Analysis of the Effect on Denture Base Metal of Cleaning with Denture Cleanser Using the Quartz Crystal Microbalance Method

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Abstract: Denture plaque control for the prevention of aspiration pneumonia is very important. The pellicle is the major cause of denture plaque adhesion. Few basic studies have evaluated the effectiveness of denture cleansers for pellicles composed of salivary proteins. The adhesion of salivary proteins formed on denture base metal and the removal rate were quantitatively analyzed using the QCM method after denture cleanser injection. This is the first study to compare the cleaning effects of denture cleanser on denture base metal using the QCM method. Au and Ti sensors were employed as the denture base metals. Albumin was used for the adsorption of salivary proteins. The results showed that no significant difference was found between Au and Ti in the amounts of albumin adsorbed, and the rate of albumin removal from Ti was significantly higher than that of Au.

In this study, the cleaning effectiveness of denture cleanser was confirmed based on the adsorbed amount and the removal rate of salivary proteins adsorbed onto denture base metals. Thus, the QCM method was suggested to be a useful tool for removing the effects of salivary proteins from denture cleaning agents on denture base metal.

Keywords: quartz crystal microbalance; denture plaque control; denture cleanser; salivary protein; denture base metal

1. Introduction

Aspiration pneumonia is a major problem in nursing and medical care settings. It is caused by the aspiration of oral and pharyngeal secretions containing food and oral bacteria [1]. In recent years, oral health care has seemed to play an important role in the prevention of aspiration pneumonia in frail elderly people [2,3]. Yoneyama et al. studied a group that received professional oral care and a group that did not, consisting of elderly patients in 11 nursing homes in Japan. This study investigated whether professional oral care might reduce pneumonia-associated death as compared with typical oral care during a 24-month follow-up period [4]. Denture cleaning is an important part of oral care for the elderly. Dentures replacing missing teeth are foreign substances in the mouth. Dentures are less self-cleansing than residual teeth and residual ridges. The wearing of removable dentures can lead to the colonization of oral pathogenic bacteria and/or poor oral hygiene [1–3]. Because of the high mortality rate of aspiration pneumonia in the elderly, the prevention of aspiration pneumonia in denture wearers should also be carefully considered. Sumi et al. reported that the bacterial flora in the denture plaque and that in the pharynx were 68.5% congruent. The importance of controlling denture plaque for the prevention of aspiration pneumonia cannot be overemphasized [5]. Basic research on denture plaque formation has been conducted since 1974.
Mukai et al. analyzed bacteria and proteins that adhere to denture base materials such as pure titanium, Co–Cr alloy, silver–palladium–copper–gold alloy, denture base resin, and hydroxyapatite. There were no significant differences in microbiota and protein adherence in hydroxyapatite as compared to the dental materials [6]. Ninaki et al. investigated bacterial adhesion by wearing an experimental resin base in the mouth. Microorganisms began to adhere to the basal surface of the experimental resin base on the second day after insertion of the denture, and division and proliferation were observed on the third day. Additionally, although plaque formation varied to some extent, the basis for plaque was confirmed to be formed on the third day after insertion of the denture [7]. Using scanning electron microscopy, Irahara et al. observed the process of denture plaque formation over time in patients with partial dentures. They reported that no bacterial adhesion was observed one day after installation, bacterial colonies formed two days later, extensive bacterial colonies were observed three days later, bacteria proliferated over the entire denture base mucosal surface one week later, and the bacterial layer became thicker two weeks later [8]. Nikawa et al. reported that denture plaque is formed when microorganisms adhere to the denture pellicle, a thin salivary film of 0.1–0.7 µm consisting of salivary proteins, and multiply and aggregate on the surface of the denture material [9]. From these research reports, it can be clarified that salivary proteins adhere to the denture base surface on the first day after denture installation, and that denture plaque is formed by subsequent bacterial adhesion.

Denture-cleaning methods include mechanical and chemical cleaning. The cleaning effect is improved by using mechanical cleaning such as brushing and ultrasonic cleaning in combination with chemical cleaning methods [10–12]. However, mechanical cleaning requires significant time and effort, and it has also been reported that incorrect use can cause denture wear by brushing [13,14]. Sadamori et al. stated that it is not the dentist who cleans dentures every day, but the patient himself; therefore, a simple method is preferable, and the use of denture cleansers significantly reduces the incidence of denture stomatitis. The use of appropriate denture cleansers is simple and effective [15]. There have been many reports on the cleaning effect of denture plaque; the ease with which denture plaque adheres to framework alloys using denture bases [16] and the cleaning effect of denture cleaning agents on adhered denture plaque have also been investigated [13]. However, since denture plaques are still composed of many bacteria and proteins and have a complex composition, no uniform cleaning method has been found. In addition, most studies on denture cleaning have been conducted on bacteria, and few basic studies have evaluated in detail the cleaning effects of denture cleansers on pellicles composed of salivary proteins [17].

