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Surface Characterization, Cytotoxicity, and Antimicrobial Activities of a Denture Base Resin Coated with *Cnidium Officinale* Extract

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Abstract

This study was aimed at investigating the surface characterization, cytotoxicity, and antimicrobial activities of a denture base resin coated with *Cnidium officinale* extract.

Cnidium officinale extracts were prepared at different concentrations, and the surface characterizations such as wettability, microhardness, and color changeof surfacewere measured. To evaluate the in vitro cytotoxicity, MTT assay was carried out in accordance with ISO 10993-5 standards. For the microbial analysis, two major oral microbes; *Streptococcus mutans* and *Candida albicans* were used in this study. All measurement data were analyzed with one-way ANOVA and Tukey's statistical test (p=0.05).

There were no significant differences in the surface characterizations and cell viability between the experiment group and the control group (p>0.05). However, there were significant differences in the antimicrobial activities between the experimental group and control group to *Streptococcus mutans*

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and *Candida albicans* (p<0.05). Denture base resins coated with *Cnidiumofficinale* extracts can function as antimicrobial denture base resins in dentistry.

Keywords: Antimicrobial activity;*Cnidium officinale;*Cytotoxicity;Denture base resin;Surface characterization

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INTRODUCTION

Due to the rapid increase of the aging society, oral health care for the elderly has emerged as an important issue in the dental field(Mirizadeh *et al*., 2018). The use of dentures (dentures) to replace lost tooth in elderly people with tooth loss is steadily increasing. However, denture stomatitis is known to occur in 67% of patient with denture, reaching a more serious level in patients with reduced immune function(Lee *et al*., 2020).Keeping dentures clean is important not only for aesthetic and the removal of bad breath, but also for maintaining a good oral health(Lee *et al*., 2020). However, majority of elderly with dentures have a poor oral hygiene, thus, increasing the infection rate of pathogenic microorganism in the oral cavity(Acosta-Torres *et al*., 2012). In addition, poor oral health in the elderly is associated to an increase in morbidity and the mortality rate among the elderly(Lee *et al*., 2020). Furthermore, even if the dentures are properly cleaned, the deposition of food residues, tartar, or pigments on the dentures can irritate the oral mucosa, denture stomatitis, or the palatal mucosal tissues(Choi *et al*., 2020). Denture stomatitis is causedby the *Candida albicans* species present in the microflora of

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the oral cavity; therefore, it is important to prevent the growth of *C. albicans*(Lee *et al*., 2020, Yang *et al*., 2020). In addition, for partial dentures, the prevention of *Streptococcus mutans* associated with secondary caries is important for the maintenance of residual teeth(Lee *et al*., 2019).

To prevent the growth of these bacteria on the denture, several researches have been carried out to introduce antibacterial substancesinto a denture base materials(Lee *et al*., 2018). Chlorhexidine is an antimicrobial substance that inhibitsthe colonization of bacteria;however, the long-term use of chlorhexidine is toxic to normal cells, destroys the balance of normal strains, and causes side effects such as discoloration(Al-Haddad *et al*., 2014). In addition, a quaternary ammonia composite used as an antimicrobial substance in denture based materials also causes discoloration at a high concentration(Beigi Burujeny *et al*., 2015). Therefore, to minimize the side effects caused by these synthetic substances, there is a need to utilize natural antimicrobial materials in denture base materials.Accordingly, several researches have been conducted to explore and develop natural antimicrobial substances(Lee *et al*., 2018, Lee *et al*., 2020).

Cnidium officinale, a medicinal plant, locally known in Korea as ‘Cheongung’, is a natural substance used as a medicinal material resource(Tran *et al*., 2018). *C. officinale*is a rich source of volatile compounds with a characteristic conspicuous oriental flavor.Several researcheshave reported the pharmacological activity of *C. officinale* such as its antioxidant, anti-inflammatory analgesic, and antibacterial activities(Jeong *et al*., 2009). However, there are few researches on denture base resin containing*C. officinale*.Therefore, in this study, we attempted to modify the surface of denture base materials using resin coated with*C. officinale*extract to preventdenture stomatitis caused by oral microbes. Hence, weinvestigated the surface characterization, cytotoxicity, and antimicrobial activities of *C. officinale* coating of denture base resin.

