In Vitro Antimicrobial Activity of Effervescent Denture Tablets on the Components of Removable Partial Dentures

Glenda Lara Lopes Vasconcelos, DDS, MSc
Patrícia Almeida Curylofo, DDS, MSc
Flâvia Cristina Targa Coimbra, DDS, MSc
Viviane de Cássia Oliveira, BS, MSc
Ana Paula Macedo, BS, MSc, PhD
Helena de Freitas Oliveira Paranhos, DDS, MSc, PhD
Valéria Oliveira Pagnano, DDS, MSc, PhD

Department of Dental Materials and Prosthetics, Ribeirão Preto School of Dentistry, Universidade of São Paulo, Ribeirão Preto, SP, Brazil.

Purpose: To evaluate the antimicrobial activity of effervescent tablets on the surfaces of cobalt-chromium (Co-Cr) and heat-polymerized resin. Materials and Methods: From a metal matrix, 55 circular wax patterns (Ø 12 × 3 mm) were obtained and cast in Co-Cr alloy. Muffles for acrylic resin were prepared from circular wax patterns (Ø 20 × 5 mm). The metal specimens were positioned in the muffle, and the resin was pressed into its surroundings to simulate the composition of a removable partial denture (RPD). The mixed specimens were sterilized and contaminated with Strep tococcus mutans, Staphylococcus aureus, Candida albicans, and Candida glabrata, composing a multispecies biofilm, and subsequently immersed according to the manufacturer’s instructions in four cleansing solutions: Polident 3 Minute denture cleanser (P3M), Polident for Partials (PP), Corega Tabs (CT), and NitrAdine (Ni); as well as distilled water (positive control) and no contamination (negative control). After cleansing, viable microorganisms were quantified by counting the number of colony-forming units (CFU/mL). From the CFU values, log10(CFU + 1) values were calculated for statistical analysis. Kruskal-Wallis test with Dunn post hoc test were performed (α = .05). Results: There was a significant reduction (P = .001) of S. mutans after immersion in Ni (median [95% CI] 3.27 [2.92; 3.45]) compared to the CT (3.86 [3.75; 4.01]) and control (4.08 [3.73; 4.22]) groups, while the PP (3.63 [3.28; 4.11]) and P3M (3.83 [3.61; 4.04]) groups presented an intermediate action. The effervescent tablets did not present antimicrobial action against S. aureus (P = .537), C. albicans (P = .795), or C. glabrata (P = .519). Conclusion: Ni exhibited moderate antimicrobial action. The effervescent tablets did not promote reduction of multispecies biofilm, and their daily use should be carefully considered. Int J Prosthodont 2020;33:315–320. doi: 10.11607/ijp.6436

The demand for oral rehabilitation treatments has increased in dentistry. A removable partial denture (RPD) is often indicated as a quick treatment of moderate cost when compared to implants or fixed prostheses.1 Effective hygienic measures are necessary to ensure the longevity of the rehabilitative treatment, as the biofilm formation and subsequent application of hygiene methods affect the durability of the acrylic resin and the metal constituents of the RPD, mainly due to the processes of surface roughness change2 and corrosion.3

The process of biofilm formation leads to changes in electrolyte concentration and pH and oxygen levels, which can lead to a loss of the mechanical properties of dental materials.4 In addition, it is important to consider that the retentive regions of RPDs, such as clamps, allow for the accumulation of biofilm and therefore may favor the occurrence of inflammatory processes and lesions in the underlying tissues5 and remaining teeth.6

An investigation of the oral microbial profile in RPD users showed a high incidence of Streptococcus, Staphylococcus, and Candida spp, among others.7 Candida spp is related to the occurrence of prosthetic stomatitis8; Streptococcus spp is
associated with carious lesions on the abutment teeth; and *Staphylococcus* spp is associated with several virulence factors, including the production of toxins capable of dribbling into the immune system to invade and destroy the host tissue.

Considering the interactions, spatial distributions, and cellular responses of microorganisms, it is important to study the effect of denture cleansers against the most prevalent microorganisms in the oral cavity, such as *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida* spp. In addition, there is evidence that microbial interactions are important in the development and stabilization of multispecies biofilms. An example of this is the interaction among the mentioned species that can lead to changes in the pathogenicity of *Candida* spp in the biofilm. Thus, when reproducing oral biofilms, it is important to include mixed species of microorganisms to replicate some of these interactions.

