Abstract

Background and Aim: Bacterial colonization occurs following contamination of impression materials with blood and saliva. However, not all disinfecting agents are compatible with the impression materials and may cause changes in the resultant casts. The purpose of this study was to assess the impact of various immersion time of addition silicon impressions in different disinfecting solutions on the surface hardness and detail reproduction of gypsum casts.

Materials and Methods: In this in vitro experiment, 84 addition silicon impressions were divided into seven groups (n=12). One group served as the control and the other groups were disinfected with 1% sodium hypochlorite, 2.4% glutaraldehyde, and 5% povidone-iodine for 5 and 30 minutes. Impressions were poured with type IV dental stone. The Knoop hardness value (KHN) was measured by microhardness tester. Surface detail reproduction, casts were inspected under a light microscope at X10 magnification. The surface hardness data was analyzed using two way ANOVA, Tukey’s test, Dunnett’s, One way ANOVA and t-test. The detail reproduction results were analyzed using the Kruskal-Wallis test.

Results: Immersion in povidone-iodine for 5 minutes and in sodium hypochlorite and glutaraldehyde for 30 minutes significantly lowered the KHN of resultant casts (P=0.001). No other significant differences were noted (P>0.05). No significant difference was found in detail reproduction between groups (P>0.05).

Conclusion: Increasing the immersion time of impressions in povidone-iodine increased the hardness of casts. Thus, 5% povidone-iodine is a more suitable disinfecting solution for longer immersion time given that other properties of the casts are not affected.

Key Words: Gypsum, Hardness, Disinfectants, Dental impression materials
microorganisms such as hepatitis virus, human immunodeficiency virus, and Mycobacterium tuberculosis [3,5,7,10,12,13]. Disinfection of the impression is the most efficient technique used in dental clinics and laboratories with the aim of eliminating microorganisms from the surface of impression [9,14]. One side effect of disinfecting the dental impression is their dimensional changes that occur due to chemical or physicochemical reactions between the disinfecting agent and the impression material [15,16]. In 1998, the Food and Drug Administration emphasized that all impressions must be cleaned and disinfected prior to be sent to the laboratory and the dentists are responsible to do so [4,7,10,14-18]. Sodium hypochlorite, glutaraldehyde, iodophor, and phenol in different concentrations are commonly used as disinfecting solutions in dentistry [15]. It has been reported that in 1978, Dr. Zanarotti accidentally noticed dimensional changes in casts as a result of the use of disinfecting agents [19]. Not all disinfecting agents are compatible with all impression materials and they may cause changes in surface detail, hardness, dimensional stability, and surface roughness of casts retrieved from impressions [9,10].

The working casts and dies, which usually are fabricated with dental stone and gypsum, should have dimensional stability and high strength and must be wear resistant. They should also be able to well reconstruct surface details [20] because even seemingly insignificant changes in cast surface could result in a prosthesis with low quality [11]. At present, chemical disinfectants are incorporated in the impression materials or gypsum powders [11]. Disinfectants are also available as sprays to use for decontaminating impressions and casts [9,18] and are highly popular especially for sanitizing the dental impressions [21]. However, immersion in a disinfecting agent is a more effective method for decontaminating [15,22,23]. Several studies have assessed the effect of disinfecting agents on surface properties of impressions and casts. One study reported 0.525% sodium hypochlorite, which caused 0.3% dimensional changes, a suitable disinfecting agent for polyvinyl siloxane while another study reported the loss of surface details and surface porosities in casts retrieved from impressions disinfected with sodium hypochlorite [9,13]. However, other studies reported that 0.525% sodium hypochlorite (1:10 dilution) has the least effect on surface hardness, surface erosion, compressive strength, and detail reproduction of casts compared to other disinfectants [12]. Furthermore, Ivanovski et al. [18] reported that 2% glutaraldehyde is the most effective disinfecting agent with minimal adverse effects on physical properties of casts.

