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Original Article

Effect of mixing techniques on bacterial attachment and disinfection time of polyether impression material

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ABSTRACT

Objective: The aim of this study was 2-fold. The first aim was to evaluate the effects of mixing technique (hand-mixing or auto-mixing) on bacterial attachment to polyether impression materials. The second aim was to determine whether bacterial attachment to these materials was affected by length of exposure to disinfection solutions. **Materials and Methods:** Polyether impression material samples ($n = 144$) were prepared by hand-mixing or auto-mixing. *Escherichia coli*, *Staphylococcus aureus* and Pseudomonas aeruginosa were used in testing. After incubation, the bacterial colonies were counted and then disinfectant solution was applied. The effect of disinfection solution was evaluated just after the polymerization of impression material and 30 min after polymerization. Differences in adherence of bacteria to the samples prepared by hand-mixing and to those prepared by auto-mixing were assessed by Kruskal-Wallis and Mann-Whitney *U*-tests. For evaluating the efficiency of the disinfectant, Kruskal-Wallis multiple comparisons test was used. **Results:** *E. coli* counts were higher in hand-mixed materials ($P < 0.05$); no other statistically significant differences were found between hand- and auto-mixed materials. According to the Kruskal-Wallis test, significant differences were found between the disinfection procedures ($Z > 2.394$). **Conclusion:** The methods used for mixing polyether impression material did not affect bacterial attachment to impression surfaces. In contrast, the disinfection procedure greatly affects decontamination of the impression surface.

Key words: Bacterial contamination, hand-mixing, polyether auto-mixing

INTRODUCTION

The risk of infection transmitted by saliva and blood has led to an increased concern for and attention to infection control in dental practice.[1,2] Virus and bacteria in saliva and blood can contaminate impressions and increase the risk of infection.[3-8] Infectious materials can be spread to prostheses and appliances of other patients if contaminated items enter the dental laboratory environment.[9] A common medium of transmission of infectious diseases among dental practitioners, patients and dental technicians is impression material contaminated with patients' saliva or blood.[10,11]

In dental practice, various impression materials are used for different purposes in dentate and edentate patients. These materials include irreversible and reversible hydrocolloid, polysulfide, condensation silicone, polyvinyl siloxane and polyether materials.

Polyether is an elastomeric impression material that is a copolymer of 1,2-epoxy ethane and tetrahydrofuran that is reacted with an α,β-unsaturated acid, such as crotonic acid, to produce the final polymer and an aromatic sulfonate through cationic polymerization.[12] Polyether is useful for impression of inlay, onlay, single crown and multi-unit fixed partial dentures; laminate veneer; functional impressions and implant impressions.

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Polyether is packaged in two tubes using a much larger volume of base than the accelerator and can be prepared using 1 of 2 methods: hand-mixing or auto-mixing. If the material is prepared using hand-mixing, it is often difficult to thoroughly blend and incorporate the catalyst with the base because the materials are highly viscous. It is virtually impossible to achieve a homogeneous, void-free mix by hand. Auto-mixing involves mixing of the impression materials using an automatic mixing machine (Pentamix, 3M ESPE, Minneapolis, MN, USA). Auto-mixing produces uniform, homogenous mixes of material with fewer bubbles in the impression material.[13] Mixing techniques result in materials that are more or less porous, producing surfaces with different levels of porousness. Surface properties determine how well microorganisms attach to impression materials and subsequently affect disinfection of these materials. Thus, surface properties, as determined by mixing techniques, are important in the transmission of microorganisms.

Disinfection is a critical component of dental practice. Current disinfection protocols vary markedly in type, time and concentration of disinfectants. Five types of chemical disinfectants are in practical use. These are chlorine compounds, combination synthetic phenolic compounds, glutaraldehydes, iodophors and phenolic/alcohol combinations.[12] Physical methods of disinfection, including microwave irradiation and ultraviolet light, are also popular.[3] An alternative procedure is the addition of a disinfectant directly into an impression material. Agents used for this procedure include iodophors, chlorhexidine, phenolics and inorganic ions (such as copper or fluoride).[9]

