

Candida albicans Denture Biofilm and its Clinical Significance

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Abstract

Fungi belonging to *Candida* genus, especially *C. albicans* play an important role in microflora of oral cavity. Microbial colonisation process taking place within oral cavity is inseparably related to formation of multispecies biofilm, *i.e.* dental and denture plaque. A mature fungal biofilm is a heterogeneous three-dimensional dense conglomeration of mixture of different morphological forms: blastospores, germ tubes, pseudohyphae and hyphae surrounded by the extracellular polymeric matrix. Composition and specific properties of substratum, saliva and yeasts as well as multiple intricate interactions between all of them influence the ability of *Candida* spp. isolates to adhere and colonise both natural and artificial surfaces, followed by biofilm formation. Obviously, specific complex host-pathogen interactions also should not be neglected. A lot of additional factors like poor oral and denture hygiene, low pH under prosthesis, sufficient concentration of sugar and iron or antibody titres influence *Candida* adhesion and colonisation of acrylic resin base. *C. albicans* is capable of inducing a variety of superficial diseases of the oral mucosa. The most common clinical form of oral candidal infection related to biofilm formation affecting a great deal of denture wearers is denture-associated stomatitis, also known as chronic atrophic candidiasis or erythematous candidiasis. Development of *C. albicans* biofilm on a denture surface constitutes a difficult and hard to resolve problem which may concern every single prosthesis-wearer. Thus, careful oral and denture hygiene is highly recommended for the population of artificial teeth wearers.

Key words: *Candida albicans*; denture acrylic resin; denture-associated stomatitis; denture biofilm

I. Introduction

Oral cavity is a specific environment naturally colonised by a large variety of different groups of microorganisms: bacteria, fungi, protozoa and viruses (Coulthwaite and Verran, 2007). About 10^9 CFU (Colony Forming Units) of microbes are found in 1 ml of saliva (Busscher *et al.*, 2010). Despite significant predominance of bacteria, fungi belonging to *Candida* genus also exert very important role in oral ecosystem. Amongst them, the most frequently isolated species is *Candida albicans*, detected in oral cavity of *ca.* 20% of healthy population (Ruby and Barbeau, 2002), followed by *Candida glabrata*, *Candida tropicalis*, *Candida kefyr*, *Candida parapsilosis*, *Candida krusei*, *Candida dubliniensis*, *Candida famata*. The carriage rate of *C. albicans* may be considerably increased by hospitalization, immunosuppression, broad spectrum antibiotic treatment, xerostomia (dry mouth), AIDS, usage of steroids inhalers, wearing of dentures or some other orthodontic appliances. Additionally, the factors compromising the physiological conditions of the host

frequently initiate the transformation of *C. albicans* isolates from commensal organisms to pathogenic ones (Coulthwaite and Verran, 2007; Busscher *et al.*, 2010; Ruby and Barbeau, 2002; Kaczała *et al.*, 2008). Microbial colonisation process taking place within oral cavity is inseparably related to dental and denture plaque. Dental plaque can be defined as a “diverse community of microorganisms found on the tooth surface as a biofilm” (Marsh, 2004) whereas denture plaque can be defined as a “biofilm that is formed on the surface of the denture and contains 10^{11} – 10^{12} microorganisms per 1 g of wet weight” (Kawasaki *et al.*, 2011). *Candida* spp. isolates are detected more often in the material obtained from denture plaque than from the plaque developed on the natural teeth (Coulthwaite and Verran, 2007; Lamfon *et al.*, 2005). The fitting surface of the upper denture is the primary site of *Candida* spp. harbouring as compared to the palatal mucosa which is in contact with the denture (Coulthwaite and Verran, 2007; Ruby and Barbeau, 2002; Lamfon *et al.*, 2005; Pusateri *et al.*, 2009; Daniluk *et al.*, 2006). It was reported that oral *Candida* spp. are capable of infiltrate into the dental

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resin material as well (Kawasaki *et al.*, 2011). Under physiological conditions, the flushing action of saliva along with mechanical cleaning by a tongue efficiently detach microorganisms that are not firmly attached to the oral surfaces. Unfortunately, the area beneath the movable prosthesis is isolated, thus considerably devoid of these natural defence mechanisms of the oral cavity (Coulthwaite and Verran, 2007; Lamfon *et al.*, 2005; Daniluk *et al.*, 2006; Pereira-Cenci *et al.*, 2008). Furthermore, increased temperature and humidity under the prosthesis supports adhesion of microorganisms (Kaczała *et al.*, 2008).