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MATERIALS & METHODS

Preparation of samples

C. officinale cultivated at the Sobaek mountains, which is purchased from an official herbal shop. To prepare the *C. officinale* extract, 500 g of *C. officinale* was grounded, immersed in 5 L of 70% methanol solution, and extracted at room temperature after 2 d. The extract was filtered using a #2 filter paper. Then, the filtered *C. officinale* extract was evaporated and concentrated in a vacuum evaporator, and prepared as a dry extract using a freeze dryer device. Acommercially available Jet denture (Lang, U.S.A.) was used as the denture resin and Plaquit solution (Plaquit, Dreve, Germany) was used asthe surface coating solution with the as-prepared *C. officinale* extract. The powdered *C. officinale* extract (0, 200, 400, and 600 μg/mL) was dissolved in Plaquit solution and then applied to a denture base resin,andcured with light(3M ESPE, USA). The resulting denture base resin with *C. officinale* coatings weresplited into four groups following the concentration of *C. officinale:* 200 μg /mL (C 200), 400 μg/mL (C 400), and 600 μg/mL (C 600), and the control group (C 0).

Surface wettability

The contact angle was measured to evaluate changes in the hydrophilicity of the specimens coated with the extract. The experimental group and the control group (uncoated, only plaquit coated) were dropped on 5 μL of distilled water using a contact angle measuring device, and the contact angle was measured immediately.

Surface microhardness

Vickers hardness was measured to confirm the difference in hardness between the experimental group and the control group (uncoated, only plaquit coated). To measure the Vickers hardness, a 0.09 MPa load was applied over the specimen surface for 20 s. The measurements were taken three times for each specimen.

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Color change

To examine the difference in the color changes, color measurement was performed using a spectrophotometer. A standard white plate was set as the standard for the measurement of color saturation, and the L*, a*, and b* values of each specimen were obtained, and then, the ΔE* value, which is the color difference value, was calculated. The L* value represents the brightness of the specimen, and the a* valuestands for the degree of green or red,while the b* value stands for the degree of blue and yellow. The formula used for calculating the△E* is as follows. The measurements were taken three times for each specimen.

Cell cytotoxicity

For the MTT cytotoxicity test, 100 μL of L929 cells wereseeded on 96-well plate, and of the as-prepared extract was distributed into the wells and incubated for 24 h.Then, 100 μL of the as-preparednatural extract diluted indiverse concentrations was applied to the cells for 24 h. RPMI 1640 was used as control. Afterward, the extract was removed, and the cells were refilled with 100 μL of Dulbecco's phosphate buffered saline solution. After washing, the DPBS was removed, and 50 μl/well was added and cultured with 1 mg/mL of MTT for 2 h. To deliquate the formed MTT formazan, 100 mL of isopropanol was insertedinto 100 μl/well and allowed to react for 20 min. Subsequently, the absorbance was determined at 570 nm using a spectrophotometer.

Antimicrobial activity

To measure antimicrobial activity, the effect of the extracts on growth inhibition for both strains was examined. The strains used in this test were *S. mutans*, and *C. albicans*. The *S. mutans* was cultured in a brain heart infusion, while the*C. albicans*was cultured in yeast mold, and then incubated at 37 °C for 24 h. The samples were then extracted in 600 uL of phosphate buffer solution and incubated for 24 h. The bacterial culture fluid was diluted so that the optical density measured at 600 nm value was 0.4-0.6. The mixture of solution and bacterial culture

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waspreparedin 1:1 ratio, and incubated at 37 °C for 24 h and 48 h. The antimicrobialactivity of the extracts was determinedfollowing the OD values in each well using an ELISA reader at 600 nm.

Statistical analysis

All data were analyzed by one-way ANOVA (PASW 23.0, IBM CO., USA), followed by Tuckey's statistical test (p=0.05).

