Stomatologija, Baltic Dental and Maxillofacial Journal, 17: 54-66, 2015

Candida albicans importance to denture wearers. A literature review

Alvydas Gleiznys, Eglė Zdanavičienė, Juozas Žilinskas

SUMMARY

Opportunistic oral fungal infections have spred, especially in denture wearers. Denture stomatitis is a common inflammatory reaction, multifactorial etiology, which is usually associated with *Candida* species, particularly *Candida albicans*, due to its high virulence, ability to adhere and form biofilms on oral cavity tissues and denture surfaces. This article highlights the pathogenesis, clinical presentation, and management strategies of *Candida*-associated denture stomatitis commonly encountered in dental practice.

Key words: *Candida albicans*, *Candida*-associated denture stomatitis, biofilm, antifungal drugs, denture.

INTRODUCTION

Longer life expectancy has led to an increase in the ageing population in developed countries. This growth in the number of elderly may lead to an increase in the number of people requiring removable dentures (22, 40). The current rates of edentulism have been estimated to be between 7 percent and 69 percent of the adult population internationally (45). For those older adults lacking the appropriate manual dexterity to eliminate oral plaque from denture appliances and teeth, there might be more susceptibility to opportunistic oral mucosal infections, particularly bacterial and fungal (37). Infection due to fungi have increased dramatically in recent years and are of prime importance because of the rising number of immunocompromised patients, such as cancer patients receiving chemotherapy, and human immunodeficiency virus infected patients (8, 12, 49). It has a high morbidity amongst the latter group with approximately 85% of patients being infected at some point during the course of their illness (8).

A spongy denture tissue surface, full of nutritive substances, is an ideal incubator for species such as *Candida albicans* (14). *Candida albicans* is a commensal in the oral cavity of 45-65% of healthy individuals with a higher prevalence found in children and young adults (48). In denture wearers, the prevalence of *Candida* increases to 60-100% (1, 13, 15, 20, 28, 31, 32, 50) and the organism can be opportunistic, which can be explained by the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic microenvironment that favours yeast overgrowth. Additionally, *Candida* has affinity for the acrylic surface of dentures and non-renewing surfaces such as teeth, dental fillings (25, 29, 34, 38, 39, 50). Surface characteristics of denture base acrylic resins, such as hydrophobicity, have generally been acknowledged to be one of the factors contributing to the adhesion, which is a crucial step in biofilm formation (23, 24, 27). *Candida albicans* biofilms are frequently associated with the occurrence of denture stomatitis (10, 24, 34), but non-albicans species, such as *C. glabrata, C. tropicalis, C. krusei, C. parapsilosis* and *C. dubliniensis*, can also contribute to the development of this infection (1, 6, 18, 19, 22, 26, 48). Its primary location is the posterior tongue and other oral sites as the mucosa, while the film that covers the dental surfaces is colonized secondarily (23). Cells in this unique environment are equipped to withstand host defenses and survive antifungal therapy (6, 36). Effective removal of biofilm is a significant challenge by both chemical and mechanical methods (36).

Historically, the ancient Greek physician Hippocrate (460–370 BC) described oral candidiasis as "disease of diseased" (16, 52). This infection was clas-

[*]*Clinical Department of Dental and Maxillofacial Orthopedics, Faculty of Odontology, Medical Academy, Lithuanian University of Health Sciences*

***Alvydas Gleiznys**[*] – D.D.S., PhD., assoc. prof.*
***Eglė Zdanavičienė**[*] – D.D.S.*
***Juozas Žilinskas**[*] – D.D.S., PhD, lect.*

Address correspondence to Dr. Alvydas Gleiznys, Clinical Department of Dental and Maxillar Orthopaedics, Lithuanian University of Health Sciences, Kaunas, Lithuania.
E-mail address: egluteskrip@gmail.com

sified according to Newton in 1962, based exclusively on clinical criteria, into three clinical types: type I is characterized by localized simple inflammation or pin-point hyperaemia. Type II, the most common type, is presented as diffuse erythema and oedema of palatal mucosal areas covered by dentures. Type III is described as granular surface or inflammatory papillary hyperplasia in central palate (11, 17, 51). Classic study has shown that this oral inflammation may occur on the maxilla and mandible; however, it is more often associated with the maxilla, sometimes found under maxilla partial dentures, but only rarely beneath mandibular dentures (33, 34, 44, 51).

Patients with candidiasis may display various symptoms including burning, painful sensation, change of taste, and swallowing difficulty, but most often are asymptomatic (5, 21, 41). In addition to its high incidence in denture wearers, there is a concern that *Candida* species from the oral cavity may colonize the upper gastrointestinal tract in immunosuppressed patients and lead to septicaemia, which can cause a 40% mortality rate or longer hospitalisation time, leading to a higher cost to the health system (30, 42, 44, 46). Finding Helicobacter pylori inside the yeast *Candida* could play a significant task in the bacterial reinfection of stomach and could be the reservoir of Helicobacter pylori in transmission to a new host. This might explain the persistence of Helicobacter pylori in the gastrointestinal tract (31).

ASPECTS OF *CANDIDA ALBICANS* BIOFILM FORMATION

Candida is present in the oral cavity in two distinct forms, as floating planktonic cells (blastopores, blastoconidia) and/or in an organized biofilm. Poor oral hygiene, practices such as failure to remove the denture whilst sleeping and poor denture cleansing allows the accumulation of biofilm (2, 16, 49), which is defined as structured microbial community that is attached to a surface, consisting of more than 10^{11} microorganisms per gram of dry weight (1, 2) and surrounded by a self-produced extracellular matrix. Biofilms are found adhering to living tissue such as mucosal surfaces or to abiotic surfaces such as implanted medical devices, intravascular catheters, and oral prostheses (52). It has been recognized that unlike dental biofilm, the biofilm that forms on denture materials harbours a much larger population of yeasts (46). Furthermore, *Candida* has been reported to form mixed biofilms with both *Staphylococcus* and *Streptococcus* during denture and catheter infection (6, 33, 47). These interactions between biofilm inhabitants in mixed-species communities are commensalism and antagonism within subpopulations (3). For example, *Streptococcus mutans* biofilm, which is considered one of the primary colonizers of oral biofilm, and is heavily involved in the initial stages of biofilm formation, producing extra cellular matrix polysaccharide and facilitating the attachment of other microorganisms such as *Candida albicans* (29). Proliferation of bacteria and fungi can cause bad breath, acrylic resin pigmentation and staining, formation of calculus deposits and the development of chronic atrophic candidiasis, also known as denture stomatitis (2).

Many factors affect adhesion and biofilm formation of *Candida* on acrylic surfaces, such as surface roughness of the inner surface of the prosthesis, salivary pellicle, hydrophobic and electrostatic interactions, receptor-ligand binding (1, 23, 31, 33, 39, 44, 51). The process of protein adsorption and bacterial adhesion seem to be more important than mechanical retention for the colonization by microorganisms (33). There are several reports to suggest the relationship between surface roughness and *Candida albicans* adherence to denture materials. Verran et al. reported that significantly higher number of *Candida albicans* was observed on roughened than on smooth surfaces, and Radford et al. reported significantly greater adhesion of *Candida albicans* to rough than smooth surfaces (44). Denture base cracks may become to be one of the best sites for microorganism propagation and provides protection from shear forces, even during denture cleaning (43, 51). This result may be due to the fact that roughness increases the surface area and may act as niches for microorganisms (16, 30). Furthermore, it has been reported that ageing processes and thermal cycling, in particular, may lead to a significant increase in *Candida albicans* adhesion to the artificially aged specimens (39). The abiotic surface of the denture means that the ability to remove adherent microbes through selfrenewal of surface layers does not occur as would be encountered on living mucosa (16).