It has been stated that immersion the impression in disinfecting solutions over the recommended time, causes changes in the impression material which could affect the quality of resultant casts [15]. This study aimed to compare the Knoop hardness number (KHN) and surface detail reproduction of casts retrieved from addition silicone impressions disinfected by immersing in 1% sodium hypochlorite, 2.4% glutaraldehyde and 5% povidone-iodine for five and 30 minutes. The null hypothesis was that type of disinfecting agent and duration of immersion would have no significant effect on hardness or surface detail reproduction of casts retrieved from the impressions.

Materials and Methods

This in vitro experiment was conducted on 84 samples and disinfecting solutions were 1% sodium hypochlorite (Clorax, CLX, USA), 2.4% glutaraldehyde (Behsadex, Behsa Pharmaceuticals, Iran), and 5% povidone iodine (Behsadin, Behsa Pharmaceuticals, Iran). This study protocol was approved by the Ethical committee of Islamic azad University (Protocol No.24847). In order to fabricate the specimens, an original stainless steel test block was used according to ANSI/ADA No.19 and in accordance with the International standard ISO4823 [23,24]. The test block consisted of three parts including a stainless steel die with 30 mm diameter and 15 mm height which had three straight lines with 2.5 mm distance from each other. Lines were engraved perpendicular to the surface and had 60-degree angles and 25μ, 50μ and 75μ widths [8,14] (Figure 1).

The base and activator of a low viscosity addition silicone impression material (Panasil, Kettenbach, Germany) were mixed according to the manufacturer’s instructions (Table 1). The metal mold was filled with the mixed material and a
plastic plate was put over the mold followed by placing 1 kg weight over it in order to simulate the pressure during taking an impression [13]. Then the whole set was transferred to a water bath at 35±1°C to simulate the oral cavity’s environment. After impression material was set, the assembly was removed from the water and the metal mold along with impression material was separated from metal die [13]. The specimens were randomly divided into seven groups of 12 as follows:

In group I, samples were not immersed in any disinfecting solution and served as control group. Specimens in group II and III were immersed in 1% sodium hypochlorite for 5 and 30 minutes respectively. In group IV and V, samples were dipped in 2.4% glutaraldehyde for 5 and 30 minutes respectively. While specimens in group VI and VII were submerged in 5% povidone-iodine for 5 and 30 minutes respectively [13,15]. In all groups, the disinfecting solution was kept at room temperature during the immersion time. Next, the impressions were rinsed under cold water for 10 seconds and dried at room temperature conditions. In the control group, impressions were only rinsed with sterile water and were not disinfected. A slurry of type IV stone (Snow Rock / MUNGYO, Table 1. Properties of the impression materials and gypsum used

<table>
<thead>
<tr>
<th>Impression material</th>
<th>Brand Name/Manufacturer</th>
<th>Mixing time</th>
<th>Setting time</th>
<th>Powder/Liquid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional silicone Light Body</td>
<td>Panasil / Kettenbach, Germany</td>
<td>1min &lt;</td>
<td>&gt; 2.5min</td>
<td>---</td>
</tr>
<tr>
<td>Velmix type IV</td>
<td>Snow rock / MUNGYO, Korea</td>
<td>30-60s by manual 20-30s by vacuum</td>
<td>2hr</td>
<td>20 ml / 100gr</td>
</tr>
</tbody>
</table>
Korea) was prepared according to the manufacturer’s instructions (24g of gypsum per 100 ml of water) and vibrated for 30 seconds [13]. All impressions were poured within one hour of making them and the obtained casts were separated from the molds after two hours [11,13]. The casts were then coded.

A microhardness tester (Wolpert Wilson Instruments, Instron Deutschland GmbH, Aachen, Germany) was used to measure the surface hardness [11,13,18]. In order to measure KHN, a 3N load was applied to the indenter followed by measuring the diameter of indentation on the display monitor. KHN was calculated by placing the length of the long axis of indentation in the below formula:

\[ HK = \frac{P}{C_p \cdot L^2} \]

where:

- \( HK \) = Hardness number
- \( P \) = Load (kgf)
- \( C_p \) = Correction factor related to shape of indenter, ideally 0.070279
- \( L \) = length of indentation along its long axis

Three indentations with a minimum of 1.5 mm distance were made in each cast and the mean value of calculated hardness was reported as each specimen’s KHN [25].

