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Effect of Hypochlorous Acid on Acrylic Resins in Preventing Cross-infection Between Clinic and Laboratory

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ABSTRACT

Objective: The COVID-19 pandemic and monkeypox disease have highlighted the importance of preventing cross-infection between dental laboratories and clinics. Thus, the aim of this study was to evaluate the effects of hypochlorous acid (HOCL), a natural disinfectant that is effective on bacteria and viruses, on polymethylmetacrylate (PMMA) to prevent cross-infection.

Methods: A total of 120 PMMA (60 heat-activated and 60 three dimensionally printed) samples were prepared. The samples were immersed in HOCL and ortophtalaldehyde (OPA) disinfectant twice for 30 minutes to simulate the travel time to clinic and the dental laboratory. 10 samples from each group were kept in deionized water as a control group. Flexural strength and surface roughness measurements of the samples were performed. T-test and Kruskal Wallis H test were used for statistical evaluation (α=0.05).

Results: There was no significant difference in flexural strength or Ra values between the HOCL and OPA groups.

Conclusion: HOCL PMMA may be considered an alternative disinfectant for preventing cross-infection between dental laboratories and clinics in dentures and orthodontic appliances. Further research is required to evaluate its biological efficacy and long-term use.

Keywords: *Acrylic resin, COVID-19, Disinfectants, Hypochlorous acid, Infection control*

1. INTRODUCTION

Removable prostheses and orthodontic appliances will be sent from the clinic to the laboratory and back several times during the period from taking impressions to being applied to the patient. During this process, they may be contaminated with blood and saliva in the clinic, and with equipment such as felt, disks, pumice or dirty hands in the laboratory. (1) Therefore, it is important for patients, physicians, dental assistants and dental technicians to prevent cross-infection between dental clinics and dental laboratories.

Polymethyl methacrylate (PMMA), used in the manufacturing of prostheses and orthodontic appliances, has been widely used in dentistry for many years. The advantages of PMMA resins include ease of application, acceptable aesthetic appearance, physical and mechanical properties, low molecular weight, and a reasonable price. However, they have low thermal conductivity and low resistance to fatigue (2,3)

During the past years, three-dimensional (3D) printed materials have become popular in PMMA production, as well as in dentistry in general. 3D PMMA materials have the advantages of higher accuracy, less material waste, and lower infrastructure costs in less time. The disadvantages of the additive manufacturing process include the inability to use all materials, the staircase effect caused by layer-by-layer production, repeatability, and the need for support structures (4-5)

Various studies have investigated the use of disinfectants like sodium hypochlorite, chlorhexidine, and glutaraldehyde for disinfecting PMMA materials (6,7). However, limited research exists on the effects of orthophthalaldehyde (OPA) and hypochlorous acid (HOCl)—the disinfectants used in this study—on acrylic resin materials (8,9).

Orthophthalaldehyde is a highly effective chemical agent commonly employed for high-level disinfection, especially for medical instruments that cannot undergo heat sterilization (10).

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Hypochlorous acid (HOCl) is a versatile disinfectant renowned for its broad-spectrum microbicidal properties. As a strong oxidant, it is easy to use, cost-effective, and highly effective against a wide range of microorganisms, including bacteria and viruses (11,12). It is listed among the World Health Organization's (WHO) recommended biocides for combating coronaviruses and is also included in the U.S. Environmental Protection Agency's 'N' list of disinfectants effective against emerging pathogens, including SARS-CoV-2 (13,14). Studies have highlighted its utility in diverse applications, such as cleaning implant surfaces and disinfecting dental plaster models (15,16).

The aim of this study was to evaluate the effect of hypochlorous acid and orthophthalaldehyde disinfectants on the fracture resistance and surface roughness of heat-activated polymerization (HA) and 3D printed PMMA resins. The null hypothesis of the study was that short-term use of OPA and HOCL to prevent cross-infection would have no effect on the fracture resistance or surface roughness of PMMA.