Adherence of *Candida albicans* to host epithelial cells is a critical first step in the infection process (1, 7, 12, 23, 26, 49, 51, 52). It is essential for both colonization and subsequent induction of mucosal disease (7). These microorganisms can stick and proliferate through the hard and soft tissues of the oral cavity (51). The fact that both *Candida* and epithelial cell surface are negatively charged means that there are repulsive forces retarding their adhesion. Nevertheless, there are other attractive forces such as Lifshitz-van der Waals forces, hydrophobic interactions, and Brownian movement forces. The sum of these nonspecific forces will determine whether the initial nonspecific adhesion between fungal and epithelial cells will be established (23, 52). An ill-fitting denture may cause frictional irritation of the palatal mucosa and this facilitates

invasion of *Candida* into the superficial layers of the epithelium. On occasions, a denture soft liner may be used to cushion the hard acrylic material of the denture against the mucosa. Unfortunately, silicone rubber (the most frequently employed material for soft liners) is also a surface that *Candida* can readily colonize and actually invade (16). The fact that the oral epithelium is continually replenished means that, in order to colonize the oral mucosa, *Candida* must be present in the mouth in sufficient numbers and with a high enough growth rate to allow their continued persistence (16). Moreover, colonization of mucosal surfaces is a known risk factor for disseminated candidiasis (7).

Candida albicans biofilm development progresses in three distinct developmental phases:Phase I is an early stage and occurs within 1-11 hours. *Candida* cells adhere to a substrateor surface within two hours. Glycoproteins are expressed to facilitate stronger adhesion. Microcolonies appear at 3 to 4 hours, and after 11 hours; aggregations of *Candida albicans* appear on substratum irregularities. Phase II is intermediate/developmental stage and occurs within 12-30 hours. The *Candida* biofilm community can be seen as a bilayer composed of yeasts, germ tubes and young hyphae with a matrix of extracellular polymeric substances.

Phase III is the last stage and represents the maturation which appears within 38-72 hours. Extracellular material enlarges with incubation time, while *Candida albicans* yeasts, pseudohyphae and hyphae are fully embedded in a matrix (22, 35, 51).

Candida albicans is able to utilize special mechanisms that allow it to be able to switch back and forth from one form to another (from budding yeast form to pseudohyphal, to hyphal forms) (27, 48, 52). Morphologic transition from the yeast to hyphal state is one of the key factors in the pathogenic activity of *Candida albicans*. In the hyphal form, *Candida*, by producing acid proteinases, which cause damage in oral mucosa, has been considered more pathogenic than its yeast form (15, 31). For this reason, the formation of *Candida* biofilms creates serious clinical injurious effects because of their increased resistance to antifungal therapy; the cells within biofilms also carry the capability to resist immune defenses of the host (52). *Candida* biofilms were first shown to exhibit resistance to antifungals by Hawser & Douglas, and this has been reported by numerous other researchers (16, 18, 51). Antifungal drug resistance increases during biofilm development (51). Dietary habits also may influence resistance of fungi in biofilms to antifungal agents since biofilms on acrylic surfaces exposed to sugars showed higher *Candida* counts, phospholipase activity, and increased production of extracellular matrix substance (metabolic activity) (52).

HOST DEFENSE AGAINST *CANDIDA ALBICANS*

The host defenses include mechanical barriers to fungal penetration such as epithelial surfaces, soluble antimicrobial factors, and innate and adaptive cellular immune mechanisms (68). They are discussed below.

The mucosal epithelium is the first line of defence against *Candida* species. It has been long acknowledged that the epithelium has a function as a passive physical barrier to restrain *Candida* from invasion of the underlying tissue. However, recent studies have broadened our knowledge about the active role played by epithelial cells in triggering immune responses (69, 70). It should be noted that stratified epithelia (non-keratinized and keratinized) in the oral cavity, which are often colonized by *Candida albicans*, are less likely to be able to endocytose hyphae because most, if not all, cells covering these epithelial surfaces are dead (71).

Detection of pathogens at mucosal surfaces is primarily an innate immune-mediated event, using a multitude of detection systems. Detection almost invariably involves recognition of pathogen-associated molecular patterns – either secreted by or present on the surface of microbes – by a large group of receptors termed pattern recognition receptors (PRRs). These PRRs include the well-known Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), and the Nod-like receptors (72-75). Nevertheless, it is thought that there is a threshold for the amount of *Candida albicans* that is tolerated by the host. The host must keep the fungal burden below this threshold and distinguish non-pathogenic *Candida albicans* cells from invasive and potentially life-threatening cells of the same fungus to maintain homeostasis (74).

Innate immunity in mucosal infection involves many cell types: neutrophils, monocytes/macrophages, Natural Killer (NK) cells, dendritic cells (DC), certain CD4+ and CD8+ T cells, non-MHC restricted T cells such as γδ-T-cells, mucosal epithelial cells, stromal cells and keratinocytes. One function of these cells is to provide a primary protective effect via direct anti-fungal activities such as phagocytosis or secretion of microbicidal compounds that neutralize fungal particles (76). Phagocytes are believed to be the most effective cell type for controlling and clearing *Candida* infection (70). In addition, innate cells also instruct the adaptive arm of the immune response via production of pro-inflammatory cytokines and chemokines, costimulatory signals and antigen uptake and presentation. Saliva is also a major source of IgA antibodies, many of which recognize *Candida albicans* and other potential pathogens. Saliva is highly enriched in

antimicrobial proteins such as lysozyme, lactoferrin, histatins, cathelicidins, calprotectins and defensins, which are key factors that help to control *Candida albicans* growth and attachment to the oral epithelium (76-78). Importantly, many of these molecules are present in rather low concentrations in whole saliva; however, it should be considered that their effects are cumulative and/or synergistic, resulting in an efficient molecular defence network of the oral cavity (76).

PREDISPOSING FACTORS FOR *CANDIDA*-ASSOCIATED DENTURE STOMATITIS

The transition of *Candida* from commensal to pathogen is often associated with predisposing factors (45). The most relevant are discussed below.

Systemic host factors:

- ***Endocrine disorders***

While some studies have demonstrated an increased oral colonisation of *Candida albicans* of diabetic subjects (4, 48), comparable studies by Manfredi et al. and Al-Karaawi et al. present contradictory findings with reduced rates of candidal carriage in diabetic patients compared with healthy controls. The level of glycaemic control appears to be a more significant factor than the presence of the disease itself, because poor control can reduce salivary flow and pH, increase salivary glucose level. These factors facilitate oral candidal growth and colonization (1, 4, 8, 11, 16, 27, 32, 34, 37, 41, 48). The saliva of diabetics favors the growth of *Candida albicans* in vitro and it has been shown that on the denture surfaces of diabetic there are more elevated counts of colonies of the yeast by comparison with the nondiabetic subjects (23). Thus, diabetic patients are more likely to acquire oral candidal infections than non-diabetic patients (48).

- ***Immunologic disorders***

Frequently, when the host defence system suffers because of any alterations, like immunodeficiency, *Candida albicans* become virulent and generate candidiasis, that can be manifested through various clinical forms, involving one or more oral sites, up to affect the whole oral cavity and to disseminate into invasive forms (23).

Candidosis occurs in over 60% of HIV-infected patients, and more than 80% of patients diagnosed with AIDS. Candidosis is also a common manifestation of a variety of other immunodeficiencies, including severe combined immunodeficiency syndrome, DiGeorge syndrome, hereditarymyeloperoxidase deficiency and Chediak-Higashi syndrome (6, 8, 11, 12, 15, 16, 21, 23, 27, 32, 35, 41, 48).

- ***Kidneys affections***

These affections are frequent in individuals of advanced age. The repeated treatments with antibiotics and sulphonamides can be predisposing factors because of the microbial alterations that they provoke in the oral cavity (1).