To assess detail reproduction, lines reproduced on the casts were evaluated under a light stereomicroscope (SHZ10, Olympus, Tokyo, Japan) at \( \times 10 \) magnification and photographed. Results of detail reproduction were graded into four categories: grade I when Lines were well-defined, sharp and continuous; grade II represent continuous V-shape lines with some loss of sharpness; grade III characterized the loss of continuity of the V-shape lines; and grade IV was given when a failure occurred to reproduce the lines [3,13,14,24].

Collected data from surface hardness was analyzed using two way ANOVA, Tukey’s test and Dunnett’s test. Since the interaction between groups was significant, One way ANOVA was applied for comparison of disinfectant materials after certain times and t-test for comparison of effect of time on each disinfectant material. The results of detail reproduction were analyzed using the Kruskal-Wallis test. All statistical analyses were carried out using SPSS version 22 considering type one error of 5%.

Results

**Surface hardness:**

Two-way ANOVA showed that surface hardness of samples was affected by the interaction of immersion time and disinfectant type (\( P=0.0001 \)), however, the impact of each parameter separately, was not significant (\( P=0.193, P=0.362 \) respectively). The Dunnett’s test showed that 5 min. immersion in 5% povidone-iodine, significantly reduced the surface hardness of resulting cast compared to control group (\( P=0.001 \)). However, immersion in 2.4% glutaraldehyde or 1% sodium hypochlorite for 5min, did not have a significant impact on KHN of retrieved casts (\( P=0.985 \) and \( P=0.998 \), respectively).

After 30 minutes, 1% sodium hypochlorite and 2.4% glutaraldehyde groups had a significantly lower KHN than the control group (\( P=0.031 \) and \( P=0.0001 \), respectively). However, the difference between 5% povidone-iodine and control group was not significant (\( P=0.997 \), Table 2).

According to one way ANOVA, there existed significant difference between surface hardness of samples after 5min (\( P=0.001 \)) and after 30 min (\( P=0.002 \)). According to t-test, surface hardness of each group (1%sodium hypochlorite, 2.4% glutaraldehyde, 5% povidone-iodine) based on time (5 min or 30 min) were significantly different (\( P=0.0001 \)).

Tukey’s test was used to assess the effect of immersion time on KHN in each disinfecting solution. The results of the comparison between two immersion times for each disinfectant are summarized in Table 3. It indicates that when impressions disinfected with 1% sodium hypochlorite, the surface hardness of casts retrieved after 5 minutes immersion (KHN=24.4) was significantly higher (\( P=0.001 \)) than those retrieved from impressions disinfected for 30 minutes (KHN=21.56). A similar result obtained from disinfecting with 2.4% glutaraldehyde; immersion for 5 min. resulted in casts with the higher KHN compared to 30 min. disinfection (\( P=0.007 \)). On the contrary, the mean surface hardness of casts retrieved from impressions...
Table 2. Results of Dunnett’s test to find differences between the case and control groups

<table>
<thead>
<tr>
<th>Groups/KHN</th>
<th>Mean</th>
<th>MD</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% sodium hypochlorite for 5 minutes</td>
<td>24.40</td>
<td>-0.235</td>
<td>0.627</td>
<td>0.998</td>
</tr>
<tr>
<td>1% sodium hypochlorite for 30 minutes</td>
<td>21.56</td>
<td>-3.071</td>
<td>0.627</td>
<td>0.0001*</td>
</tr>
<tr>
<td>2.4% glutaraldehyde for 5 minutes</td>
<td>24.28</td>
<td>-0.352</td>
<td>0.627</td>
<td>0.985</td>
</tr>
<tr>
<td>2.4% glutaraldehyde for 30 minutes</td>
<td>22.87</td>
<td>-1.762</td>
<td>0.627</td>
<td>0.031*</td>
</tr>
<tr>
<td>5% povidone iodine for 5 minutes</td>
<td>22.08</td>
<td>-2.550</td>
<td>0.627</td>
<td>0.001*</td>
</tr>
<tr>
<td>5% povidone iodine for 30 minutes</td>
<td>24.89</td>
<td>0.255</td>
<td>0.627</td>
<td>0.997</td>
</tr>
</tbody>
</table>

