Effect of ozonated water on the surface roughness of dental stone casts

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Infection control of dental stone cast is an important issue. Ozone is effective for disinfection against microorganisms and inactivation of viruses. However, there is little information regarding the use of ozone. We prepared 4 types of gypsum specimens and 3 types of disinfectants (4–5 ppm Ozonated water [OZW], 2% glutaraldehyde [GL], and 1% sodium hypochlorite [SH]). Gypsum specimens were immersed in each disinfectant for 5 and 10 min, and surface roughness was then examined using laser scanning microscopy. Surface microstructure was investigated using scanning electron microscopy. Immersion of gypsum specimens in SH, GL, and OZW increased the surface roughness to a maximum of 1.04, 0.37, and 0.30 μm, respectively, based on the difference between the average values of surface roughness before and after the disinfection procedure. The effects of OZW and GL were comparable. OZW is useful as a candidate for relatively safe disinfection of material for dental stone casts.

Keywords: Ozonated water, Dental stone cast, Disinfectant material, Glutaraldehyde, Sodium hypochlorite

INTRODUCTION

Infection control has become an important issue for dental clinics and dental laboratory personnel in recent years. Dental prostheses, appliances, and other items (e.g., impressions, occlusal rims, and bite registrations) become contaminated with the patient’s saliva, bacterial plaques, and blood. Patients at the dental clinic and dental healthcare personnel can be exposed to viruses and bacteria.1-9.

Maintaining the surface quality of impressions made with irreversible hydrocolloid impression materials and disinfecting them are difficult.4,9. Dental prostheses, dental stone casts, or impressions sent to laboratories from dental clinics may be contaminated with microorganisms from patients’ saliva and blood.6-9. As a countermeasure against infectious diseases, a method of immersing a dental stone cast in a disinfectant solution and disinfecting it has been investigated.5,10. However, these dental stone cast disinfection processes increase the surface roughness of the dental stone cast.9,11,12.

Various studies have been conducted on disinfection of dental stone casts. Glutaraldehyde (GL) has gained wide acceptance as a high-level disinfectant and chemical bactericide.13. GL and sodium hypochlorite (SH) have been studied as dental stone cast disinfectants.8,10. However, there are concerns regarding the effect of GL on human health.14. Methods for heating disinfectants for dental stone models using microwaves have been investigated.10. Additionally, ozonated water (OZW) has been investigated in various fields worldwide.16-18. Previous study reported that OZW for disinfecting dental units,17 dental impressions,19, dental plates20 and dental surgery materials was potency enough.

Ozone gas is produced when O2 is energized and split into 2 monatomic (O1) molecules. These monatomic oxygen molecules then collide with O2 molecules to form ozone (O3). Therefore, ozone consists of O2 with a loosely bonded third oxygen atom that is readily available to attach to and oxidize molecules.13.

OZW has a strong oxidizing potential and is effective for disinfection against microorganisms and inactivation of viruses.13,18,22. However, there is little information on the effect of OZW on surface roughness of dental stone casts. Therefore, we investigated the effects of OZW on surface roughness of dental stone casts from the viewpoint of biomaterial science as a disinfectant material.

MATERIALS AND METHODS

OZW preparation

Ozone gas was produced from 99.5% oxygen gas by an ozone generator (Prity ozone, Fuji Electric, Mie, Japan). An ozone-oxygen mixture of gas was generated by an electrical discharge on 99.5% oxygen gas. OZW was prepared by bubbling ozone gas (ozone gas concentration of 1×102 mg/L) through ultrapure water to prepare a concentration of 3–4×10 mg/L (3–4×10 ppm) each time for experiments. Ozone concentrations in water were measured using indigo methods.23. OZW was immediately diluted to the appropriate final concentration (4–5 ppm)24 with a total volume of 500 mL using ultrapure water before the experiment. To inhibit decomposition,

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of OZW, all manipulations of OZW were performed with glassware or a glass beaker and covered with plastic wrap.