Spray and immersion are the two most widely used techniques in clinical practice, although they have some disadvantages. These techniques may cause loss of surface detail and affect the dimensional accuracy of impressions. Sprayed materials are irritants, so inhalation of disinfectant vapors may cause health problems.[14] Early studies concluded that alginate and polyether surfaces should be disinfected by spray alone. However, later studies recommended immersion of alginate, polyether and (newer) hydrophilic polyvinyl siloxane impression materials in disinfectant.[13] Effective infection control procedures should be established to break the chain of transmission of infection and reduce cross-contamination. Such procedures should include disinfection of all impressions before delivery to dental laboratories.[1]

To eliminate bacteria from impression surfaces, disinfection time is an important factor. Disinfection of the impression immediately after removal from the mouth is the most effective way to avoid transmission of infectious agents.[11,13,15] The American Dental Association (ADA) recommends that the disinfection time for impression materials such as polysulfide, condensation silicone, polyvinyl siloxane, polyether and agar hydrocolloid should not exceed 30 min.[12]

The aim of this study was to evaluate the effect of mixing techniques on bacterial attachment and the efficiency of exposure time of disinfection solution on polyether impression materials prepared by hand-mixing and auto-mixing. We sought to determine whether hand-mixing causes surface porosities and encourage attachment of bacteria to impression material surfaces.

MATERIALS AND METHODS

Bacterial isolates and disinfectant

In this study, *Escherichia coli* (*E. coli*; ATCC strain number 25922), *Staphylococcus aureus* (S. aureus; ATCC strain number 29213) and *Pseudomonas aeruginosa* (*P. aeruginosa*; ATCC strain number 27853) were used. These strains are susceptible to antibiotics and disinfectants. The efficiency of the disinfectant Descosept AF in spray form (Dr. Schumacher GmbH, Melsungen, Germany) was evaluated. The composition of Descosept AF per 100 g is as follows: 20 g ethanol, 28 g 1-propanol and 0.056 g quaternary ammonium compounds.

Sample preparation

Polyether impression material samples were prepared by two different methods, namely, hand-mixing and auto-mixing, according to the manufacturer's instructions. For auto-mixing, ImpregamPenta (3M ESPE, Seefeld/Oberbay, Germany), a medium-bodied consistency polyether impression material manufactured for the Pentamix mixing device (3M ESPE), was used. Hand-mixing and auto-mixing procedures were performed according to the manufacturer's recommendation.

A total of 144 samples, 72 hand-mixed and 72 auto-mixed, were prepared by the same operator using a standardized and highly polished stainless steel master model containing 49 holes, each 5 mm in diameter and 5 mm deep. The impression materials were allowed to set at room temperature for 3 min. Template molds, petri dishes, glass rods, mixing pads and other necessary items were sterilized before use

and between uses. All mixing procedures were carried out in a laminar flow biosafety cabinet. Surface photos were obtained under light microscopy in order to observe surface characteristics of the hand-mixed and auto-mixed samples [Figure 1].

Microbiological procedures

Comparison of bacterial adherence to polyether samples

To compare bacterial adherence, two groups of samples were used. Polyether samples prepared by hand-mixing constituted the first group while the second group comprised polyether samples prepared by auto-mixing. Bacterial suspensions were adjusted to one by McFarland turbidity standards in nutrient broths. Polyether samples were placed into these bacterial suspensions using a sterile needle wires and incubated overnight at 37°C. Following overnight incubation, adherent bacteria were transferred into new nutrient broths with a sterile needle wires. Nutrient broths were vortexed for 30 s and then 10 µl from each suspension was inoculated onto blood agar media (LAB M, Ltd., Lancashire, UK) and spread with a sterile Drigalski spatula. After overnight incubation at 37°C, colonies were counted.

Disinfectant efficiency test

In order to test the efficiency of the disinfectant, 3 subgroups (Groups 1, 2 and 3) were designed for each of the hand-mixing and the auto-mixing groups.

Six samples were used for each bacterial species, resulting in a total of 18 samples for each type of bacteria. A total of 54 samples (18 samples for each type of bacteria) were used for hand-mixing and 54 samples were used for auto-mixing. The remaining 18 samples for each mixing group were previously evaluated for bacterial attachment in order to ensure that the materials had been prepared under sterile conditions. Group 1 of these samples was the control group, in which no disinfectant was used. For Groups 2 and 3, the effect of the disinfectant, applied in spray form, was evaluated immediately after polymerization of impression material and 30 min after polymerization, respectively. For each Group, 6 samples were used for each type of bacterium.

