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Antimicrobial Efficacy, Surface Characterization, and Biocompatibility of a Denture Relining Material Coated with *Glycyrrhiza Uralensis* Extract

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Abstract

The purpose of present study was to confirm the antimicrobial efficacy against *Streptococcus mutans* and *Candida albicans*, surface characteristics, and biocompatibility of a denture relining material coated with *Glycyrrhiza uralensis* extract.A polymerized denture relining disk was coated with *Glycyrrhiza uralensis* extract (200, 400, and 600 μg/ml). Coated disk specimens incubated with each microbial were evaluated based on spectrophotometric growth inhibitory assay. The contact angle, microhardness, and color change of coated specimens were measured. Coated specimen was soaked in distilled water for one day. Extracts were analyzed using a UV/VIS spectrometer to determine the amount of polyphenol content. Biocompatibility of the coated specimens was assessed with MTT assay. Obtained values were analyzed with one-way ANOVA and Tukey's statistical test.*Glycyrrhiza uralensis* coated specimen showed a significant growth inhibitory effect on each microbial, demonstrating antimicrobial efficacy. Water contact angle, microhardness, and delta*E** values did not show significant differences between the groups. When the content of *Glycyrrhiza uralensis* extract was increased, the level of polyphenol was significantly increased. None of the experimental groups had cytotoxicity.A coating material containing *Glycyrrhiza uralensis* extract is a promising candidate for an antimicrobial dental resin. The newly developed coating resin will not degrade the surface or the biocompatibility properties of the denture relining material.

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Introduction

Seven hundred varieties of microorganisms exist in the human mouth (Zijnge et al., 2010). *Streptococcus mutans* (*S. mutans*) are attached to the tooth surface and metabolize acid from fermentable carbohydrates. Through the process of producing the acids, they cause dental caries. *Candida albicans* (*C. albicans*) also adhere to the denture surface or oral mucosa. This causes diseases such as denture stomatitis in denture patients. Therefore, to prevent dental caries and dental stomatitis, it is necessary to apply an antimicrobial agent that can inhibit the proliferation of the bacteria, or the attachment of microbes, to the tooth surface in an oral cavity.However, microorganisms like *S. mutans* and *C. albicans*, easily adhere to, and make biofilms on, both poly(methyl methacrylate) and poly (ethyl methacrylate) liner surfaces (Karakis et al., 2016). To improve oral hygiene, cleaning of the denture surface and oral health care are effective methods for preventing aspiration pneumonia and denture stomatitis. Mechanical cleaning of denture surfaces is efficient in improving the denture hygiene. However, mechanical brushingcan induce the degrade the denture materials, creating a rough surface (Oliveira et al., 2007). Therefore, the application of antimicrobial agents that can prevent infection from oral microorganisms, without deteriorating the denture surface may help oral hygiene management.Applying coating resins to denture surfaces has proven beneficial for biological resistance against oral pathogens, improved mechanical and chemical properties, and stain resistance (Zuo et al., 2016). Studies on the modification of denture materials have attempted to reduce colonization by pathogenic oral microorganisms, thereby demonstrating an improvement in antimicrobial activity (Monteiro et al., 2012). Studies have also investigated preventing the adhesion of microbes to the surface and suppressing the activity of oral pathogenic microorganisms (Hirasawa et al., 2018). Previous studies either developed an antibacterial coating material or synthesized a chemical substance exhibiting antibacterial activity on the

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denture surface. In addition, denture base resins were mixed with a chlorhexidine agent (Procopio et al., 2018). These antimicrobial agents inhibited the growth of oral pathogens, but cytotoxicity to human gingival fibroblasts was observed.Naturally derived *Glycyrrhiza uralensis* Fisch extract is highly valued for its several medicinal properties, including its antibacterial effects (He et al., 2006). Additionally, its biological activity and chemical constituents have been explored in previous studies. The polyphenol component in *Glycyrrhiza uralensis* extract inhibits the activity of oral pathogenic microorganisms (Yang et al., 2020). These previous studies have only tested for antimicrobial activity, without investigating the extract as a coating resin on denture relining materials for improving oral health. Therefore, this study investigated modifying the surface of denture base materials using a coating resin containing naturally derived *Glycyrrhiza uralensis* extract. The investigation would aid in the prevention denture stomatitis from oral pathogenic microorganisms. The present study evaluates the antimicrobial efficacy against two types of oral microorganisms. The study also investigated the surface characterization and the biocompatibility of the *Glycyrrhiza uralensis* extract coating on denture relining material. Null hypotheses were that the *Glycyrrhiza uraeuses* extract would not produce significant differences in the antimicrobial efficacy, contact angle, microhardness, color change, amount of released polyphenol, and cell viability, compared to the pure resin coating.

