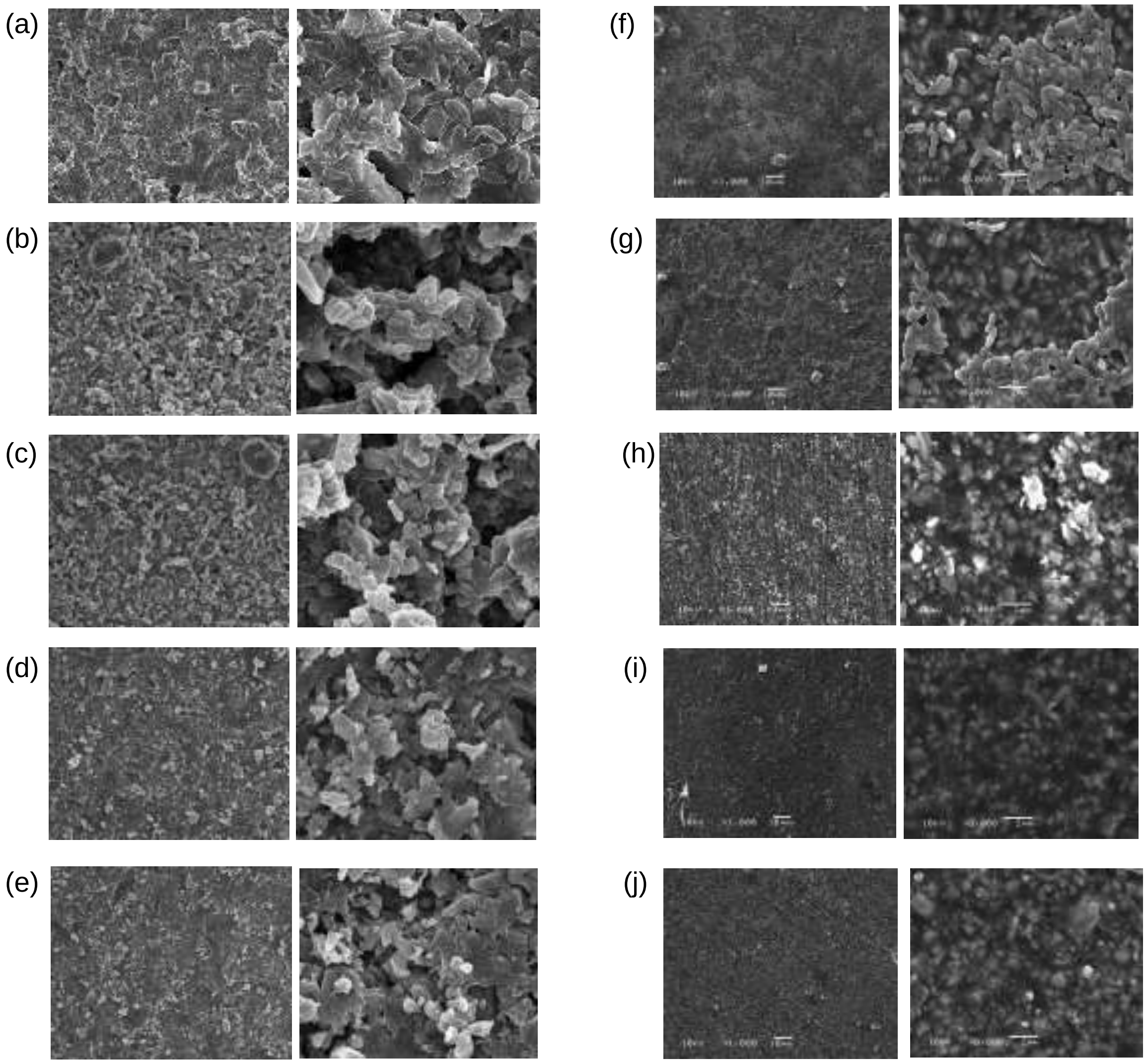


Fig.5 - SEM images of *S.mutans* biofilms formed on CPC-Mont30 and 7 resin disk surfaces after 7 days cultivation. (a - e) 2 to 7.5 wt.% CPC-Mont 30 resin disk, (f - j) 2 to 7.5 wt.% CPC-Mont 7 resin disk.