There are several methods for analyzing protein adsorption, such as infrared reflection spectroscopy [18], ellipsometry [19], surface plasmon resonance (SPR) [20], enzyme-linked immunosorbent assay (ELISA) [21], and microcalorimetry [22,23]. Although these methods are effective for detecting small amounts of protein adsorption, it is difficult to follow the temporal changes of adsorption. Therefore, few studies have evaluated proteins quantitatively and over time.

Hayakawa et al. used the quartz crystal microbalance (QCM) method to quantify protein adsorption on the surface of a material and to monitor the change in the amount of adsorbed protein in real time [24]. The QCM method used a quartz crystal resonator. A quartz crystal resonator is when a quartz crystal is cut into very thin plates and metal thin films are attached to both sides. When an AC electric field is applied to each metal thin film, the quartz crystal resonator vibrates at a certain frequency. When a nanogram of a substance is adsorbed on a metal thin film, the resonance frequency decreases in proportion to the mass of the substance so that it can be used as a microbalance. The QCM method is used not only in dentistry but also in various forms of research [25–27]. The QCM technique is a straightforward method, has few technical errors, and has excellent reproducibility for detecting the adsorption of proteins onto a material surface by measuring differences in the oscillating frequency of the quartz cell [26–28]. Protein adsorption experiments
in the dental field for QCM analysis have been conducted mainly on dental implant materials [29–34] and metal materials for denture bases [35–37]. Hirota et al. used the QCM method to compare the adsorption behavior of salivary proteins on various denture base metal materials [36]. However, no study has been found that compares the cleaning effects of denture cleansers on denture base metal materials using the QCM method. In this study, salivary proteins were first adsorbed onto the surface of metal materials. The effect of denture cleansers on the removal of salivary proteins was then analyzed quantitatively and over time. We aimed to investigate the adsorption of salivary proteins on metal materials for denture bases and the removal effect of denture cleaning agents on salivary proteins. This is the first study to compare the cleaning effects of denture cleanser on denture base metal materials using the QCM method.

2. Materials and Methods
2.1. QCM Apparatus and Sensors
The adhesion of salivary proteins formed on denture base metals and the effectiveness of denture cleanser on salivary proteins were analyzed by the QCM method. The principle of the QCM method is that a crystal oscillator vibrates at a certain frequency (resonant frequency) when an AC electric field is applied to the metal electrode thin film attached to both sides of a crystal plate. When a nanogram of protein substance is adsorbed on the metal electrodes, the resonance frequency decreases in proportion to the weight of the substance, and vice versa. From the frequency changes, the amount of salivary protein adsorbed and the amount of salivary protein removed by denture cleansers were calculated using the Sauerbrey equation [38].

$$ΔF = \frac{2F_0^2 Δm}{A\sqrt{ρ_γμ_γ}}$$

where:
- $ΔF$: frequency changes (Hz);
- $Δm$: mass change (g);
- $F_0$: fundamental frequency of the quartz crystal ($27 × 10^6$ Hz);
- $A$: electrode area (0.049 cm$^2$);
- $ρ_γ$: density of quartz (2.65 g cm$^{-3}$);
- $μ_γ$: shear modulus of quartz ($2.95 × 10^{11}$ dyncm$^{-2}$).

