A statistical comparison of denture sanitation using a commercially available denture cleaner with and without microwaving

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Poly(methyl-methacrylate) dentures, worn by patients for periods ranging from 12 days to 48 years, were cultured and found to be heavily contaminated with a variety of microorganisms both externally and internally. A commercially available denture sanitizer, used as prescribed by the manufacturer, was ineffective at decontaminating the dentures. This study examined the effectiveness of this denture sanitizer when used in combination with a microwaving procedure. Statistical methods were used to compare the decontamination results of the denture sanitizer applied with and without microwaving. The statistical results indicated that the dentures were decontaminated most effectively when the denture sanitizer was used in conjunction with a two-minute microwave procedure.

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A comprehensive review by Iacopo and Wathen pointed out that denture-related stomatitis often is associated with a combination of Candida albicans and altered cell-mediated immunity (CMI). More recent studies have reinforced the findings that Candida species play an important role in denture-related stomatitis and have been associated with increased levels of cytokines. Additional studies have also implicated a variety of other microorganisms including gram-negative bacilli, respiratory pathogens, and periodontopathogens.

An in vitro study by Glass et al demonstrated that poly(methyl-methacrylate) denture materials could be contaminated not only on the surfaces but also within the porosities of the materials. Additional studies revealed that as little as eight hours of contact between denture materials and media containing known quantities of microorganisms contaminated the materials beyond the point where they could be sanitized by commercial denture cleaners. Dixson et al have reported that microwaving denture materials in vitro for five minutes killed C. albicans and that while repeated five-minute irradiation did not alter the heat-polymerized acrylic resin denture base material, it did harden PermAsoft denture soft-lining material significantly.

A recent in vivo study by Glass et al compared the effectiveness of two denture-sanitizing systems and tap water on complete dentures that had been worn for periods of time ranging from 12 days to 48 years. An experimental product that was dissolved in water and subsequently microwaved for two minutes (System A) was overwhelmingly more effective than either a commercially available denture cleaner (System B) or soaking the dentures for five minutes in tap water; it eliminated nearly all of the microorganisms found both on the denture surfaces and within the porosities (depths). This method of decontamination left the dentures virtually germ-free, placing the denture wearer at a considerably lower risk of infectious diseases.

The superior efficacy of System A raised the question of whether the effectiveness of a commercially available denture cleaner could be enhanced by using the same microwaving protocol for the experimental product.

Materials and methods

Denture evaluation

Figure 1 shows a typical complete denture, used in both the original study by Glass et al and this one. The original denture study used the entire mandibular denture and the right half of the maxillary denture from 20 complete dentures. The present study used the remaining (left) half of the upper denture arch, which had been stored at -80°C for approximately 18 months. The frozen fragments were allowed to thaw and equilibrate to ambient temperature before use. Matching the original study protocol, the first 10 denture segments were sectioned from posterior to anterior; the other 10 were sectioned from anterior to posterior.

Standard aseptic techniques, such as flame-sterilization of all cutting and handling instruments, were used throughout these experiments.

The baseline level of contamination in these untreated denture fragments (labeled System B) was established by rolling the external surfaces and touching the freshly cut edges of the fragments to three different culture media three times in succession. The media used and their respective microbial target populations were sabouraud dextrose agar (yeasts), chocolate agar (fastidious microorganisms), and brain heart infusion (BHI) reduced blood agar (anaerobes and facultative anaerobes). The inoculated media were incubated at 37°C either in candle jars (sabouraud dextrose and chocolate agars) or anaerobically in BBL GasPak jars (BD, Lyman, MA; 800/592-6544) (BHI reduced blood agar). The same protocol was used to culture the surfaces and freshly cut depths of treated denture fragments, which were sectioned between each of three subsequent treatments described below.

After the establishment of the baseline level, the remaining denture fragments were immersed in a solution of System B denture cleaner (Polident with Polshield, GlaxoSmithKline, Philadelphia, PA; 888/825-5249) which had been prepared in accordance with the manufacturer's guidelines. The immersed denture fragments were subjected to two minutes of microwave treatment in the same manner as System A. The fragments were removed from the receptacle and washed...
with sterile water. The resultant denture sections were cultured as described above. The remaining fragments were returned to fresh denture cleaner each time prior to treatment. This protocol was repeated to yield a total of four sampling: no treatment, treatment 1, treatment 2, and treatment 3.