- ***Xerostomia***

Qualitative and quantitative alterations of the salivary flow in elderly patients is probably secondary to the assumption of drugs, above all the antihypertensive ones, rather than a primary functional deficit. Such reduction has been shown to act as predisposing factor to the virulence of the *Candida* species (1, 8, 11, 12, 15, 16, 23, 27, 32, 34). Furthermore, ethanol and caffeine reduce salivary flow, and influence the pathogenesis of oral candidosis. Primary and secondary Sjogren's syndrome patients have a higher incidence of oral yeast carriage regardless of oral hygiene measures and denture wearing. Patients with chronic hepatitis C virus and HIV infection may develop lymphocytic sialadenitis with resultant salivary gland hypofunction and oral candidosis. Sialadenosis in alcoholics and patients with diabetes mellitus and other metabolic disorders can cause xerostomia and predispose to candidosis (48).

- ***Blood dyscrasias and malignancy***

Solid organ or haematological malignancies, their treatment with cytotoxic chemotherapy, radiotherapy impair host defence mechanisms and frequently develop oral candidosis. The incidence of oral candidosis in this patient group has been reported to range between 30 to 94%. In these patients, antifungal prophylaxis is important to prevent the development of oral mycoses, systemic fungal infection and potential mortality. By the way, nystatin appears ineffective in patients undergoing treatment for cancer (11, 35, 48).

- ***Deficiency of nutritional factors***

Some authors report that protein-energy malnutrition, high levels of cholesterol, deficiencies of iron, folate, vitamin C, vitamin B12 and possibly vitamin A may result in reduced host defenses and a loss of mucosal integrity, potentially facilitating hyphal invasion and infection. Conversely, carbohydrate rich diets might represent a risk factor that increases the adherence of *Candida* species to epithelial cells (1, 8, 11, 16, 23, 27, 32-34, 37, 48).

- ***Drug therapy***

Several classes of pharmacologic agents, such as broad-spectrum antibiotics, immunomodulatory drugs and xerogenic drugs, predispose to oral candidosis. A large number of prescribed drugs, such as corticosteroids, antidepressants, antipsychotics, anticholinergics, antihypertensives and antiadrenergics, elicit xerostomic side effects (6, 8, 11, 12, 15, 16, 27, 32, 33, 35, 37, 41, 48).

- ***Extremes of age***

The elderly generally suffer from systemic illnesses, changes in nourishment and their salivary char-

acteristics (32). Advancing age is also a risk factor for denture stomatitis in the elderly, because cell-mediated immunity, which provides protection against candidal infection declines with age. According to Ryu et al. , some oral environmental factors, such as unstimulated salivary flow rate and age of subjects, are associated with higher numbers of microbes in the saliva of complete denture wearers. Those authors suggest that a reduction in the salivary flow rate with aging induces an increase in concentration of microbes in saliva (17).

Local host factors:

- ***Traumas***

Nyquist considered traumas as the main liable to determine *Candida*-associated denture stomatitis with none association with the microbial communities and the presence of denture (1, 6, 10, 15, 23, 27, 32). Subsequently, Cawson showed that traumas and *Candida* infection are together responsible for the pathogenesis of the denture stomatitis. The latest study pointed out that trauma alone does not induce pictures of generalized denture stomatitis but, rather, it could be the cause of localized forms. Instead, in the generalized forms the principal pathogenetic role is played by *Candida albicans*. Furthermore, mechanical trauma from a poorly fitting denture increase the risk of tissue penetration and colonisation by *Candida* (1, 50).

- ***The saliva***

The role of the saliva in the colonization of *Candida albicans* is still controversial (1, 23, 28, 30). Saliva shows a physical cleaning effect and innate defence molecules, including lysozyme, hystatin, lactoferrin, calprotectine and IgA (1, 8, 23, 31). However, it has been shown that salivary proteins as the mucines and the statherins may act as adhesion receptors used by the mannoproteins present in the *Candida* species (1, 23). The decrease or the complete absence of saliva in individuals with xerostomy induces the change and the imbalance of the normal microbial communities favouring the proliferation of bacteria as Staphylococcus aureus, that inhibits the normal adaptation of the commensals. Besides, the presence of a low level of pH and of a high oxygen tension reduces the growth of some commensals while increases the proliferation of *Candida* species, Streptococcus mutans and Lactobacillus (1).

- ***pH of the oral cavity***

In the oral cavity, the pH is maintained near neutrality (6.7 to 7.3) by saliva in two mechanisms. First, the flow of saliva eliminates carbohydrates that could be metabolized by bacteria and removes acids produced by bacteria. Second, acidity from drinks and foods, as well as from bacterial activity, is neutralized by the buffering activity of saliva (33). Low levels of pH can favour the adhesion and the proliferation of *Candida* yeast. In fact, a pH equal to 3 is optimal not only for the adhesion of the yeasts, but also for the enzymatic activity of the proteinases that, together with the lipases, are the most important factors of virulence of *Candida* because of their cytotoxic and cytolytic effects. Moreover, high levels of carbohydrates present in the saliva can act as an additional nourishing source for the *Candida* yeasts, that, by metabolizing these sugars, produce acid metabolic products and contribute to maintain low the environmental pH. Samaranayake and McFarlane have demonstrated that carbohydrates in the diet help the biofilm formation of *Candida albicans* on acrylic materials (1, 10, 15, 32, 33, 37).

The mean value of pH was lower in complete denture wearers than in nondenture wearers. Therefore, dentures obstruct the salivary flow from minor salivary glands and the free exchange of oxygen. In support of this, changes of salivary pH were found after the insertion of complete denture and dentures supported by implants. It has been shown that oral *Candida* colonisation can increase up to six-fold in denture-wearers (4, 33). Furthermore, decreased salivary gland hypofunction and pH changes was noted in the elderly. The pH of the oral mucosa in the elderly appears to be more acidic than the oral pH of younger people, that helps denture stomatitis development (12, 32).

- ***Permeability of the acrylic resins***

Initially, the adhesion of *Candida* depends on the microporosity present on the surface of the denture. Such irregularities of surface make possible the yeasts to nest and make difficult to eliminate bacteria (1, 23).

- ***Presence of microbial plaque***

Among the predisposing local factors the main one is the accumulation of microbial plaque on the surface of the denture in contact with the mucosa (1). Both the plaque accumulated on the denture and the poor oral hygiene contribute to the virulence of *Candida*, offering the clinical picture of *Candida*-associated denture stomatitis (1, 11, 15, 21, 27, 32, 34, 37, 41, 50).

- ***Age of the denture***

Denture age is shown to be an important factor as a result of poor fit, roughness, inadequate hygiene, and accumulation of plaque due to aging of denture (11, 27, 34). It was reported, that aging of the denture and release of residual monomer with time results in poorer fit which affects the contamination of the denture. Moreover, denture age was proportional to *Candida* colonization and not to degree of inflammation (11, 13). Only 25% of individuals using dentures for <1 year were diagnosed with denture stomatitis, whereas >84% of those using dentures for >5 years had the disease (26). Besides, poor denture quality is also important as the age of the denture (11).

- ***Allergy***

Allergic and primary irritant reactions to the denture base material also play a parts (11, 23, 37).

- ***Uninterrupted denture wearing***

Denture wear at night should be considered as one of predisposing factor (32, 37).

Other factors:

- ***Smoking***

Whether tobacco smoking should be considered as one of predisposing factor is still a matter of debate. Several previous studies have reported that tobacco smoking, either alone or in combination with other systemic or local factors, is associated with changes in the oral cavity, increased oral *Candida* colonization or with the development of oral candidosis. Specifically, it has been shown that cigarette smoke interferes with Streptococcus mutans and *Candida albicans* adhesion, resulting in biofilm formation on dental restoration materials, which suggests that cigarette smokers are more susceptible to life-threatening oral infections including candidiasis (10, 35). Though, other studies have not shown this association. In addition, tobacco smoking appeared to increase the prevalence of erythematous candidosis among denture wearers and patients with candidal leukoplakia. There was a marginally significant positive correlation between the number of cigarettes smoked per day and the density of *Candida* growth in oral rince cultures (10, 35). So, cigarette smoke can be labeled as an infection-promoting agent (15, 16, 27, 32, 35, 48).