2. METHODS

A total of 120 samples and two different disinfectant solutions (HOCL and OPA) were used in this study. For the 3D bending test, 60 samples with dimensions of 65×10×3 mm (in accordance with ADA specification no. 21) were prepared. Of these, 30 samples were made of 3D-printed PMMA, and the remaining 30 were made of HA PMMA. Similarly, for the surface roughness test, another 60 samples with dimensions of 20×20×1.5 mm were prepared, evenly split between 30 samples of HA PMMA and 30 samples of 3D-printed PMMA (17).Each group of 30 samples was further divided into three subgroups: Group I: OPA disinfectant group Group II: HOCL disinfectant group Group III: Control group (Distilled water)

2.1. Fabrication of 3D PMMA Resin Specimens

Specimens for the three-point bending test and surface roughness test were designed and saved as Standard Tessellation Language (STL) files. The 3D-printed specimens were fabricated using these STL files on a 3D printing unit (Ackuretta FreeShape 120, Taiwan) with a cross-sectional thickness of 100 microns, following the manufacturer's instructions (Dentona Denture 385, Dortmund, Germany). (Ay design, Ankara, Türkiye)

2.2. Fabrication of HA PMMA Resin Specimens

Wax specimens were prepared using 3D PMMA specimens as molds. They were polymerized using heat-polymerizable PMMA (Meliodent, Heraeus Kulzer GmbH Co, Germany) according to the manufacturer's recommendations and cooled on the bench for 30 minutes at room temperature. A precision scale (Kern ABJ 220-4 NM, Germany) was used to determine the ideal ratio of polymer (powder) to monomer (liquid).

The same finishing and polishing process was applied to both HA and 3D samples. First, they were smoothed with a tungsten carbide router at low speed. Then, each surface was finished under water with 400 grit silicon carbide papers (Met Rolls; Kemet, London, England) for 30 seconds (18) For polishing a mixture of dental pumice and water was used with felt for 90 seconds, then universal polishing paste (Universal Polishing Paste, Ivoclar Vivadent, Schaan) was applied. (Figure 1)

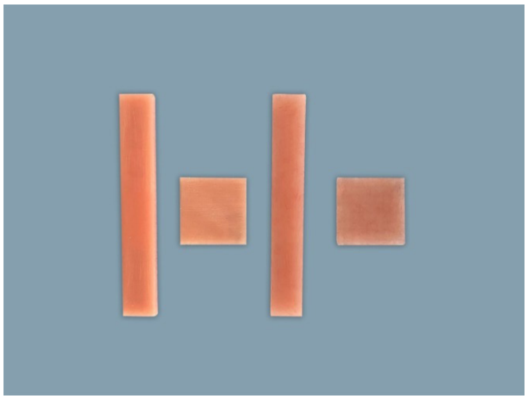

Figure 1. *3D and HA PMMA specimens.*

The dimensions of the PMMA specimens were checked using a digital caliper (Shinwa Co, Osaka, Japan). 30 specimens in each group were randomly divided into 3 groups (HA PMMA for 3D bending test, HA PMMA for surface roughness test, 3D PMMA for 3D bending test and 3D PMMA for surface roughness test) (n=10). Ten specimens from each group were used as a control group. All specimens were stored in distilled water at 37°C to maintain dimensional stability throughout the study.

2.3. Disinfection Method

Specimens were kept in disinfectant twice for 30 minutes each, simulating the circulation between dental laboratory and the clinic. They were kept in distilled water for 5 minutes between the two disinfections. Orthophthalaldehyde (OPA) (Cidex OPA, Johnson & Johnson Medical, Norderstedt, Germany) and hypochlorous acid (HOCL) (SuperOx, Anolit, Yenimalle, Ankara) were used as disinfectants. Specimens in the control group were additionally soaked in distilled water for 30 minutes twice and removed. In order to provide the same conditions as the disinfectant-exposed specimens, they were soaked in distilled water again for 5 minutes between the two half-hour periods (18).

2.4. Three-Point Bending Test

Flexural strength of disinfected specimens was determined using a 3-point bending testing in a universal testing machine (Lloyd LRX, Lloyd Instruments Ltd, Fareham, Hampshire, England). Each specimen was placed horizontally on two vertical supports with a 50 mm distance, and the head speed was 5 mm/min. The load was applied to the center of the specimens where fractures occurred (19) The fracture force was recorded in Newtons (N). Flexural strength values were calculated using the formula S= 3FL/2bd2 (S: flexural strength (N/mm2), F: Load recorded at break (N), L: Distance between supports (50 mm), b: Specimen width (10 mm), d: Specimen thickness (3 mm) (20).