According to the equation, a decrease of 1 Hz frequency corresponds to 0.61 ± 0.1 ng/cm$^2$ adsorption on the sensor, and an increase of 1 Hz frequency corresponds to 0.61 ± 0.1 ng/cm$^2$ removal from the sensor. A 27 MHz QCM (AFFINIX QN, ULVAC, Inc., Kanagawa, Japan) with 550 µL sensor cells was used in this study (Figures 1 and 2). Au and Ti sensors were employed on the assumption of denture base metals. The Ti sensor was prepared using sputtering deposition (CS200, ULVAC Inc., Kanagawa, Japan) under argon gas with a Ti target for Au electrode. The temperature was maintained at 25 ± 1 °C, and the solution in the cells was stirred during the measurements. Peter et al. stated that the QCM method is greatly affected by temperature. One degree of temperature change results in a frequency shift of about 40 Hz. In this study, the temperature in the apparatus was kept at 25 °C to prevent changes in the frequency due to temperature changes [39]. Bovine serum albumin (BSA, Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a salivary protein for adsorption. Two denture cleansers, namely Dentmousse (Bee Brand Medico Dental, Osaka, Japan) and Polident for partial dentures (GlaxoSmithKline PLC, London, UK) were used in this study. Dentmousse contains cetylpyridinium chloride, which is a cationic surfactant. Similarly, Polident contains peroxide and proteolytic enzymes.
The amount of BSA adsorbed was calculated by substituting the difference between the vibration frequency before BSA adsorption and the vibration frequency before the injection of denture cleanser using the Sauerbrey equation. The amount of protein removed from the sensor was calculated by dividing the amount of BSA removed by the amount of BSA remaining after the injection of the denture cleanser.

2.2. Measurements of Contact Angle

To evaluate the wettability of the metal used in the sensor cell, we measured the contact angle. The contact angles were measured using a Ti seat (1 mm × 10 mm × 20 mm, Ti, Furuuchi Chemical Corp., Tokyo, Japan) and an Au seat (1 mm × 10 mm × 20 mm, Au, Furuuchi Chemical Corp., Tokyo, Japan) after ultraviolet irradiation. The contact angle of each sensor to distilled water was measured using a contact angle meter (DMe-201, Kyowa Surface Science, Saitama, Japan) under the water drop volume of 0.5 µL at 10 s after the water was dropped, and each measurement was performed 10 times.

2.3. QCM Analysis

After ultraviolet irradiation of Au or Ti, the sensor cell was mounted in the QCM apparatus, and the sensor cell was filled with 300 µL BSA solution (10 mg/mL). After BSA was adsorbed onto the sensor for 24 h, the sensor cell was rinsed with distilled water, the solution was removed, and it was dried. The sensor cell was then filled with 450 µL of PBS, and 50 µL of denture cleanser was injected into the PBS solution in the sensor cell. The frequency increase was monitored for 2 h using both Au and Ti sensors. Three runs of QCM measurements were performed.

The amount of BSA adsorbed was calculated by substituting the difference between the vibration frequency before BSA adsorption and the vibration frequency before the injection of the denture cleanser using the Sauerbrey equation. The amount of protein removed from the sensor was calculated by dividing the differences between frequencies before the injection of denture cleanser and two hours after injection using the Sauerbrey equation. The BSA removal rate was calculated by dividing the amount of BSA removed by the amount of BSA adsorbed. The change in frequency after the injection of denture cleanser was detected as changes in the frequency curve. To examine the cleaning effect of the denture cleansers by soaking time, the BSA removal rate for one hour after injection of the denture cleanser (hereafter, time period $\text{①}$) and one hour after that (hereafter, time period $\text{②}$) were compared. The removal rate of $\text{①}$ was calculated by dividing the amount of BSA removed after one hour by the amount of BSA adsorbed at the injection of the denture cleanser.
and the removal rate of $\circ$ was calculated by dividing the amount of BSA removed by the amount of BSA remaining after the injection of the denture cleanser.

2.4. Statistical Analysis

All data were statistically analyzed using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test at a significance level of 0.05 ($p < 0.05$). All statistical analyses were performed using a statistical software program (IBM SPSS Statistics v23; IBM Corp, New York, NY, USA).

3. Results

3.1. Contact Angles

Contact angles of the Au and Ti sensors are listed in Table 1. There were significant differences in contact angles ($p < 0.05$).

| Sensor | Contact Angle ($^\circ$) |
|--------|--------------------------|
| Au     | 40.1 $\pm$ 1.1 $^a$     |
| Ti     | 21.3 $\pm$ 1.6 $^b$     |

Different superscript letters indicate a statistically significant difference ($p < 0.05$).

3.2. QCM Analysis

Figure 3 shows the typical frequency change curve after using BSA solution. A decrease in the frequency over time was observed by injecting BSA solution into both Au and Ti sensors.

Figure 4 shows the amount of BSA adsorbed on Au and Ti sensors calculated from the Sauerbrey equation. The decrease in frequency was observed by injection of the BSA solution in all sensors measured. There was no significant difference in the amounts of BSA adsorbed by Au and Ti sensors ($p < 0.05$).
Typical change in frequency curves by cleaning time with Dentmousse and Polident are shown in Figures 5 and 6. Using both denture cleansers, increased frequencies and typical $\Delta F$ of the removal of BSA were observed.