- ***Blood group***

Studies have demonstrated that the blood group H antigen functions as a receptor for *Candida albicans*. Therefore, individuals of blood group O (who have increased H antigen on their cell surfaces) may be at higher risk of developing oral candidosis (48).

- ***Pregnancy***

Commensal *Candida* carriage is increased during pregnancy (15, 48).

- ***Gender***

It has been reported that oral *Candida* colonisation is significantly higher in females compared to males; however, this relationship remains debatable (4, 11, 32).

CLINICAL SPECTRUM OF *CANDIDA*-ASSOCIATED STOMATITIS

Infection with *Candida albicans* presents mainly in any of four forms: pseudomembranous candidiasis, hyperplastic candidiasis, erythematous candidiasis, or angular cheilitis. Patients may exhibit one or a combination of any of these presentations (8, 12, 16). Each type of infection is associated with characteristic clinical signs and symptoms that are influenced by a range of predisposing factors (16). These forms of infection are discussed below.

Pseudomembranous candidosis is synonymous with the term oral thrush , and is reported in neonates and the elderly at rates of 5–10 % (8, 16). The infection is characterized by the presence of superficial whitish-yellow plaques on the surface of the oral mucosa and tongue, resembling cottage cheese, that are easily removed by gentle rubbing of the lesion. The underlying mucosa may be erythematous, but ulceration would not be expected (8, 12, 16, 48). While symptoms are typically mild for this form of infection, patients may complain of a slight tingling sensationora foul taste. The plaques consist of necrotic material, desquamated epithelial cells, fibrin and fungal hyphae (8, 48). The ability to remove these plaques is a diagnostic feature that differentiates pseudomembranous candidosis from other forms of white patch lesions of the oral mucosa (16). This form of candidosis is most common in immunocompromised individuals (8, 48). Infact, the presence of pseudomembranous candidiasis in a seemingly healthy adult may be an indication of underlying systemic disease, such as infection with the human immunodeficiency virus (HIV) (8).

Acute erythematous candidosis is a clinical form of *Candida albicans* characterized by localized erythema of the oral mucosa, with or without associated symptoms. The clinical signs and symptoms of acute erythematous candidosis are therefore a direct consequence of an ecological shift in the normal homeostatic balance of the microbial community. It commonly occurs on the dorsum of the tongue and the palate, and less commonly the buccal mucosa. This variant was previously known as antibiotic sore mouth, and is associated with chronic use of broad-spectrum antibiotics and corticosteroids (8, 16, 48). Cessation of antibiotic therapy results in a return to normal levels of bacteria, which subsequently resolves the candidosis without intervention. It is also present in patients with HIV infections (16, 48).

Commonly known as *Candida*-associated denture stomatitis, *chronic erythematous (atrophic) candidosis* exhibits a diffusely reddened, often relatively dry mucosa beneath the fitting surface of a denture. The infection may develop under any acrylic denture or intra-oral appliance, but is almost exclusively encountered on palatal tissues and gingiva (8, 12, 16, 32, 48). Lesions are frequently asymptomatic, however, patients may complain of slight soreness or burning sensations (48). Chronic erythematous candidosis is the most prevalent form of oral candidosis, with up to 75 % of denture wearers having clinical signs of this condition, although the sufferer is often unaware of the presence of infection (16). Furthermore, atrophic

denture stomatitis is common patients with systemic illnesses (32).

Hyperplastic candidiasis has been also referred to as "candidal leukoplakia". It clinically appears as a well demarcated, slightly elevated, adherent white lesion of the oral mucosa ranging from small translucent lesions to large, dense opaque plaques, that cannot be wiped away by the clinician. The most common location of such lesions is the post-commissural buccal mucosa, and less frequently the tongue, and the palate posterior to upper dentures (8, 12, 16, 20, 48, 52). Hyperplasic candidiasis development is especially of importance in terms of squamous cell carcinoma development in that area. Because, *Candida albicans* induces neoplastic developments by inducting the production of carcinogenic nitrosamines in the saliva (16, 20).

Two clinical types of hyperplastic candidosis have been described based on the appearance of the lesion. Homogeneous hyperplastic candidosis is described as having smooth white lesions that are notably distinct from those of heterogeneous hyperplastic candidosis in which areas of erythema occur resulting in a nodular, speckled appearance. It has been suggested that heterogeneous lesions have a greater likelihood of malignant transformation. Moreover, hyperplastic candidiasis has a strong association with tobacco smoking in addition to the other well-known risk factors. Complete resolution appears to be dependent on cessation of smoking in addition to the other therapeutic measures (16, 48, 52).

Angular cheilitis presents as cracking, peeling, or ulceration involving the corners of the mouth (8, 12, 16, 48). It is often symptomatic and bilateral. It will frequently be seen in combination with erythematous candidiasis in denture wearers (8, 12). Facial skin folds and wrinkling along the labial commissures and nasolabial folds may cause saliva accumulation and a chronic moist environment that predisposes infection (8, 48). Patients with a reduced vertical dimension of occlusion, secondary to severe attrition or worn dentures, are particularly susceptible to the development of angular cheilitis (8). Nutritional factors have an important aetiologic role in the development of these lesions. It is reported that this disease may be caused by candidiasis (20%), mixed candidial bacterial infections (60%), or bacteria alone (20%) (3, 8, 48).

OTHER ORAL CANDIDOSES

Candida has also been implicated in *median rhomboid glossitis* (16). This is a chronic mucosal condition that characteristically presents as a symmetrically shaped lesion on the midline of the dorsum of the tongue (16). This area represents atrophy of the filiform papillae (12, 16, 48).

Chronic mucocutaneous candidosis is a rare, heterogeneous disorder characterized by persistent or recurrent candidal infection of the skin, nails and mucosa. More than 90% of all of these patients exhibit oral candidosis. It is often associated with a variety of endocrinopathies and immunodeficiencies (16, 48).

Candida infections must be differentiated from other lesions with similar clinical presentations. In pseudomembranous variants, differentiation from other lesions with a pseudomembrane is important. These include chemical burns, traumatic lesions, syphilis, and other white keratotic lesions. Solitary erythematous lesions such as erythematous candidosis (acute and chronic forms) should be differentiated from thermal traumatic lesions, erosive lichen planus and lichenoid reactions, lupus erythematosis, erythema multiforme, pernicious anaemia, and epithelial dysplasia (48).

DIAGNOSTICS OF ORAL CANDIDOSIS

Generally, the diagnosis of oral candidosis is based on the clinical recognition of a particular form of *Candida* in conjunction with a thorough medical history (12, 48). Provisional diagnoses are often confirmed through further laboratory testing of clinical specimens. A number of methods for sampling the oral mucosa for the presence of *Candida* have been developed. Such methods include a swab, an imprint culture, the collection of whole saliva, an oral rinse sample and incisional biopsy. Each sampling method has individual advantages and disadvantages, and the choice of technique is governed by the nature of the lesion to be investigated (8, 15, 21, 48).

In clinical practice, two tests are essential to diagnose oral candidiasis. Oral swab obtained from the lesion is usually cultured on the selective medium, for example, Sabouraud's agar, and incubated aerobically for approximately 48 hours. This is combined with oral smear test and direct microscopy following rapid staining. It is widely accepted clinically that combining the presence of the clinical signs suggestive for oral candidiasis and positive results of swab and smear tests is confirmatory for the clinical candidal infection (52).

Differentiation between strains of *Candida* may be required in refractory lesions or in immunocompromised individuals. While some media may differentiate between several species through macroscopic colony characteristics, complete differentiation between different strains of *Candida* requires advanced techniques, such as fluorescent and optical brightening, automated blood culture, molecular methods (polymerase chain

reaction (PCR), nucleic acid sequense-based amplification (NASBA), amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA analysis (RAPDA)) (15). In addition to strain identification, antifungal sensitivity testing may be useful in resistant lesions to determine susceptibility and to direct definitive antifungal therapy (15, 48).