Materials and Methods

Five hundred grams of *Glycyrrhiza uralensis* roots was ground, and extracted in 70% methanol solutionfor 48 h. Filtered *Glycyrrhiza uralensis* was concentrated with a vacuum evaporator (EYELA, Tokyo, Japan). Extract was dried with a freeze dryer device (Ilshin Lab, Gyeonggi-do, Korea). To create experimental coating resins, *Glycyrrhiza uralensis* extract powder was dispersed in a light-curable coating resin material (Plaquit, Dreve, Germany)using a magnetic stirrer for 24 h. The following experimental and control groups (weight of *Glycyrrhiza uralensis* extract powder/volume of coating resins) were prepared: 200 µg/mL (GU 200), 400 µg/mL (GU 400), and 600 µg/mL (GU 600). A coating resin without *Glycyrrhiza uralensis* extract was used as a control (GU 0).A stainless-steel mold 0.1 cm in thickness and 1 cm in diameter, was positioned onto a slide glass covered with polyester film. The powder and liquid components of the auto-polymerizing denture relining material (Tokuyama Rebase II, TOKUYAMA, Japan) were mixed referring to the manufacturers' instructions. Components were mixed until they were the consistency of dough state. The mold was filled with the relining material at this consistency. A polyester

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film was positioned on denture relining material with a microscope slide glass. Polymerized denture relining material was carefully separated from the mold.After than, 10 µL of experimental coating resin was spread on the cured disks and then polymerized using the light curing unit (Visio Beta Vario unit,3M ESPE, USA)for 10 min.

Antimicrobial activity

A growth inhibition test was performed on the experimental specimens coated with *Glycyrrhiza uralensis* extract. This was completed to assess the antimicrobial effect of the coating treatment against two types of oral microorganisms, *S. mutans* and *C. albicans*. *Streptococcus mutans* (ATCC 25175) and *C. albicans* (ATCC 10231) were cultured in each media, respectively, and then incubated at 37 °C. The microbial fluids were diluted to obtain an optical density (OD600) value of 0.4-0.6. The coated specimen was then immersed in microbial fluids for 48 h at 37℃ to achieve the extraction ratio suggested in ISO 10993-12. Afterwards, the coated specimens were removed and the OD value of microbial fluids was observed at 600 nm with an ELISA device (Epoch, BioTeck, VT, USA).

Surface wettability

To evaluate the surface wettability of the coated specimens, contact angle (Phoenix 300, SEO, Korea) was measured. A micro pipette was used to drop the distilled water on experimental and control specimen surfaces, and water contact angles was observed after 3 s.

Surface microhardness

To observe the hardness of the coated surface, Vickers microhardness number (DMH-2, Matsuzawa Seiki Co., Tokyo, Japan) was measured under 0.09 MPa load for a dwell time of 20 s.

Color characteristic

To examine the surface color change of the coated specimens, color was measured before and after the coating treatment using a spectrophotometer (Lamba20, Perkin Elmer, Orwalk, CT, USA). Color change value (*ΔE**) before and after coating was estimated by this equation $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$.

Analysis of extracts

The coated specimen was immersed in distilled water for 7 days at 37℃. This was completed according to the extraction ratio suggested in ISO 10993-12. To evaluate the released polyphenol content, 50 µL of Folin-Denis reagent and 50 µL of the extract solution were dropped to 650 µL of distilled water. After then, 150 µL of distilled water

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and 100 µL of 10% Na_2CO_3 solution were added to make the total 1 mL volume. This mixed solution was stored at 37 °C for 1 h. Absorbance of the solution was observed with UV/VIS spectrophotometer (X-ma 1200 Spectrophotometer, Human, Korea)at 725 nm. Polyphenol content was estimated from the 20, 60, and 100 µg/mL standard curves obtained using garlic acid solution (Sigma Aldrich, MO, USA).