MD= Mean difference  
SD= Standard deviation  
* The MD is significant at the 0.05 level

Table 3. Results of Tukey’s test to assess the effect of material on hardness

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Mean KHN</th>
<th>MD</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% sodium hypochlorite</td>
<td>5 min</td>
<td>24.40</td>
<td>2.836</td>
<td>1.576</td>
<td>Pv=0.001*</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>21.56</td>
<td>1.894</td>
<td>1.654</td>
<td></td>
</tr>
<tr>
<td>2.4% glutaraldehyde</td>
<td>5 min</td>
<td>24.28</td>
<td>1.265</td>
<td>1.033</td>
<td>Pv=0.007*</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>22.87</td>
<td>1.033</td>
<td>1.245</td>
<td></td>
</tr>
<tr>
<td>5% povidone iodine</td>
<td>5 min</td>
<td>22.08</td>
<td>1.666</td>
<td>1.701</td>
<td>Pv=0.0001*</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>24.89</td>
<td>1.701</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MD= Mean difference  
SD= Standard deviation  
* The MD is significant at the 0.05 level

This study showed that increasing the immersion time of impressions in disinfecting solutions caused changes in surface hardness of casts. Thus, our null hypothesis was rejected. Bacterial contamination via exposure to blood and saliva during dental procedures is inevitable. When casts are in contact with impression material, bacterial colonies can transfer from the impression to cast. Thus, proper disinfection of impressions is necessary to prevent cross-contamination from the clinical setting to the laboratory [4,5,8-11,13]. On the other hand, disinfecting agents should not change the properties of impressions because any slight changes disinfecting with 5% povidone-iodine for 30 minutes (KHN:24.89) was higher (P=0.0001) than those retrieved from impressions disinfected for 5 minutes (KHN=22.08) in 5% povidone-iodine.  

Detail reproduction:  
Analysis of detail reproduction data showed no significant difference between samples immersed for 5 and 30 minutes (P=1 for 50μm and 75μm and P=0.801 for 25μm). In other words, type of disinfecting agent and duration of immersion had no effect on detail reproduction up to 25μm (Diagram 1).

Discussion

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in the dental impression might transfer to the resultant cast and results in inaccuracy in consequent prosthesis [14]. Several methods have been suggested to minimize microbial contamination such as adding the disinfecting agent to the mixing water in the alginate preparation, disinfecting the impression by spraying or immersion, and mixing gypsum with a disinfecting solution which leads to a disinfected cast [3,11,14-22]. Nevertheless, spraying and immersion of impressions in disinfecting solution are among the most commonly used techniques [9,21].

In the present study, we used three commonly used disinfecting solutions namely 1% sodium hypochlorite, 2.4% glutaraldehyde, and 5% povidone-iodine for disinfecting impressions by immersion. These materials were selected for our study since evidence has shown that they have the ability to eliminate human immunodeficiency virus and hepatitis virus in 10 minutes [7]. Also, disinfection of impressions by immersion is more reliable as recommended by the American Dental Association [1,26-28]. Moreover, low viscosity addition silicone impression material and type IV dental stone was used in our study because addition silicone has higher volumetric and dimensional stability than conventional silicone and is among the most commonly used impression materials in fixed prosthodontics [1]. Type IV dental stone has optimal strength and hardness and minimal setting expansion and can reproduce the details of impressions in order to create a hard and accurate surface for fabrication of wax model [26]. Based on the results of the present study, the surface hardness of casts increased after 30 minutes of immersion in povidone-iodine compared to 5 minutes immersion and became closer to the KHN of the control group. Furthermore, the mean value of surface hardness in group III was higher than other disinfected groups. Surface hardness decreased in glutaraldehyde and sodium hypochlorite groups after 30 minutes immersion and had a significant difference with the control and povidone iodine group.

It has been shown that impregnating the set gypsum with substances like epoxy resins or a light-cured dimethacrylate resin could increase the scratch resistance of the dental stone casts by 15% to 41% though it decreases its compressive strength [27]. The exact mechanism of this phenomenon has not been completely explained however infused materials might induce some changes in surface crystals of gypsum cast. Probably similar circumstance occurred in the casts of the povidone-iodine group after 30 minutes and resulted in increasing the hardness.
Abdelaziz et al. [14] showed that addition of disinfecting agents to gypsum increases the size of gypsum crystals and increases roughness. This was more prominent when impressions were disinfected with povidone iodine, glutaraldehyde, and sodium hypochlorite [14].