Table 2 shows the effect of BSA removal by the application of various denture cleansers to Au and Ti sensors. Significantly greater removal of BSA on Ti was found than that of Au in both Dentmousse and Polident at two hours ($p < 0.05$).

Tables 3 and 4 indicate the BSA removal rate in time periods ① and ②. Comparing the BSA removal rate with Dentmousse between two time periods, Ti demonstrated a rate significantly higher than that of Au in ① ($p < 0.05$), and there was no significant difference between Au and Ti in ② ($p > 0.05$). In the comparison of metal sensors, no significant difference was observed between ① and ② for Au ($p > 0.05$); however, ② for Ti showed a removal rate significantly lower than that of ① ($p < 0.05$). The BSA removal rate by Polident at different time periods was significantly higher for Ti than for Au in both ① and
Within the metal sensors, the removal rate of both Au and Ti was significantly lower in 2 than in 1 (p < 0.05).

Figure 4. Amount of BSA adsorbed on Au and Ti sensors. Error bars represent the standard deviation (SD).

Typical change in frequency curves by cleaning time with Dentmousse and Polident are shown in Figures 5 and 6. Using both denture cleansers, increased frequencies and typical ΔF of the removal of BSA were observed.

Figure 5. Typical frequency change curve after using Dentmousse.

Figure 6. Typical frequency change curve after using Polident.

Table 2. BSA removal rate of Dentmousse and Polident for Au and Ti.

| Denture Cleanser | Sensor | Removal Rate (%) |
|------------------|--------|------------------|
| Polident         | Au     | 15.8 ± 3.4       |
|                  | Ti     | 69.1 ± 4.6       |
| Dentmousse       | Au     | 4.8 ± 0.9        |
|                  | Ti     | 9.8 ± 1.3        |

Different superscript letters indicate a statistically significant difference (p < 0.05).

Table 3. BSA removal rate by Dentmousse injection by time period.

| Time Period | Sensor | Removal Rate (%) |
|------------|--------|------------------|
| 1          | Au     | 3.2 ± 0.9        |
|            | Ti     | 9.4 ± 1.5        |
| 2          | Au     | 1.4 ± 0.3        |
|            | Ti     | 0.7 ± 0.6        |

1 For one hour after denture cleanser injection; 2 between one and two hours after injection of denture cleanser. Different superscript letters indicate a statistically significant difference (p < 0.05).

Table 4. BSA removal rate by Polident injection by time period.

| Time Period | Sensor | Removal Rate (%) |
|------------|--------|------------------|
| 1          | Au     | 10.9 ± 2.6       |
|            | Ti     | 54.3 ± 3.7       |
| 2          | Au     | 4.8 ± 2.0        |
|            | Ti     | 32.2 ± 4.5       |

1 For one hour after denture cleanser injection; 2 between one and two hours after injection of denture cleanser. Different superscript letters indicate a statistically significant difference (p < 0.05).

4. Discussion

The major advantage of the QCM method is that it can be used to monitor the adsorption and removal of materials onto biomaterials simply and in real time [24]. In this study, we compared the removal behavior and rate of denture cleansers using Dentmousse and
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Polident with BSA-adsorbed Au and Ti sensors. The contact angle of Ti was significantly smaller than that of Au. It was suggested that Ti has higher wettability than Au.

Generally, the surface wettability affects serum protein adsorption [40], while the adsorption of proteins onto metal materials is controlled by many factors, such as electro-static interaction [33,41], hydrogen bond, hydrophobic–hydrophilic interaction [40,42], and protein orientation and tertiary structure [9,43]. The adsorption of proteins on materials can be influenced by multiple factors, including the contact angle and surface roughness. In this study, the adsorbed amounts were the same for Au and Ti sensors. At two hours, there was significantly greater removal of BSA in Dentmousse and Polident for Ti than for Au. In comparing the BSA removal rate by denture cleanser injection at between these time points, Ti showed a significantly higher removal rate one hour after denture cleanser injection than one to two hours after denture cleanser injection. In comparison within each metal sensor, no significant difference was observed between one hour after denture cleanser injection and one to two hours after denture cleanser injection for Au.

