The effect of disinfectants on the accuracy, quality and surface structure of impression materials and gypsum casts: A comparative study using light microscopy, scanning electron microscopy and micro computed tomography

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The aim of this study was to compare the effect of several commercially available disinfectants on the accuracy of various types of impression materials and their compatibility with gypsum including surface quality and structure evaluation. Four alginate and three elastomeric impression materials in combination with disinfectants Aseptoprint Liquid, Zeta 7 solution, Silosept and Dentaclean Form were tested. The dimensional changes, detail reproduction, the compatibility with gypsum and surface/subsurface morphology were evaluated using light microscopy, scanning electron microscopy and micro computed tomography. Two alginate materials disinfected in Dentaclean Form exhibited the most significant differences (p<0.0001). The loss of detail on some alginate impressions in combination with this disinfectant including deterioration and change of morphology of gypsum surfaces was observed. Porosity in subsurface area and exposed large particles were detected. It was confirmed that the desired properties of impressions may be negatively affected in combination with some disinfectants.

The aim of this work was to investigate the influence of several commercially available disinfectants recommended for the disinfection of dental impression materials and their compatibility with gypsum including surface quality and morphology observation of alginate, silicone and polyether impression materials and their gypsum casts.

Null hypothesis supposed that the tested disinfectants have no influence on the accuracy, quality and surface structure of impression materials and gypsum casts.

Keywords: Dental impression material, Disinfectant, Dimensional accuracy, Detail reproduction, Surface and subsurface morphology
disinfectants had no effect on the parameters observed.

MATERIALS AND METHODS

Materials selection and specimen preparation

The alginate impression materials differing in gypsum pouring time recommended by their manufacturers and in color change properties, low-viscosity addition and condensation silicone and polyether impression materials in medium consistency were chosen as representative materials (Table 1). All the materials were mixed according to the manufacturer’s recommendations (Table 1). The alginates Alligat fast set (AFS), Elastic Cromo (EC), Kromopan (K), Phase Plus (PP) and elastomers Xantopren L blue (XLB) and Impregum Soft (IS) were hand mixed at the weight mixing ratio. Variotime Medium Flow (VMF) was delivered in the cartridge auto mixing versions. The impressions were poured with two dental gypsums, type 3 Mramorit Blue (MB) and type 4 BegoStone plus (BSP). The distilled water was used to mix alginate and gypsum powders to prevent the effect of water hardness on their properties.

The impressions were disinfected in Aseptoprint Liquid (Oro Clean Chemie, Fehraltorf, Switzerland), Zeta 7 solution (Zhermack, Badia Polesine, Italy), Silosept (Kettenbach, Eschenburg, Germany) and Dentaclean Form (L.C. PLIWA, Malsfeld-Ostheim, Germany)-commercially available disinfectants, compatible with all types of dental impression materials. Before disinfection they were freshly prepared according to their instructions for use by dilution of the concentrates or dissolution of the powder in tap water to the concentrations required (Table 2).

The specimens for dimensional stability, detail reproduction and compatibility with gypsum and quality surface tests were prepared according to the technical standards ISO 21563 and ISO 4823[12,13]. For this purpose, the stainless-steel test block with three parallel grooves of triangular 60° cross section of 20, 50 and 75 μm in width and 25 mm length on its surface was used (Fig. 1). The mixed impression material was applied within the working time to the cavity created by seating a metal ring mold on the test block. The impression material was pressed by the polyethylene-covered glass plate to expel the excess material. The whole assembly was then placed in the water thermostat with 37°C for the time recommended by the manufacturer for leaving the impression in the mouth. The assembly was then removed from the water bath and the impression was separated from the mold. Its surface was rinsed with tap water, gently streamed with clean air and immediately investigated and recorded with a light stereo microscope Olympus SZM-2T (Olympus, Tokyo, Japan) at 6.3× and 25× magnification equipped with a digital camera.

