This clinical study investigated if daily immersion in denture cleansers reduces microbial counts on removable partial denture’s (RPD) biofilm. Twenty-five RPD wearer volunteers were selected and instructed to complement the hygiene of their dentures by immersing them in an enzymatic peroxide-based denture cleanser (Polident® 3 minute) once a day for 3 min for a period of 15 days. The biofilm was collected from RPD surfaces with a swab immediately before (baseline) and after the experimental period. The samples were placed in sterile saline solution, sonicated at 7 W and then plated on specific culture media to quantify total microorganisms, total streptococci and Candida spp. counts. Data from both collections were compared by paired t-test (α=0.05). It was observed a significant reduction on total microorganisms’ counts in RPD biofilm after denture cleanser use (p=0.007). This reduction was also observed for total streptococci (p=0.0428), but no difference was observed on Candida spp. counts. It was concluded that daily use of denture cleanser improved denture hygiene by reducing total microorganisms and total streptococci from RPD surface but had no effect on Candida spp. population.

Introduction
Poor oral hygiene and consequent biofilm accumulation on removable partial dentures (RPD) can increase the incidence of tissue inflammation and caries on abutment teeth (1). In addition to local diseases, biofilm is a well recognized reservoir of microorganisms that can also cause important systemic infections (2). Therefore, the establishment of an adequate oral hygiene is of great importance for oral and systemic health and should contemplate an adequate control of biofilm on RPD surfaces.

Brushing is a simple and widely used method to clean removable dentures (3,4). However, the frequent visual and manual limitations presented by some patients (5), combined with the difficulties imposed by RPD design, can impair complete biofilm removal by brushing. Thus, the use of chemical solutions has been recommended as complementary method for dentures hygiene (6). Denture cleansers are commercially available solutions efficient in controlling biofilms (7,8) and, when introduced in patients’ oral hygiene routine, they are able to improve biofilm control as observed in clinical studies with complete denture wearers (6,9,10).

Regarding partial dentures, although some in vitro studies have investigated the use of denture cleansers for RPD hygiene, the main focus has been on investigating the possible harmful effects on prosthesis components, especially metallic framework (11-13). For biofilm control, while many studies evaluated complete dentures and found favorable results, little is known about its effectiveness in controlling biofilm in RPD. Thus, the aim of this clinical study was to evaluate if RPD daily immersion in a denture cleansing solution in combination with the routine method of cleansing could reduce microbial counts on biofilm.

Material and Methods
The study population consisted of patients that were rehabilitated with RPD at the University Dental Clinic. Subjects were eligible to participate if they presented good oral and systemic health conditions, did not use any antimicrobial in the last 3 months, had ability to comply with the experimental protocol, were continuously using the RPD and used brushing as the only method for denture hygiene. The selected volunteers signed the informed consent and were submitted to anamnesis, clinical examination and the first denture biofilm collection was performed (baseline).

The volunteers were instructed to brush their RPD as usual complementing hygiene by immersing their dentures in an enzymatic peroxide-based denture cleanser presented in tablets (Polident® 3 Minute; GlaxoSmithKline, Philadelphia, PA, USA) once a day for 3 min, after the nocturnal brushing. The cleansing solution was prepared by dissolving one tablet of denture cleanser in 200 mL of warm tap water. Dentures should be rinsed in running water to ensure complete biofilm removal.
water before reinsertion into the oral cavity. The volunteers were asked to keep on brushing their dentures as usual, and no additional hygiene instructions were provided to avoid any bias. After 15 days, the volunteers returned for the post-treatment biofilm collection.

For both baseline and post-treatment biofilm collections, RPDs were removed, gently washed with sterile distilled water to remove debris and saliva and then a sterile swab was rubbed onto the whole denture inner and outer surfaces always in the same way – first clasps followed by teeth and acrylic resin base. The swab was immediately placed in a polypropylene tube containing 3 mL of sterile saline solution (NaCl 0.9%) and then sonicated at 7 W for 30 s. The resulting suspension containing biofilm was 10-fold diluted and inoculated in triplicate by the drop-counting technique in the following culture media: Columbia Blood Agar (Difco BD, Franklin Lakes, NJ, USA) supplemented with 5% (v/v) defibrinated sheep blood, Mitis Salivarius Agar (MSA; Difco BD) and CHROMagar™ Candida (Difco BD) for total microorganisms, total streptococci and Candida spp., respectively. The plates were incubated for 48 h at 37 °C in aerobiosis (CHROMagar™ Candida) and 10% carbon dioxide (blood agar and MSA) and the number of colony-forming units (CFU) was quantified using a stereomicroscope (Coleman ST30-2L; Coleman Equipamentos, Santo André, SP, Brazil) in the dilutions containing between 6 and 60 CFU.

The data from both collections were compared by paired t-test with a significance level fixed at 5% (SAS v. 9.0; SAS Institute, Inc., Cary, NC, USA).

