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Antibacterial and antifungal properties of polyether impression materials

Bilge T. Bal[1)], Handan Yılmaz[1)], Cemal Aydın[1)], Caner Yılmaz[1)] and Funda D. Al[2)]

[1)]Department of Prosthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey
[2)]Department of Medical Microbiology, Faculty of Medicine, Gazi University, Ankara, Turkey

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Abstract: The aim of this study was to evaluate the antibacterial and antifungal properties of polyether impression materials using the agar diffusion test. Three different types of polyether impression materials (P2, Penta Soft and Penta) were tested to determine their ability to inhibit the growth of *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The areas of inhibition zones were measured visually with a 0.1-mm incremental Boley gauge. In all groups, none of the samples of the P2 polyether impression material exhibited antibacterial or antifungal activity against any of the microorganisms. All Penta Soft and Penta samples exhibited antibacterial activity against *E. faecalis* and *S. aureus*, and only Penta samples exhibited antifungal effect against *C. albicans*, which decreased progressively as the setting time of the material increased. (J. Oral Sci. 49, 265-270, 2007)

Keywords: polyether impression material; microbiology; antibacterial activity.

Introduction

Dental impressions and prostheses that have been inserted into the mouth are contaminated with potentially infectious microorganisms (1). Some pathogenic contaminants include bacteria such as *E. coli*, *S. aureus*, *P. aeruginosa*, *S. mutans* and the yeast *C. albicans*. *S. aureus* is related to tonsillitis and respiratory infection and has been isolated from the mouth (2). *E. faecalis*, a facultative anaerobic Gram-positive coccus, is a normal commensal adapted to ecologically complex environments in the oral cavity and gastrointestinal tract (3). Fungi such as *C. albicans* and coliforms including *P. aeruginosa* are robust organisms, considerably more difficult to disinfect than viruses (4). Impression materials in contact with oral tissues, saliva, and possibly blood may act as media for potential transfer of organisms from patients to dental personnel (5). Disinfection of an impression immediately after removal from the mouth, as recommended by the American Dental Association and Centers for Disease Control and Prevention to avoid possible transmission, is now considered a routine procedure in dental offices and laboratories (6,7).

The most commonly used non-aqueous elastomeric impression materials in fixed prosthodontics are classified as polysulfide, addition-reaction silicone and polyether (8,9). Polyether impression materials are widely used in dentistry due to their high rate of accuracy and high dimensional stability. These materials have been shown to be unstable under conditions of high humidity in aqueous solutions (10). They are more hydrophilic in nature (11), and disinfection procedures may affect their physical properties. Therefore it would be advantageous if such materials possessed effective antibacterial properties.

Microbiologic testing of a material is an essential step towards its acceptance for clinical use, besides testing of its physical and biological properties. Some studies have examined the effects of disinfectants on the mechanical and physical properties of impression materials (12-15), but the dental literature has little information on the antibacterial and antifungal properties of polyether impression materials. This study assessed the antibacterial and antifungal properties of three different types of

Correspondence to Dr. Bilge Turhan Bal, Department of Prosthodontics, Faculty of Dentistry, Gazi University, 06510, Emek, Ankara, Turkey
Tel: +90-312-2034192
Fax: +90-312-2239226
E-mail: bilgeturhan@gmail.com

polyether impression materials.

Materials and Methods

Sample preparations

Three different types of polyether impression materials were tested [P2 (polyether, Heraeus Kulzer, Hanau, Germany), Impregum Penta Soft (polyether, 3M Espe, Seefeld, Germany), Impregum Penta (polyether, 3M Espe, Seefeld, Germany)]. Each impression material was mixed by an automatic mixer (3M Espe Pentamix, Seefeld, Germany) according to the manufacturer's instructions. Samples were prepared using aluminum plate molds containing 8 holes, each 10 mm in diameter and 1 mm deep under sterile conditions by one operator. Solid aluminum plates were placed under the molds and mixed impression material was placed into the holes. Solid aluminum plates were then positioned over the molds. The impression materials were allowed to set at room temperature for 3 min. The set disks were pushed into glass petri dishes using glass rods. Template molds, petri dishes, glass rods and other necessary items were cleaned and then sterilized by steam autoclaving between uses.