Table 1  Dental impression materials and dental gypsums used

<table>
<thead>
<tr>
<th>Material</th>
<th>Type</th>
<th>Lot no.</th>
<th>Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impression materials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alligat fast set (AFS) (Heraeus Kulzer, Hanau, Germany)</td>
<td>alginate</td>
<td>H121210</td>
<td>W/P 11.5 mL: 5 g 30 s hand mixing</td>
</tr>
<tr>
<td>Elastic Cromo (EC) (SpofaDental, Jicin, Czech Republic)</td>
<td>alginate</td>
<td>2551073</td>
<td>W/P 10 mL: 4.5 g 30 s hand mixing</td>
</tr>
<tr>
<td>Kromopan (K) (Lascod, Sesto Fiorentino, Florence, Italy)</td>
<td>alginate</td>
<td>0164370145.105</td>
<td>W/P 10 mL: 4.5 g 45 s hand mixing</td>
</tr>
<tr>
<td>Phase Plus (PP) (Zhermack, Badia Polesine, Italy)</td>
<td>alginate</td>
<td>205849</td>
<td>W/P 9 mL: 4.5 g 45 s hand mixing</td>
</tr>
<tr>
<td>Xantopren L blue (XLB) (Heraeus Kulzer)</td>
<td>C-silicon</td>
<td>R400510: base</td>
<td>B/A 4 g:0.3 g 30 s hand mixing</td>
</tr>
<tr>
<td>Variotime Medium Flow (VMF) (Heraeus Kulzer)</td>
<td>A-silicon</td>
<td>R390514: activator</td>
<td>cartridge system; gun with mix. Tip</td>
</tr>
<tr>
<td>Impregum Soft (IS) (3M ESPE, St. Paul, MN, USA)</td>
<td>polyether</td>
<td>565545: base</td>
<td>B/C 1:1 45 s hand mixing</td>
</tr>
<tr>
<td>Dental gypsums</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BegoStone plus (BSP) (Bago, Bremen, Germany)</td>
<td>type 4</td>
<td>8556133</td>
<td>W/P 3 mL: 15 g 30 s hand mixing</td>
</tr>
<tr>
<td>Mramorit Blue (MB) (SpofaDental)</td>
<td>type 3</td>
<td>SD00205</td>
<td>W/P 4.5 mL: 15 g 30 s hand mixing</td>
</tr>
</tbody>
</table>

W/P: Water/Powder; B/A: Base/Activator; B/C: Base/Catalyst
Table 2  Disinfectants, their composition and application

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Chemical composition*</th>
<th>Lot no.</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptoprint Liquid</td>
<td>in 100 g: 30 g benzalkonium chloride, surfactants, excipients</td>
<td>10042</td>
<td>1% solution 2 min</td>
</tr>
<tr>
<td>Zeta 7 solution</td>
<td>in 100 g: 7.7 g dimethyl-didecyl-ammonium chloride, 15 g phenoxethanol, tensides, additives, excipients, water</td>
<td>89638</td>
<td>1% solution 10 min</td>
</tr>
<tr>
<td>Silosept</td>
<td>in 100 g: 45% pentapotassium-bis-(peroxymonosulphate) bis(sulphate), 5–15% sodium dodecylsulphate, &lt;5% iso decanol ethoxylate, &lt;5% natriumcarbonate</td>
<td>1255161</td>
<td>2% solution 10 min</td>
</tr>
<tr>
<td>Dentaclean Form</td>
<td>2.5–10% isopropylalcohol, 2.5–10% polyglycolether of fatty alcohols, 2.5–10% butyldiglycol, 2.5–10% N,N-didecyl-N-methyl-poly(oxyethyl) amoniumpropionate, &lt;2.5% N,N-bis(3-aminopropyl)-dodecylamine, &lt;2.5% cocopropylendiamine-1,5-bis-guanidiniumacetate</td>
<td>W1112</td>
<td>6% solution 15 min</td>
</tr>
</tbody>
</table>

*available information from the original packaging and safety data sheets

Fig. 1  Test block.