For comparison, 25% GL (25% C2H5O2, Lot no EPH0574, Wako, Osaka, Japan) and 10% SH solution (10% NaOCl, Lot no A9049, Sigma-Aldrich, Tokyo, Japan) were used in this study. They were diluted using ultrapure water to 2%\textsuperscript{11,20} and 1%\textsuperscript{13}, respectively, for disinfection of gypsum specimens immediately before use.

Gypsum specimen preparation

Four types of dental gypsum, the die stone New Fujirock (Lot no. 1003251, GC, Tokyo, Japan), Hybrid Rock (Lot no. 950, Noritake, Aichi, Japan), the dental stone New Plastone (Lot no. 1003151, GC), and Vel-Mix Classic (Lot no. 0-21090, Kerr, Orange, CA, USA) were used in this study. We prepared a stainless steel test die according to Japanese Industrial Standards (JIS) (T6505: 2005) as specified in the ISO Specification 1563. Silicone molds were replicated from the stainless steel test die using Duplicon (Lot no. 650852, Shofu, Kyoto, Japan). Custom-made rubber molds (Kinki-rubber, Kyoto, Japan) were prepared to make gypsum specimens.

A silicone mold and a custom-made rubber mold were combined in this study to make the gypsum specimens (Fig. 1). Two specimens of approximately 11 (width)×12 (length)×3 (thickness) mm were made by using a combined silicone mold and rubber mold per manipulation. Three horizontal lines and 2 vertical lines were transferred onto the gypsum specimens using a silicone mold taken from the stainless steel die. Intersection of these lines was used as a marker to measure the surface roughness of the same location as possible before and after disinfection (Fig. 2). Dental gypsum was mixed according to the manufacturer’s instructions, poured into the combined rubber mold, and separated from the mold 1 h later. Gypsum specimens were prepared using multiple molds. Gypsum specimens were dried overnight at room temperature (23±3°C).

Disinfection procedure

All disinfectants were prepared fresh each time for use. Four pieces of gypsum specimens were immersed with the disinfectant as carefully and as quickly as possible for the prescribed period of time. After disinfection, the gypsum specimen was rinsed with ultrapure water for 10 s. The remaining water on the surface of the gypsum specimen was completely removed using a hand blower. Gypsum specimens were then dried overnight at room temperature (23±3°C). Gypsum specimens without disinfection were only rinsed with ultrapure water for 10 s. Thereafter, the remaining water on the surface of the gypsum specimen was completely removed using a hand blower. All of the experimental instruments used in this disinfection procedure were glassware and beakers covered with plastic wrap during the experiment.

Measurement of surface roughness of gypsum specimens

Surface roughness of the gypsum specimens was measured in almost the same location before and after disinfection of the gypsum specimen using laser scanning microscopy is 0.01 μm.
microscopy (VK-8500, Keyence, Osaka, Japan). The average surface roughness of gypsum specimens was calculated using the surface roughness of 4 independent, prearranged 100×100 μm² areas (Fig. 2).

Subsequently, the difference (DIF) between the surface roughness values of each before and after disinfection procedure were calculated and averaged.

**Observation of the crystal structure using scanning electron microscopy (SEM)**

To evaluate the effectiveness of disinfectant on the crystal structure, the crystal structure of the gypsum specimen surface was observed using SEM (VE-8800, Keyence) with 3,000× magnification.

**Statistical analysis**

Descriptive statistics including the mean, standard deviation, and the minimum and maximum values were calculated for each test group. The data were analyzed by the two-tailed Student’s t-test and Tukey’s test. For detecting the time dependent effects, the specimen was disinfected for 5 or 10 min to compare the difference using t-test. To detect the statistically significant differences among the three disinfectants in the same gypsum specimen was used by Tukey’s test. We compared OZW with SH and GL to evaluate effects of OZW. The critical value for rejecting the null hypothesis was set at p<0.05.

**RESULTS**

**Changes in surface roughness by the disinfectants**

In this study, we evaluated the change in surface roughness at the same location as possible before and after disinfection with each disinfectant.