2.5. Surface Roughness Evaluation

After exposure to the disinfectant solutions, surface roughness measurements were conducted using a profilometer (Perthometer M2, Mahr GmbH, Göttingen, Germany). Three measurements were taken from each sample, and the average of these values was calculated and recorded as the surface roughness (Ra) in micrometers (µm).

2.6. Statistical Analyses

Statistical analyses were performed using the Statistical Package for Social Science Software (SPSS, version 23.0, IBM Corp, Chicago, USA). For comparisons between three or more groups, Kruskall Wallis H test was used because the data were not normally distributed. For comparisons between groups, a t-test was used due to the normal distribution of data (α=.05).

2.7. Scanning Electron Microscopy (SEM) Analysis

In order to evaluate the surface properties, the homogeneity of the samples was examined under 1000 X magnification using a scanning electron microscope (SEM) (Evo 40, Carl-Zeiss, Oberkochen, Germany). Gold coating was applied using a sputter coating machine (Emitech K550X, Richmond Scientific, ORE, England).

3. RESULTS

The results for flexural strength, surface roughness, and SEM analysis of the samples exposed to both disinfectants are presented below.

3.1. Flexural Strength

The flexural strength (MPa) values and standard deviations (SD) of the HA PMMA and 3D PMMA resin groups are shown in Table 1. The flexural strength values of the HA PMMA resin group were statistically significantly higher than 3D PMMA group in all three groups (DW, HOCL and OPA) (P= .0001). Differences between rows within columns are displayed with lowercase superscripts in Table 1.

Table 1. *Mean flexural strength (MPa) values and standard deviations (SD)*

	Mean (MPa)-(SD) DW	Mean (MPa)-(SD) HOCl	Mean (MPa)-(SD) OPA
HA PMMA	147.92 (13.73)^{Aa}	143.09 (12.80)^{Aa}	142.03 (9.05)^{Aa}
3D PMMA	56.42 (19.55)^{Ab}	55.20 (16.26)^{Ab}	56.99 (14.13)^{Ab}

HA PMMA: Heat activated polymethyl methacrylate 3D PMMA: Three dimensionally printed polymethyl methacrylate DW: Distilled water, HOCL: Hypochlorous acid, OPA: Ortho phthalaldehyde. Superscript lowercase letters incate the statistically significant differences between studiedresin groups for disinfectants and control (p<0.05). Superscript capital letters incate the difference between disinfectant groups within each resingroup (p>0.05).

No significant difference was found between the flexural strength (MPa) values of HOCL, OPA disinfectant applications and the control group for each resin group. The differences between columns were shown with a capital letter superscript in Table 1. The p values were *(p>0,05)* for both HA PMMA and 3D PMMA.

3.2. Surface Roughness

The surface roughness measurements of the two resin groups were compared using a t-test (Table 2). The Ra value differences between HA PMMA and 3D PMMA resins were not statistically significant when disinfected with HOCl and OPA *(p HOCl=0.111, pOPA=0.852).* Exposure with three different solutions did not cause a significant difference in Ra values in both the HA PMMA *(p=0.464)* and 3D PMMA *(p=0.307)* groups.*(p>0,05).*

Table 2. *The mean surface roughness (Ra) values and standard deviations (SD)*

Ra	Mean (SD) DW	Mean (SD) HOCl	Mean (SD) OPA
HA PMMA	.64(.20)^{Aa}	.63(25)^{Aa}	.52(.21)^{Aa}
3D PMMA	.42(.14)^{Ab}	.46(.20)^{Aa}	.54 (.17)^{Aa}

HA PMMA: Heat activated polymethyl methacrylate 3D PMMA: Three dimensionally printed polymethyl methacrylate DW: Distilled water, HOCL: Hypochlorous acid, OPA: Orthophthalaldehyde. Superscript lowercase letters incate the statistically significant differences between studied resin groups for each disinfectant and control (p<0.05). Superscript capital letters incate the difference between disinfectant groups within each resin group (p>0.05).

