

Dental Journal

Majalah Kedokteran Gigi



Effects of herbal medicine components on the physical properties of trial denture adhesives • The role of active ingredients Nano-powder *Stichopus hermannii* gel to bone resorption in tension area of orthodontic tooth movement • The effects of *Anadara granosa* shell-*Stichopus hermannii* on bFGF expressions and blood vessel counts in the bone defect healing process of Wistar rats



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Effects of herbal medicine components on the physical properties of trial denture adhesives

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ABSTRACT

Background: Denture adhesive is widely used in elderly people who wear complete dentures. Chinese herbal medicine has long been used for the treatment of oral disease. The addition of herbal medicine to denture adhesive might be used to develop an adhesive which is effective for xerostomia patients. **Purpose:** To examine the effects of herbal medicine components on the physical properties of denture adhesive. **Methods:** This study used a combination of 35wt% PVM-MA, 20wt% CMC, 40wt% white petrolatum and 5wt% liquid paraffin as base materials. Three kinds of herbal medicine components: Maimendong (*Ophiopogon*), Fuling (*Hoelen*) and Dongkuizi (*Cluster mallow seed*) were added to base materials of 1wt%, 5wt% and 10wt%. The initial viscosity was measured using a controlled-stress rheometer (AR-G2). The adhesive strength was quantified according to ISO-10873 recommended procedures. All data was analyzed independently by one-way ANOVA combined with a Turkey's multiple comparison test at a 5% level of significance. **Results:** Significant differences were observed between materials in initial viscosity ($p < 0.05$). Specifically, samples containing 5wt% and 10wt% of Maimendong (*Ophiopogon*) showed higher values compared with the control samples. The larger the amounts of herbal medicine components, the greater the changes in the adhesive strength of denture adhesives over time. The denture adhesives containing herbal medicine components do not have a cytotoxic effect and are safe for use in actual clinical practice. **Conclusion:** The study showed that the addition of herbal medicine components does not affect physical properties (i.e. initial viscosity and adhesive strength).

Keywords: *Ophiogon; Hoelen; Cluster mallow seed; herbal medicine; denture adhesives; initial viscosity; adhesion strength*

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INTRODUCTION

According to the 2017 Annual Report on the Aging Society in Japan,¹ the over-65s account for 27.3% of the entire country's population. This constitutes the highest number of elderly in its history. Japan has, undisputedly, become regarded as a super-aging society, with the number of Japanese above the age of 80 surpassing 10 million. Many elderly people wear partial or complete dentures, with the rate of complete denture use known to increase with age.² Denture function begins from the moment dentures are placed in the oral cavity. Therefore, having comfortable dentures is crucial to improving the quality of life of the elderly. However, the latter face numerous problems, such as xerostomia, anatomical limitations

and psychological anxiety. The number of patients using denture adhesives to stabilize and maintain dentures is increasing worldwide. According to a census of American academic prosthodontists regarding denture adhesives, the general attitude toward their use has changed greatly over the last 10 years.³ Despite the range of disagreements regarding the clinical significance of denture adhesives, the fact remains that improvements in denture function, stability and maintenance have been observed following their application.⁴⁻⁷

Denture adhesives are broadly divided into two types, liner (home reliners) and glue, based on their mechanism of action.⁸ Liner type denture adhesives fill the gap between the denture and the oral mucosa, thereby improving adhesion. However, the damage (i.e. deviation, bite raising) caused

by these materials has long raised concerns regarding their use.^{9–12} In addition, liner type denture adhesive can change the occlusal vertical dimension and promote ridge resorption, with the result that dental professionals pay little attention to such materials.

On the other hand, glue type denture adhesives are mainly composed of water-soluble polymers that absorb the saliva between the denture base and the oral mucosa, thereby improving adhesion. These types of denture adhesives are broadly divided into powder-form, cream-form and sheet-form. Of these, the cream-form and powder-form varieties are advantageous in that they are thin and do not affect the occlusal vertical dimension. Cream-form denture adhesives are also advantageous in terms of their durability.¹³