### Table 1 Results of measured surface roughness of dental gypsum specimens

<table>
<thead>
<tr>
<th>Time</th>
<th>Disinfectant</th>
<th>Surface roughness&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DIF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time</th>
<th>Disinfectant</th>
<th>Surface roughness&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DIF&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>Treated</td>
<td></td>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>5 min</td>
<td>OZW</td>
<td>1.20±0.15</td>
<td>1.13±0.11</td>
<td>-0.07±0.07</td>
<td>5 min</td>
<td>OZW</td>
<td>1.37±0.05</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>1.27±0.11</td>
<td>1.31±0.15</td>
<td>0.05±0.20</td>
<td>5 min</td>
<td>GL</td>
<td>1.36±0.08</td>
</tr>
<tr>
<td></td>
<td>SH</td>
<td>1.32±0.25</td>
<td>1.14±0.14</td>
<td>-0.18±0.10</td>
<td></td>
<td></td>
<td>1.14±0.02</td>
</tr>
<tr>
<td>10 min</td>
<td>OZW</td>
<td>1.25±0.14</td>
<td>1.34±0.31</td>
<td>0.08±0.20</td>
<td></td>
<td>OZW</td>
<td>1.50±0.06</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>1.14±0.09</td>
<td>1.13±0.09</td>
<td>-0.02±0.04</td>
<td></td>
<td>GL</td>
<td>1.30±0.09</td>
</tr>
<tr>
<td></td>
<td>SH</td>
<td>1.31±0.14</td>
<td>1.15±0.05</td>
<td>-0.16±0.16</td>
<td></td>
<td>SH</td>
<td>1.04±0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are expressed as mean for n=4 (μm) and mean±standard deviation; <sup>b</sup>Data are expressed as the mean of after calculated each difference (treated-untreated) (DIF) in μm. *p-value<0.05.
and after disinfection by using a mark on the gypsum specimen that was decided in advance. As a result, we were able to measure the surface roughness of a wide area by measuring 4 points in an independent 100×100 μm area for each specimen by using this method.

Surface roughness of all gypsum specimens was less than 2.08 μm (Hybrid Rock, SH for 10 min) after the disinfection procedure (Table 1). As shown in Table 1, tendency of the DIF value of New Plastone, Hybrid Rock, and Vel-Mix Classic immersed in SH increased in a time-dependent manner. Tendency of the DIF value of Hybrid Rock immersed in GL increased in a time-dependent manner. The DIF value of Vel-mix Classic immersed in OZW significantly decreased in a time-dependent manner. The DIF value of Vel-Mix Classic immersed in OZW for 10 min was larger than immersed in GL and OZW. Similarly, the DIF value of Hybrid Rock immersed in OZW for 10 min was significantly smaller than immersed in SH for 10 min. The maximum DIF value of Hybrid Rock immersed in SH for 10 min was 1.04 μm. The maximum DIF value of Hybrid Rock immersed in GL for 10 min was 0.37 μm. The maximum DIF value of Vel-Mix Classic immersed in OZW for 5 min was 0.30 μm. DIF values for immersion in GL and OZW tended to be smaller than those for immersion in SH. DIF values for immersion in GL and OZW were similar. The average value of DIF for all gypsum specimens was 0.16 μm. Some DIF values of New Fujirock were negative. This result did not occur in other gypsum specimens.
Fig. 4 Results of representative scanning electron micrographs of 4 types of dental gypsum specimens immersed in 4–5 ppm OZW for 5 and 10 min. Scale bars=3.33 μm.

**SEM observations of the crystal profiles**

SEM micrographs showed that immersion of Hybrid Rock and Vel-Mix Classic in SH affected the crystal grains of gypsum specimens in a time-dependent manner (Fig. 3A). In particular, immersion of Hybrid Rock and Vel-Mix Classic in SH for 5 or 10 min resulted in collapse of the shape of the rectangular crystal grains depending on the immersion time. This result was consistent with the trend in DIF values (Table 1). The crystal grains of New Fujirock and New Plastone immersed in SH for 5 and 10 min were well collapsed, and we could not determine the breakage of crystal grains by time dependent manner. (Fig. 3A). However, the crystal grains of Hybrid Rock, New Fujirock and New Plastone immersed in GL did not show distinct breakage by time-dependent immersion (Fig. 3B). Weak, eroded crystal grain boundaries were observed on the surface of Vel-Mix Classic immersed in GL by SEM (Fig. 3B). The results of Vel-Mix Classic observation by SEM micrographs and DIF values (Table 1) were in good agreement. SEM micrographs showed that (Fig. 4), except for Hybrid Rock, all types of gypsum specimens that were immersed in OZW for 5 or 10 min did not show distinct breakage or erosion of crystal grains by time-dependent immersion. However, weak, eroded crystal grain boundaries were observed on the surface of Hybrid Rock.