PREVENTION AND TREATMENT

Candida-associated denture stomatitis, even if asymptomatic, should be treated as it may act as reservoir for infections more extensive and encourage the resorption of the alveolar bone. The treatment is difficult and complex due to its multifactorial etiology, common recurrences, as well as lack of antifungal drug efficacy (1, 6, 23, 32, 51). The therapeutic strategy includes the use of topical and systemic antifungal drugs, the use of preservatives and disinfectants, the irradiation with microwaves and the scrupulous removal and control of the plaque present on the denture and on the oral mucosa (1, 23, 51). Therefore, management should be directed at identifying and correcting, if possible, any underlying factors that may predispose or contribute to oral candidosis through a thorough history taking (17, 32, 48).

Antifungal drugs

Recommended antifungal agents for treatment of oral candidosis: topical administration (Nystatin, Amphotericins, Miconazole, Clotrimazole), Systemic administration (Ketoconazole, Fluconazole, Itraconazole) (1, 8, 12, 16, 17, 20, 23, 26, 27, 29, 31, 32, 34, 37, 38, 41, 46, 51, 52). Newer antifungals in the prophylaxis and maintenance of oral candidiasis therapy for invasive fungal infections include Posaconazole, Ravucanazole, and Echinocandins such as Caspofungin, Micafungin, and Anidulafungin (12, 20). Drug choice is dictated by several factors, including the patient's medical history, oral symptoms and predicted compliance with application method (48). Identification of the causative species is essential for the rapid treatment initiation with an appropriate antifungal agent (50). The standard treatment for denture stomatitis is nystatin, which is accompanied with complications such as a bitter taste (41). Intraoral appliances should be removed during the treatment as the medication works topically and must be in contact with the tissue (8). It is widely clinically accepted rule that the patient has to use the nystatin or topical amphotericin B double the time needed for resolution of the clinical signs of infection (52). Note that some studies indicate nystatin to be ineffective for candidal lesions in cancer patients (48). Furthermore, medications specifically for the treatment of angular cheilitis include nystatin ointment, miconazole cream (2%), and clotrimazole cream (1%) (12, 20). However, the diluent effect of saliva and the cleansing action of the oral musculature tend to reduce the concentration of these agents to sub-therapeutic levels (8, 26, 27). Patient education on the use of antifungal therapy is also essential (52).

If treatment is still unsuccessful, treatment with systemic antifungal agents may be deemed necessary (48). Systemic antifungal drugs are expensive and may present some limitations in terms of toxicity and drug interactions (26). Moreover, in patients with reduced saliva production, therapeutic drug levels are difficult to achieve in the oral cavity with systemic therapy (25).

Treatment regimens tend to be prolonged and recurrence rates are high. The major problem associated with the prolonged or recurrent use of antifungal drugs is the development of resistant species. This makes it necessary to seek new therapeutic approaches (17, 26, 27, 34).

Plants and their essential oils

Alternative in using plants and their essential oils is a new trend in treatment *Candida* associated denture stomatitis. These plants are *Pelargonium graveolens*, *Satureja hortensis*, *Zataria multiflora*, *Punica granatum*, *Salvia officinalis*, *Streblus asper*, *Boesenbergia pandurate*, *Phyllanthus emblica*, *Scutellaria baicalensis*, *Azadirachta indica*, *Melaleuca alternifolia*. Natural compounds from essential oils : eugenol, farnesol, geraniol, linalool, menthol, menthone, terpinen- 4-ol, α-terpineol and tyrosol, carvacrol, expressed strong antifungal in vitro activity (19, 51). Their fungistatic and fungicidal activities might convert them into promising alternatives for the topic treatment of oral candidiasis and denture stomatitis, some of them can be included in mouthrinses or toothpastes. (19). Moreover, garlic has a number of physiological effects with lack of side effects, so it could be a suitable substitution for nystatin in healing of oral candidosis, particularly related to denture stomatitis (41, 51). However, potential intolerance and/ or toxic effects of some of these compounds should be taken into consideration (19, 51).

Preservative and disinfectant agents

The chemical method is considered to be the most effective for inhibiting *Candida albicans* infection and denture biofilm formation (44). Chemical denture cleaners are classified into various groups such as alkaline peroxides, alkaline hypochlorite, acids, disinfectants, and enzymes (2, 16, 17, 34, 41, 47). Chlorhexidine gluconate is an antiseptic and disinfectant agent, which is active against various bac-

teria, viruses, bacterial spores, and fungi, and is used in the treatment of chronic erythematous candidosis (8, 12, 16, 25, 47, 48). However, there are reports of reduced efficacy of nystatin when used in combination with chlorhexidine gluconate, and therefore it is often advocated that nystatin treatment be delayed for 30 min after use of chlorhexidine mouthwash (1, 16). The clinical and microbiological efficacy of chlorine dioxide (ClO_2) as a topical antiseptic and disinfectant agent also used for the treatment of chronic atrophic candidiasis in geriatric patients has been assessed. It was discovered that all of the alkaline peroxide tablets reduced *Candida albicans* colonies, but did not completely eliminate them (47). The highest efficacy for the removal of *Candida albicans* biofilms was identified for sodium hypochlorite (NaOCl) (gold standart solution) (36, 39, 48). Unfortunately, sodium hypochlorite may not be used for an indeterminate period of time according to its ability to damage the prosthetic handiwork (1, 36, 39, 45). It was demonstrated that citric acid denture cleanser is effective in reducing *Candida albicans* cell viability in a mature biofilm, immediately after treatments. However, this cleansing solution does not completely remove the biofilm and does not prevent its recolonization after 48 h (36). However, the routine use of denture cleansers has been known to cause adverse effects on physical characteristics of denture materials and resilient liners (34, 44, 45). Goll et al. reported that resilient liners increased discolouration, porosity, surface and size changes, and solubility by the use of denture cleanser for 30 days (44).

Microwave irradiation

As an alternative to regular brushing, irradiation with microwave has been proposed for the denture disinfection (1, 26, 34, 37, 39). Microwave irradiation has proved to be a safe, simple, easy to use , effective, and inexpensive disinfection method that can be used not only to disinfect dentures, but also for the treatment of denture stomatitis (1, 26, 34, 37, 42). The use of a microwave oven does not require special storage, nor does it has an expiry date. It does not induce resistance for fungi or other microorganism and seems not to alter the colour or smell of the dentures (26, 42). Its use prevents the undesirable effects of using antifungal agents, such as nausea, vomiting, and hepatotoxic and nephrotoxic effects (26). Although it cannot be used if the appliances contain metal components (37).

Maximum effectiveness is achieved when the substrate colonized by microorganisms is irradiated immersed in water (8, 42, 45). It has been established that 650 W of microwave irradiation for 3 min is effective against microorganisms that become attached within 24 h, where the extracellular polymeric matrix has not yet structured the biofilm (34, 42, 45). Dentures with larger biofilm areas require longer irradiation exposure to be disinfected (42).

However this treatment is responsible to produce conformational changes on the denture (1). It appears that the detrimental effect of irradiation on a denture's mechanical properties depends on the time of exposure, power of the microwave oven, solution of immersion, frequency of use, and whether or not the denture resin has been auto or microwave polymerised when fabricated (26, 37). Several studies have found that a voltage of 850 W and higher, and an irradiation time of 6 min or longer may have a harmful effect on the mechanical and chemical properties of the acrylic resin and reline materials (16, 26, 37).

Scrupulous removal of denture plaque

The poor oral and denture hygiene are fundamental in the onset of disease, demonstrating the importance of the cleanliness of the denture through mechanical, chemical methods (1, 2, 8, 9, 16-18, 34, 38, 39, 45, 47). Therefore, the treatment must primarily be directed towards the denture and secondarily to the mucosa (26, 42). Mechanical methods comprise brushing, sonic vibrators and ultrasonic (2, 18). Ultrasonic devices are mechanical aids, generally used by professionals. The mechanical cleansing activity of the device is complemented with the concomitant use of a chemical solution. Ultrasonic cleansing presented a discrete antimicrobial effect and was less effective than cleansing tablets for complete denture disinfection (2, 18, 45).