Xerostomia, which is particularly common among complete denture users, is treated with humectants. Currently, no other treatments are available and no radical solution has been identified. However, symptoms such as xerostomia have long been treated with Chinese herbal medicines. In particular, the herbal medicines Maimendong (*Ophiopogon*), Fuling (*Hoelen*) and Dongkuizi (*Cluster mallow seed*), as written in *Shennong Bencao Jing* (The Classic of Herbal Medicine) and *Bencao Gangmu* (The Compendium of Materia Medica), have long been considered effective in curing illnesses characterized by dry mouth or tongue, conditions wherein the tongue dries and takes on a burnt color, and in treating the dry mouth in patients with head and neck cancers.¹⁴ In addition, Maimendong (*Ophiopogon*) has been used to improve xerostomia in patients with Sjögren's syndrome.¹⁵

Many researchers reporting the use of denture adhesive *in vitro* and *in vivo* have confirm its effectiveness in improving denture retention and stability, increasing bite force, improving taste discrimination and taste perception among other benefits.^{16–20} A number investigated the initial viscosity and adhesive strength of denture adhesives and the effect of components on the mechanical properties of denture adhesives *in vitro*.^{21,22} However, the effect of herbal medicine on the initial viscosity and adhesive strength remains unclear. A combination of herbal medicine and denture adhesive might be used to develop denture adhesive which proves effective in cases of xerostomia.

The purpose of the present study was to examine the effects of herbal medicine components on the initial viscosity and strength of denture adhesive, to examine the cytotoxicity of herbal medicine components on the

fibroblast cell and to develop cream-form denture adhesives containing herbal medicines. The null hypothesis adopted was that the addition of herbal medicine components neither affects initial viscosity and adhesive strength nor induces cytotoxicity in fibroblast cells.

MATERIALS AND METHODS

The primary denture adhesive components used in the study consisted of polyvinyl methyl ether-maleic acid (PVM-MA) copolymer alkali salt and sodium carboxymethyl cellulose (CMC). In addition, white petrolatum and liquid paraffin were used as ointment bases. The composition, formulation and code are shown in Table 1 and Table 2, respectively. After stirring a proportion of 40 wt% white petrolatum and 5 wt% liquid paraffin, 35 wt% PVM-MA and 20 wt% CMC were added. The ingredients were mixed for two minutes using a vacuum mixer (VM-II, GC Co., Tokyo, Japan) to produce a control sample for use as a base. A powdered herbal medicine was subsequently added to the prepared bases at different concentrations of 1wt%, 5wt%, and 10wt% (Table 2). The vacuum-mixed products were transferred to hermetically sealed containers and placed in a dark room at 23°C for 24 hours to yield

Table 2. Formulations of components

Code	Base materials	Herbal medicine components
BMT01	PVM-MA 35wt%; CMC	Maimendong (<i>Ophiopogon</i>) 1wt%
BMT05	WPL 40wt%; LP 5wt%	Maimendong (<i>Ophiopogon</i>) 5wt%
BMT10	WPL 40wt%; LP 5wt%	Maimendong (<i>Ophiopogon</i>) 10wt%
FKR01	PVM-MA 35wt%; CMC	Fuling (<i>Hoelen</i>) 1wt%
FKR05	WPL 40wt%; LP 5wt%	Fuling (<i>Hoelen</i>) 5wt%
FKR10	WPL 40wt%; LP 5wt%	Fuling (<i>Hoelen</i>) 10wt%
TKS01	PVM-MA 35wt%; CMC	Dongkuizi (<i>Cluster mallow seed</i>) 1wt%
TKS05	WPL 40wt%; LP 5wt%	Dongkuizi (<i>Cluster mallow seed</i>) 5wt%
TKS10	WPL 40wt%; LP 5wt%	Dongkuizi (<i>Cluster mallow seed</i>) 10wt%

Table 1. Primary denture adhesive components used

Polymer	Manufacturer	Lot. No.
Methoxy ethylene maleic anhydride copolymer (PVM-MA)	ISP Japan LTD., Tokyo, Japan	CC600150446
Sodium carboxymethyl cellulose (CMC)	Daiichi Kogyo Seiyaku Co., LTD., Kyoto, Japan	353847
White petrolatum (WPL)	Nikko Pharmaceutical Co., LTD., Hatori, Japan	669319
Liquid paraffin (LP)	Sigma-Aldrich Japan Co. LLC., Tokyo, Japan	A7568

experimental samples containing each of the three herbal medicines Maimendong (*Ophiopogon*), Fuling (*Hoelen*), and Dongkuizi (*Cluster mallow seed*); (Uchida Wakanyaku Ltd., Tokyo, Japan) (Table 2).