Olympus E-520 (Olympus). The specimen was then placed to the metal ring with sufficient space above the impression created by the slit mold and immediately poured with the gypsum using a vibrating platform. Elastomeric materials were poured no earlier than 30 min after impression making. The gypsum cast was separated from the impression one hour after pouring. The surfaces of both impression and cast were investigated and recorded by the digital camera as before. For each combination impression material and disinfectant six specimens were prepared, three were poured with gypsum type 3, three by gypsum type 4, which gave a total amount of 168 impression specimens and the same number of casts. Forty-two other specimens and casts prepared in the same way but without being disinfected served as a control group. All the specimen preparation and testing were performed at 23±2°C and RH 50±10%.

**Tests methods**

The linear dimensional change was established as a percentage change of the 25 mm length between two lines perpendicular to the line 75 μm reproduced on impressions and on the stainless-steel test block according to\(^{12}\):

\[\Delta L=100\times\left(\frac{L_2-L_1}{L_1}\right)\]

where \(L_1\) is the distance between diagonal lines on the test block and \(L_2\) is the distance between diagonal lines on the impression. The distances were measured on the images of the block and impression surfaces with an accuracy of ±0.001 mm using Quick Photo Industrial (Olympus) calibrated before the measurements with light stage micrometer (Pyser Optics, Edenbridge, UK). The results were statistically analyzed using a two-factor ANOVA (factors: type of impression material, type of disinfectant) and a one-way analysis ANOVA with Tukey HSD post-hoc tests with Statistica 12 (StatSoft, Tulsa, Oklahoma, USA) at a significance level \(\alpha=0.05\). The detail reproduction and compatibility with gypsum defined as the ability of an impression material to reproduce 20-μm line in its whole length and to transform 50 μm line to the cast surface was evaluated at magnification 25×. The clean separation from the gypsum cast poured against impression was examined. A modified scoring system of line integrity and surface quality of a cast with the rating score from 1 to 4 was also used\(^{8,14}\): (1) sharp continuous line, smooth cast surface; (2) continuous line, loss of sharpness and small pits on the surface; (3) deterioration of line detail, more extensive cast surface pits and porosity; (4) rough appearance, loss of line continuity, pronounced porosity and deterioration of surface.

The morphology of impression and cast surfaces were analyzed by scanning electron microscopy (SEM; JSM 5500-LV, Jeol, Tokyo, Japan) in the backscattered mode. The specimens were slowly dried at 37°C for 14 days and then sputter-coated with gold (JFC-1200 Fine coater, Jeol) before analysis. Cast surface and its subsurface structure to the depth 30–40 μm was investigated using a micro computed tomography (micro-CT; SkyScan 1272, Bruker, Kontich, Belgium) equipped with a 16 Mp X-ray detector and processed with original
linear dimensional change

While the control alginate specimens showed only small dimensional changes (−0.13 to 0.33%), after disinfection they exhibited significant (p<0.0001) expansion depending on the impression material and disinfectant combination. Fig. 2. The highest expansion was found in Dentaclean Form reaching 0.60% for alginate AFS and 0.87% for PP and significantly higher values up to 1.50–1.61% for alginates K and EC. Except for AFS the lowest dimensional changes after disinfection were observed for Silosept, reaching expansion up to 0.25%.

The elastomeric impression materials showed shrinkage after setting. The control specimens of silicone impression materials —XLB and VMF and of polyether IS showed only small (−0.05 to −0.14%) statistically non-significant dimensional changes (p≥0.99). After disinfection shrinkage of XLB was the most pronounced, reaching approximately −0.31 to −0.50%. With this material a significant increase in shrinkage over the control group was found for Silosept (p<0.01) and Dentaclean Form (p<0.0003, Fig. 2). VMF impression specimens disinfected in all tested products shrunk up to −0.29% (Fig. 2), the significant increase compared to control group was found for disinfection in Zeta 7 solution and Aseptoprint Liquid (p<0.03). With polyether IS only a maximum shrinkage up to −0.19% was measured in Dentaclean Form, where this shrinkage significantly (p<0.03) exceeded the shrinkage in all other disinfectants and control group.

