An Initial Longitudinal Prospective Clinical Study Showed that the Aid of Oral Antiseptics Combats Microbial Growth

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Abstract

Introduction

Yeasts are unicellular fungi, that is, formed only by a single cell. Yeasts, especially Candida albicans, are organisms commonly found in the oral cavity of healthy or sick individuals. The most frequently affected region is the dorsum of the tongue, but C. albicans can also be found on the cheek, gingiva, palate, periodontal pockets, and refractory lesions.

Objective

To evaluate and identify the best antiseptic to combat microorganisms. Three patients were analyzed and gingival fluid samples were collected from the patients.

Methods

We analyzed 3 patients 1 smoker and 2 nonsmokers who sought the Dentistry clinic for dental treatment. Gingival fluid samples were collected from patients with chronic periodontal disease. A culture was performed to identify the types of pathogens that colonize the shallow and deep periodontal pockets. Agar Nutrient was prepared and the swab was used for smear application with light hand on the ready agar.

Results

The results obtained directly by the products 1- Colgate Plax® (soft mint), 2- Alcohol 70.0%; 3- Cepacol , 4- Colgate PerioGard® / alcohol were satisfactory, although with a reduced number of patients.

Conclusion

All products used in the research are efficient for combating yeasts, especially Colgate Plax® (Soft Mint).

Keywords

Antiseptics; Yeasts; Oral Diseases; Treatment Screening

Introduction

The prevalence and severity of periodontal disease increases with age, at age 45, 97.0 - 100.0 % of individuals present some form of periodontal disease [1]. The increased severity associated with age is reflected by loss of alveolar bone. Several microbiological examinations can be applied in bacterial plaque recognition, among them culture media, optical and electronic microscopes, immunological tests and DNA probes [1, 2].

The dental biofilm presents as a determining
agent of dental caries and periodontopathies, which are characterized as the main problem in the field of sanitary dentistry [2]. For the effective combat of the dental biofilm, the procedures of a mechanical nature (brush and dental floss) are used that stand in the difficulties presented by the patients. Although there are several studies focusing on the chemical control of the dental biofilm, with the use of several substances, none of them was able to replace the brushing [3].

The microscopic architecture of plaque is well defined, with bacterial cells being arranged in groups or columns of microcolonies [3, 4]. This structure is permeable due to its porosity, allowing saliva, gingival fluid and diet liquids to infiltrate the plaque. The involvement of microorganisms in the etiology of periodontal disease is well established; however, a complete identification of all microbial agents involved with periodontal disease has not yet been fully defined [4, 5].

The supragingival plaque presents a columnar arrangement of bacterial species morphologically distinct from the dental surface and the subgingival plaque often characterized by a zone of Gram negative and / or mobile species located adjacent to the epithelial lining of the pouch, whereas Gram positive rods and coccus appear to form a Layer of organisms firmly adhered on the enamel and root surface [5, 6].

Bacteria that initially colonize the tooth surface are predominantly Gram-positive facultative microorganisms, such as Actinomyces viscosus and Streptococcus sanguis [1, 6]. These adhere to the film through adhesins, which interact with specific receptors on the dental film. Other mechanisms determining selectivity in film colonization are surface structures of certain bacteria, such as Actinobacillus viscosus, called fimbriae, which aid in initial adhesion [6, 7].

In this context of yeast findings, the yeasts can be oval (Hansenula), round (Saccharomyces), cylindrical (Kloeckera), triangular (Trigonopsis), apical (Kloeckera) and ogives (Bretanomyces). Reproduction of yeasts can occur in two ways: New cell formation: This type of yeast reproduction occurs in fermenting musts. In this process occurs the formation of small protuberances on the surface of the cell that, after developing, detach themselves, happening to own life; sporulation of new cells: this type of reproduction consists of the formation of spores inside the cells, which become free by the rupture of the cells [2-5].

In the ecological succession of bacterial plaque, a transition from the aerobic environment, initially characterized by facultative Gram-positive species, to a medium deprived of oxygen, with the predominance of gram-negative anaerobic microorganisms [8, 9]. In secondary colonization, the different species of microorganisms (Prevotella intermedia, Prevotella loescheii, species of Capnocytophaga, Fusobacterium nucleatum, Porphyromonas gingivalis), adhere to the pre-existing bacterial plaque. The plaque virulence factors depend on the presence or increase of specific microorganisms, which produce substances that mediate the destruction of host tissues [9, 10].

In 1988, Socransky et al. Reported that microbial interactions are important in different periodontal conditions, thus, they may result in health or periodontal disease [11, 12]. It seems likely that certain associations may favor the colonization of potentially virulent species (positive association), or even be antagonistic to this colonization (negative association) [13]. In 1996, Zambon described microbial factors in periodontal diseases, considering host protection factors, such as epithelium and gingival fluid flow, cellular immunity and rapid tissue replacement, which make it difficult to fix possible periodontopathogens [14, 15].

Yeasts are unicellular fungi that is, formed only by a single cell. Due to this feature they grow and reproduce more rapidly [1]. This category of fungi are visualized only with the aid of a microscope. They are much larger than most existing bacteria and were first visualized in 1680 by Antony van Leeuwenhoek [3]. The objective of the present study was to evaluate and identify the best oral antiseptic in the fight against microorganisms, as adjuvant to periodontal treatment.

**Methods**

**Seleção Dos Pacientes**

We analyzed 3 patients, 1 smoker and 2 nonsmokers who sought the Dentistry clinic of Unorp de São José do Rio Preto-São Paulo for dental treatment. Gingival fluid samples were collected with swab rods from patients with chronic periodontal disease. A culture was performed to identify the best oral antiseptic in the fight against microorganisms, as adjuvant to periodontal treatment.