***Candida albicans* biofilm development**

Introduction of a removable prosthesis, that is a foreign body, into mouth alters environmental conditions of the oral cavity which imbalances the local microflora (Lamfon *et al.*, 2005; Daniluk *et al.*, 2006; Zomorodian *et al.*, 2011). Synthetic polymers (acrylic resins), that dentures are made of, present an additional hard porous surface with high rate of adsorption that supports fungal growth, adherence and development of plaque (Coulthwaite and Verran, 2007). The adherence of *C. albicans* to host cells and abiotic surfaces as denture acrylic resin or soft lining materials is the first, early stage (0–11 h) of the whole 3-phases process of biofilm formation. Non-specific bonds such as electrostatic and Van der Waals forces, hydrogen and covalent linkages along with the surface tension play the essential role at the initial attachment of *Candida* cells to biomaterials. Some of *Candida* spp. cell wall proteins coded by for example genes from ALS (agglutinin-like sequence) family serve as receptors that recognise host ligands in the conditioning film of saliva or serum (Kaczała *et al.*, 2008; ten Cate *et al.*, 2009). After approximately 3–4 h of the initial adherence, formation of microcolonies on the colonised surface takes place (Coenye *et al.*, 2011). During the second, intermediate stage (12–30 h) proliferation of the attached cells and development of extracellular matrix is observed. The last stage, referred to as biofilm maturation (30–72 h), is characterised by further development of extracellular matrix and production of yeast colonies (Nett and Andes, 2006; Emira *et al.*, 2011; Ramage *et al.*, 2005) as well as their transition to filamentous forms (pseudohyphae and hyphae). Filamentous forms provide biofilm integrity and enable to form a multilayered spatially organised structure (Ramage *et al.*, 2005).

A mature fungal biofilm is a heterogeneous three-dimensional dense conglomeration of mixture of different morphological forms: blastospores, germ tubes, pseudohyphae and hyphae surrounded by the extracellular polymeric matrix of polysaccharides, carbohydra-

tes, proteins and other unknown components. It possesses a complicated system of channels that enables flow of water and nutrients as well as efflux of metabolites or waste products. Yeast cells retained within a biofilm differ from their planktonic counterparts in physiological and metabolic properties as well as in reduced mobility (Kaczała *et al.*, 2008; Coenye *et al.*, 2011; Nett and Andes, 2006; Ramage *et al.*, 2005; Thein *et al.*, 2009). They are able to detach from the biofilm surface, become free-floating and colonise new places. This phase is known as biofilm dispersal (Coenye *et al.*, 2011).

Oral biofilms are not random mixtures of microorganisms (Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009). Despite the fact that yeasts constitute only a small percentage of all strains isolated from a denture plaque, their mass within the plaque biofilm is significant due to their size (Coulthwaite and Verran, 2007). *C. albicans* isolates are able to co-aggregate with oral streptococci (Ruby and Barbeau, 2002; ten Cate *et al.*, 2009) and colonise acrylic surfaces more easily if bacteria such as *Streptococcus mutans*, *S. gordonii*, *S. sanguinis*, *S. salivarius* already settle the area (Ruby and Barbeau, 2002). Several studies confirmed that bacterial adhesion enhances subsequent adhesion of *Candida* (Busscher *et al.*, 2010; Lamfon *et al.*, 2005; Daniluk *et al.*, 2006; Pereira-Cenci *et al.*, 2008). After introduction of an acrylic surface into the oral cavity or after cleaning it, bacteria colonise the material as first, within hours. Later, after days, yeasts appear. It was found that *C. albicans* is one of the microorganisms that are able to bind to the antigen I/II, a cell-wall-anchored protein receptor of the majority of commensal oral *Streptococcus* species (Busscher *et al.*, 2010; Pereira-Cenci *et al.*, 2008). In the mixed bacterial-yeast communities microorganisms are able to interact *via* extraction of quorum sensing (QS) small soluble molecules which accumulate in the extracellular matrix and are responsible for coordination of physiology and homeostasis. Biofilm development, existence, disintegration along with both intercellular and host-microorganisms interactions are determined by this quorum-sensing phenomenon to a significant extent. When the concentration of QS molecules is sufficiently high, they induce a suitable genetic response from the local cells (Kaczała *et al.*, 2008; Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009; Nett and Andes, 2006).