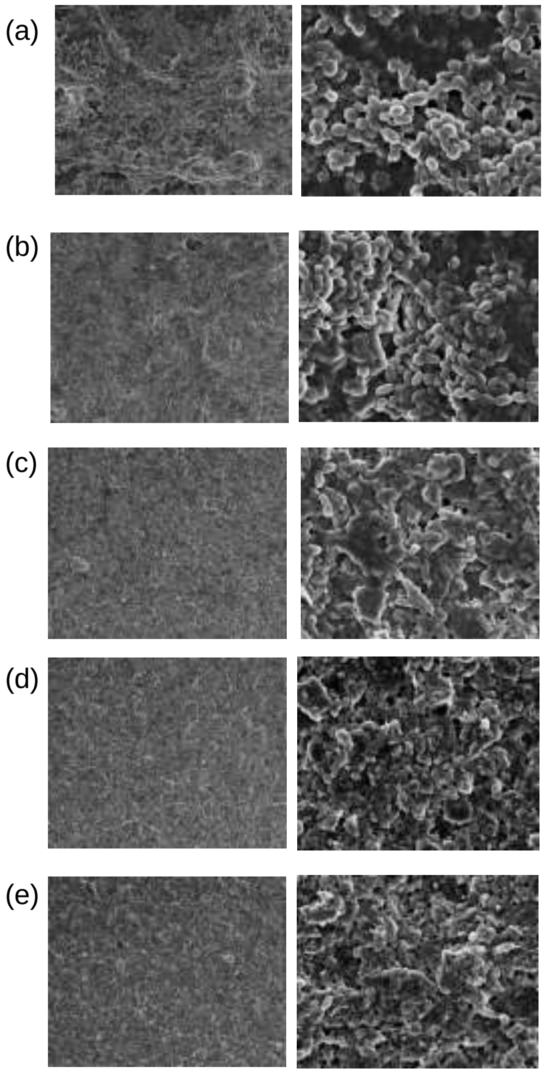


Fig.6 - SEM images of *S.mutans* biofilms formed on CPC-Mont7 resin disk surfaces after 30 days cultivation. (a - e) 2 to 7.5 wt.% CPC-Mont 7 resin disk.

4. Discussion

In aging society, prevention of secondary caries in restoration treatment is becoming increasingly important for extending the life of teeth and maintaining oral function.

Many dental cements, such as carboxylate cements, glass ionomer cements, and resin cements are used for various clinical cases, among them resin cements have excellent characteristics in mechanical property and bonding performance compared to other dental cements [11,20].

In this study, the two different particle size of anti-microbial compound CPC-Mont were synthesized, and the performances of the experimental resin cement formulations containing the CPC-Mont at several concentrations were investigated.

The particle size of filler significantly affects the mechanical properties of particulate-polymer composites. Addition of larger microparticles reduces flexural strength. However the composites reinforced with small microparticles have high flexural strength values due to the increase of surface area, which results in high surface energy at the filler-matrix interface [21].

The flexural strength of the experimental resin cement formulations with 30μm CPC-Mont decreased with increasing its concentrations, but no significant reduction were observed in elastic modulus, and thus the fracture toughness tended to decrease. In the case of the formulations with 7μm CPC-Mont, the flexural strength and elastic modulus were higher than those of the control in the low concentration range. Although the values decreased slightly with increasing the CPC-Mont additive rate, there was no significant difference between control and the high concentration formulations. Furthermore, the fracture toughness of the 7μm CPC-Mont formulations was comparable to that of the control with no significant difference.

de Menezes et al. reported that the addition of montmorillonite to the bonding material improved its mechanical property, and this improvement was caused by the dispersion of the clay platelets and the larger polymer/nanofiller interfacial area [19]. They also reported that the clay initially makes them act as microparticles, but after reaching a particular concentration, they tend to form larger agglomerates that act as fracture points, causing the material's failure when subjected to mechanical stress [19]. In this study, higher mechanical properties were obtained when the small particle CPC-Mont were added at low concentration, which were attributed to the increased polymer/filler interaction and the suppression of agglomerates that acts as fracture points.