Cell viability test

To evaluate the biocompatibility of control and experimental specimens, they were soaked in cell culture media (RPMI 1640, Gibco Laboratories, NY, USA) for 24 h at 37 ℃. This was done according to the extraction methods suggested in ISO 10993-12. To examine the cell viability of the extracts, an MTT test was performed referring to ISO 10993-5. The L929 mouse fibroblast cells were seeded in well plate and stored in an incubator at 37 ℃ in the presence of 5% CO_2. After one day, the culture media was carefully removed, 100 µL of the extract was dispensed on to the attached cell and stored in an incubator at 37 ℃ in the presence of 5% CO_2. Then, after 24 h, the 100 µL extract solution was replaced with 50 µL of 1% MTT solution. Well plate was stored in an incubator at 37 ℃ in the presence of 5% CO_2. After 3 h, MTT solution was suctioned and 100 µL isopropanol was added and then positioned on a shaker for 20 min. Well plate was measured at 570 nm with an ELISA device (Epoch, BioTek, VT, USA).

Statistical analysis

Antimicrobial activity, surface wettability, microhardness, color characteristic, polyphenol contents, and cell viability tests were repeated in five separate experiments. All of the test results were analyzed using one-way ANOVA (IBM SPSS statistics 25.0, IBM Co., NY, USA) followed by Tukey's statistical test. The significance levels were fixed at 0.05.

Results and Discussion

In the present study, the potential of a denture relining material coated with *Glycyrrhiza uralensis* extract on the microbial activity, surface characterization and biocompatibility were evaluated. Many natural medicines derived from plant extracts, have been studied to explore their anti-inflammatory and antibacterial efficacy. Natural medicine is currently being considered as a component in oral hygiene.

Microbes used in the present study are the most important bacteria and fungi in oral cavity. *Streptococcus mutans* and *C. albicans* adhere to the enamel surface or denture base materials (Koch et al., 2013; Olsson et al., 1990). The evaluation of the antimicrobial efficacy against these bacteria and fungi is a valuable clinical result. In our study, the

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experimental specimens that contained *Glycyrrhiza uralensis* extract significantly affected the growth of oral pathogens ($p<0.05$). This is shown in Fig. 1. These results demonstrate that a denture relining resin coated with *Glycyrrhiza uralensis* extract prevents activity of *S. mutans* and *C. albicans*. Antimicrobial activity of three different concentrations of *Glycyrrhiza uralensis* extract was not significantly different for the two microbes.