Factors determining denture biofilm formation by *C. albicans*

Composition and specific properties of substratum, saliva and yeasts as well as multiple intricate interactions between all of them influence the ability of *Candida* spp. isolates to adhere and colonise. Obviously, specific complex host-pathogen interactions also

should not be neglected (Kaczala *et al.*, 2008; Pereira-Cenci *et al.*, 2008; Nett and Andes, 2006; Emira *et al.*, 2011; Salerno *et al.*, 2011).

Nearly all *in vivo* studies indicate that rough surfaces attract more biofilm than smooth ones (Busscher *et al.*, 2010); 0.2 µm is a threshold roughness value proposed by Quirynen *et al.* (1990), as the point below which no effect on the adhesion should be expected. Surface irregularities entrap *Candida* spp. cells, allow them to settle and protect fungal cells while denture is being cleaned, giving them enough time to attach irreversibly (Pereira-Cenci *et al.*, 2008). Besides, presence of a hard removable prosthesis facilitates development of local microtrauma which leads to inflammatory reaction and additionally increases colonisation of yeasts (Kaczala *et al.*, 2008; Zomorodian *et al.*, 2011). In order to prevent irritation of the mucosa and improve patient's comfort, some dentures are enriched in soft lining materials such as silicone. However, a porous structure of silicone material increases microbial adhesion (Coulthwaite and Verran, 2007; Pereira-Cenci *et al.*, 2008; Zomorodian *et al.*, 2011; ten Cate *et al.*, 2009; Hahnel *et al.*, 2012). It was demonstrated that *C. albicans* retention was greater on silicone liners than on denture base acrylics (von Fraunhofer and Loewy, 2009; Hahnel *et al.*, 2012). Attached fungi destroy the surface of the liner and deprive it of the cushioning properties. As a consequence, local tissues irritation occurs, partially because of increased surface roughness and partially because of high concentration of exotoxins and metabolic products of fungal cells (Coulthwaite and Verran, 2007; Pereira-Cenci *et al.*, 2008; Zomorodian *et al.*, 2011; ten Cate *et al.*, 2009).

Hydrophobicity of acrylic resin along with surface charge and surface free energy also implicate in the adhesion of *Candida* spp. (Kaczala *et al.*, 2008; Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009). It was found that the higher surface free energy, the higher adhesion of microorganisms is observed and the more hydrophobic surface the less adherence is expected (Pereira-Cenci *et al.*, 2008).

Sometimes, the salivary pellicle layer may be more relevant than the surface properties of the dental material itself (Pusateri *et al.*, 2009; Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009; Nett and Andes, 2006). Although some materials do not support biofilm growth, after introduction into mouth they are at once covered by a salivary conditioning film that can mask their properties and allow microorganisms to form a biofilm (Kaczala *et al.*, 2008; Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009). On the other hand, formation and composition of the conditioning film may be specific to the host and to the dental material, since the substratum characteristics influence the response of host proteins (Pereira-Cenci *et al.*, 2008; Nett and Andes, 2006).

According to the results of a number of performed studies, the role of saliva coating may be divergent – several researches indicated that it reduces the adherence of *C. albicans* to acrylic resin, the others showed the opposite, while the further investigators found no effect at all (Pusateri *et al.*, 2009; Pereira-Cenci *et al.*, 2008; ten Cate *et al.*, 2009; Nett and Andes, 2006; Salerno *et al.*, 2011). Additionally, a dynamic effect depending on the morphological phase of *C. albicans* was observed – at first, adherence was increased but after 24 h it was decreased. Such surprising outcomes probably result from the dissimilarities of the studies conditions, that is different incubation periods and saliva temperatures, the presence/absence of nutrients, use of filtered/whole saliva. The very components of saliva interact differently with *Candida* species: lysozyme, histatin, lactoferrin, calprotectin and IgA decrease their adherence and colonisation of oral surfaces, whereas mucins, statherin and proline-rich-proteins facilitate both *C. albicans* adherence to resins and the subsequent biofilm formation (Kaczala *et al.*, 2008; Pusateri *et al.*, 2009; Pereira-Cenci *et al.*, 2008; Nett and Andes, 2006; Salerno *et al.*, 2011). The use of stimulated versus unstimulated saliva results in different protein composition and viscosity hence different study outcomes were obtained. Similarly, denture-wearers inter-individual variations in saliva composition and its secretion rate should also be taken into consideration. Busscher *et al.* (1997) concluded that the low molecular weight proteins are supposed to be related to the adherence level of *Candida* spp. Higher *Candida* spp. counts was found in patients with low or impaired salivary flow or/and composition comparing with normal salivary flow. This is in agreement with another *in vivo* study demonstrating that different subjects present different biofilm formation rate, architecture and density (Pereira-Cenci *et al.*, 2008). Apart from its influence on the adhesion process, saliva conditioning may facilitate the diffusion of nutrients into a biofilm system (Pusateri *et al.*, 2009; Pereira-Cenci *et al.*, 2008).

