Influence of ozone and paracetic acid disinfection on adhesion of resilient liners to acrylic resin

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PURPOSE. The aim of this study was to evaluate the effect of paracetic acid (PAA) and ozone disinfection on the tensile bond strength (TBS) of silicone-based resilient liners to acrylic resins. **MATERIALS AND METHODS.** One hundred and twenty dumbbell shaped heat-polymerized acrylic resins were prepared. From the mid segment of the specimens, 3 mm of acrylic were grinded off and separated parts were reattached by resilient liners. The specimens were divided into 2 control (control1, control7) and 4 test groups of PAA and ozone disinfection (PAA1, PAA7, ozone1 and ozone7; n=10). While control groups were immersed in distilled water for 10 min (control1) and 7 days (control7), test groups were subjected to PAA (16 g/L) or ozone rich water (4 mg/L) for 1 cycle (10 min for PAA and 60 min for ozone) per day for 7 days prior to tensile tests. Measurements of the TBS were analyzed using 3-way ANOVA and Tukey's HSD test. **RESULTS.** Adhesive strength of Mollosil decreased significantly by application of ozone disinfection. PAA disinfection had no negative effect on the TBS values of Mollosil and Molloplast B to acrylic resin. Single application of ozone disinfection did not have any negative effect on TBS values of Molloplast B, but prolonged exposure to ozone decreased its adhesive strength. **CONCLUSION.** The adhesion of resilient liners to acrylic was not adversely affected by PAA disinfection. Immersion in ozonated water significantly decreased TBS of Mollosil. Prolonged exposure to ozone negatively affects adhesion of Molloplast B to denture base materials. ***[J Adv Prosthodont 2016;8:290-5]***

KEYWORDS: Resilient liner; Ozone; Peracetic acid

INTRODUCTION

Tissue-born partial and full dentures may need relining with silicone based resilient tissue liners for enhancing patient comfort. Relining dentures with silicones could be very useful for treating patients with ridge atrophy or resorption, bony undercuts, bruxing tendencies, congenital or acquired oral defects requiring obturation, xerostomia, and dentures opposing natural dentition.[1,2] Despite its ability to prevent discomfort, inherent surface roughness of resilient liners can cause bacterial and fungal colonization.[3] One of the most serious problems concerning resilient denture liners is the loss of adhesion with denture base. This failure can further increase plaque and calculus formation and bacterial growth, hence responsible for denture related stomatitis and even more serious infection in geriatric patients[4]. Well organized routine cleaning with soap and brush and further with chemical cleanser may control microbial colonization. However, for elderly people, especially for disabled denture wearers, denture cleaning may be challenging.[4] Therefore, more effective disinfectants that are non-toxic to the patient and to the environment without any negative side effects on dentures and resilient liners are needed.

Various types of disinfectants were used in dental practice like glutaraldehyde, formaldehyde, sodium hypochlorite, hydrogen peroxide, chlorhexidine and mixture of different chemicals. Despite their strong disinfection capabilities, aldehyde solutions are not the material of choice due to their high toxic effects. Chlorhexidine and hydrogen peroxide are shown to have a minor effect, especially on Candida

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species.[5] Sodium hypochlorite solutions are very effective in various concentrations against microorganism. However, prolonged application may cause bleaching of the denture base and denture liners.[6] Microwave irradiation can also be used to disinfect dentures. However, microwave disinfection might negatively affect the physical properties of the denture base materials.[7]

Ozone is a naturally occurring compound consisting of three oxygen atoms (triatomic oxygen or trioxygen). It can be found in the form of gas in the stratosphere in concentration of 1-10 ppm or it can be produced by ozone generators. Ozone, in the gaseous and aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses. These properties make ozone an important therapeutic agent in infectious and inflammatory diseases.[8] Furthermore, ozone decomposes rapidly to O_2 and OH radicals in water solutions.[9,10]

PAA is generated by the reaction of tetraacetylethylenediamine in the presence of an alkaline hydrogen peroxide solution. It has broad antimicrobial efficacy against bacteria, fungi, protozoa, and viruses and it is not affected by protein residues. PAA solution decomposes to acetic acid and oxygen in 24 hours and is drained to sewage after use with little to no effect on the environment.[11,12] Peracetic acid-based disinfectants have been widely used in food industry and water treatment facilities, as well as for decontamination and sterilization of thermosensitive medical and hospital equipment and devices such as endoscopes and UV lenses.[11,13]

The research about PAA and Ozone disinfection is mainly focused on their antimicrobial efficacy. The effects of their use on dental materials have rarely been studied. To date, there are no studies in the literature regarding their effect on adhesion of resilient liners to the acrylic resins. Therefore, the aim of this study was to evaluate the effect of PAA and ozone disinfection on the tensile bond strength (TBS) of heat and self-polymerized silicone-based resilient denture liners to denture base acrylic resins.