RESULTS AND DISCUSSION

Numerous bacteria exist in the oral cavity as a colony of microflora consisting of over 500 species of bacteria, viruses, and fungi.Particularly, *C. albicans,* which is present in the oral cavity causes opportunistic infections that appears as a disease(Yang *et al*., 2020). In addition, *S. mutans* is known to have great influence on secondary caries(Lee *et al*., 2019). To prevent the attachment of these disease causing bacteria to denture base materials, antimicrobial denture base materials have been extensively studied;however, the cytotoxicity and surface change limits the application of these materials(Al-Haddad *et al*., 2014). In this study, to prepare denture base resins with antimicrobial properties, *C. officinale* was added to the denture base resins in concentrations of 0, 200, 400, and 600 μg/mL. The changes in the surface properties, antibacterial properties, and cytotoxicity at each concentration was evaluated. The surface hardness of denture base resins is an important factor indicating its resistant to forces exerted during authoring(Lee *et al*., 2020). A low surface hardness indicates that the stress distribution due to the masticatory force was not uniformly achieved. Furthermore,color changes in denture base resin is an important factor for aesthetics, and greatly influence the use of dentures(Lee *et al*., 2020).There were no significant differences in the surface characterization such as the contact angle, microhardness, and the surface color change between the experimental groups and the control group (Table 1) (p>0.05).

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Table 1. Surface characterizations of denture base resin coated with different concentration of *C. officinale* extract

Group	C 0	C 200	C 400	C 600
Contact angle	73.45 ± 5.44 [a]	72.96 ± 7.29 [a]	74.55 ± 6.05 [a]	67.46 ± 7.82 [a]
Microhardness	26.5 ± 1.7 [a]	24.6 ± 3.1 [a]	27.0 ± 1.5 [a]	24.9 ± 2.4 [a]
Color change	1.05 ± 0.30 [a]	1.08 ± 0.27 [a]	1.10 ± 0.33 [a]	1.14 ± 0.40 [a]

It is crucial to evaluate the cytotoxicity of denture base materialdue to its direct contact with the oral mucosa(Yang *et al*., 2020). Cytotoxicity assessment is planned to investigate the biological response of cells in vitro utilizingsuitable biological parameters. To determinethe cytotoxicity of the samples, an MTT assay was conducted, that is an approved method for examining biocompatibility, as specified in iso 10993-5(Lee *et al*., 2020).There were no significant differences in the cytotoxicitybetween the experimental groups and control group (Table 2) ($p>0.05$).

Table 2. Cytotoxicity of denture base resin coated with different concentration of *C. officinale* extract

Group	C 0	C 200	C 400	C 600
Cell viability	109.47 ± 4.34 [a]	103.77 ± 1.08 [a]	99.06 ± 2.31 [a]	102.95 ± 5.82 [a]

Microbial analysis showed that the OD of the experimental samples significantly decreased at 48 h for both *S. mutans* and *C. albicans*(Table 3) ($p<0.05$).

This result is similar to that of our previous study, which reported that the antimicrobial properties of *C. officinale*may be associated to the presence of a phenolic compound, a reference material exhibiting antibacterial activity in *C. officinale*. Therefore, the antibacterial properties of the experimental groups to *S. mutans* and *C. albicans*can be attributed to the phenolic compounds (polyphenol and flavonoid) components of *C. officinale*(Jeong *et al*., 2009). However, the antibacterial mechanism of *C. officinale* is uncertain, but these results confirmed that these

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compounds may exhibit effective antimicrobialactivities. To understand theantibacterial mechanism of *C. officinale*,further research should be conducted to investigate their antibacterial mechanisms and their long-term effects.

Table 3.Antimicrobial activities of denture base resin coated with different concentration of *C. officinale*extract

Group	C 0	C 200	C 400	C 600
S. mutans	0.612 ± 0.008 [a]	0.477 ± 0.100 [a,b]	0.440 ± 0.075 [a,b]	0.359 ± 0.088 [b]
C. albicans	0.855 ± 0.003 [a]	0.726 ± 0.220 [b]	0.705 ± 0.213 [b]	0.678 ± 0.362 [b]

CONCLUSION

In conclusion, the results of this study were summarized as follows.

1. *C. officinale* extract coated on the denture base resin, had no significant effect on the surface characterizationssuch as surface wettability, microhardness, and color change of the denture base resin (p>0.05).

2. There were no significant differences in the cytotoxicity between the experimental group and the control group (p>0.05).

3. There were significant differences in the antimicrobial activities between the experimental group and the control group (p<0.05).

These results confirm the potential*C. officinale* extract as an antimicrobial agent in denture base resin coating materials.Therefore, denture based resin coated *C. officinale* can be effectively used for application as an antimicrobial dental materials.

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