Microbial adhesion also depends on physicochemical properties of the surface of microbial cell that is the surface charge and hydrophobicity (Pereira-Cenci *et al.*, 2008; Emira *et al.*, 2011; Ramage *et al.*, 2005). Higher adherence of some non-*albicans* species (*C. tropicalis*, *C. glabrata*, *C. dubliniensis*) is explained by their relative surface free energy values. Similarly, more hydrophobic microorganisms like *C. glabrata* seem to be more adherent to acrylic surfaces than *C. albicans* (Pereira-Cenci *et al.*, 2008). On the other hand, *C. albicans* isolates produce enzymes which help them to adhere to the host tissues or prosthesis and subsequently form a biofilm. Slime production is another virulence factor of *Candida* strains that contributes to microbial colonisation (Emira *et al.*, 2011).

A lot of additional factors like poor oral and denture hygiene, low pH under prosthesis, sufficient concentration of sugar and iron or antibody titres influence *Candida* adhesion and colonisation of acrylic resin base (Kaczala *et al.*, 2008; Pereira-Cenci *et al.*, 2008; Emira *et al.*, 2011).

Clinical significance of *C. albicans* denture biofilm and its prevention

Facilitated biofilm formation on the abiotic prosthesis causes high-risk of oral diseases among denture-wearing population. High risk population includes elderly people, often physically or mentally infirm, malnourished, staying in long-term hospitals or in nursing homes, who are not able to care about their oral hygiene by themselves and have to depend on healthcare professionals or family. Malnutrition and poor denture hygiene like inaccurate denture cleaning, lack of regular check-ups, smoking, not removing denture at night are known factors responsible for diverse health problems associated with denture wearing (Coulthwaite and Verran, 2007; Gendreau and Loewy, 2011).

C. albicans is capable of inducing a variety of superficial diseases of the oral mucosa. Indeed, they are not serious but frequent, recurring and troublesome. The most common clinical form of oral candida infection affecting a great deal of denture wearers is denture-associated stomatitis (DAS), also known as chronic atrophic candidiasis or erythematous candidiasis (Busscher *et al.*, 2010; Zomorodian *et al.*, 2011; Emira *et al.*, 2011; Thein *et al.*, 2009; Salerno *et al.*, 2011; Gendreau and Loewy, 2011). The disease may be promoted by poorly fitting dentures, trauma, low levels of pH (the most optimal pH is equal to 3), qualitative and quantitative alterations of the salivary flow, age, smoking, immunosuppression, repeated treatment with antibiotics and sulphonamides, diabetes, HIV infection or poor general health (Coulthwaite and Verran, 2007; Ruby and Barbeau, 2002; Kaczala *et al.*, 2008; Pusateri *et al.*, 2009; Zomorodian *et al.*, 2011). Although *C. albicans* is still recognised as the most predominant etiological agent, the other *Candida* species, *i.e.* *C. glabrata*, *C. dubliniensis*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* along with bacteria from several genera may be implicated in the pathogenesis (Busscher *et al.*, 2010; Lamfon *et al.*, 2005; Daniluk *et al.*, 2006; Salerno *et al.*, 2011). These inflammatory conditions affecting the mucosa under the movable prosthesis may be associated by leukoplakia, pseudomembrane formation and erythema, and sometimes is accompanied by other forms of candidiasis: angular cheilitis, median rhomboid glossitis, chronic hyperplastic candidiasis (Lamfon *et al.*, 2005; Daniluk *et al.*, 2006; Zomorodian *et al.*, 2011). Relationship between dura-

tion of denture use and increased occurrence rate of candidosis was noted (Zomorodian *et al.*, 2011). There are reports that sideropenic anaemia and high level of cholesterol contribute to development of candidiasis. Moreover, high level of carbohydrates in saliva is considered as an additional nourishing source for *Candida* yeasts (Salerno *et al.*, 2011).