3.3. (SEM) Analysis

SEM images of 3D PMMA specimens are displayed in Figure 2. DW immersed specimens have a smooth structure. The amount of roughness in the specimens disinfected with OPA was observed to be higher than the specimens disinfected with HOCL. HA PMMA specimens have a rougher appearance than 3D PMMA specimens in all three solutions (Figure 3). The findings are in parallel with the numerical values.

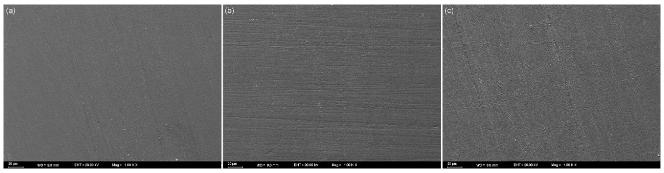

Figure 2. *SEM images of DW (a), OPA (b) and HOCL(c) of treatment of 3D PMMA original magnification 1000 X*

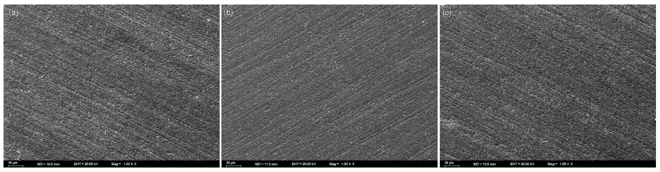

Figure 3. *SEM images of DW (a), OPA (b) and HOCL(c) of treatment of HA PMMA original magnification 1000 X*

4. DISCUSSION

In this study, the effect of two different disinfectants (HOCL and OPA) on the flexural strength and surface roughness of HA and 3D PMMA specimens were evaluated. Compared to the OPA and DW groups, HOCL was found to have no negative effect on the flexural strength and surface roughness of PMMA specimens and the null hypothesis that it can be used for a short time to prevent cross-infection between the laboratory and clinic was accepted.

Disinfection of impressions, models, orthodontic appliances and prostheses by dentists and dental technicians during transfer between clinic and laboratory is a routine procedure. Antibacterial chemicals used in spray and dip disinfection can influence mechanical properties such as flexural strength, hardness and roughness (18,21,22).

The effect of various disinfection methods and solutions on the physical properties of HA PMMAs has been evaluated in the literature (23,24). However, there are limited studies on the impact of disinfection protocols on the mechanical properties of 3D PMMAs and data on HOCL disinfection, an innovative approach (9,19,25).

In this study, the flexural strength values (DW, HOCL and OPA) of the 3D PMMA resin group were found to be significantly lower than HA PMMA group in all three immersion solutions *(P= 0.0001).* This result is consistent with previous studies (19,26). Akhaltham et al. concluded that 3D resins had lower flexural strength and elastic modulus than HA PMMA resins. Researchers have reported that the choice of appropriate disinfectant and material is effective on the life of the prosthesis and orthodontic appliances (19).

Although the results are similar, the difference between the immersion times should not be ignored. In the literature, the factors affecting the strength of 3D PMMA resins include water storage, immersion in chemicals and aging (27). Resin materials easily absorb water due to their polarity. The amount of water absorption is related to the amount of residual monomer and the diffusion coefficient of the resin. The diffusion coefficient of the resin depends on the time it takes for the material to saturate with water. Therefore, the immersion time of the resin material in the disinfectant has an effect on the results (28). In this study only one-hour long findings were evaluated, which simulated the travel time between the laboratory and the clinic. The lack of difference in flexural strength values between disinfectants in both resin groups can be attributed to the fact that the time was limited to only one-hour. The decreased flexural strength of 3D printed resins after disinfection was explained by the higher water absorption rate of 3D printed resins compared to HA resins (29).