MATERIALS AND METHODS

A dumbbell shaped master model was produced according to ASTM D-412 standards for tensile test of elastic materials. A silicone mold was produced by embedding a master model into the type A silicone impression material (Panasil, 154081, Kettenbach GmbH & Co KG, Eschenburg, Germany). Modeling wax was melted and poured into the silicone mold. Having been set and reached to room temperature, wax patterns were demolded and inspected for any defects and voids. Wax patterns were invested in dental stone (Triastone Type 4, 1302055, Triadent, Istanbul, Turkey) in a stainless steel metal flask to construct the molds. Following the setting of the dental stone, boil-out procedure was performed. (Fig. 1) The heat-polymerized (Meliodent, 10JAN051, Heraeus Kulzer, Hanau, Germany) dumbbell shape specimens were polymerized according to the manufacturer's instructions in a manner similar to that used in conventional denture construction. Two different resilient denture liners from the same manufacturer were selected: Mollosil (Mollosil, 150401, Detax, Ettlingen, Germany) auto-polymerized and Molloplast B (Molloplast B, 140731, Detax, Ettlingen, Germany) heat-polymerized silicone-based resilient liner. From the mid segment of the dumbbell shaped resin specimens, 3 mm of acrylic were grinded off. The segments to be grinded off were marked with a digital caliper (Mitutoyo Corp, Kanogawa, Japan) and removed with a diamond bur. The grinded surfaces were conditioned according to the manufacturer recommendations for proper adhesion between acrylic resin and liner. The sectioned acrylic specimens were relocated in the flask where they were previously polymerized. The resilient liners were prepared according to manufacturer instructions, and the upper portions of the flasks were closed after application of the resilient liner. (Fig. 2) For the Molloplast B specimens, the adhesive was applied 2 times over the grinded acrylic surfaces and left to bench dry for 60 minutes. The ready-to-use resilient liner was applied over the acrylic

Fig. 1. (A) Master model and embedded wax patterns in flask before and after boil-out, (B) Master model and embedded wax patterns in flask before and after boil-out, (C) Master model and embedded wax patterns in flask before and after boil-out.

Fig. 2. Dumb-bell shaped test specimen reattached with silicone soft liner.

Fig. 3. Tensile testing procedure of test specimens.

surfaces with a clean spatula. The resilient liner and acrylic resin assembly was covered with thin foil and the flask was closed and placed under the hydraulic press for 4 minutes. The flask was opened and the foil and excess material were removed with a sharp scalpel. The flask was closed and relocated under the hydraulic press during 15 minutes. The flask was located in cold water and heated slowly up to 100°C and boiled for 2 hours. After bench cooling, the specimens were removed from the flasks. For the Mollosil specimens, the adhesive was applied over the grinded acrylic surfaces and left to bench dry for 1 minute. Equal amount of base material and catalyst were mixed for 30 seconds and applied over the acrylic surfaces with a clean spatula. The flask was closed and placed under the hydraulic press for 30 minutes. The excess material was removed with a sharp scalpel and the specimens were removed from the flasks.

One hundred and twenty dumbbell shaped heat-polymerized denture base resin were prepared: 60 for Mollosil and 60 for Molloplast B. They were reattached in mid-segment with a 3 mm long resilient liner. The specimens were divided into 2 control groups (control1, control7) and 4 test groups of PAA (Actosed, 0910026, Acto GmbH, Braunschweig, Germany) and ozone disinfection (PAA1, PAA7, ozone1 and ozone7; n = 10) (Table 1). Tensile tests were performed after immersion in distilled water (37°C) for 10 minutes (control1) and seven days (control7) for the control groups. The specimens of PAA and ozone test groups were subjected to PAA (16 g/L) or ozone rich water (4 mg/L). The exposure cycle was 10 minutes for PAA and 60 minutes for ozone. Following each cycle, test specimens were rinsed under running water and stored in distilled water until the next cycle. Immediately after one cycle of PAA1 and Ozone1 and seven cycles (just one cycle per day) of PAA7 and Ozone7, test specimens were subjected to tensile test using an universal testing machine (M500 25kN; Testometric Co., Rochdale, UK) with a crosshead speed of 5 mm/sec until debonding or rupture occurred. (Fig. 3) The tested specimens were observed with a magnifying loupe (× 4) (Eyemag Pro S; Carl Zeiss AG, Germany) and the type of failure and TBS values were recorded. The failure types were classified as: adhesive (no liner remnant on the acrylic surface), cohesive (both acrylic surfaces covered with liner) and mixed (acrylic surfaces partially covered with liner). Measurements of the TBS were analyzed using ANOVA and Tukey's HSD test.