In this study, the initial viscosity was measured using a stress-controlled rheometer (AR-G2, TA Instruments Ltd., Tokyo, Japan) and a parallel plate measuring 2 cm in diameter with a gap between the plates of 54 μm (Figure 1). The instrument was used in a constant strain mode with an angular velocity of 10 rad/s at $37 \pm 2^\circ\text{C}$. Five specimens were measured for each material.

Measurements were obtained using the ISO 10873⁸ prescribed sample holder I and a pressure-sensitive axle (Figure 2). Sample holder I was fabricated from methacrylic resin (Acron Lot. No. powder 0308011; liquid 0308092, GC Co., Tokyo, Japan). The hole of sample holder I was filled with 500 ± 5 mg of sample which was subsequently immersed in 300 ml of distilled water at $37 \pm 2^\circ\text{C}$ for 0, 1, 10, 30, 60, 180 or 360 minutes. A load of 9.8 ± 0.2 N was applied to the sample using a constant load compression testing machine (A-001, Japan Mecc Co. Ltd, Tokyo, Japan) at a pressurizing velocity of 5 mm/min using a pressure sensitive knob for 30 seconds. The sample was then pulled in the reverse direction with tensile velocity using a materials testing machine (Model 5565, Instron Co., Canton, MA, USA) at a crosshead speed of 5 mm/min. Measurements were taken five times for each combination.

A methacrylate board prescribed by ISO 10873⁸ and measuring 5.0 5.0 cm was fabricated using heat-polymerized denture base resin (Acron Lot. No. powder 0308011; liquid 0308092, GC Co., Tokyo, Japan). Samples were applied evenly on the methacrylate board, immersed in distilled water at $37 \pm 2^\circ\text{C}$ for one hour and, finally, scrubbed 20 times with a denture brush under running water. The methacrylate board was subsequently observed visually and the presence or absence of residue clumps assessed five times. The sample was considered to have passed the test if no clumps of residue were observed during at least four of the five assessments.

Following degradation of 1.00 ± 0.05 gram of sample by the addition of 5.00 ± 0.05 g of propylene glycol, 300 ml of distilled water were stirred in, before the sample was agitated further. Then, the electrode of a pH meter

(Series 2368, Beckman Coulter Inc., Tokyo, Japan) was inserted and the pH value displayed three minutes later was recorded. The same measurement was recorded five times and the values were averaged.

Following culture with a human gingival fibroblast cell line (p10-13) in a 24-well plate (110^4 /well) for 24 hours, the sample was placed in cell culture inserts so that it would account for 10% of the entire culture medium (1500 μl /well). Culture was performed again at 37°C under 5% CO_2 for 24 hours. Then, OD at 450 nm spectrophotometry ($n=5$) was measured using a WST-8 cell counting kit (Sigma-Aldrich Japan, Tokyo, Japan). For the apoptosis measurement, the cells (110^4 cells/well) were exposed to specimens for 24 hours in a 24-well plate. Following exposure, the cells were trypsinized, centrifuged and resuspended in 50 μl FBS containing 10 $\mu\text{g}/\text{ml}$ Hoechst 33342 and 10 $\mu\text{g}/\text{ml}$ propidium iodide (Sigma, MO, USA). After incubation for 30 minutes in the dark at room temperature, the cells were spread onto microscope slides. A minimum of 300 cells were counted using a fluorescence microscope (BZ-9000, Keyence, Japan) with an excitation filter 340–380 nm, and cells were classified as apoptotic, necrotic or viable.

All data was statistically analyzed using one-way analysis of variance (ANOVA) combined with a Tukey's multiple comparison test at a 5% level of significance. All analyses were computed with PASW Statistics for Windows (PASW Statistics 18, SPSS Japan Inc., Tokyo, Japan).

RESULTS

The initial respective viscosity of each sample is shown in Figure 3. Significant differences ($p < 0.05$) were observed between materials. More specifically, samples containing 5wt% and 10wt% of Maimendong (BKT05 and BKT10) showed higher values compared with the control samples, while samples containing Dongkuizi (TKS01, TKS05 and TKS10) showed lower viscosity values compared with the control samples. In general, the addition of herbal medicines tended to increase the initial viscosity.

The adhesive strength prior to immersion for all samples is shown in Figure 4. All samples indicated values higher

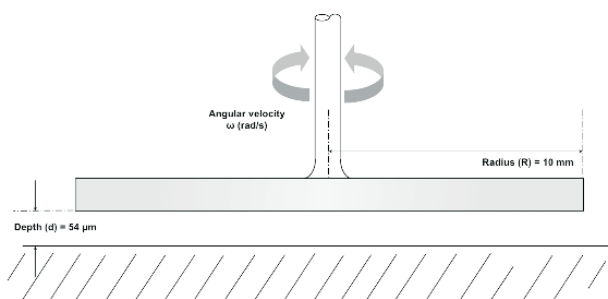


Figure 1. Block diagram of viscosity test jig.