**Ethics**

All procedures were performed in accordance with ethical standards and were submitted to the approval of the Ethics Committee of this Institution.
Preparação Do Material
Agar Nutrient: 2.3 g of Nutrient Agar was prepared for 100.0 ml of distilled water. Dissolved Agar was stripped from the sterilized plates. And it took in the pressure in half an hour. Sterile Agar was then inserted into the refrigerator.

Análise Microbiana Da Bolsa Periodontal
Several methods have been proposed for collecting bacteria, choosing the swab. With it we rub as close to the gingival margin of the 3 patients and in the sequence we applied by smearing with the light hand in the ready agar. And we put it in the greenhouse for 3 days. Four products have been used to check whether it is effective in combating micro-organisms:

1- Colgate Plax® (soft mint) - Cetylpyridinium chloride (0.075% - Contains 225 ppm Fluorine);
2- Alcohol 70.0%;
3- Cepacol - cetylpyridinium chloride: 0.500 mg - tartrazine yellow dye;
4- Colgate PerioGard® / alcohol - Oral solution based on 0.16% Chlorhexidine Gluconate. - Mechanism of action specific and active against gram-positive and gram-negative bacteria.

5.0 mL of each product was used. We apply individually on the plate. We waited another 3 days and got the results, sufficient time to perceive the total decline of microorganisms A sterile wipe was used to scrape the bacteria that was isolated and applied in four sterile glass plates, which was due in two groups, one group with four glass plates 2A and the other group with four glass plates 2B. A drop of 0.91 sodium chloride was dripped onto the plate, then rubbed the bacterium onto the plate by smearing and finally passed the plate through the flame to dry.

The next step of the laboratory part was to make GRAM staining, as follows: 1 - Violet crystal: 30 seconds, 2 - Lugol: 1 minute, 3 – Alcohol and Acetone: cover the blade until the color turns purple, 4 - Wash With tap H₂O, 5 - Fuchsin: 30 seconds, 6 - Rinse with tap H₂O, 7 - Dry the slide. To classify in positive and negative we use the scale: Blue color: positive; Red color: negative. After drying the slide, the microscope was analyzed.

Result
Microscopic analysis revealed that the microorganisms present were yeasts. No microorganism other than yeast was found in this analysis. The results obtained directly by the products were satisfactory (figure 1). Thus, on plates 2A, Colgate Plax® (Soft Mint) obtained a halo diameter of 2.0 cm, Alcohol 70.0% obtained a halo of 0.7 cm, Cepacol® obtained a halo of 2.0 cm and Colgate PerioGard® without alcohol Obtained a halo of 1.5 cm.

Figure 1: Culture Showing Yeast Growth

Figure 2: Culture Showing the Effect of Antiseptics on Yeast Growth in Halos
On plates 2B Colgate Plax® (Soft Mint) obtained a halo of 3.5 cm, Alcohol 70.0% obtained a halo of 1.0 cm, Cepacol® obtained a halo of 1.0 cm and Colgate PerioGard® without alcohol obtained a halo of 1.5 cm (Table 2 and Figure 2). There was no statistically significant difference in the growth of halo in the smoker patient in relation to the other nonsmoking patients.

Also, there was no statistically significant difference between each sample of each product used, with p-value <0.05 for all correlations.

### Discussion

In the present study it was observed that the microorganisms present were yeasts. The results showed that in both plates 2B and 2B, Colgate Plax® (Soft Mint) obtained the best halo diameter of 2.0 cm, followed by Colgate PerioGard®. There was no statistically significant difference in the growth of halo in the smoker patient in relation to the other nonsmoking patients. Also, there was no statistically significant difference between each sample of each product used, with p-value <0.05 for all correlations, according to non-parametric correlation.

The main species of human pathogen yeasts are: Cryptococcus neoformans: this species is a human pathogen and causes several systemic mycoses (such as cryptococcosis); Candida albicans: it is also a human pathogen and causes superficial and deep mycoses [6-9]. Candidiasis, a very common disease, is caused by this species; Blastomyces spp: Responsible for the cause of blastomycosis, this species also causes deep mycoses (systemic, invasive of organs and tissues); Paracoccidioides brasiliensis: Human pathogen, causing various superficial and deep mycoses [8, 9].

Yeasts, especially Candida albicans, are organisms commonly found in the oral cavity of healthy or sick individuals [10-14]. The most frequently affected region is the dorsum of the tongue, but C. albicans can also be found on the cheek, gingiva, palate, periodontal pockets and refractory lesions. These regions are considered important entry points for systemic infections, therefore the prevention of oral colonization by Candida deserves great attention [16-19].

They establish a balance with the host called symbiosis, when this balance is broken due to changes in the defense mechanism and oral environment (chemotherapy and radiotherapy, HIV-positive, etc.), these yeasts can cause opportunistic oral infections, if not treated can become generalized and / or disseminate systemically causing fungemia [20-23].

Oropharyngeal candidiasis presents as its main etiological agent C. albicans, common in immunocompromised patients and is usually preceded by colonization of oral mucosal surfaces [22, 23]. Systemic candidiasis, which is related to high mortality rates, may occur due to previous colonization of the oral cavity or oropharynx, therefore the prevention of oral colonization by Candida is of great importance in the prevention of oropharyngeal and systemic candidiasis, especially in patients with Neutropenia [12, 15].

### Conclusion

We concluded that all the products used in the research were efficient to combat yeasts, although with a reduced number of patients [24]. All had satisfactory results, especially Colgate Plax® (Soft Mint), which obtained the best performance for this study, followed by Colgate PerioGard®.

### Limitations

This present study was an initial pilot study, therefore further studies will be required to confirm the results obtained.
Conflict of Interests
There is no conflict of interest between authors.

References


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