Abass [5] disinfected gypsum casts with calcium hypochlorite and his results were similar to the results obtained by Abdelaziz et al. [14]. In Abass’s study, calcium hypochlorite caused changes in size and shape of calcium sulfate dihydrate crystals which increased the porosity of the casts. This increase was observed in all concentration of calcium hypochlorite [5].

Sabouhi et al. [11] evaluated the effect of calcium hypochlorite on setting expansion and surface hardness of type V dental stone. They have found that presence of calcium hypochlorite disinfecting agent led to an increase in setting expansion while hardness was decreased [11]. Therefore, it is important to select an appropriate material to disinfect impressions and casts which has the least adverse effects on physical properties of the resultant casts. Moslehifard et al. [25] evaluated the surface hardness of casts after spraying with Virkon and 0.525% sodium hypochlorite, and they have shown that even though both disinfectants reduce the casts’ hardness, this decrease was less with the Virkon [25]. Another study revealed that sodium hypochlorite decreases the hardness of casts by 5.7% [29]. In our study, immersion in povidone-iodine for 30 minutes increased the hardness compared to other tested groups.

Ivanuvski et al. [17] added disinfecting solutions to the die stone mix and measured the tensile and compressive strength of resultant types III and IV casts. They have reported a reduction in mechanical properties of casts [17]. In the present study, the assessment of detail reproduction showed that no significant difference existed between samples over time. However, disinfection for 30 minutes seemed to have a better result.

According to ANSI/ADA No19, dental casts retrieved from alginate impressions must be able to reproduce details by 75μ; casts retrieved from silicon impressions must be able to reproduce details by up to 25μ [23] In the current study, the goal was to reproduce all the lines especially 24μ line, which was well reconstructed in all samples; overall, 79.8% of the casts were grade 1, 16.7% were grade 2, 3.6% were grade 3 and none of them were grade 4. In all specimens, 50μm and 75μm lines were sharp, continuous, and well defined (grade I). Although it seemed that 2.4% glutaraldehyde solution for five minutes resulted in better surface detail reproduction than 1% sodium hypochlorite and 5% povidone-iodine, the difference was not statistically significant. In general, silicon materials including condensation silicon and addition silicon, have better surface integrity than other materials. These substances are hydrophobic and their surface has high resistance to hydrophilic disinfecting agents (irrespective of their type or duration of contact with the disinfecting agent) [7]. Ahila et al. [15] disinfected addition silicon impression with 4% sodium hypochlorite, 2.45% glutaraldehyde, and 5% povidone-iodine for 10, 30, and 60 minutes by spray and immersion method. They have shown that although glutaraldehyde groups recorded surface details better than sodium hypochlorite and povidone iodine groups, none of the disinfectants had an adverse effect on surface details [15]. In a study by Amin et al. [13], four impression materials namely, alginate, zinc oxide eugenol, addition silicon, and condensation silicon were immersed in 0.5% and 1% sodium hypochlorite, Corsodyl, and Hexane for one hour. They have observed that disinfected alginate and zinc oxide eugenol had poor surface quality while casts retrieved from addition silicon were not significantly different from control casts in terms of surface details [13].

In another study, impressions were immersed in 0.525% sodium hypochlorite for 30 minutes for several times. It has been found that disinfecting by immersion had no significant effect on surface properties of casts, and changes were within the ADA/ANSI specifications [3].

**Conclusion**

Within the limitations of this study, it may be concluded that if there is a time limitation, 1% sodium hypochlorite and 2.4% glutaraldehyde would be suitable choices since they have fewer effects on surface hardness of casts. However,
when no time restriction is required, 5% povidone-iodine could be a suitable choice given other cast properties remained stable. Duration of immersion and type of disinfecting agent had no significant effect on surface detail reproduction of casts; although 2.4% glutaraldehyde for five minutes seemed to record the details better, this difference was not statistically significant.

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References
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