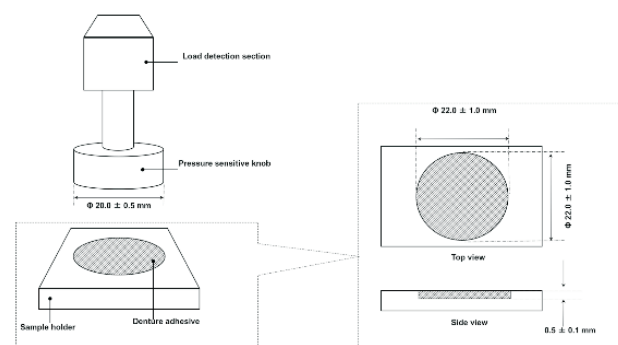


Figure 2. Block diagram of sample holder for adhesive strength test.⁸

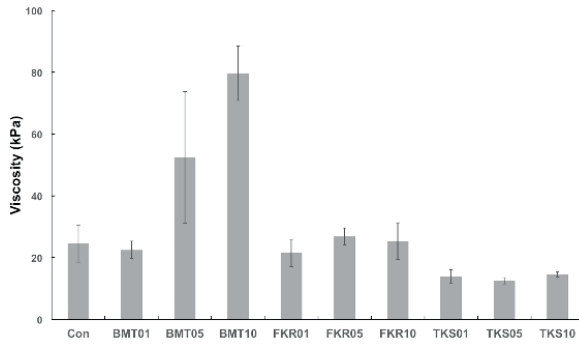


Figure 3. Mean initial viscosity with standard deviation.

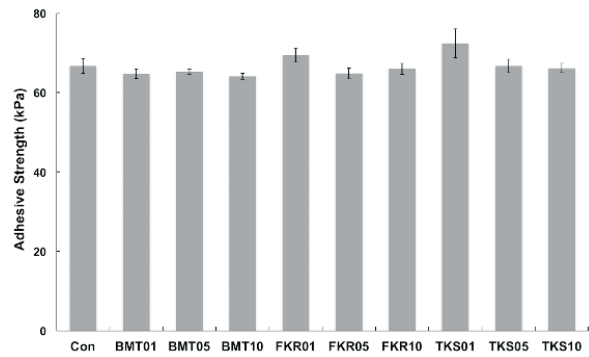


Figure 4. Adhesive strength before immersion.

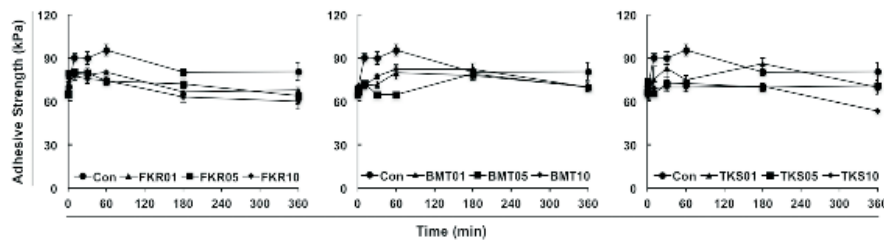


Figure 5. Variations in adhesive strength.

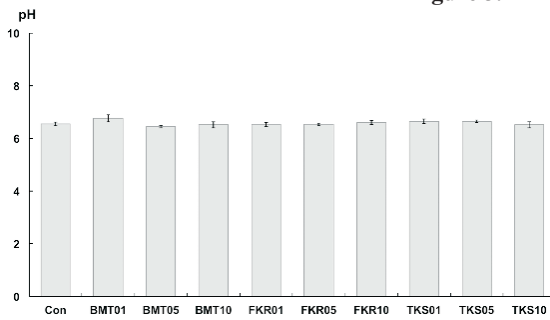


Figure 6. pH value of materials.