Previous studies have reported that NaOCl immersion decreases flexural strength. The lower flexural strength value was associated with the absorption of NaOCl aqueous solution and the active chlorine content. It was reported that the active chlorine content increased the residual monomer solubility, which was compensated by more water absorption, affecting the PMMA strength (19,30,31). In this study, the use of hypochlorous acid for a short time to prevent cross-infection may have prevented the negative effects of active chlorine content.

Freitas et al (32) evaluated the mechanical properties and anti-biofilm formation of CAD/CAM milled, 3D printed denture base resins and conventional resins. It was concluded that 3D printed specimens had the lowest flexural strength and the highest surface roughness values. A value (57.23 ± 9.07*; $p < 0.05$*) below the required resistance (65 MPa) recommended by ISO 20795-1:2013 for denture base resins was observed for 3D printed specimens. Similarly, in this study, 3D printed resin flexural strength values were 56.42 ± 19.55, 55.20 ± 16.26 and 56.99 ± 14.13 for DW, HOCL and OPA, respectively. These results are in line with the study of Prpić et al. (26). (Max: 84.5 MPa and Min: 60.0 MPa)

The low flexural strength value of 3D PMMA resin may also be related to the polymerized material, polymerization time and printing structure (26,33). In other words, the flexural strength value is not only related to the production method of the material but also to the selected material itself. In a previous study, the lower mechanical performance of 3D printed denture resins was associated with a lower conversion rate compared to conventional PMMA resins (34).

When evaluated in terms of surface roughness values, the results of this study are similar to previous studies (32,35). Al Dwairi et al (35) concluded that HA PMMA surface roughness values were higher than three different brands of 3D PMMA materials. The difference between the values of three different brands supports the idea that the material and printing structure used are effective for mechanical properties. Freitas et al (32) similarly concluded that the surface roughness values of HA PMMA resin were higher than the 3D printed group.

Unlike previous studies on PMMA disinfection, HOCL was used as a disinfectant in this study. HOCl is an easy-to-use, cost-effective, and non-toxic disinfectant that combines most of the desired effects of an ideal disinfectant. It has been shown to inactivate a variety of viruses, including coronaviruses, in less than 1 min. The American Food and Drug Administration (FDA) has approved the use of hypochlorous acid in eye and dental applications and as a rinse-free disinfectant for fruits and vegetables, demonstrating its biological safety (10,11,12).

Gessi et al (36) found that HOCl-based disinfectant did not damage floor surfaces even after more than a thousand applications and was also safe for sensitive surfaces used as controls. They concluded that unlike bleach or chlorine solutions, HOCl is not corrosive to surfaces or equipment due to its slightly acidic pH conditions. The study achieved a satisfactory level of antimicrobial decontamination and at the same time reported CO2 emission savings of approximately 30% per square meter.

According to the data obtained from this study, hypochlorous acid can be used as an alternative disinfectant to prevent laboratory-clinic cross-infection for 3D and HA-PMMA resin prostheses and orthodontic appliances. On the other hand, care should be taken to ensure that there is a balance between the effectiveness of disinfectants and their effect on the resin.

The current study has certain limitations. Because of the artificial travel time between the clinic and laboratory, the amount of time that PMMA materials were exposed to the disinfectant was restricted. Future studies may focus on the effects of longer exposure to HOCl on the mechanical properties and color of PMMA materials or the microbiological activity of viruses such as Sars Cov-2 and monkey pox. Thus, the use of biological disinfectants such as HOCl, which do not have negative effects on mechanical properties and the environment, may become widespread.

5. CONCLUSION

1. Immersing 3D and HA-PMMA resins in disinfectant solutions (HOCL and OPA) for 1 hour resulted in no significant difference in flexural strength values.

2. Immersion of 3D and HA PMMA resins in DW and disinfectant solutions (HOCL and OPA) for 1 hour did not produce a significant difference on Ra values.

3. 3D printed PMMA resins showed lower flexural strength values than HA PMMA resins.

4. The surface roughness values of HA PMMA resins immersed in disinfectant solutions were higher than the surface roughness values of 3D PMMA resins.

5. Hypochlorous acid showed no adverse effects on the mechanical properties of 3D and HA-PMMA resin prostheses and orthodontic appliances when used as an alternative disinfectant to prevent cross-infection between the laboratory and the clinic.

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