Detail reproduction, compatibility with gypsum, surface quality

The 20-μm line was fully reproduced on the surface of all the control alginate impression specimens and was also clearly visible on the surface of impression specimens after their disinfection in Aseptoprint Liquid and Zeta 7 solution. As the 50-μm line was fully reproduced on the gypsum surfaces in the entire length, the requirements of the ISO 21563 were fulfilled suggesting that these two disinfectants do not affect neither the surface quality of alginate tested or their gypsum casts (Fig. 3).

The 20-μm line was also fully visible on the AFS and K impression surfaces disinfected in Silosept, but more pronounced deterioration of gypsum surface was observed when poured. Small pores, loss of sharpness and discontinuations of the 50-μm line (rating score=2, 3) were observed on the surface of gypsum casts (Figs. 4B, C). The most serious deterioration effect on the impression and gypsum surfaces was observed for combination of Dentaclean Form —AFS (Figs. 3, 5). After disinfection, the lines on the impression surface were hardly visible and the whole surface was matte (Fig. 5B). The cast surface was rough, covered with numerous pits so that 50-μm line was not visible (Fig. 5C, rating score=4) and the impression surface after its separation from the cast, was covered with an incoherent layer of white powder debris regardless of gypsum used. A lesser degree of surface deterioration was found...
with combination of other alginate materials and this disinfectant, rating score=2 (Fig. 3).

Very good resistance to the disinfectants was found with elastomeric impression materials. All of which reproduced 20-μm line and clearly and sharply transferred 50-μm line on the gypsum casts in its full length (Fig. 6). Impressions could easily be separated from the cast without any debris on their surface. No differences were observed between the two gypsum materials BSP and MB.

**Surface morphology**
The detailed examination of alginate impressions and of the corresponding cast surfaces with pronounced changes in their morphology after disinfection (rating score=3, 4) was performed by SEM. On the surface of control non-disinfected impressions, large disk-like porous particles of diatomaceous earth (DEP) could easily be recognized even after their pouring with the gypsum (Fig. 7A). Opposite this, on the surface of impressions disinfected in Dentaclean Form separate from the cast, DEP was
not visible, and the surface was covered by particles with shape and size similar as in the gypsum powder (Figs. 7B, 8). The gypsum cast surface morphology completely changed in comparison to control cast surface (Fig. 9). Regular structures were lost (Fig. 9B) and the same particles, similar as in the gypsum powder, were also identified on the most deteriorated surfaces (Fig. 9C).

The surface and subsurface structure of gypsum casts with rating scores 1 and 3, 4 analyzed using micro-CT are depicted in Fig. 10. With the casts poured against control impressions fine structure and regular particle distribution without porosity and other defects in its surface and subsurface areas were observed (Figs. 10A1–3). On the other hand, porosity was found in the
subsurface areas of casts poured against disinfected impressions with slightly damaged surface observed with the light microscopy and SEM (Figs. 10B1–3). In the most deteriorated casts prepared from AFS disinfected in Dentaclean Form porosity in deep subsurface area of app. 30–50 μm and exposed large particles were detected (Figs. 10C1–3).

