THE FUNCTION OF ENZYMES IN REMOVING CANDIDA ACCUMULATED ON DENTURE PLAQUE

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SUMMARY: The denture plaque that is a cause of denture stomatitis has Candida albicans to a great extent. The plaque accumulated should be removed in order to prevent infections related to Candida albicans. Since chemical denture cleansing agents have some inconveniences in application and are less effective, in recent years the use of enzyme containing cleansers has become popular. The aim of this study was to evaluate the efficacy of enzymes in removing C. albicans from the denture plaque. The plaque was accumulated on polymethyl-methacrylate plates by the strain of C. albicans and the effect of enzymes such as papain, trypsin and amylase were investigated by the cell-counting procedure. Papain, a proteolytic enzyme, was found to be effective (80%).

Key Words: Candida albicans, denture plaque, enzymes.

INTRODUCTION

The hygiene of denture has been overlooked by patients and physicians for unknown reasons. However, colonies of bacteria and fungi grown on denture plaque give rise to denture stomatitis. According to Neill the soiling of denture occurs in 3 phases (11): first mucin and food debris accumulate upon the surface of denture, then plaque builds up, this plaque acts as a matrix and then calcification begins.

There is a close relationship between denture stomatitis seen in most of the patients who are denture wearers and Candida albicans (3, 6, 8,12,13). Thus, the presence of Candia albicans has been shown on the denture plaque by studies (14). For this reason, Candida should be removed from the surface of denture.

Another uneasy cosmetic concern is stains which accumulate on the surface of denture. They are formed as a result of various habits such as consumption of colored foodstuffs and smoking. The satins adhere biologically to the surface of the denture in the same way as plaque (1). This situation causes odor and also impairs the esthetic quality of denture.

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Mechanical denture cleansing does not always yield good results. The use of chemical denture cleansers produces more effective results especially in geriatric patients and in people who have problems in wearing dentures. An effective denture cleanser must have the ability to remove microbial plaque formed and prevent its rebuild. In addition, it must be capable of removing mucin, food debris, calculus and stains. In the meantime, however, it should not cause harm to denture base material (4).

Chemical denture cleansers are classified into various groups such as alkaline peroxides, alkaline hypochlorites, acids, disinfectants and enzymes (4, 8,11,14,16).

Alkaline peroxides are particularly effective on new plaque and satins formed. To be effective they must remain in contact with denture for a long period. These cleansers cause bleaching of the acrylic resin base when routinely used.

Alkaline hypochlorites remove stains on denture and decompose mucin and other organic substances. They are effective against both bacteria and fungi and dissolve the organic matrix of denture plaque by acting directly. Besides, these materials cause some harmful effects such as tarnish and corrosion of the metal components of dentures. The routine use of alkaline hypochlorites may also give rise to bleaching of the acrylic resin base.

Effective against calculus or stains on dentures, acids

may corrode the metal component in dentures. Disinfectants such as dilute solution of chlorhexidine gluconate or salicylate reduce visibly the amount of denture plaque. However, when the treatment is discontinued, a relapse is seen. They are effective especially against fungi. As their daily use leads to coloring of denture, they have a limited field of application.

Enzymes breakdown the macromolecules of glycoproteins, mucoproteins and mucopolysaccharides found in denture plaques into less adhesive small units (6). They are effective against both bacteria and fungi. Enzymes are very suitable in removing the heavy deposits from denture plaque and they also prevent new plaque formation. Their use has no harmful effect on the denture base material and its other component. In this study, the cleansing-ability of some enzyme containing solutions, which are superior to chemical disinfectants, to remove Candida albicans from acrylic resin denture plaque were investigated.

MATERIALS AND METHODS

Preparation of test specimen

Fifty specimens, 10x10x2 mm in size, were prepared from polymethyl-methacrylate (DeTrey Dentsply QC-20 Denture Material, France) which was used as denture base material and both of the surfaces were polished by standard polishing procedures (Figure 1). Specimens were kept in distilled water to prevent dehydration.

Preparation of fungal suspension

The strain of Candida albicans (RSKK 81-623) used in this study was supplied by Ankara Refik Saydam Hifzissihha Institute from Institute Pasteur. The cultures were grown in peptone-yeast extract glucose (PYG) medium containing 1% peptone, 0.5% yeast extract and 2% glucose. Ten ml suspensions of these cultures (5x106 cfu/ml) were prepared. Acrylic resin plates were immersed in these suspensions and incubated for 12 hours at 37°C.

Figure 1: Standard acrylic plates (10x10x2 mm).