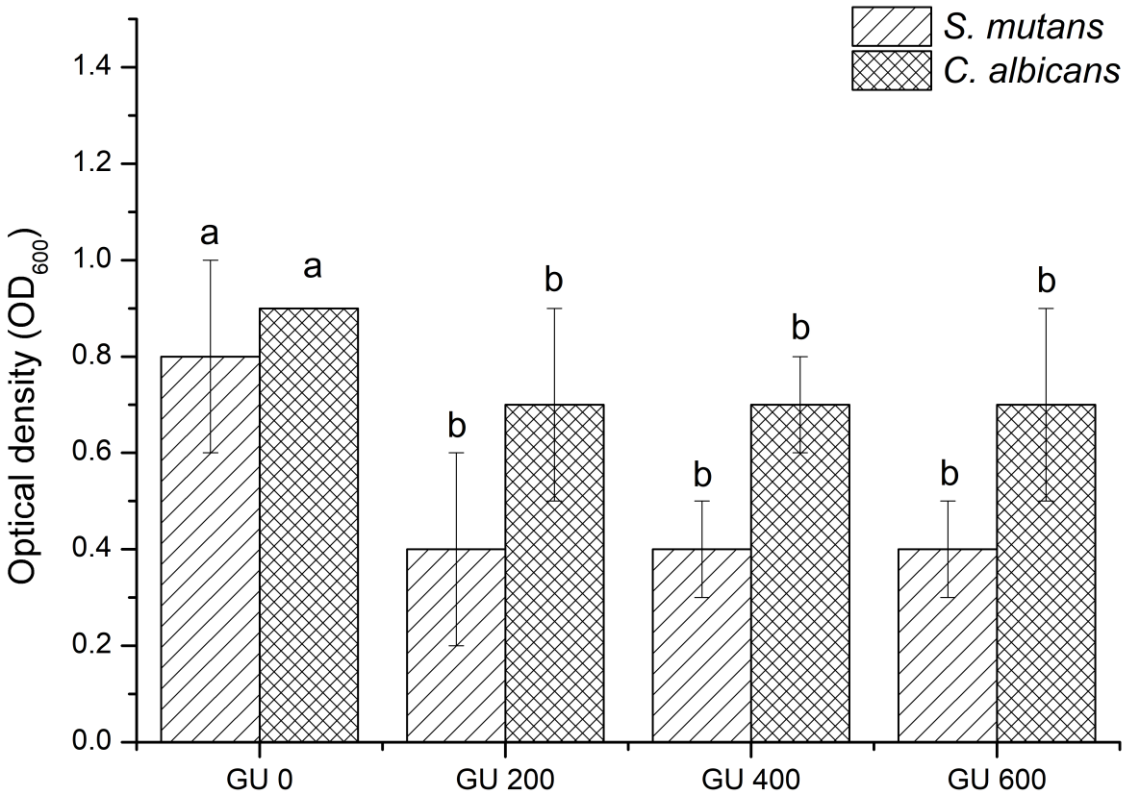

Figure 1: Optical density value of control and experimental groups for each microbial. The lowercase letter shows no significant differences between experimental and control groups for each microbe ($p>0.05$).

During the analysis, polyphenol was confirmed in the extracts of coated specimens that contained *Glycyrrhiza uralensis*($p<0.05$). This is shown in Fig. 2d. These results imply that the *Glycyrrhiza uralensis* extract in the coating materials had been leached out of the polymerized coating resin in the liquid environment. Phenolic metabolites are widely distributed in plants and show useful biological activities such as antimicrobial efficacy. The antimicrobial effects of plants are also known to be related to phenolic compounds(Yang et al., 2020). The released phenolic compounds from the *Glycyrrhiza uralensis* extract may contribute to the inhibition of microbial growth. Therefore, *Glycyrrhiza uralensis* extract could be applied to prevent microbial-associated denture stomatitis.

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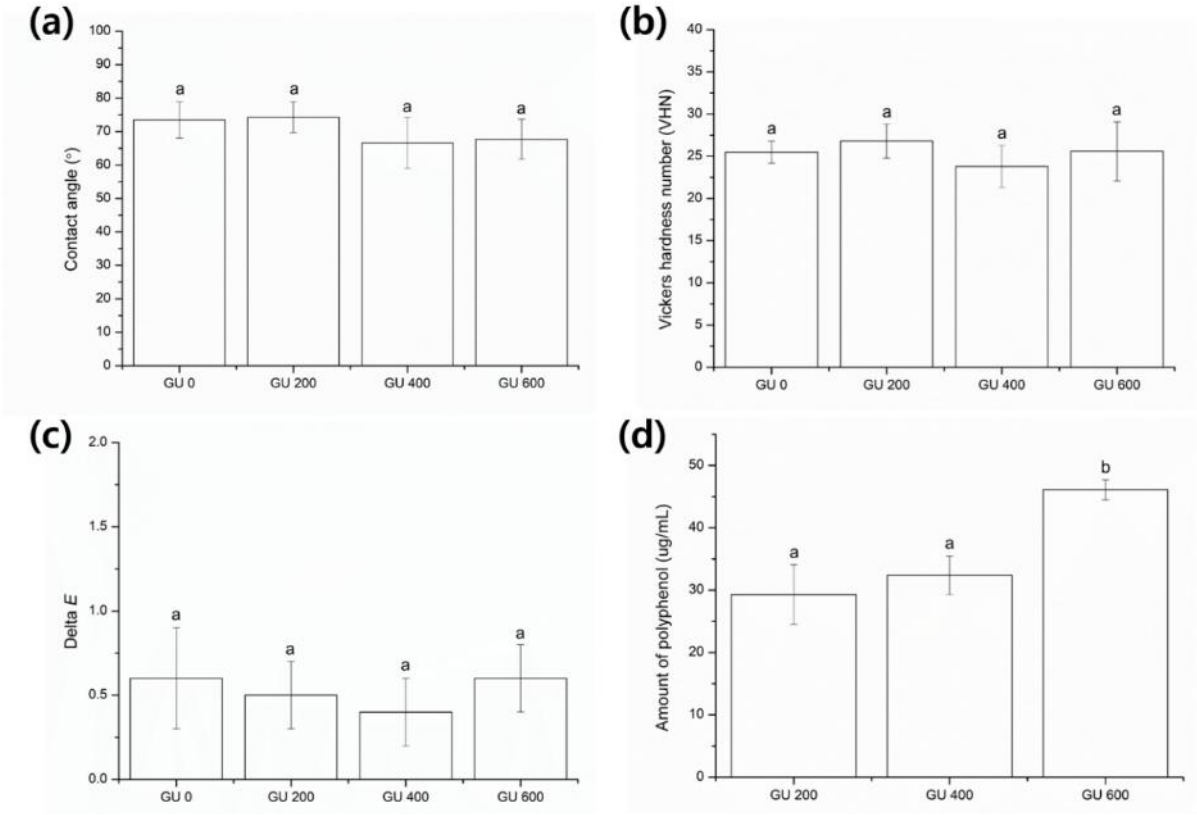