Adequate denture hygiene remains the most important and certain procedure in edentulous patients. They are usually elderly and have difficulty for brushing their teeth (1, 2, 18, 27, 37, 38, 42, 47). A good oral hygiene, including toothbrushing and the use of mouthwashes, can be alone effective in treating denture stomatitis as well as when it is adopted in association with systemic and topical antifungal drugs (1, 2, 8, 16, 23, 27, 34). Moreover, brushing is the most widespread, simple, inexpensive and effective method (2). The hygiene control of denture is also essential , because the porous material or surface biofilm can serve as a reservoir of fungal microorganisms and contribute to relapse or reinfection (1, 2, 8, 16, 23, 27, 34). Both the prosthesis that oral mucosa in contact with it must be involved in procedures for oral hygiene through brushing them after each meal with water or an effective, nonabrasive chemical agents (1, 45). Toothbrushing offers a physical means to combat biofilms, but may be limited to accessible sites within the oral cavity and can have deleterious effects on acrylic

denture surfaces if abrasive toothpastes are used. In such cases, the resulting roughened acrylic surface could, in theory, be more conducive to subsequent biofilm formation (8, 16, 37, 39, 45).

The patients should also be instructed to remove the denture during night and to immerse in a suitable antimicrobial cleansing agent (1, 16, 26, 37). The use of cleaning tablets for acrylic denture overnight storage reduces denture biofilm mass and pathogenicity when compared to dry and water preservation, and may contribute to the overall systemic health (Duyck et al., 2013) (34). Dentures should always be thoroughly rinsed after soaking and brushing with denture-cleansing solutions prior to reinsertion into the oral cavity (16, 45). In addition, during therapy for stomatitis, the prosthesis should be removed for at least two weeks (1).

Probiotic therapy

Recent developments in the treatment of oral candidiasis include the use of probiotic bacteria which are known to reduce the growth of pathogenic microbes. A recent study used cheese containing a mixture of probiotics such as *Lactobacillus rhamnosus GG*, *Lactobacillus rhamnosus LC705*, *Propionibacterium freudenreichii* and *Shermanii JS*. The use of probiotic cheese revealed the reduction of the prevalence of oral *Candida* in the elderly and its use also allowed for the reduction of the prevalence of hyposalivation (3, 12, 16).

Correction predisposing factors

Successful management of patients with oral candidosis requires identification, and where possible correction, of the specific underlying predisposing factors in an individual patient. Without this recognition, subsequent treatment using antifungal therapy may only result in the temporary relief of infection, with relapses inevitably following (16).

All patients should be advised on the importance of reduction or cessation of any smoking habits (16). Any pharmacologic agents that may contribute should be identified, and if practical, substituted for an alternative drug (48). Individuals who use corticosteroid asthma inhalers must rinse their mouths with water after each use to reduce their chances of developing oral candidiasis (8). Any identified nutritional deficiency should be corrected, and advice provided on dietary habits such as appropriate carbohydrate intake (16, 48). Saliva testing should be performed to determine hydration and salivary gland function (48).

Treatment of denture stomatitis also includes denture replacement, especially when dentures are very old (17, 26, 27). This result is in agreement with that of Neppelenbroek et al. , Zomorodian et al. and Figueiral et al. , who found that the time of denture use was related to denture stomatitis. From this standpoint, the provision of new dentures should also be considered during the management of denture stomatitis (27). The prosthetic options for this purpose may include the fixed implant-prosthesis and the implant-retained overdenture as an alternative to conventional dentures, for a better treatment outcome, with a significant improvement of oral function and quality of life (53, 66, 67). Despite the prosthesis on implants has numerous advantages such as better balance, increased functional efficiency, safer mastication, diminished ridge resorption, improved aesthetics, elimination the fear of detachment in speech or mastication (unpleasant aspects, particularly in situations when patients are in the company of others), but it is not a risk-free process and has high costs (66).

Future strategies for management of candidal biofilms

One strategy is to modify the surfaces of biomaterials with components which may reduce the adhesion and inhibit the growth of *Candida* (16, 23). Approaches have included pre-coating biomaterials such as silicone rubber or denture acrylic with chemicals such as silanes, chlorhexidine, histatins, metal nanoparticles (TiO_2, Fe_2O_3, AgNPs) and other surface-modifying groups (14, 16, 54-56). The findings support the use of chlorhexidine-impregnated self-cured PMMA chair-side resin as a new dosage form for the treatment of denture-induced stomatitis. It was indicated that a chlorhexidine-supplemented drug-release device has a powerful capacity to inhibit growth of *Candida albicans* (57).

In addition, thin-film polymer formulations with incorporated antifungals (nystatin, amphotericin, fluconazole, clotrimazole) have also recently been shown to inhibit *Candida albicans* biofilm growth on denture materials (16, 58).

Moreover, resin containing nanoparticles showed a lowered *Candida albicans* adhesion (14). For example, the modified denture base acrylic combined with silver nanoparticles displayed antifungal properties and acted like latent antifungal material itself with low-releasing Ag^+ using tongue and chin muscle action, however, the improvement of poor color stability was still required (38, 54-56, 62, 63). Thus, it was concluded that the addition of silver-zinc zeolite to polymethylmethacrylate can be a valuable alternative for reducing candidal contamination of heat cure acrylic resin denture bases. The addition of 0.5% of silver-zinc zeolite to heat polymerized acrylic resin was enough to provide antifungal activity against the test strains of *Candida albicans* (60). It is important

to mention, that hydrophilic surface modification by the silica coating reduces *Candida albicans* adherence and could contribute to daily denture care (64).

Park, et al. incorporated methacrylic acid into an acrylic denture base resin and observed a reduction in the number of *Candida albicans*. This could be considered as a potential preventive treatment for denture stomatitis (30, 59, 61, 65).

One potential alternative is Photodynamic Therapy (PDT), which uses a photosensitizing agent and light of appropriate wavelength. The interaction between the photosensitizer and light in the presence of oxygen produces reactive species, which cause cell damage and death. Organisms resistant to conventional antifungal agents could be successfully killed by PDT, and it seems unlikely that they will develop resistance to such a therapy. PDT is effective against oral species and may not promote damage to host cells and tissues. Investigations have shown that *Candida* spp. are susceptible to photoinactivation, including resistant strains Therefore, PDT seems to be a promising method for the treatment of denture stomatitis (17, 24, 27).

Recurrence of the oral candidal infection

Recurrence of the oral candidal infection constitutes a frustration and disappointment for both the clinician and the patient. One study estimated that around 20% of patients with oral candidiasis experience infection recurrence. If the superficial oral candidal infection was not well managed in severe immunosuppression, the patient may become susceptible to esophageal spread of infection or to the potentially lethal systemic candidemia (52). Systemic antifungal therapy is usually not sufficient to treat candidosis, because of re-infection commonly caused by the use of a contaminated denture (17, 19, 26, 27, 37, 42). Moreover, the widespread use of antifungal agents has resulted in the development of resistant species (17). A common malpractice is that once a predisposing factor was identified the treating dentist may not follow up other factors which may lead to unsatisfactory treatment and persistence of the infection (34, 52).

CONCLUSIONS

Candida albicans is the most common causative species in *Candida*-associated denture stomatitis. This inflammatory reaction is popular in general dental practice. It manifests in a variety of clinical presentations. Management of oral candidosis is difficult, complex and should always include a thorough investigation of underlying predisposing conditions to avoid its recurrence or systemic spread.