Clinical studies have shown that the symptoms of denture stomatitis often return soon after cessation of treatment, suggesting that denture plaque may serve as a niche for *C. albicans* isolates resistant to antifungal drugs (Salerno *et al.*, 2011; Chandra *et al.*, 2001). Furthermore, the continuous swallowing and aspiration of microorganisms from denture plaque may be dangerous particularly for the people in poor general health who are at risk of development of systemic diseases and remote infections (Coulthwaite and Verran, 2007). *Candida* infections are probably involved in the development of caries, root caries and periodontitis of the remaining teeth (Coulthwaite and Verran, 2007; ten Cate *et al.*, 2009; Andruccioli *et al.*, 2004). Microbial colonization and/or their secreted metabolites are also likely to cause allergic reactions (Zomorodian *et al.*, 2011).

Despite the fact that removable prosthesis may be subjected to stricter cleaning procedures than natural teeth or non-removable orthodontic appliances, lots of denture wearers neglect oral hygiene. Brushing with a paste or soap is the most common form of denture cleaning. Apart from that, there are a number of denture cleansers containing alkaline peroxides, alkaline hypochlorites, dilute acids, disinfectants and enzymes. Amongst mechanical methods of denture cleaning microwaving, ultrasonication along with abrasive pastes or powders are applied (Coulthwaite and Verran, 2007). Unfortunately, most available denture cleaning products are not very efficient in the control and removal of denture biofilm (Andruccioli *et al.*, 2004). Alkaline peroxide denture cleansers appeared to be unsuccessful in removing *Candida* spp. biofilm from denture liner as well as in preventing biofilm recolonisation. Although elimination of *C. albicans* cells was obtained after use of sodium hypochlorite solution (Coelho Vieira *et al.*, 2010; da Silva *et al.*, 2011; Dahlan *et al.*, 2011; Hahnel *et al.*, 2012), this product is not destined for daily denture immersion since it may bleach the denture base and cause corrosion of the metal elements of the partial prosthesis (Coelho Vieira *et al.*, 2010). In spite of the fact that brushing removes denture biofilm more effectively than immersion cleansing, using a toothbrush with abrasive dentifrices should not be recommended since it increases both the number of surface scratches and roughness which subsequently promotes the attachment of the early colonisers, *Streptococcus oralis* (von Fraunhofer and Loewy, 2009). However, promising effects were obtained in the research con-

ducted by Andruccioli *et al.* (2004), when an experimental paste containing mildly abrasive silica, surfactants at relatively high concentrations and antiseptic substances along with a soft-bristle toothbrush were used. The denture was cleaned properly without any damage to the acrylic resin.

Removal of denture biofilm as well as fighting off potential biofilm-associated fungal infection are very difficult (Busscher *et al.*, 2010; Lamfon *et al.*, 2005) because this complex and heterogeneous structure protects biofilm-embedded microbial cells from noxious environmental factors, including a vast majority of known antimicrobial agents (Kaczała *et al.*, 2008). The exact mechanism of the increased resistance of biofilm has not been recognised yet. Most probably it is conditioned by a number of different factors, including the above-mentioned protective effect of the matrix material which acts as a specific barrier retarding the diffusion of antifungal agents, biofilm high density and integrity, phenotypic changes of the sessile cells, their decreased growth rate and nutrient limitation, drug efflux regulated by *Candida* drug resistance (CDR) and multiple drug resistance (MDR) genes and ability to generate the so-called persister cells that survive at the concentrations of antifungal drugs higher than the MIC values (Kaczała *et al.*, 2008; ten Cate *et al.*, 2009; Ramage *et al.*, 2005; Chandra *et al.*, 2001). Table I summarises the susceptibility *in vitro* of *C. albicans* grown as denture-associated biofilms to different antifungals and antiseptics (Dahlan *et al.*, 2011; de Andrade *et al.*, 2012; Chandra *et al.*, 2001). Chandra *et al.* reported the increased resistance of *C. albicans* biofilms grown on denture acrylic to fluconazole, amphotericin B, nystatin and chlorhexidine (Chandra *et al.*, 2001). It was also demonstrated that *C. albicans* cells after re-suspension from a biofilm, though more susceptible than an intact biofilm are still more resistant to antifungals in comparison to the free-floating cells (Pusateri *et al.*, 2009; Chandra *et al.*, 2001).