The need to standardize a safe and effective cleansing protocol, as well as one that does not cause damage to the constituents of the RPD, is necessary. Effervescent tablets have been widely disclosed as a complementary method to the mechanical method for hygiene, especially for the elderly or for those with motor or visual limitations. These products are widespread among denture users, mainly for their simplicity of use and pleasant odors. Although manufacturers do not accurately disclose the compositions of these products, the mechanism of action of effervescent tablets appears to be associated with the actions of oxidizing and chelating and of surfactant agents. It has been proposed that the interaction of these compounds removes organic material from the prosthetic surfaces and causes structural damage to the cell membranes of the microorganisms, increasing permeability and leading to cellular lysis.

The literature has reported the effectiveness of these tablets on the surface of acrylic resin. However, there are few quantitative and qualitative studies that report their antimicrobial efficacy on resin and metal simultaneously in order to simulate an RPD. Thus, it is fundamental to study the antimicrobial actions of these effervescent tablets, concomitantly, in relation to the two constituent materials of RPDs in a multispecies biofilm model.

The null hypothesis of this study was that there would be no difference in antimicrobial action among the effervescent cleaning tablets in the RPD constituent materials.

**MATERIALS AND METHODS**

**Confection of Specimens**

A total of 55 disk-shaped wax patterns (12-mm diameter × 3-mm thickness) were obtained from a circular metallic matrix. To obtain the dental casting model, the wax patterns were positioned in flow channels with a large sprue former (6 mm; Cerafix) and included in the phosphate investment (Micro-Fine 1700; Talladium do Brasil). The casting of the cobalt-chromium (Co-Cr) metal alloy (DeguDent, Dentsply Sirona) was performed in an electronic vacuum induction machine with a standardized temperature of 1,380°C and a constant torque centrifuge. After casting, the metal specimens were finished with 220-, 400-, 600-, and 1,200-grit sandpaper (Norton Abrasives, Saint-Gobain) in a polishing machine (AROTEC) under water cooling.

To standardize the initial surface roughness, all specimens were measured (in μm) in three areas using a surface analyzer (Surftest SJ-201P, Mitutoyo) calibrated to a specimen length of 0.8 mm, with a 4.0-mm percussion of measure and a speed of 0.5 mm/second. At the end, the arithmetic mean of these three measurements was calculated (roughness average [Ra]), and the roughness interval was standardized between 0.04 and 0.08 μm.

To simulate the composition of an RPD, the metal disks were placed in conventional muffle molds (no. 7, OGP) and fastened with double-sided tape (3M ESPE). Subsequently, the heat-polymerized acrylic resin was manipulated, inserted into the molds, and pressed (Vh-Grupo Midas Dental Products) in 1.200 kgf for 30 minutes. Polymerization of the acrylic resin was performed with the water bath method described by Arruda et al.

Afterwards, the specimens were disinfected and the excess acrylic resin removed (Minicut) following previously described polishing protocols (Fig 1).

The mixed specimens were immersed in distilled water and kept in an oven (Odontobrás) at 37 ± 1°C for 24 ± 2 hours for the removal of the residual monomer. The specimens (n = 55) were then packed in special envelopes with a plastic face (Thermosealable Surgical Grade Envelope, SisPack) and disinfected with ethylene oxide gas sterilization (Accel). The following groups (n = 9 each) were formed: Polident 3 Minute denture
For the cleaning protocol, the specimens were placed in a stainless steel basket (6 mm long x 3 mm wide x 2 mm high). The set was immersed in 200 mL of warm, sterilized, distilled water containing an effervescent tablet of the cleansers used according to the manufacturer's instructions (Table 1). For the control groups, the specimens were immersed in sterile distilled water for 15 minutes under the same conditions previously mentioned (Fig 2).

After immersion, the specimens were removed from the basket and rinsed three times sequentially in sterile distilled water. Each specimen was individually placed in polypropylene tubes (Techno Plastic Products) with 10 mL of Letheen Broth medium (HiMedia Laboratories), which was sonicated (200 W, 40 KHz) (Altsonic Clean 9CA) for 20 minutes in order to detach the microorganisms. Serial dilutions (100 to 10⁻³) of the suspension were seeded in Petri dishes with culture media selective for each microorganism.