This result suggested that salivary proteins adsorbed on Ti were removed by denture cleaning agents earlier than those on Au. Gaurav et al. studied the adsorption of proteins on metal materials and their removal effect [42]. They found that BSA is adsorbed better by Au than by Ti. In Au, protein–protein interactions were dominant, and the removal of protein layers was limited. In this study, there was no significant difference in the removal effect of denture cleansers at different times of day for Au, and the removal rate of BSA by denture cleansers was significantly greater for Ti than for Au for Dentmousse and Polident, which is consistent with the results of this study. Barberi et al. studied the adsorption of proteins on titanium and stated that they adsorb on hydrophobic material surfaces [44]. This is because water is more easily displaced from the surface, and hydrophobic interactions between ammino acid residue and a surface can be strong. On the other hand, it has been reported that high wettability increases the adsorption of proteins. Matsumoto et al. investigated protein adsorption on modified Ti surfaces using contact angle measurement and QCM analysis [45]. UV treatment or plasma irradiation on Ti improves wettability. UV-treated and plasma-treated Ti showed higher BSA adsorption than untreated Ti. This result is inconsistent with the findings of the present study, which compared the amount of BSA adsorbed by Au, which has low wettability, and Ti, which has high wettability.

Protein adsorption is such a complex matter that a full and comprehensive explanation of it is still missing. The effects of surface properties such as roughness, morphology, chemical properties, surface energy, wettability, and charge need to be investigated further when considering protein adsorption.

In this study, Dentmousse and Polident were used as denture cleansers. Dentmousse acts through cetylpyridinium chloride, a cationic surfactant with a large surfactant effect. Polident is a peroxide and enzyme-based cleaning agent. Matei et al. reported that albumin indicates the interaction of albumin with Au through sulfur-containing amino acid residues [46]. Sousa et al. reported that sulfur, present in disulfide bonds in albumin adsorption to Ti, was observed for concentrations of protein higher than 0.30 mg/mL [47]. Polident contains peroxide which leads to an oxidation of sulfide resulting in BSA splitting off from the Au and Ti surface.

Because the two denture cleansers have different action mechanisms, differences in the removal behavior of BSA were observed.

However, in this study, we assumed that saliva would adhere to the denture base metal, so we injected a phosphate buffer solution with a pH of 7.4 into the sensor cell and then used a denture cleanser.

Although the QCM method has the advantages of simplicity and the ability to check the attachment and detachment of substances over time, it also has some constraints. One is the difficulty of analyzing the mechanical cleaning method using the QCM method alone because the metal sensor may damage the crystal plate by mechanical stimulation. Therefore, in order to investigate the removal effect of mechanical cleaning methods for
denture plaque control, a broader range of knowledge can be obtained by combining with other methods that can quantify proteins.

In this study, the effect of denture cleaning agents on salivary proteins was investigated using a QCM system, assuming for denture base metal, and using Au and Ti sensors. Many types of QCM sensors were used in the previously reported QCM analysis. Co–Cr sensors are prepared by sputtering on Au sensors [36], and PMMA can also be prepared by spin coating on Au sensors [32]. In this study, BSA was used as a salivary protein. Salivary proteins include not only albumin but also proline-rich proteins and cystatins, and pellicles are composed of multiple proteins [6]. In the future, it will be possible to examine the effectiveness of denture cleansers on the sensors of other materials used as denture base materials and on other salivary proteins composed of the pellicle. The aim of future studies is to find the most effective combination of denture base materials and denture cleaning agents to efficiently remove denture plaque and reduce the risk of denture stomatitis and aspiration pneumonia.

5. Conclusions

Using a 27 MHz QCM system, the cleaning effects of denture cleaning agents could be confirmed based on the adsorbed amount and removal rate of salivary proteins adsorbed on denture base metal materials. By quantifying the adsorbed amount of BSA and using denture cleansers, the following conclusions were drawn about the removal efficiency of salivary proteins on Au and Ti by various denture cleansers.

1. The contact angle of Au was significantly higher than that of Ti. Second item.
2. There was no significant difference between the amounts of BSA adsorbed by Au and Ti sensors.
3. The frequency increased when using Dentmousse and Polident, and frequency shifts were observed in the removal of BSA after using various denture cleansers.
4. The BSA removal amount of Ti was significantly higher than that of Au when using Dentmousse and Polident.
5. The comparison of BSA removal rates of denture cleansers at different times of day showed that the removal rate of Ti was significantly greater than that of Au within one hour after injection of the cleaning agents, but the removal rate decreased after one hour.

Based on these results, the QCM method was shown to be a useful tool for measuring the removal of salivary proteins from denture base metals with denture cleaning agents.

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