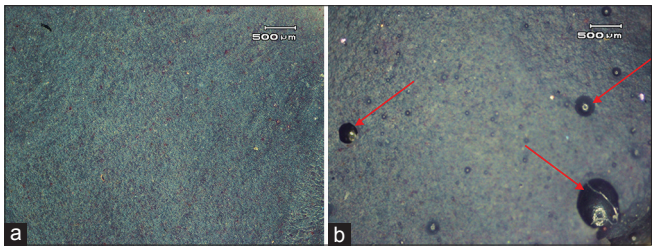

Figure 1: Microscopic images of the sample surfaces: (a) Auto-mixed and (b) hand-mixed (original magnification, ×40)

The bacterial attachment procedure was carried out as mentioned above. After overnight incubation at 37°C, colonies were counted. Subsurface bubbles in the impression material were counted using a light microscope (Olympus SZ 61, Olympus Corporation Tokyo/Japan).

Statistical analyses

Differences in adherence of bacteria to samples prepared by hand-mixing and by auto-mixing were assessed using the Kruskal-Wallis and Mann-Whitney *U*-tests. A $P \leq 0.05$ was considered as statistically significant. For evaluating the efficiency of the disinfectant, the Kruskal-Wallis multiple comparisons test was used. A $Z > 2.394$ was considered statistically significant.

RESULTS

Adherent bacteria counts for each mixing type and disinfection procedure are presented in Tables 1-3.

E. coli counts were higher in hand-mixed materials in Group 1 ($P < 0.05$) [Table 1]. No other statistically significant differences resulting from mixing methods were observed.

According to the Kruskal-Wallis test, there was a significant difference among the disinfection procedures (no disinfection used, disinfection assessed just after polymerization and disinfection assessed 30 min after polymerization). To evaluate the difference among the groups, *post-hoc* Kruskal-Wallis multiple comparisons (Dunn's *Z*-test) were applied

Table 1: Effect of disinfection time on hand-mixed and auto-mixed samples for *E. coli*

Mixing Methods	Median (min-max)			Z^b
	Group 1	Group 2	Group 3	
Auto-mixing	40×10^5 (25×10^5-50×10^5)	12.5×10^3 (10×10^3-25×10^3)	100 (0-3000)	>2.394
Hand-mixing	60×10^5 (40×10^5-80×10^5)	53×10^3 (0-90×10^3)	0.00 (0-200)	>2.394
P^a	<0.05	>0.05	>0.05	

[a]Mann-Whitney *U*-test applied and *P values* calculated, [b]Kruskal-Wallis multiple comparisons test applied and *Z* values calculated, *E. coli*: *Escherichia coli*

Table 2: Effect of disinfection time on hand-mixing and auto-mixing samples for *S. aureus*

Mixing Methods	Median (min-max)			Z^b
	Group 1	Group 2	Group 3	
Auto-mixing	22.5×10^5 (11×10^5-27×10^5)	150 (0-8500)	0.00 (0-700)	>2.394
Hand-mixing	23.5×10^5 (95×10^4-30×10^5)	650 (0-4400)	0.00 (0-300)	>2.394
P^a	>0.05	>0.05	>0.05	

[a]Mann-Whitney *U*-test applied and *P* values calculated, [b]Kruskal-Wallis multiple comparisons test applied and *Z* values calculated; *S. aureus*: *Staphylococcus aureus*

Table 3: Effect of disinfection time on hand-mixed and auto-mixed samples for *P. aeruginosa*

Mixing Methods	Median (min-max)			Z^b
	Group 1	Group 2	Group 3	
Auto-mixing	55×10^5 (40×10^5-60×10^5)	8750 (900-20×10^3)	50 (0-3600)	>2.394
Hand-mixing	55×10^5 (50×10^5-60×10^5)	6000 (200-20×10^3)	150 (0-600)	>2.394
P^a	>0.05	>0.05	>0.05	

[a]Mann-Whitney *U*-test applied and *P* values calculated, [b]Kruskal-Wallis multiple comparisons test applied and *Z* values calculated, *P. aeruginosa*: *Pseudomonas aeruginosa*

and Z values were calculated. Statistically significant differences were found between Groups 1 and 2, Groups 1 and 3 and Groups 2 and 3 ($Z > 2.394$).