REFERENCES

1. Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. Candida-associated denture stomatitis. *Med Oral Patol Oral Cir Bucal* 2011;16:e139-43.
2. Cruz PC, Andrare IM, Peracini A, Souza-Gugelmin MCM, Silva-Lovato CH, Souza RF, et al. The effectiveness of chemical denture cleansers and ultrasonic device in biofilm removal from complete dentures. *J Appl Oral Sci* 2011;19:668-73.
3. Thein ZM, Seneviratne CJ, Samaranayake YH, Samaranayake LP. Community lifestyle of Candida in mixed biofilms: a mini review. *Mycoses* 2009; 52:467-75.
4. Javed F, Klingspor L, Sundin U, Altamash M, Klinge B, Engstrom PE. Periodontal conditions, oral Candida albicans and salivary proteins in type 2 diabetic subjects with emphasis on gender. *BMC Oral Health* 2009; 9:12.
5. Arirachakaran P, Piboonratanakit P, Kiatkroekkrai P, Sornmai M, Srimart N. Prevalence of oral Candida carriage in denture wearers. *CU Dent J* 2009;32:101-12.
6. Nett JE, Marchillo K, Spiegel CA, Andes DR. Development and validation of an in vivo candida albicans biofilm denture model. *Infec Immun* 2010;78:3650-9.
7. Zhu1 W, Filler SG. Interactions of Candida albicans with epithelial cells. *Cell Microbiol* 2010;12:273-82.
8. Dangi YS, Soni ML, Namdeo KP. Oral candidiasis: a review. *Int J Pharm Pharm Sci* 2010;2:3641.
9. Ryu M, Ueda T, Saito T, Yasui M, Ishihara K, Sakurai K. Oral environmental factors affecting number of microbes in saliva of complete denture wearers. J Oral Rehabil 2010;37: 194-201.
10. Darwazeh AMG, Al-Dwairi ZN, Al-Zwairi AA. The Relationship between tobacco smoking and oral colonization with Candida species. *J Contemp Dent Pract* 2010;11:17-24.
11. Naik AV, Pai RC. A Study of factors contributing to denture stomatitis in a North Indian Community. *Int J Dent* 2011; 2011:589064.
12. Abraham CM. Advances and emerging techniques in the identification, diagnosis and treatment of oral candidiasis. *Open Path J* 2011;5: 8-12.
13. Zomorodian K, Haghighi NN, Rajaee N, Pakshir K, Tarazooie B, Vojdani M, et al. Assessment of Candida species colonization and denture-related stomatitis in complete denture wearers. *Med Mycol* 2011;49:208-11.
14. Acosta-Torres LS, Lopez-Marin LM, Nunez-Anita RE, Hernandez-Padron G, Castano VM. Biocompatible metal-oxide nanoparticles: nanotechnology improvement of conventional prosthetic acrylic resins. *J Nanomaterials* 2011;2011:941561.
15. Ghani F, Chughtai MA, Shah SA. Biochemically assessed pathological activity of oral Candida in denture and non denture wearers. *J Postgrad Med Inst* 2011;25:188-98.
16. Williams DW, Kuriyama T, Silva S, Malic S, Lewis MAO. Candida biofilms and oral candidosis: treatment and prevention. *Periodontology 2000* 2011; 55:250-65.
17. Mima EGO, Pavarina AC, Silva MM, Ribeiro DG, Vergani CE, Kurachi C, et al. Denture stomatitis treated with photodynamic therapy: five cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;112:602-8.
18. Andrade IM, Cruz PC, Silva CHL, Souza RF, Paranhos HFO, Candido RC, et al. Effervescent tablets and ultrasonic devices against Candida and mutans streptococci in denture biofilm. *Gerodontology* 2011;28:264-70.
19. Marcos-Arias C, Eraso E, Madariaga L, Quindos G. In vitro activities of natural products against oral Candida isolates from denture wearers. *BMC Complement Altern Med* 2011;11:119.
20. Abaci O, Haliki-Uztan A. Investigation of the susceptibility