### Table 1 Effervescent Tablet Characteristics

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Composition</th>
<th>Time of immersion (min)</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polident 3 Minute denture cleanser</td>
<td>Sodium bicarbonate, citric acid, sodium carbonate, potassium monopersulfate, sodium perborate, sodium benzoate, polyethylene glycol (PEG) 180, ethylenediaminetetraacetic acid (EDTA), lauryl sulfocacetate, polyvinylpyrrolidone/vinyl acetate (VP/VA) copolymer, aroma</td>
<td>5</td>
<td>GSK</td>
</tr>
<tr>
<td>Polident for Partials</td>
<td>Sodium benzoate, citric acid, sodium carbonate, potassium monopersulfate, sodium perborate, PEG-100, EDTA, lauryl sulfocacetate, VP/VA copolymer, flavoring</td>
<td>5</td>
<td>GSK</td>
</tr>
<tr>
<td>Corega Tabs</td>
<td>Sodium bicarbonate, citric acid, sodium carbonate, potassium monopersulfate, sodium perborate, sodium benzoate, sodium polyphosphate, sodium lauryl sulfocacetate, sodium stearate, PEG</td>
<td>5</td>
<td>GSK</td>
</tr>
<tr>
<td>NitrAdine</td>
<td>Citric acid, sodium lauryl sulfate, lactose monohydrate, sodium bicarbonate, sodium chloride, hydrogen potassium monopersulfate, sodium carbonate, peppermint flavoring, polyvinylpyrrolidone</td>
<td>15</td>
<td>bonyfAG</td>
</tr>
</tbody>
</table>

### Antimicrobial action
- **Candida albicans**
- **Candida glabrata**
- **Streptococcus mutans**
- **Staphylococcus aureus**

![Fig 2 Flowchart of the study protocol. P3M = Polident 3 Minute Cleanser; PP = Polident for Partials; CT = Corega Tabs; Ni = NitrAdine; CFU = colony-forming units.](image-url)
RESULTS

Table 2 presents the results of the statistical analyses of the species in the multispecies biofilm after immersion in the effervescent tablets. After comparisons, a significant difference among the effervescent tablets was observed only for *S. mutans* (*P* = .001). In this group, a significant reduction of CFU counts after immersion in NitrAdine was noticed, while P3M and PP showed an intermediate action (Fig 3).

None of the effervescent tablets tested presented antimicrobial action against *S. aureus* (*P* = .537), *C. albicans* (*P* = .795), or *C. glabrata* (*P* = .519).

All test tubes containing the test specimens were turbid after 24 hours of incubation.

DISCUSSION

This study evaluated the antimicrobial actions of four denture cleaning solutions on RPD constituents contaminated with multispecies biofilms composed of *C. albicans*, *C. glabrata*, *S. aureus*, and *S. mutans*. Based on the results, the null hypothesis was rejected, since there was a difference among the effervescent tablets used.

A multispecies biofilm was chosen to evaluate the effectiveness of the tablets on the microorganisms to aim for a situation closer to that found in the oral cavity. These results showed the ineffectiveness of the denture cleaning against *C. albicans*, *C. glabrata*, and *S. aureus*. This fact can be explained by the protection factor caused by extracellular polymeric substances (EPS). This structure directly supports microbial adhesion and increased tolerance to antimicrobials while forming a polymeric matrix that enhances the

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Table 2: CFU/mL in log10(CFU + 1) for Strains in the Multispecies Biofilm

<table>
<thead>
<tr>
<th>Group</th>
<th><em>S. mutans</em></th>
<th><em>S. aureus</em></th>
<th><em>C. albicans</em></th>
<th><em>C. glabrata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>P3M</td>
<td>3.83 (3.61, 4.04)</td>
<td>2.04 (1.38, 3.23)</td>
<td>4.94 (2.22, 5.80)</td>
<td>5.08 (2.21, 5.64)</td>
</tr>
<tr>
<td>PP</td>
<td>3.63 (3.28, 4.11)</td>
<td>2.60 (1.5, 3.49)</td>
<td>4.23 (0.71, 4.63)</td>
<td>4.08 (2.58, 5.04)</td>
</tr>
<tr>
<td>CT</td>
<td>3.86 (3.75, 4.01)</td>
<td>2.08 (1.33, 2.83)</td>
<td>3.88 (1.65, 4.94)</td>
<td>4.77 (2.90, 5.40)</td>
</tr>
<tr>
<td>Ni</td>
<td>3.27 (2.92, 3.45)</td>
<td>2.45 (1.38, 3.15)</td>
<td>4.23 (2.21, 5.03)</td>
<td>4.08 (2.69, 5.10)</td>
</tr>
<tr>
<td>Control</td>
<td>4.08 (3.73, 4.22)</td>
<td>2.94 (1.84, 3.62)</td>
<td>4.18 (2.56, 5.37)</td>
<td>4.77 (4.23, 5.2)</td>
</tr>
</tbody>
</table>