Table 1. Distribution of test specimens (n = 10)

	Mollosil	Molloplast B
Control	Control1 - 10 min. Control7 - 7 days	Control1 - 10 min. Control7 - 7 days
PAA	PAA1 - 10 min. PAA7 - 10 min/day for 7 days	PAA1 - 10 min. PAA7 - 10 min/day for 7 days
Ozone	Ozone1 - 60 min. Ozone7 - 60 min/day for 7 days	Ozone1 - 60 min. Ozone7 - 60 min/day for 7 days

RESULTS

The mean TBS values of the control and test groups are presented in Table 2. Molloplast B showed greater adhesive strengths compared to Mollosil for all test conditions. PAA disinfection had no negative effect on the TBS values of Mollosil and Molloplast B. No statistically significant differences were detected between the mean TBS values of the control and PAA tests groups for both materials.

On the other hand, adhesive strength of Mollosil decreased significantly by application of ozone disinfectant. A statistically significant decrease was found between the control and both Ozone test groups. Also, a further decrease

Table 2. Mean TBS values (in MPa)

	Control1	Control7	PAA1	PAA7	Ozone1	Ozone7
Mollosil	1.28 (0.21)^{Aa}	1.18 (0.13)^{Aa}	1.22 (0.14)^{Aa}	1.11 (0.19)^{Aa}	1.02 (0.15)^{Ab}	0.90 (0.09)^{Ac}
Molloplast B	1.76 (0.23)^{Ba}	2.37 (0.27)^{Bb}	1.85 (0.16)^{Ba}	2.15 (0.25)^{Bb}	1.78 (0.30)^{Ba}	1.82 (0.25)^{Ba}

Different superscripted lowercase letters indicate statistically different means within each row ($P < .05$).
Different superscripted uppercase letters indicate statistically different means within each column ($P < .05$).

Table 3. Failure type of Mollosil

	Mixed	Adhesive	Cohesive
Control1	2	1	7
Control7	1	1	8
PAA1	1	1	8
PAA7	1	1	8
Ozone1	3	2	5
Ozone	1	2	7

Table 4. Failure Type of Molloplast B

	Mixed	Adhesive	Cohesive
Control1	1	1	8
Control7	1	3	6
PAA1	3	3	4
PAA7	3	3	4
Ozone1	4	3	3
Ozone7	3	4	3

was detected by prolonged exposure of Mollosil to ozone. Single application of ozone disinfectant (Ozone1) did not have any negative effect on TBS values of Molloplast B; however, prolonged exposure to ozone (Ozone7) decreased its adhesive strength.

The types of failures are represented in Table 3 and Table 4. Mollosil mostly had a cohesive type of failure in test and control groups. Even distribution was seen in terms of failure type for Molloplast B, except control groups in which cohesive type of failure dominated.

DISCUSSION

There are mainly two types of resilient denture liners used in dental practice: acrylic and silicone based liners. Acrylic liners have similar composition to acrylic base resin and can create a chemical bond with denture base. After a certain amount of time, softening agents in acrylic liners wash off and harden the liner. On the other hand, silicone based resilient liners cannot create a chemical bond with denture base due to their different chemical composition; yet their softness remains long.[14,15] Despite its relatively weak bonding strength, silicone resilient liner's greater long term structural stability over acrylic liners made them the material of choice in the current study.

Various test methods have been used for testing the adhesion between resilient liners and denture base resins including tensile, peel and shear tests.[16] The tensile bond strength test has been widely used by researchers.[17,18] Due to lower tear strengths of silicone based resilient liners, the use of tensile bond strength test method to evaluate their adhesion to acrylic resins is recommended.[19]

In order to achieve bond between silicone liners and denture for a reasonable clinical life, surface conditioning of the denture base is mandatory. Although adequate bond strength can be achieved with surface treatments, oral environment with pH, temperature changes and use of various chemicals for cleaning and disinfecting dentures further diminish bond strength and can cause bond failure eventually.[20] Therefore, disinfectants with little or no effect to the bonding properties of resilient liners to the acrylic are important for long term clinical use. Oxidizing agents are usually very effective disinfectants and act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death.[12] A large number of disinfectants operate in this way, including Ozone, Peracetic acid, and sodium hypochlorite. Paracetic acid and ozone, despite their effective disinfecting capabilities, are safe materials for the patient and environment. They are non-toxic and non-allergenic at low concentrations and decompose to water, oxygen, carbon, and OH, which are biocompatible products present in nature.[12] The PAA has been considered an effective and safe alternative to glutaraldehyde by international reference institutions, such as the US Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC) and the Association for Professionals in Infection Control and Epidemiology (APIC).[11]