**DISCUSSION**

Physical and chemical treatments, including the use of high temperatures, microwave irradiation, and chemical reagents, have been used for disinfection of dental stone casts. Disinfection using OZW has also been studied in various fields.

In our study, except for New Fujirock, immersion of all gypsum specimens in SH suggested that the surface roughness of the gypsum specimens increased in a time-dependent manner (Table 1). Hybrid Rock and Vel-Mix Classic immersed in GL showed a tendency for increased surface roughness of gypsum specimens in a time-dependent manner. Immersion of gypsum specimens in SH showed a bigger increase in surface roughness in a time-dependent manner than immersion in OZW and GL. We could not determine the reason why the surface roughness of New Fujirock immersed in disinfectants took minus values (Table 1) in our study.

Our study suggested that crystal grains of gypsum specimens immersed in SH tended to be more destroyed in a time-dependent manner compared with immersion in GL and SH (Fig. 3). These results are in good agreement with the trend of increasing surface roughness shown in Table 1. Our results suggested that the difference in destruction of crystal grains depended on the type of disinfectants. We could not determine the reason why SEM observations (Fig. 3B) of Hybrid Rock immersed in GL were not in good agreement with DIF values (Table 1) in our study. There was no significant destruction of the crystal structure on the surface of gypsum specimens immersed in OZW in a time-dependent manner (Fig. 4).

Ozone in water degrades to oxygen. Ozone decomposition in water decreases OZW concentrations. As a preliminary experiment, five pieces of gypsum specimens of a similar size in our study were immersed in 500 mL of OZW, and a decomposition test of OZW was performed. We found that 3 ppm of OZW charged with the specimens reached 2.4 ppm after 5 min, 2 ppm after 10 min, and 1.8 ppm after 20 min. OZW concentrations became approximately half by 20 min. These findings suggested that the reaction of gypsum material used in this study with OZW did not cause decomposition of OZW. In our study, the effect of disinfectant on the surface roughness of dental gypsum under static water was evaluated. The effect of rinsing with ultrapure water for 10 s on the surface roughness of gypsum specimens was less than 0.02 μm. We did not observe distinctly destroyed crystal structures on the surface of specimens immersed in OZW by SEM. OZW for disinfection of dental stone casts is an excellent disinfectant material. This can be expected to prevent further effects on surface roughness of the dental stone cast after disinfection because rinsing the dental stone cast after disinfection is not necessary to remove OZW.
Previous study reported that using OZW for disinfection needs for 10 s\textsuperscript{16} or 1 min\textsuperscript{20}. In clinical cases, it is not to avoid that organic substances adhere on dental stone casts\textsuperscript{1-5}. It has been known from previous experiments that most organic solutes (impurities) promote or inhibit the decomposition of ozone\textsuperscript{27}. Considering the above, further study must be necessary using the dental stone cast adhering organic matter to know proper OZW concentration, immersion time and OZW volume as a clinical application model.

CONCLUSION

Our results reveal that effects on the surface roughness of gypsum specimens immersed in OZW and GL are similar. These results indicate that the influence of crystal grains of gypsum specimens immersed in OZW or GL leads to milder erosion than SH immersion.

Because of the limitations of this study, the decomposition of OZW, the optimum OZW concentration, the volume of OZW, using the gypsum specimen adhering organic matter model and the disinfection time must be determined in future research. However, OZW is a useful candidate for relatively safe disinfection material for dental stone casts in contrast to conventional disinfection chemicals, such as GL\textsuperscript{16} and SH, in which there were concerns about their effects on the human body. There was no large difference in the surface roughness value among the 3 disinfectants used in this study.

REFERENCES