of Candida species isolated from denture wearers to different antifungal antibiotics. *Afr J Microbiol Res* 2011;5:1398-403.
21. Hoshi N, Mori H, Taguchi H, Taniguchi M, Aoki H, Sawada T, et al. Management of oral candidiasis in denture wearers. *J Prosthodont Res* 2011;55:48-52.
22. Coenye T, Prijck KD, Nailis H, Nelis HJ. Prevention of Candida albicans biofilm formation. *Open Mycol J* 2011;5:9-20.
23. Hoshing C, Dixit S, Mootha A, Diwan N. Role of Candida albicans in denture stomatitis. *J Indian Acad Oral Med Radiol* 2011;23:617-9.
24. Lazarin AA, Zamperini CA, Vergani CE, Wady AF, Giampaolo ET, Machado AL. Candida albicans adherence to an acrylic resin modified by experimental photopolymerised coatings: an in vitro study. *Gerodontology* 2014;31:25-33.
25. Salim N, Moore C, Silikas N, Satterthwaite J, Rautemaa R. Candidacidal effect of fluconazole and chlorhexidine released from acrylic polymer. *J Antimicrob Chemother* 2013; 68:587-92.
26. Silva MM, Mima EGO, Colombo AL, Sanita PV, Jorge JH, Massucato EMS, et al. Comparison of denture microwave disinfection and conventional antifungal therapy in the treatment of denture stomatitis: a randomized clinical study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;114:469-79.
27. Mima EG, Vergani CE, Machado AL, Massucato EMS, Colombo AL, Bagnato VS, et al. Comparison of photodynamic therapy versus conventional antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial. *Clin Microbiol Infect* 2012;18: 380-8.
28. Kanaguchi N, Narisawa N, Ito T, Kinoshita Y, Kusumoto Y, Shinozuka O, et al. Effects of salivary protein flow and indigenous microorganisms on initial colonization of Candida albicans in an in vivo model. *BMC Oral Health* 2012;12:36.
29. Marra J, Paleari AG, Rodriguez LS, Leite ARP, Pero AC, Compagnoni MA. Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. *J Appl Oral Sci* 2012;20: 643-8.
30. Lazarin AA, Machado AL, Zamperini CA, Wady AF, Spolidorio DMP, Vergani CE. Effect of experimental photopolymerized coatings on the hydrophobicity of a denture base acrylic resin and on Candida albicans adhesion. *Arch Oral Biol* 2013;58:1-9.
31. Loster BW, Loster J, Wieczorek A, Ryniewicz W. Mycological analysis of the oral cavity of patients using acrylic removable dentures. *Gastroenterol ResPract* 2012;2012:951572.
32. Chopde N, Jawale B, Pharande A, Chaudhari L, Hiremath V, Redasani R. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. *J Contemp Dent Pract* 2012;13:456-9.
33. Dandekeri S, Prasad K, Shetty M, Hegde C, Sowmya MK, Jagadeesh M. Occurrence of streptococcus and Candida species and salivary ph in patients wearing complete denture. *Int J Health Rehabil Sci* 2013;2:198-203.
34. Dantas APFM, Consani RLX, Sardi JCO, Mesquita MF, Silva MCVS, Sinhoreti MAC. Biofilm formation in denture base acrylic resins and disinfection method using microwave. *J Res Pract Dent* 2014;2014:112424.
35. Semlali A, Killer K, Alanazi H, Chmielewski W, Rouabhia M. Cigarette smoke condensate increases C. albicans adhesion, growth, biofilm formation, and EAP1, HWP1 and SAP2 gene expression. *BMC Microbiol* 2014;14:61.
36. Faot F, Cavalcanti YW, Bertolini MM, Pinto LR, Silva WJ, Del Bel Cury AA. Efficacy of citric acid denture cleanser on the Candida albicans biofilm formed on poly(methylmethacrylate): effects on residual biofilm and recolonization process. *BMC Oral Health* 2014;14:77.
37. Brondani MA, Samim F, Feng H. A conventional microwave oven for denture cleaning: a critical review. *Gerodontology* 2012;29:6-15.
38. Nam KY, Lee CH, Lee CJ. Antifungal and physical characteristics of modified denture base acrylic incorporated with silver nanoparticles. *Gerodontology* 2012;29:413-9.
39. Hahnel S, Rosentritt M, Burgers R, Handel G, Lang R. Candida albicans biofilm formation on soft denture liners and efficacy of cleaning protocols. *Gerodontology* 2012;29:383-91.
40. Yasui M, Ryu M, Sakurai K, Ishihara K. Colonisation of the oral cavity by periodontopathic bacteria in complete denture wearers. *Gerodontology* 2012;29:494-502.
41. Bakhshi M, Taheri JB, Shabestari SB, Tanik A, Pahlevan R. Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouthwash in denture stomatitis. *Gerodontology* 2012; 29:680-4.
42. Senna PM, Silva WJand, Del Bel Cury AA Denture disinfection by microwave energy: influence of Candida albicans biofilm. *Gerodontology* 2012;29:186-91.
43. Sahin C, Ergin A, Ayyildiz S, Cosgun E, Uzun G. Effect of biofilm formation, and biocorrosion on denture base fractures. *J Adv Prosthodont* 2013;5:140-6.
44. Huh JB, Lim Y, Youn HI, Chang BM, Lee JY, Shin SW. Effect of denture cleansers on Candida albicans biofilm formation over resilient liners. *J Adv Prosthodont* 2014;6:109-14.
45. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, et al. Evidence-based guidelines for the care and maintenance of complete dentures: A publication of the American College of Prosthodontists. *J Am Dent Assoc* 2011;142:S1-20.
46. Offenbacher S, Barros, Altarawneh S, Beck JD, Loewy ZG. Impact of tooth loss on oral and systemic health. *J Gen Dent* 2012; 6:494-500.
47. Uludamar A, Ozkan YK, Kadir T, Ceyhan I. In vivo efficacy of alkaline peroxide tablets and mouthwashes on Candida albicans in patients with denture stomatitis. *J Appl Oral Sci* 2010; 18:291-6.
48. Farah CS, Lynch N, McCullough MJ. Oral fungal infections: an update for the general practitioner. *Aust Dent J* 2010;55:48-54.
49. Bhat V, Sharma SM, Shetty V, Shastry CS, Rao V, Shenoy SM, et al. Prevalence of Candida associated denture stomatitis (CADS) and speciation of Candida among complete denture wearers of south west coastal region of Karnataka. *NUJHS* 2013;3:59-63.
50. Sampaio-Maia B, Figueiral MH, Sousa-Rodrigues P, Fernandes MH, Scully C. The effect of denture adhesives on Candida albicans growth in vitro. *Gerodontology* 2012;29:348-56.
51. Petrovic M, Kostic M, Kostic M, Krunic N, Igic M, Pesic Z, et al. Therapeutic alternatives of natural compounds in treatment of Candida-associated denture stomatitis. *Acta Med Medianae* 2014;53:73-9.
52. Darwazeh AMG, Darwazeh TA. What makes oral Candidiasis recurrent infection? a clinical view. *J Mycol* 2014;2014:758394.
53. Stilwell C. Risk management in clinical practice. Part 6b. Identifying and avoiding medico-legal risks in removable dentures. *Br Dent J* 2010;209:339-50.
54. Acosta-Torres LS, Mendieta I, Nunez-Anita RE, Cajero-Juarez M, Castano VM. Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. *Int J Nanomedicine* 2012;7:4777-86.
55. Monteiro DR, Silva S, Negri M, Gorup LF, Camargo ER, Oliveira R, et al. Silver nanoparticles: influence of stabilizing agent and diameter on antifungal activity against Candida albicans and Candida glabrata biofilms. *Lett Appl Microbiol* 2012;54:383-91.
56. Chladek G, Mertas A, Barszczewska-Rybarek I, Nalewajek T, Zmudzki J, Krol W, et al. Antifungal activity of denture soft lining material modified by silver nanoparticles - a pilot study. *Int J Mol Sci* 2011;12:4735-44.
57. Ryalat S, Darwish R, Amin W. New form of administering chlorhexidine for treatment of denture-induced stomatitis. *Ther Clin Risk Manag* 2011;7:219-25.
58. Salim N, Moore C, Silikas N, Satterthwaite J, Rautemaa R. Candidacidal effect of fluconazole and chlorhexidine released from acrylic polymer. *J Antimicrob Chemother* 2013; 68:587-92.
59. Cao Z, Sun X, Yeh CK, Sun Y. Effects of Methacrylic Acid on physical/mechanical properties and biocompatibility of urethane-based denture biomaterials. *Mater Sci Appl* 2011; 2: 1070-5.
60. Mutneja P, Raghavendraswamy KN, Gujjari AK. Antifungal activity of heat cure acrylic resin after incorporating different persentages of silver zinc zeolite - an in-vitro study. *Int J Clin*

Cases Invest 2012;4:49-54.
61. Paleari AG, Marra J, Pero AC, Rodriguez LS, Ruvolo-Filho A, Compagnoni MA. Effect of incorporation of 2-tert-butylaminoethyl methacrylate on flexural strength of a denture base acrylic resin. *J Appl Oral Sci* 2011;19:195-9.
62. Monteiro DR, Gorup LF, Takamiya AS, Camargo ER, Filho ACR, Barbosa DB. Silver distribution and release from an antimicrobial denture base resin containing silver colloidal nanoparticles. *J Prosthodont* 2011;21:7-15.
63. Busscher HJ, Rinastiti M, Siswomihardjo W, Mei HC. Biofilm formation on dental restorative and implant materials. *J Dent Res* 2010;89:657-65.
64. Azuma A, Akiba N, Minakuchi S. Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on Candida albicans adherence. *J Med Dent Sci* 2012;59:1-7.
65. Cao Z, Sun X, Yeh CK, Sun Y. Rechargeable Infection-responsive Antifungal denture materials. *J Dent Res* 2010;89:1517-21.
66. Melescanu Imre M, Marin M, Proteasa E, Tancu AM, Proteasa CT. Two implant overdenture - the first alternative treatment for patients with complete edentulous mandible. *J Med Life* 2011;4:207-9.
67. Shahmiri RA, Atieh MA. Mandibular Kennedy Class I implant-tooth-borne removable partial denture: a systemic review. *J Oral Rehabil* 2010;37:225-34.
68. Naglik JR, Moyes DL, Wachtler B, Hube B. Candida albicans interactions with epithelial cells and mucosal immunity. *Microbes Infect* 2011;13: 963-76.
69. Weindl G, Wagener J, Schaller M. Epithelial cells and innate antifungal defence. *J Dent Res* 2010; 89(7): 666-675.
70. Cheng SC, Joosten LAB, Kulberg BJ, Netea MG. Interplay between Candida albicans and the Mammalian Innate Host Defence. *Infect Immun* 2012;80:1304-13.
71. Dalle F, Wachtler B, L'Ollivier C, Holland G, Bannert N, Wilson D, et al. Cellular interactions of Candida albicans with human oral epithelial cells and enterocytes. *Cell Microbiol* 2010;12:248-71.
72. Naglik JR, Moyes D. Epithelial cell innate response to Candida albicans. *Adv Dent Res* 2011;23:50-5.
73. Gomes PS, Fernandes MH. Defensins in the oral cavity: distribution and biological role. *J Oral Pathol Med* 2010;39:1-9.
74. Gow NAR, Veerdonk FL, Brown AJP, Netea MG. Candida albicans morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* 2012;10:112-22.
75. Gauglitz GG, Callenberg H, Weindl G, Korting HC. Host defence against Candida albicans and the role of pattern-recognition receptors. *Acta Derm Venereol* 2012;92:291-8.
76. Fabian TK, Hermann P, Beck A, Fejerdy P, Fabian G. Salivary defense proteins: their network and role in innate and acquired oral immunity. *Int J Mol Sci* 2012;13:4295-320.
77. Conti HR, Gaffen SL. Host responses to Candida albicans: Th17 cells and mucosal candidiasis. *Microbes Infect* 2010;12:518-27.
78. Zhu W, Filler SG. Interactions of Candida albicans with epithelial cells. *Cell Microbiol* 2010; 12:273-82.

Received: 14 09 2014
Accepted for publishing: 26 06 2015