Different concentrations of ozone solutions were tested for eradication of different microorganisms in literatures.[8,21-23] Generally, as the concentration of ozone increases, the antimicrobial efficacy increases as well. According to studies of Arita *et al.* and Nagayoshi *et al.* concentration of 4 mg/L ozonated water was effective for killing gram-positive and gram-negative oral microorganisms and oral Candida albicans in 60 minutes.[8,21] Ozonated water can be generated by various ozone generators. Hyper Medozon comfort (Herrmann Apparateau GmbH, Germany) ozone generator was used in the current study for preparation of ozonated water. The concentration was controlled by device and in this study it was adjusted to 4 mg/L. On the other hand, different concentrations of paracetic acid solutions have been used in dental and medical fields depending on the application. In the current study, the paracetic acid concentration was determined by the manufacturers' instructions.

Before testing procedure, a pilot study was performed. It was calculated that bond strength test procedure was extended to 60 minutes for each group. During test procedure of control groups, the specimens were left in distilled water to prevent drying, which extended the duration of the specimens in water from 10 minutes to 60 minutes. It has been shown that storing Molloplast B and Mollosil resilient liners in water one day to one week has no effect on bond strength to acrylic resins.[24] Therefore, the authors decided not to have two separate control1 (10 minutes for PAA and 60 minutes for Ozone) groups for Mollosil and Molloplast B.

In the current study, Mollosil had lower bond strength than Molloplast B, which is in accordance with the previous studies.[25] Due to uncontrolled polymerization process and cross-link density, self-polymerized polymers generally have weaker mechanical properties.[26] Besides the differences in the type of polymerization, the compositional differences might alter the characteristics of the resilient liners. The ratio of the matrix to the filler particles of the silicone-based resilient liners directly affects the physical and mechanical properties of the materials. The increase in filler content improves the strength.[26] Mollosil showed mostly cohesive failures in test and control groups. The inherent lower mechanical properties of the self-polymerized Mollosil may be related to the material's failure.

PAA disinfection showed no effect on bond strength of resilient liners being tested in the current study. On the other hand, the ozone disinfection affected the bonding strength of resilient liners differently. TBS values of Mollosil decreased significantly after exposure to Ozone in both test groups. However, single exposure of Molloplast B to Ozone solution had no effect on TBS values while multiple exposures decreased TBS. These findings might be explained by the high oxidizing capabilities of Ozone solution, which may deteriorate the bond between the liner and denture base.

It is well documented that polymerization process continues after deflasking of heat-polymerized silicones.[27,28] The TBS values of Molloplast B in Control7 group were significantly higher than those of Control1. It is possible that the polymerization process of Molloplast B might have continued after deflasking and increased its bonding strength.

There are few studies investigating the effect of disinfection techniques on the bond strength of resilient liners to denture base resins.[20,29,30] Garcia *et al.*[29] and Pisani *et al.*[20] evaluated the influence of denture cleansers on resilient liner adhesion to denture bases by tensile test method and reported no adverse effects. Also, Machado *et al.*[30] found no negative effects on the peel bond strength of resilient liner to denture base resins when disinfected by microwave irradiation.

There are various TBS values in the literature for proper adhesion between resilient liners and acrylic base. It has been reported by different researchers that this value should be between 0.45-0.9 MPa.[31-33] Considering these threshold values, all test and controls groups had satisfactory TBS results. Nevertheless, prolonged exposure of Mollosil to Ozone (Mollosil-ozone7; 0.9 MPa) had high risk of decreased TBS to clinically unacceptable values.

Ozone generators are relatively expensive and they could be massive. Their use can be objected due to their cost-effectiveness in terms of everyday use for home-care. Using ozone generators in dental laboratories or dental clinics seems to be more suitable for disinfection of dentures instead of home-care for now. However, their price and dimensions will decrease and in time they will be more affordable and suitable for daily use.

Within the limitations of this *in vitro* study, it has been shown that PAA and Ozone can be an alternative to disinfection of dentures with resilient liners. While PAA disinfection is safe in terms of bonding strength of silicone to acrylic base, multiple disinfection of Mollosil with ozone has high risk of de-bonding. Further studies are needed for investigation of the longer-term effect of PAA and Ozone on TBS values of resilient liners to acrylic resins.

CONCLUSION

The adhesion of resilient liner materials being tested is not influenced by PAA disinfection. Prolonged Ozone exposure might adversely affect the adhesion of self-curing and heat-curing resilient liners to denture base material.

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