Figure 2: Results of the contact angle (a), microhardness (b), color change (c), and amount of polyphenol (d) of each group. For each test, the lowercase letter indicates no significant differences between groups (p>0.05).

Coating treatment for denture base materials should be have crack resistance, high hardness, stable mechanical and physical properties, and inhibit negative effects in an aqueous environment(Zuo et al., 2016). Previous studies have recommended that denture retention could be improved by maintaining the surfacewettability(Sipahi et al., 2001). Therefore, contact angle tests on coated surfaces can imply the capability of denture retention. To observe the wettability of the experimental specimens in the presence of *Glycyrrhiza uralensis* extract, contact angles were measured using distilled water. As shown in Fig. 2a, the contact angle was not significantly different between control and experimental groups ($p>0.05$). Our result suggests that components of *Glycyrrhiza uralensis* extract do not any effect on the wettability of the coating resin.

Surface hardness could indicate the wear resistance of a prosthesis(Lappalainen et al., 1989). To confirm the hardness of the coated denture relining materials in the presence of *Glycyrrhiza uralensis* extract, a surface hardness test was conducted with a Vickers microhardness tester. As shown in Fig. 2b, the surface microhardness was not significantly different between the control and experimental groups ($p>0.05$). Our present result implies

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that *Glycyrrhiza uralensis* did not have any adverse effect onsurface hardness of denture relining materials coated with *Glycyrrhiza uralensis*extract.

Glycyrrhiza uralensis extract should not affect the color or aesthetics of the prosthesis. To evaluate the surface color characteristics of experimental specimens coated with *Glycyrrhiza uralensis* extract, a spectrophotometer was used. This equipment had the advantage of quickly measuring the intensity and color difference of transmitted light reflected by the specimen, with high accuracy and reproducibility(Koksal and Dikbas, 2008). As shown in Fig. 2c, color changes (before and after coating treatment) did not show significant differences between the control and experimental groups ($p>0.05$). Thus, it was found that *Glycyrrhiza uralensis* extract had no adverse effect on the color of the coating surface.

Biological risk from medical devices can be partly estimated by *in vitro*cell viability tests(Procopio et al., 2018). Our study assessed the effect of *Glycyrrhiza uralensis* extract coating material on cell viability. This result shows that the *Glycyrrhiza uralensis* extract does not have a toxicity on the cell.