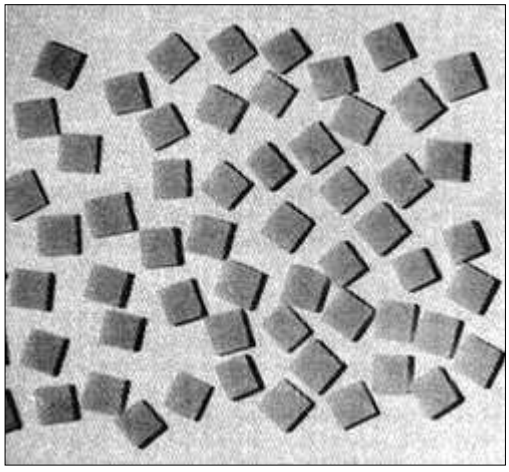
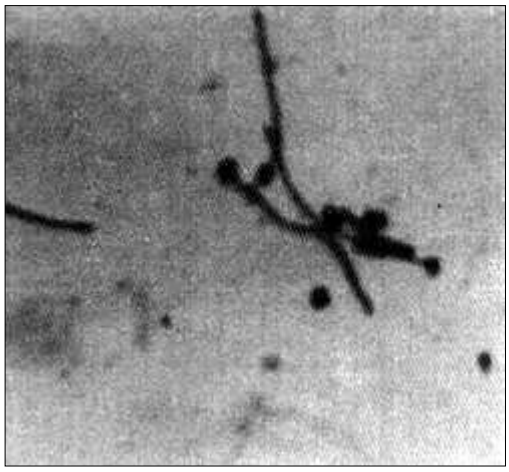


Figure 2: Candida species seen on the acrylic plates (x320).



Enzymes

The standards of enzymes, Papain, Trypsin and Amylase were obtained from Ankara Refik Sydam Hifzissihha Institute. 0.1 mg/ml solutions of these enzymes in PBS (pH 7.2) were prepared. For each enzyme five 10ml tubes were prepared. Tubes having only 10 ml PBS were used as control.

Removing of Candida

Plates having plaque as a result of incubation with Candida albicans were placed in the solutions of enzymes. Tubes were incubated at 37°C for 8 hours. After incubation, plates were washed ten times in saline solution. They were fixed in methanol and stained with crystal violet. After drying, fungal cells adhered to the plates were counted by microscopy (Figure 2) and the mean of each count was used. By comparing with the control plates, the Candida-removing percentage by enzymes was determined.

RESULTS AND DISCUSSION

The Candida-removing ability of enzymes from acrylic plates was illustrated in Table 1. According to these results, amylase was ineffective for removing Candida albicans. Proteolytic enzymes, Trypsin and Papain were effective 5 and 80% respectively.

Candida albicans as a significant cause of denture stomatitis can be isolated not only from the tissue surface on which the denture fits but from the inner surface of denture as well (5,10). During the growth and metabolism of Candida species organic acids that decrease the pH are formed. These acids may have a direct cytotoxic effect on the mucosal epithelium. Moreover, the acidic pH may activate the acid proteases or phospholipases of Candida albicans and may result in the inflammation of mucosa. As this type of inflammation increases, more Candida albicans may adhere to the surface of denture. Many studies have been conducted to evaluate the effi-

Table 1: The enzymes effective in removing Candida albicans.

Enzyme	% cell removed	Mean of the Candida albicans-cell counted	Control
α-amylase	0	100	100
Proteolytic enzyme Trypsin	5	95	100
Proteolytic enzyme Papain	80	20	100

cacy of commercial denture cleansing agents used for removing the denture plaque (1, 2, 7, 8,10,13,15,16).

However, most of them have some known disadvantages. It has been reported that enzyme -containing cleansers are less harmful and more effective. In our country this type of study with enzymes has not yet been done.

Several methods have been used in order to determine the plaque cleanser efficacy of denture cleansers. The weight method was suggested by some of the investigators (13). However, this method is not very sensitive. More quantitative results can be obtained by counting the cells grown on plaque. In our studies, we used this method.

The cells of C. albicans found diffusively on denture plaque adhere to the acrylic resin by means of proteins and polysaccharides. Consequently, this plaque can be removed from the surface of acrylic by enzymes cleaving these bonds (13).

The enzymes were found to be of optimum effect in plaque removal in 8 hours as reported by the researchers (6). We also used this period in our experiments. There are studies in which various enzymes have been used in denture cleansing. Particularly yeast lytic enzyme group and proteolytic enzymes were found to be effective. In our study from protedytic enzyme group Trypsin was effective 5% whereas Papain was effective 80%. Our results are consistent with the other studies (9,14).

As a conclusion, in denture cleaning in order to prevent Candida induced denture stomatitis, the use of proteolytic enzyme-containing solutions such as papain could be suitable. Patients were instructed to remove their dentures when they retire at night. It is our opinion that if patients could immerse their dentures in enzyme-containing solutions at night, 8 hours sleeping time would serve well to complete the cleansing procedure.

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