Microbiological procedures

Agar diffusion tests were used to assess the antibacterial and antifungal activities of the samples. The microorganisms selected for inoculation of the impression materials were *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212), which were grown aerobically on blood agar at 37°C for 24 h, and *Candida albicans* (ATCC 10231) which was grown on Sabouroud's dextrose agar at 37°C for 48 h. To prepare a standard concentration of microorganisms, two loops of microorganisms, harvested from either blood or Sabouroud agar, were inoculated into 20 ml of phosphate-buffered saline (0.1 mol/l, pH 7.2), vortex-mixed, and then diluted by a factor of 1 in 10. The resulting suspensions yielded 1-5 $\times 10^6$ CFU/ml bacterial microorganisms and 1-3 $\times 10^5$ CFU/ml *C. albicans*. Under sterile conditions seven wells of the same size as the samples were cut into nutrient agar for *S. aureus* and *P. aeruginosa*, blood agar for *E. faecalis*, and YEPD (yeast extract peptone dextrose agar) for *C. albicans* on plates previously innoculated with the appropriate microorganisms, and samples of the impression materials were placed in wells that were punched in the agar. Some of the samples were processed immediately after being released from the molds while others were processed 30 or 60 min after release. After incubation at 37°C for 24 and 48 h, the agar plates were examined and the inhibition zones of the microorganisms around the samples were measured visually with a 0.1-mm incremental Boley gauge (Salvin Dental Specialties, North Carolina, USA), all measurements being recorded in millimeters rounded to one-tenth. Control

Table 1 Samples processed immediately (0 min), 30 min and 60 min after setting at 24 h incubation time

Polyethers	Microorganisms			
	E. faecalis $\bar{x} \pm s_{\bar{x}}$	*S. aureus* $\bar{x} \pm s_{\bar{x}}$	*C. albicans* $\bar{x} \pm s_{\bar{x}}$	*P. aeruginosa* $\bar{x} \pm s_{\bar{x}}$
Penta Soft (0)	1.85 ± 0.18	2.94 ± 0.15	0	0
P2 (0)	0	0	0	0
Penta (0)	2.42 ± 0.38	3.51 ± 0.37	1.70 ± 0.26	0
Penta Soft (30)	1.74 ± 0.25	2.80 ± 0	0	0
P2 (30)	0	0	0	0
Penta (30)	2.15 ± 0.24	3.44 ± 0.31	1.60 ± 0.29	0
Penta Soft (60)	1.37 ± 0.23	2.75 ± 0.11	0	0
P2 (60)	0	0	0	0
Penta (60)	1.67 ± 0.16	3.34 ± 0.27	1.25 ± 0.18	0

Mean zone (mm) ± standard deviation of the mean (n = 7)
Continuous vertical lines between two or three materials indicate that there was no significant difference ($P > 0.05$)
Discrete vertical lines indicate that there was significant difference between the materials ($P < 0.05$)

plates containing the following were also inoculated: (a) bacterial cultures in agar without a test sample, (b) test material in agar without added bacteria, and (c) empty agar.

The data were subjected to four-way analysis with repeated measures on one factor (repeated measurement ANOVA). The interactions between incubation time, microorganisms and impression materials, and between microorganisms, impression materials and setting times, were found to differ significantly ($P < 0.01$). Therefore, the significance of differences among all the groups was examined by the Duncan test ($P < 0.05$).

Results

The mean zones of inhibition together with the standard deviations for each microorganism and material after incubation for 24 or 48 h are presented in Tables 1, 2, and 3, and statistical comparisons between the setting times (0, 30, 60) of the impression materials are shown in Table 3.

In all groups, none of the P2 samples showed antibacterial or antifungal activity against any of the microorganisms. However, Penta and Penta Soft samples exhibited measurable antibacterial activity against all of the

Table 2 Samples processed immediately (0 min), 30 min and 60 min after setting at 48 h incubation time

Polyethers	Microorganisms			
	E. faecalis $\bar{x} \pm s_{\bar{x}}$	*S. aureus* $\bar{x} \pm s_{\bar{x}}$	*C. albicans* $\bar{x} \pm s_{\bar{x}}$	*P. aeruginosa* $\bar{x} \pm s_{\bar{x}}$
Penta Soft (0)	1.88 ± 0.10	3.10 ± 0.19	0	0
P2 (0)	0	0	0	0
Penta (0)	2.54 ± 0.11	3.45 ± 0.27	1.75 ± 0.30	0
Penta Soft (30)	1.58 ± 0.36	2.81 ± 0.36	0	0
P2 (30)	0	0	0	0
Penta (30)	2.54 ± 0.52	3.45 ± 0.42	1.65 ± 0.27	0
Penta Soft (60)	1.21 ± 0.14	2.80 ± 0.22	0	0
P2 (60)	0	0	0	0
Penta (60)	1.88 ± 0.10	3.30 ± 0.20	1.11 ± 0.10	0

Mean zone (mm) ± standard deviation of the mean (n = 7)
Continuous vertical lines between two or three materials indicate that there was no significant difference ($P > 0.05$).
Discrete vertical lines indicate that there was significant difference between the materials ($P < 0.05$).