DISCUSSION

Due to the increasing number of infectious diseases posing a significant risk not only for clinical workers but also for dental laboratory staff, impression disinfection is one of the most important and frequently discussed topics. Healthcare providers are responsible for adhering to hygiene requirements in health care facilities, including the disinfection of instruments, aids and dental impressions. In addition to the basic antimicrobial efficacy of the disinfectants, it is also necessary to assess their effect on the properties of dental impression materials. Optimal disinfectants for dental impression materials should not only have a wide range of antibacterial and antiviral efficacy, but also should not adversely affect the properties and structure of the materials. As some research papers show, the latter requirement may not always be satisfied. Our results using commercially available disinfectants specifically recommended by their manufacturers for disinfection of dental impressions confirmed these findings. The null hypothesis of this work predicting no effect of disinfectants on the dimensional stability, accuracy and quality of impression material surfaces and gypsum casts was partially rejected.

One of the most important properties of impression materials is their dimensional stability which affects the accuracy of the impressions and the casts. Generally, the linear dimensional change of alginate impression materials shall not exceed 1.0%, when tested in accordance with ISO 21563 and should not be negatively affected with disinfection. Our results suggest that all alginate materials are susceptible to expansion in a water environment, with the exception of PP in combination with Silosept, where surprisingly, the shrinkage occurred in all disinfected specimens. EC and K were more sensitive to the disinfection and the type of disinfectant in comparison to other impression materials. The highest values were achieved in Dentaclean Form, where dimensional changes exceed the requirement of the normative document. Due to ignorance of detailed composition of tested materials, the explanation of behavior differences during disinfection process can only be speculative. The dimensional changes could be associated with an alginate/filler ratio, or ratio of Ca/Na ions15,16. Imbibition process could be caused by increasing free water located between fillers in alginate gel and by the concentration of surfactant in impression material16. In contrast to other alginate materials, the PP shrunk in Silosept disinfectant (~0.04%). It is reported in the literature that dimensional changes of alginate materials can be affected by different osmolarity and different pH of disinfectants as well as by the thickness of the impression17,18. In Muzaffar et al. the Hydrogum alginate material and the Perform ID disinfectants and sodium hypochlorite were tested. After the Perform ID disinfection, which had 2.5 times higher osmolarity value and a significantly lower pH than the sodium hypochlorite solution used, a larger shrinkage of alginate specimens was observed after the initial expansion, with an increase in time17. Efforts to solve problems with alginate impression materials have led, for example, to prefer disinfection by spraying method19,20, to the direct incorporation of the disinfectant into alginate powder (so called self-disinfectant irreversible hydrocolloids), gypsum or mixing water1,21,22 or to development of new, more durable alternative materials similar the structure of polyvinyl siloxanes16.

With elastomeric impression materials, linear dimensional changes can reach max. 1.5%19. Our results not exceeding 0.5% for all test materials met this requirement. However, condensation silicone impression materials tested were more prone to shrinkage after disinfection in agreement with Sinobad et al.23. These materials set via condensation reaction based on the crosslinking reaction between terminal polysiloxane OH groups and the cross-linking agent (tetraalkoxysilane) in the presence of activator (dioctyltin oxide). In addition to the limited mobility of polymer segments in the chemically crosslinked polymer network, the release of methanol or ethanol as by-products of this setting reaction contributes to the impression shrinkage. It might be speculated, that release of alcohols from the impression into the aqueous solution of disinfectants is faster than their lost from the control impression placed in air. The hydrolysis of unreacted alkoxy groups induced by water penetration into the impression material and thus increased amount of alcohols released cannot be also excluded.