Microbial growth after 16–18 hours of incubation was evaluated independently by three researchers counting and scoring colony-forming units (CFU)/touch, using the scoring system shown in the table. The scores of individual researchers were combined and averaged to give a final result. The culture plates were photographed immediately after scoring.

Statistical analyses
As with the original study, the results of the present study were statistically analyzed using a one-way within-subjects ANOVA. The dependent variable was the extent of microbial growth as observed by the three researchers. Three blocking variables were included in the total count of four independent variables: system, media, location, and treatment.

System was the focus of the study and was the only independent variable not included for blocking purposes. System was administered at four levels: Systems A, B, and C from the original study and System B' from this study. Media, treatment, and location were the three independent variables included in these analyses. These variables have well-documented abilities to affect the various types and numbers of microorganisms that can be detected in static and in dentures. Media were administered at three levels: sabouraud dextrose agar, chocolate agar, and BHl reduced blood agar. Treatment consisted of four levels: no treatment (baseline), treatment 1, treatment 2, and treatment 3. Two different locations: external surfaces and internal surfaces (depths or porosities), were evaluated for microbial growth. As with any within-subjects design, each subject was observed for the dependent variable (microbial growth expressed as CFU/touch) at every possible combination of all four independent variables.

The study compared the initial level of growth (baseline or no treatment) on and in the dentures with the three levels of treatment. The baseline for System B' was established in analogous experiments during this study in order to compare the baselines of all four systems.

Results
Statistical comparison of the relative effectiveness of System B and System B' clearly demonstrated System B's superior ability to decontaminate dentures (see table). The disparity in efficacy between System B and System B' is especially noteworthy since the only difference between these two systems is the inclusion of a microwaving step in System B'.

It should be noted that the average baseline level (no treatment) of contamination for System B' (2.4) was considerably lower than the average baseline level for the other three systems (3.2). While the original three baselines were not significantly different from each other, the comparatively lower baseline level for System B' undoubtedly was the result of 18 months of storage at -80°C with subsequent freezing and thawing cycles. However, even after prolonged storage there still were relatively high numbers of microorganisms found in the baseline level of System B' (>10–25 CFU/touch). These findings indicate that long-term cold storage may reduce the detectable numbers of microorganisms but it does not eradicate them from dentures.

Discussion
This study compared the sanitization ability of a commercially available denture cleaner used according to manufacturer's instructions with that of the same product and same method, plus a step of microwaving the dentures for two minutes. A single treatment of immersing contaminated dentures in a commercially available denture cleaner (System B) did not sanitize them. Furthermore, Mannon et al found that soaking dentures for up to four weeks reduced but did not eliminate microorganisms. These data point out the absolute necessity of combining a sanitizing agent with a microwave treatment to obtain a synergistic effect not achievable by either component alone.
Moreover, one treatment of System B was sufficient for decontamination (Fig. 2). This is in marked contrast to the ineffectiveness of Systems B and C, neither of which were capable of decontaminating dentures, even after three treatments. The successful decontamination of dentures by a single treatment of System B is of clinical significance because it approximates the level of compliance of most denture patients.

A number of studies have noted the association between poor oral and denture hygiene and the development of Candida-related denture stomatitis; additional studies have demonstrated that this denture-borne infection is associated with neglect. Several authors have noted that proactive denture hygiene can have a substantial effect on preventing denture stomatitis.

A previous in vitro study found that dentures may become heavily contaminated after eight hours of exposure to microorganisms. Therefore, it stands to reason that dentures exposed to a grossly contaminated environment, such as the human oral cavity, could be colonized significantly by potentially harmful microorganisms during the 14–16 hours each day that dentures normally are worn. Using System B on a daily basis would disrupt this potentially hazardous cycle of patients being continually re-infected by their dentures (nosocomial disease transmission).

Unpublished studies have shown that repeated microwave of dentures did not result in significant alteration of their dimensional stability. Therefore, daily applications of System B should not adversely affect the useful life of normal poly-methyl-methacrylate denture materials.

The results of the present and original studies raise the following questions: What are the health risks for patients who are harboring either opportunistic or pathogenic microorganisms in their dentures? Can these same microorganisms become the etiological agents of oral diseases such as denture stomatitis? Can the microorganisms associated with oral diseases lead to increased risk of systemic diseases involving the cardiovascular, respiratory, and gastrointestinal systems?

**Conclusion**

The addition of two minutes of a microwave procedure to the manufacturer's recommended use of a denture sanitizer markedly enhances the decontamination of dentures that have been worn for periods ranging from 12 days to 48 years.

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