Table 3 Mean differences recovered at three times (in min) post setting

Polyethers	Microorganisms											
	E. faecalis			*S. aureus*			*C. albicans*			*P. aeruginosa*		
	0	30	60	0	30	60	0	30	60	0	30	60
Penta Soft	1.87	1.66	1.29	3.02	2.80	2.77	0	0	0	0	0	0
P2	0	0	0	0	0	0	0	0	0	0	0	0
Penta	2.48	2.35	1.77	3.48	3.45	3.32	1.72	1.62	1.18	0	0	0

Mean zone (mm)
Continuous horizontal lines between two or three materials indicate that there was no significant difference ($P > 0.05$).
Discrete horizontal lines indicate that there was significant difference between the materials ($P < 0.05$).

microorganisms except *P. aeruginosa*. All Penta Soft samples exhibited no antifungal activity against *C. albicans*, whereas all Penta samples demonstrated some antifungal effect. After 24 and 48 h of incubation, larger zones of microbial inhibition were observed around Penta samples than around Penta Soft samples, and the Duncan test revealed significant differences between the three impression materials at all three post-setting times (0, 30 and 60 min) ($P < 0.05$). Furthermore, the inhibition zones of *S. aureus* were larger than those of *E. faecalis* for all Penta Soft and Penta materials in all groups.

Table 3 demonstrates the significance of differences between the three post-setting times for the polyether impression materials for each of the microorganisms. Inhibition zones of all the Penta Soft and the Penta samples processed immediately after setting were larger than those of the samples processed 30 or 60 min after setting. For *E. faecalis*, significant differences were found between the three setting times (0, 30, 60) for both Penta and Penta Soft. For *S. aureus*, there were significant differences between Penta Soft samples processed immediately and 60 min after setting, and there was no significant difference between the samples processed 30 and 60 min after setting. The differences between Penta samples processed immediately and 60 min after setting were found to be statistically significant, whereas the differences between 0 and 30 min and also those between 30 and 60 min were not significant. For *C. albicans*, significant differences were found between Penta samples processed immediately and 60 min after setting, but no significant difference was found between post-setting times of 0 and 30 min.

Discussion

Decontamination of impression materials is an essential stage in the control of cross-infection. Oral microorganisms can easily become incorporated into setting impression materials. The main concern is therefore to minimize the number of organisms present and thus reduce the chances of microbial transfer to staff members and the resulting stone casts (16).

To achieve protection and prevent physical changes to the material, the more water-impervious materials such as addition silicone or many polyether impressions need only to be rinsed and disinfected by immersion or spraying (16). Because of the hydrophilic properties of polyether impression materials, the disinfection process should be adequate but should not adversely affect the dimensional accuracy or surface detail of the impression (10,17). Merchant (18) has warned that polyether should be disinfected for short periods using disinfectants accepted by the ADA. Therefore it is important for impression materials to possess effective antibacterial properties.

Although antibacterial effects of irreversible impression materials have been investigated recently (5,16,19-22), no information is available in the literature related to antibacterial tests on polyether impression materials. In the present study, three different types of polyether impression materials were tested to determine their ability to inhibit the growth of *E. faecalis*, *S. aureus*, *P. aeruginosa* and *C. albicans*. The results revealed statistically significant differences in antibacterial effects among the three polyether impression materials after 24 and 48 h of incubation. The samples of Penta Soft exhibited no antifungal activity against *C. albicans*, whereas all Penta samples demostrated some antifungal effect after all the incubation periods. This result was of considerable interest. The difference in antibacterial activity among the polyether impression materials could be related to the differences in their chemical composition. Solubility and diffusibility in agar may also play important roles. No measurable zones of inhibition of *P. aeruginosa* were observed for any of the polyether impression materials after 24 and 48 h of incubation. The absence of antibacterial activity against *P. aeruginosa* is in agreement with data obtained by Tobias et al. (19), who investigated the antibacterial and antifungal properties of an irreversible hydrocolloid impression material impregnated with disinfectant and found an absence of activity against *P. aeruginosa*. *P. aeruginosa* can survive harsh environmental conditions and displays intrinsic resistance to a wide variety of antimicrobial agents that facilitates the organism's ability to survive in a hospital setting (23). Because of absence of information about the antibacterial properties of polyether impression materials, it is not possible to relate this investigation to similar relevant studies.

The agar diffusion method used in the present study is one of the most commonly employed techniques for evaluation of antibacterial activity (24,25). However the limitations of the agar well technique have been referred to in previous investigations (26,27). The great disadvantage of the agar diffusion test is that it does not distinguish between bacteriostatic and bacteriocidal properties of dental materials. Also, it does not provide any information about the viability of test microorganisms. However, if all of the variables for the agar diffusion test are carefully controlled, consistent and reproducible results may be obtained (19).

This preliminary study has some limitations. All testing was done in a laboratory, under controlled conditions, and the specimens were small in comparison with regular-sized impressions. Also, the study investigated only four microorganisms common to the oral environment. Further

studies are needed to develop a more realistic clinical technique for demonstrating the true efficacy of such materials.

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