*All data are reported as median (95% confidence interval). P3M = Polident 3 Minute; PP = Polident for Partial; CT = Corega Tabs; Ni = NitrAdine. *Kruskal-Wallis test for independent samples. Values denoted by the same superscript letter indicate no statistical significance (*P* > .05).
mechanical stability of biofilms.21 This hypothesis is also supported by Drake et al22 and Lucena-Ferreira et al.23 The authors suggest that polysaccharides secreted by S mutans may produce a protective barrier that limits the exposure of other microorganisms to the antimicrobial components of the denture cleanser in a mixed biofilm model. Molecular studies have shown that EPS matrix composition and structure—for example, increasing concentrations of polysaccharides24 and extracellular DNA (eDNA) released25—diverge according to the presence of different microorganisms via activation of distinct molecular pathways.26 According to Rostami et al,27 eDNA—one of the main mechanisms of resistance in biofilms—was detected in the oral biofilms of mixed species.

The results demonstrated that only the NitrAdine cleanser showed antimicrobial action against S mutans. This result is relevant because S mutans is the primary etiologic agent of dental caries; thus, it is possible that NitrAdine can help avoid higher incidence of carious processes, which compromise the oral health of the user and the longevity of the partial dentures, in the abutment teeth of an RPD.9,28 In addition, the presence of sodium lauryl sulfate (SLS) in its composition may have potentiated the antimicrobial action against S mutans, since SLS has the ability to adsorb and interact with components of the cell membrane of microorganisms, resulting in increased permeability and subsequent cell lysis.17 Petersen et al29 showed a decrease in cell viability of S mutans in the presence of SLS.

In vitro studies have evaluated the antimicrobial efficacy of NitrAdine Medical Interporous on acrylic resin specimens and reported efficacy on C albicans, C glabrata, S aureus, S mutans, Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa biofilms, contrary to the findings of the present study.19,30 Also, Glass et al31 reinforced that NitrAdine tablets contain a disinfectant formula that exercises high activity in the removal of isolated biofilms of C albicans, P aeruginosa, S aureus, and viruses. The different results of the present study can be explained by the biofilm model used, where factors in a multispecies biofilm, such as coaggregation and metabolic cooperation, may increase resistance to the antimicrobial response when compared to monospecific biofilms.32

In addition, bacterial adhesion and biofilm phenotype are altered by the chemical properties of the adhesion surface, in addition to the topography, hydrophobicity, and roughness.33 This fact can be explained by the divergence with the results obtained by Coenye et al,34 who verified a significant efficacy of NitrAdine in reducing the multispecies biofilm (C albicans, S mutans, S aureus, and P aeruginosa) formed on the acrylic resin surface after immersion for 15 minutes. It should be noted that in this study, metal was used in addition to the resin, which, according to Urushibara et al,35 provides greater adhesion of microorganisms than acrylic resin alone, demonstrating the need for an even more effective action of the cleanser. These authors suggested that the remaining monomer could have promoted the antimicrobial effects and decreased the biofilm adherence on the resin surface.

Another factor that may have contributed to the action of NitrAdine on S mutans was the immersion time. According to Coenye et al,34 the contact of the cleanser with the surface must be sufficiently long—that is, at least 10 to 15 minutes—for the disinfectant to penetrate the biofilm and present a significant reduction of microorganisms. The immersion time recommended by the manufacturers was used for all tablets, 15 minutes for NitrAdine and 5 minutes for others. Factors such as concentration, mechanisms of action of the tablets, and response of microorganisms can also considerably influence the effectiveness of a cleanser.15

In view of the results obtained, it is possible to affirm that the NitrAdine tablet had a moderate antimicrobial action; however, its recommendation should be made with caution, since its action did not cover a broad spectrum of microorganisms. The results of this study, coupled with the results of other studies that have demonstrated that denture cleanser tablets do not promote adverse effects on the surface,36–38 are expected to contribute to the selection and definition of a hygiene protocol.

Further research and in vivo studies with RPD users should be developed aiming to assess the efficacy of tablets and other cleansers on the surface of the RPD in a way that can promote safe and adequate hygiene, especially for the elderly and for those with motor difficulties.

CONCLUSIONS

Within the limitations of this in vitro study, it can be concluded that the use of the effervescent tablet NitrAdine has the potential to be indicated as an auxiliary method of hygiene for RPD users, since it was the only cleanser that presented effective action, and only in relation to S mutans.

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REFERENCES