These materials solidify a polycondensation reaction based on the polycondensation crosslinking reaction between the terminal OH groups of the polysiloxane polymer and the tetraalkoxy silane in the presence of dibutyltin dilaurate (DBTD) as the “catalyst” of the reaction. In another work (Martin et al.), dimensional changes of XLB impression material after sodium hypochlorite containing disinfectants were measured. These authors describe the shrinkage of approximately −0.2 to −0.3%7. The values we found for the same impression material corresponded to these results. The exception was the Dentaclean Form disinfectant, which showed slightly higher shrinkage, close to −0.5%. The same authors in other study also tested Impregum F polyether material with the dimensional change ±0.1%. Our results were similar, again with the exception of Dentaclean Form, where the shrinkage of the impressions was app. 0.2%. It is known that polyether compounds are more sensitive to the disinfection because of their hydrophilic properties. Disinfection in aqueous solutions can lead to higher sorption as well as to higher weight loss when kept in air3,19. However, these changes appear after a longer time, as shown by the work of Martin et
The results of the detail reproduction and the compatibility with gypsum including surface quality evaluation confirmed that mainly alginate impression materials are prone to deterioration in disinfectant solutions. AFS showed the most affected surface in Dentaclean Form leading to a rough appearance of the gypsum cast with loss of detail reproduction (rating score=4). Most detailed qualitative analysis well illustrates the impact of disinfectants on the surface of tested materials\textsuperscript{25).} Particles detected by SEM on the gypsum surface (Fig. 9C) and the impression surface after its pouring (Fig. 7B) resembled hemihydrate particles of the gypsum (Fig. 8). The deterioration of cast structure revealed up to 30–50 μm in depth was confirmed also using micro-CT analysis.

It suggests that AFS disinfected in Dentaclean Form might prevent the setting reactions of gypsum. A damaged cast surface, rating score 3, was also found for a combination of K disinfected with Silosept. The negative effect of these disinfectants results from their specific chemical composition, high concentration and long-time of immersion (15 and 10 min). In terms of antimicrobial activity, immersion disinfection is considered to be more reliable but riskier from the point of view of the monitored parameters. It can be assumed that a chemical reaction occurs between disinfecting solution and impression material or gypsum. It might be speculated that segments of alginate chains released from the surfaces of AFS and K impressions or ingredients of the disinfectants absorbed in their surface inhibit crystallization of calcium sulphate dihydrate of the gypsums.

In the case of elastomers, the low-viscosity silicone and polyether impression materials in medium consistency were chosen. These materials are in direct contact with the gypsum and as they contain surfactants to increase their hydrophilic properties, their surface may be the most susceptible to the effect of disinfectants. Polyether impression material in medium consistency is recommended for a monophase impression, where any dimensional change would have essential effect on its clinical performance. All tested elastomers did not show a change in reproduction of details or surface quality after disinfection in selected disinfectants. Compatibility with both types of gypsum was very good.

The limitation of this study may be the fact that the linear dimensional changes were detected immediately after rinsing with water or disinfecting the impressions in this study. In order to get closer to clinical practice, it would be advisable to evaluate dimensional changes in several time intervals up to the manufacturer’s recommended peak periods of pouring with model gypsum, always following the conditions for the correct method of impressions storing. In the future, we would also like to use the method micro-CT for the determination of maximal depth with deteriorated structure and for more detailed analysis of large particles or extent of porosity.

The effect of disinfectants on the accuracy and quality of gypsum casts primarily of alginate impression materials is very individual. Despite the declared universality of disinfectants, it is very important to follow the manufacturer’s recommendations related to suitable combinations of impression material and disinfectant to achieve the highest quality of dental prosthetic reconstruction.

CONCLUSIONS

The following conclusions can be made based on the results obtained:

1. The alginate impression materials, compared to elastomeric materials, are more sensitive to the disinfectants used. They can interact with certain types of disinfectants resulting in subsequently negative effects on the dimensional stability, reproduction of detail and the quality of the impression surfaces and gypsum models made from them. The results of our work show that the effect of disinfectants on the monitored properties primarily of alginate impression material is very individual.

2. From this point of view the disinfectant Dentaclean Form in combination with some alginate materials causes the largest dimensional changes and the deterioration of impression and gypsum cast surfaces.

3. All the imaging methods used in this work achieve similar results. SEM imaging method is suitable for more detailed surface analysis as a good supplement of evaluation by light microscopy. Micro-CT allows viewing into the inner structure of the material where the influence of penetrated disinfectant was proven.

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REFERENCES