For hand-mixed and auto-mixed samples, each bacterium was compared in terms of attachment capability and no statistically significant difference was found ($P > 0.05$) among bacteria.

DISCUSSION

Transmission of pathogenic microorganisms is an important issue for dental health-care workers. Dental practitioners, patients and laboratory technicians face notable risks with respect to infectious diseases. These diseases can be spread by saliva or blood present in contaminated impression material.[14] Dental impressions play an especially important role in microorganism transmission, because they are easily contaminated with blood and saliva. Blood and saliva often carry viruses, bacteria and fungi.[9]

Polyether is an impression material prepared by hand-mixing or auto-mixing. Methods of preparation of impression materials affect the surface properties of these materials. Previous studies showed that hand-mixing of polyether impression material caused air entrapment during spatulation.[13,16] This leads the formation of both surface and subsurface bubbles, which may result in inaccurate dental impressions and/or jeopardize their physical properties.[17] The amount of bacterial attachment and the effects of disinfectant solutions on polyether impressions may depend on differences in surface properties resulting from the technique used to mix the impression material. In the present study, bacterial attachment to hand-mixed polyether samples was less than that to auto-mixed samples. We speculate that the polished surfaced stainless steel mold used to prepare the samples contributed to this result. This mold produced smooth-surfaced samples, in which bubbles were probably beneath the surface. We observed that bacterial attachment to these smooth-surfaced samples, prepared by hand-mixing, was not higher than bacterial attachment to auto-mixed samples.

Previous studies on the antimicrobial effect of disinfectant solutions concluded that disinfectant solutions eliminated microorganisms from the surface of the impression.[7,10,18] The aim of the present study was to evaluate the effect of exposure time of the solution, not type of the disinfectant, on elimination of bacteria.

The presence of blood, saliva and mucosal debris on the surfaces of impression material influences the effect of disinfectants. Rinsing impressions under running water for 10-15 s, followed by application of a disinfectant, was recommended to remove potentially infectious substances.[19-21] This study was performed under laboratory conditions, so the impression surfaces were not contaminated by blood, saliva or tissue. Therefore, it was able to observe the direct effects of disinfectant on impression surfaces under controlled conditions.

The effects of disinfectant solutions on dimensional stability and surface qualities has been shown in some studies.[16,17,22,23] Disinfectant materials, exposure times and types of impression materials all play important roles in the dimensional stability and surface structure of impressions. The hydrophilic

properties of polyether impression materials require that the disinfection process should not adversely affect dimensional accuracy or surface detail of each impression.[24]

Duration of exposure to disinfectant affects the removal of organisms from surfaces of the impressions. For elastomeric impression materials, it is recommended that the disinfectant be applied for at least 30 min. In this study, impression materials were incubated overnight. Bacteria achieve optimum adherence capacities within 18-24 h of their incubation period.

In this study, three types of disinfection procedures were performed. In the first (control) group, disinfectant was not applied. In the second group, disinfectant was applied and then impressions were immediately rinsed with water. In the last group, disinfectant solution was applied and impressions were rinsed with water after 30 min. Statistically significant differences were found among the three groups. In Group 3, surface bacteria were eliminated completely from nearly all samples. We conclude that a 30-min interval between application of the disinfection solution and water rinse is appropriate, as recommended by the ADA.

One of the limitations of this study is that all samples were prepared in a laboratory, under controlled conditions. In the oral cavity, bacterial attachment on teeth and soft-tissues is affected by the saliva, oral temperature, pH, dental plaque, diet and oral hygiene. All of these conditions may affect the transfer of microorganisms from the oral cavity to impression materials.

In this study, causative agents of nosocomial infections were preferentially included. In routine procedures, disinfectants are applied to polyether impression materials in order to prevent transmission from contaminated materials to health-care workers. Given that health-care workers could transmit bacteria to patients, impression materials are possible sources of nosocomial infections.[25-27]

In order to test, the efficacy of disinfection time and mixing methods, further studies with normal oral cavity flora should be performed.

CONCLUSION

It was concluded that bacterial attachment to polyether impression surfaces is not affected by hand-mixing or auto-mixing and that the disinfection procedure is important for decontamination of impression surfaces. The findings of this study suggest that after the impression is taken, disinfectant should be applied and allowed to stay on the impression for 30 min before washing with water.

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