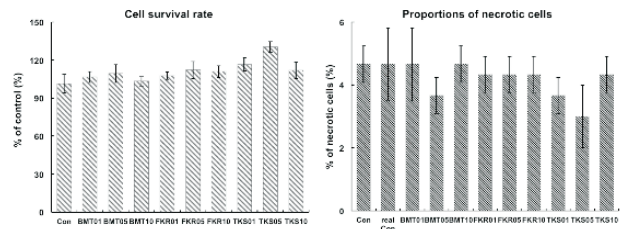


Figure 7. Results of cytotoxicity test

than 5kPa, which is the value recommended by the ISO standard for denture adhesives.⁸ Significant differences were observed between materials ($p < 0.05$). Specifically, FKR01 and TKS01 showed higher values compared with those of the control samples. The adhesive strength tended to decrease when the proportion of herbal medicine components in the sample exceeded 5wt%.

Changes in the adhesive strength for each sample are shown in Figure 5. Compared with the samples containing herbal medicine components, the control samples showed higher values over time. The adhesive strength was more stable in those samples with a lower proportion of herbal medicine components.

All samples indicated favorable cleanability. None of the methacrylate boards exhibited clumps of residue in any of the five assessments. All samples showed weakly acidic to neutral pH values (6–7) conforming to ISO standards⁸ (Figure 6). No significant differences were observed between materials ($p > 0.05$).

Figure 7 shows the cell survival rates relative to those for the control sample which was established as 100%. All

samples with herbal medicine components showed high cell survival rates, but there were no differences in cytotoxicity when compared with the control samples. The proportions of necrotic cells are shown in Figure 7. The values for all samples were lower than 5%, with no significant differences between the experimental and control samples ($p > 0.05$).

DISCUSSION

The null hypothesis that the addition of herbal medicine components affects neither initial viscosity nor adhesive strength and lacks cytotoxicity for fibroblast cells was accepted. In the study reported here, the effects of herbal medicine components possessing therapeutic effects against xerostomia on the physical properties of denture adhesive materials were examined. The development of cream-form denture adhesives containing herbal medicines that possess therapeutic effects against xerostomia was found to be feasible.

Denture adhesives, widely used by the elderly for

improving stability and retention of complete dentures²³ are broadly divided into two types: liner and glue.⁸ When patients use glue type denture adhesives (classified into cream-form, powder-form and sheet-form denture adhesives) under a dentist's supervision, these prove effective in improving denture stability and retention.⁴⁻⁷

The primary components of cream-form denture adhesives are water-soluble polymers and white petrolatum. In the oral cavity, the water-soluble polymers absorb saliva, thereby increasing viscosity and adhesion strength.^{22,24} PVM-MA and CMC, the main polymers used in denture adhesives, are the most common water-soluble polymers. Made from natural pulp, they are extremely safe and have various applications in situations where environmental and personal safety is of importance.²⁵ However, the water-soluble polymers used in current commercially available cream-form denture adhesives are degraded during use in the oral cavity, resulting in an early decrease in their adhesion strength.²⁵ Therefore, the adhesion strength of denture adhesives should be more durable. Han et al.²¹ examined combinations of water-soluble polymers to improve the durability of the adhesion strength and found consistent effects. Therefore, for the base material in the present study, we used a combination of 35wt% PVM-MA, 20wt% CMC, 40wt% white petrolatum, and 5wt% liquid paraffin. This formulation was shown to be the most durable combination in our previous study, with strong, stable, adhesive action and the promise of commercial viability as a long-acting cream-type denture adhesive.²¹

Xerostomia greatly affects oral health and causes various oral diseases. In the West, it is particularly pronounced in the elderly, with more than 20% of such individuals being reported as exhibiting subjective symptoms of xerostomia.^{26,27} In contrast, in Japan, nearly 20-40% elderly individuals reportedly exhibit subjective symptoms of this condition.²⁸ Currently, in the absence of a radical solution, the main treatment for xerostomia involves the use of humectants. Herbal medicines such as Maimendong (*Ophiopogon*), Fuling (*Hoelen*), and Dongkuizi (*Cluster mallow seed*) are considered effective in curing illnesses characterized by dry mouth or tongue. The initial viscosity, adhesion strength, cleanability and pH are the routinely assessed physical properties of denture adhesives.