Durable bonding of dental cement to dentin plays a critical role in preventing detachment of indirect restorations, microleakage, secondary caries, and tooth fractures. The micro-tensile bond strength of the experimental resin cement formulations using large particle CPC-Mont tended to decrease with increasing the additive rate. In particular, the significant reduction was observed at a concentration of 7.5 wt.%. On the other hand, no significant decrease in the tensile bond strength was observed when small particle CPC-Mont was used.

In the previous study conducted by Matsuo et al., 3 wt.% CPC incorporated in the commercial adhesive reduced the micro-tensile bond strength, which was attributed to the reduction of the degree of conversion and interaction of CPC with acidic functional monomer in the adhesive [16].

From a comparison of CPC release amount of large particle and small particle CPC-Mont cement formulations, the release behavior of CPC which might impair the bonding performance were not affected by the particle size.

Many factors could influence the bonding performance of dental cement, including degree of conversion, mechanical/chemical property, surface treatment procedure and existence of interfacial gap [16, 21, 22]. In the previous study, the increase of flexural strength improves the micromechanical locking of cement and enamel, which in turn increases the bond strength [23]. A moderate, though not significant, correlation between the fracture toughness and tensile bond strength was also reported [23].

In this study, the bonding performance of 7.5 wt.% large particle CPC-Mont formulation significantly reduced, although the CPC release amount was similar to that of 7.5 wt.% small particle CPC-Mont formulation. These result suggested that the decrease in bonding performance of the large particle CPC-Mont formulation is caused by the embrittlement of it with the large particle addition.

Many bacteria are present in oral plaque, among them, it is known that *S.mutans*. is the most common bacteria associated with caries. The synthesizing of insoluble glucans and acids by *S.mutans* is a major factor in the formation of biofilms and dental caries [24]. CPC is a cationic surfactant composed of quaternary ammonium salt, and has a wide range of anti-bacterial spectrum against many bacteria in the oral cavity by its lytic action, and also can be expected to have a higher anti-microbial capacity against Gram-positive bacteria such as *S. mutans* [25].

Namba et al. reported that the *S. mutans* biofilm formation on hydroxyapatite disk coated a bonding resin containing 3 wt.% CPC was not observed after 7 days cultivation, despite the amount of CPC released from the resin after 12 h was less than 0.11 ppm [15]. Therefore, they indicated that the CPC immobilized in resin matrix also exhibited the anti-microbial potential.

In the present study, the CPC release from the cement disks containing less than 3 wt.% CPC-Mont were calculated about 5 ppm, but the biofilm formation was partially observed after 7 days. Although the CPC release amount from the disk containing 5wt% CPC-Mont was almost the same, no biofilm formation was observed and the anti-biofilm formation capability was maintained even after 30 days. These results indicated that the anti-biofilm formation capability of the CPC-Mont cement formulations was also attributed to the immobilized CPC, as previous study conducted by Namba et al. reported. Although it is difficult to confirm clinical efficacy in vitro, we investigated that the anti-biofilm formation capability of the resin cement containing CPC-Mont greater than 5 wt.% lasted at least 30 days.

In conclusion, the mechanical properties, bonding performance and anti-biofilm formation capability of the experimental resin cement formulations modified by different particle size anti-microbial compound CPC-Mont were evaluated. The addition of large particle CPC-Mont may reduce the mechanical properties and bonding performance of the resin based cement. Therefore, it was considered that the addition of the smaller particle CPC-Mont at concentrations of 5 to 7.5 wt.% was preferable in order to demonstrate the continuous anti-biofilm activity while the mechanical properties and bonding performance of the resin cement were maintained.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

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