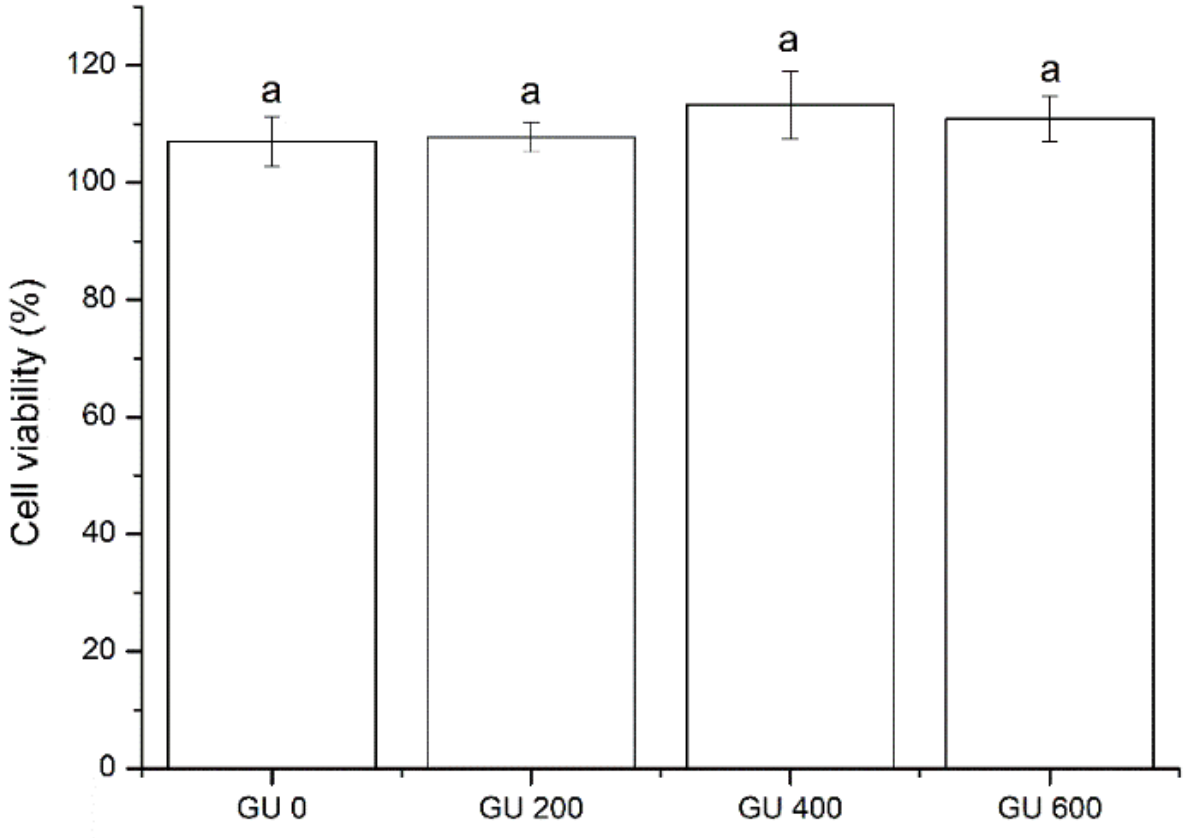

Figure 3: Cell viability of experimental and control groups. The lowercase letter indicates no significant differences between groups ($p>0.05$).

Therefore, we accept null hypothesis that the denture relining material coated with *Glycyrrhiza uralensis* extract would not produce significant differences in the water contact angle, microhardness, color change, and cell viability, compared to the pure resin coating. On the other hand, we reject the null hypothesis stating that the application of the coating material containing *Glycyrrhiza uralensis* extract does not result in significant differences in the antimicrobial activity on *S. mutans* and *C. albicans*,released polyphenol content, compared to the pure resin coating.

Conclusion

A denture relining material coated with *Glycyrrhiza uralensis* extract revealed an antimicrobial efficacy against two types of oral pathogens. Moreover, it exhibited characteristics similar to the control group when considering surface contact angle, microhardness, color characteristics, and biocompatibility. In present study, the antimicrobial efficacy of the denture relining material coated with *Glycyrrhiza uralensis* extract against oral pathogens, lasted for a few days. Therefore, it is necessary to investigate the long-term antimicrobial activity, as well as the surface characterization of this novel coating material.Nevertheless, coating materials containing *Glycyrrhiza uralensis* extract are a promising candidate for application as an antimicrobial dental material, with no lowering of the surface characterization and biocompatibility.

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