In this study, the experimental samples containing herbal medicines tended to demonstrate significantly higher initial viscosity values compared with those of the control samples. The initial viscosity of a denture adhesive is the viscosity prior to the absorption of water by the material.^{22,24} It represents properties unique to the composition of the material. Here, viscosity increased with a proportional increase in the herbal medicine components. This condition was particularly pronounced in samples containing Maimendong (*Ophiopogon*) which were more viscous than samples containing other herbal medicines. Maimendong (*Ophiopogon*) is a herbal medicine more viscous than either Fuling (*Hoelen*) or Dongkuizi (*Cluster mallow seed*). In addition, powdered herbal medicines

are more viscous than white petrolatum water-soluble polymers, which may explain why the samples containing herbal medicines demonstrated a high initial viscosity. Such initial viscosity is closely related to the application of denture adhesives. Highly viscous materials compromise the application because it becomes difficult to squeeze the denture adhesive from the tube and apply it evenly on the mucosal surface of the dentures. Thus, adding only a small amount of herbal medicine is desirable.

Samples containing herbal medicine components not only demonstrated a less pronounced water immersion-associated increase in adhesive strength compared with the control sample, but also manifested a comparatively lower adhesive strength. When a denture adhesive is immersed in water, the water-soluble polymers are degraded, thereby increasing its viscosity and adhesive strength.^{7,21,22} However, the samples containing herbal medicine components consisted of base material (the control group) with herbal medicine. When the adhesive strength was measured using the same amounts of material, the total amount of water-soluble polymer was, apparently, smaller than that in the control samples, resulting in a small water solubility-associated increase in the viscosity. This may have accounted for the marginal increase in adhesive strength.

According to ISO 10873,⁸ only those denture adhesives that exhibit the recommended standard values for adhesive strength, washing performance (cleanability) and pH can be used. All the herbal medicine-containing cream-form denture adhesives assessed in the present study complied fully with all ISO standards, indicating that these trial denture adhesives can be used in actual clinical practice. Because denture adhesives are applied to the mucosal surface of dentures and are in direct contact with the oral mucosa, they must be biologically safe.⁸ None of the trial cream-form denture adhesives used in the present study demonstrated cytotoxicity, which confirms that they are safe for use in actual clinical practice. Chinese herbs are generally classified as superior, middle and inferior. In *Shennong Bencao Jing* (The Classic of Herbal Medicine), the oldest Chinese text of pharmacology, superior herbs are described as medicines that nourish life, cause no side effects or harm and can be consumed safely, even in large quantities over extended periods. However, middle and inferior herbs entail the risk of side effects and other damage and must be used with caution. The three herbal medicines used in the present study are all classified as superior herbs. This may be a reason why no materials used in this study indicated the presence of cytotoxicity.

Chinese medicine is not suited to acute illnesses or symptoms identified by modern medicine. However, in recent years, it has been frequently used to support the postoperative recovery of physical strength. In an increasing number of cases, Chinese medicine and modern medical science are being used in combination. Chinese herbs are used for patients who complain of xerostomia. In fact, in these patients, Chinese herbs are used in far greater

amounts compared with those used in the present study. Therefore, both physical examination and a prescription from a physician are required which makes it difficult to use Chinese herbs on a larger scale. In addition, because denture adhesives can be purchased at pharmacies without a prescription, it is impractical for them to incorporate large amounts of herbal medicines. From these perspectives, it is desirable to include only small amounts of herbal medicines.

Chinese medicines typically consist of two or more herbal components used in combination. Thus, Chinese prescriptions include many components. The combination of multiple medicines also enhances potency, while reducing undesirable side-effects. Therefore, multiple herbal medicines should be used in combination, rather than a single medicine. In this study, the effects of herbal medicines on the physical properties of trial denture adhesives, each containing only one type of herbal medicine, has been examined. Even a 10% concentration of Chinese herbal medicines did not result in cell damage. However, since this study only tested the effect of the herbal medicines after a 24-hour period of exposure, testing of longer duration involving the use of animal subjects are needed to ensure their safe use. In addition to explaining the action mechanisms of herbal medicines in animal experiments, future studies should also examine how combinations of multiple herbal medicines affect the physical properties of materials and both the water channels in the luminal membrane of salivary ducts and saliva secretion in test animals.

From the standpoint of physical properties, smaller amounts of herbal medicine components are associated with a lower viscosity and, consequently, superior operability of denture adhesives. Denture adhesives containing Maimendong (*Ophiopogon*), Fuling (*Hoelen*), or Dongkuizi (*Cluster mallow seed*) are non-cytotoxic and are, therefore, safe for use in real clinical practice. The study showed that the addition of herbal medicine components does not affect initial viscosity or adhesive strength and does not cause cytotoxicity in fibroblast cells. It may be, therefore, be feasible to develop cream-form denture adhesives containing herbal medicines.

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