Results

The final sample consisted of 25 volunteers (12 male, mean age: 57±8.6 years) (Fig. 1). From the RPD analyzed, 12 were located in the maxillary arch and the mean period of RPD usage was 8.4 months. It was observed a significant reduction of total microorganisms’ counts in RPD biofilms after denture cleanser use (p=0.007; Table 1). Total streptococci also had a significant decrease (p=0.0428; Table 1) in accordance with total microorganism results. In the present sample, 11 volunteers had Candida spp. at baseline collection and no difference was observed on Candida spp. counts after denture cleanser use (p>0.005; Table 1).

Discussion

The findings of the present study showed that the daily use of a denture cleanser was effective on reducing total microorganisms’ counts on RPD surface. This finding is in agreement with those of previous studies, which have reported effectiveness of peroxide-effervescent denture cleansers in reducing microbial population on dentures surface (14,15). This effectiveness of alkaline-peroxides denture cleansers in biofilm control has been attributed to the mechanical action exerted by the effervescence produced when the product is dissolved in water; additionally, the resulting solution presents hydrogen peroxide and active oxygen, which have antimicrobial effects, and enzymes that break down proteins in biofilm (16). On the other hand, Oliveira et al. (17) have found different results in a clinical study and reported no reduction of total microorganisms after daily use of peroxide-effervescent denture cleanser. This divergence can be explained by differences in the methodological aspects, such as the investigation of patients from a long-term institution who may not have brushed properly during experimental period.

The present study also evaluated the effect of denture cleanser on total streptococci for being one of the most prevalent bacteria isolated from removable dentures biofilm; also, this genus includes some clinically relevant species related to oral or systemic diseases such as Streptococcus mutans and S. pneumoniae (2,18). The daily use of denture cleanser was able to reduce total streptococci

<table>
<thead>
<tr>
<th>Biofilm collection</th>
<th>Total microorganisms (x10⁸)</th>
<th>Total Streptococci (x10⁸)</th>
<th>Candida spp. (x10⁷)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.2 ± 2.4⁹</td>
<td>7.3 ± 8.9¹⁸</td>
<td>6.6 ± 12.8⁵</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>0.8 ± 1.1²</td>
<td>4.7 ± 6.8¹⁸</td>
<td>9.3 ± 18.2³</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant difference between baseline and post-treatment data.
population on RPD biofilm, following total microorganisms reduction. In contrast to these results, in the present study, denture cleanser had no effect on Candida spp. population. Actually, some individuals had an increase in Candida spp. counts after denture cleanser use, which is an important finding considering that some species, mainly C. albicans, are associated with the development of denture-related stomatitis (19).

The lack of effect of denture cleanser on Candida spp. goes against some results from in vitro studies that have found a significant action of this product against these microorganisms. However, it must be pointed that these studies used pure fungal biofilms (7,20,21). When confronting the results of the present study with those of in vitro and in vivo studies evaluating mixed bacterial-fungal biofilms (14,15,22), the outcomes are similar. Andrade et al. (15) assessed clinically the effect of different denture cleansing protocols and found that, after 21 days of denture cleanser use, there was a significant reduction of S. mutans but no reduction was observed for C. albicans population.

In order to explain the different response of streptococci and candida species when a mixed biofilm is exposed to denture cleanser, it has been hypothesized that streptococci may be located in more superficial layers of the biofilm and consequently are more exposed to antimicrobial agents. In addition, S. mutans produces a barrier of glucan that could limit the exposure of yeast cells to cleansing solution. Thus, in a multispecies biofilm, Candida spp. would be protected from denture cleanser action by layers of extracellular matrix and bacterial cells (15,22).

Some limitations of the present study should be pointed as the absence of a control group and the short-term evaluation. Future studies should be conducted for longer periods to investigate whether this stronger effect of denture cleanser on bacterial cells could lead to a selection of Candida spp. and if this finding could have any clinical relevance, especially to some groups of patients, like the immunocompromised individuals.

From the obtained results, it was concluded that the daily use of denture cleanser should be recommended to limit the exposure of patients to potentially pathogenic microorganisms as it reduced the counts of total microorganisms and total streptococci on RPD. However, no effect was observed on Candida spp. counts, a finding that deserves future investigations.

Resumo
Este estudo clínico investigou se a imersão diária de próteses parciais removíveis (PPR) em limpar para micro-organismos no biofilme. Vinte e cinco voluntários que utilizavam PPR foram selecionados e instruídos a complementar a higiene das PPRs uma vez por dia, por 3 minutos, durante 15 dias. O biofilme foi coletado da superfície das próteses com auxílio de um swab imediatamente antes (base) e após o período experimental. A amostra foi imersa em solução salina estéril, submetida a sonicação a 7 W, e semeada em meios de cultura específicos para quantificar micro-organismos totais, estreptococos totais e Candida spp. Os dados de ambas as coletas foram comparados pelo teste t pareado (p=0,05). Foi observada uma redução significativa das contagens de micro-organismos totais, estreptococos totais e Candida spp. Os dados de ambas as coletas foram comparados pelo teste t pareado (p=0,007). Essa redução também foi observada para estreptococos totais (p=0,028), mas não foi observada diferença nas contagens de Candida spp. Concluiu-se que o uso diário do limpar para micro-organismos totais e estreptococos totais na superfície da PPR, mas não teve efeito sobre a população de Candida spp.

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