On the other hand, studies performed by Spiechowicz *et al.* (1990) demonstrated promising, however requiring further evaluation, effectiveness of chlorhexidine

in reducing both *C. albicans* adherence and biofilm growth when applied directly to the surface of acrylic. Presumably, several factors contribute to this effect: (i) antifungal activity of chlorhexidine, (ii) its ability to adhere to salivary glycoproteins in plaque and (iii) its slow release into environment (Pusateri *et al.*, 2009). Research performed by other authors confirmed satisfactory performance of chlorhexidine-based solutions in inhibition of *C. albicans* adhesion process, as well as reduction of the viable cells of *Candida* spp. biofilms (Wójtowicz and Malm, 2010; Machado *et al.*, 2010; da Silva *et al.*, 2011; de Andrade *et al.*, 2012). However, the correlation between MIC (minimal inhibitory concentration) values and the stage of biofilm formation was noted – the more mature biofilm becomes the higher concentration of chlorhexidine is needed. Most probably there is limited ability of the chlorhexidine to penetrate the whole fungal biofilm and its activity is mostly towards the superficial biofilm layers only (Wójtowicz and Malm, 2010).

Studies performed by Kassab *et al.* (2007) revealed significant efficacy of the ethanol solution of curcumin, a yellow pigment obtained from the rhizome of *Curcuma longa*, in reducing activity of *C. albicans* biofilm that had developed on acrylic resin denture material. Its use as a novel denture cleansing agent was proposed. Interestingly, Pusateri *et al.* (2009) found that Hst 5, one of the salivary histatins – family of histidine-rich peptides produced in acinar cells of human parotid and submandibular glands, exerts a potent antifungal activity against the later stages of *C. albicans* biofilm formation on acrylic disks. The published results suggest its potential therapeutic role as a topical agent. Similarly, Samaranayake *et al.* (2009) confirmed that naturally occurring salivary lysozyme prevents *Candida* colonisation on denture acrylic surfaces. Moreover, the combination of lysozyme and polyene antifungal agents inhibits biofilms in the synergistic mode.

Incorporation of antifungal agents or antiseptics into denture's lining materials was found during *in vitro* studies to have limited effectiveness. Permanent bathing in saliva which extracts the active substances within

Table I
In vitro Susceptibility of *C. albicans* grown as denture-associated biofilms to different antifungals and antiseptics

Agent	Bioactivity	Mode of application and/or effective concentration	References
Sodium hypochlorite	antiseptic	Immersion of denture in 1:25 solution for 30 min.	Dahlan <i>et al.</i> , 2011
Chlorhexidine	antiseptic	Daily immersion of denture in 0.12% solution for 20 min or a single immersion of denture in 2.0% solution for 5 min. 128 µg/ml (50% reduction in metabolic activity)	de Andrade <i>et al.</i> , 2012 Chandra <i>et al.</i> , 2001
Amphotericin B	antifungal	8 µg/ml (50% reduction in metabolic activity)	Chandra <i>et al.</i> , 2001
Nystatin	antifungal	16 µg/ml (50% reduction in metabolic activity)	Chandra <i>et al.</i> , 2001
Fluconazole	antifungal	>64 µg/ml (50% reduction in metabolic activity)	Chandra <i>et al.</i> , 2001

a short time after introduction of the denture into mouth or dilutes their concentration below the fungicidal point is the possible cause of the failure. Besides, the *Candida* spp. isolates responsible for development of infection may be resistant to the incorporated antifungals/antiseptics (Pereira-Cenci *et al.*, 2008).

Several research groups perform studies on so-called quorum sensing quenching (QSQ) that is conceivable possibilities of disturbance of the cell-cell communication between microorganisms within a biofilm. The method is based on the inhibition of synthesis of farnesol or tyrosol – the well-described QS molecules, involvement of their agonists and antagonists, blockage of the signal transmission and reception (Kaczala *et al.*, 2008).

Busscher *et al.* (2010) suggested that even the removal of a streptococcal biofilm may constitute a prophylactic step against development of a pathogenic fungal biofilm, since *Candida* spp. isolates often adhere to the denture base *via* a layer of plaque-forming bacteria, the early colonisers (Pereira-Cenci *et al.*, 2008; Chandra *et al.*, 2001). Since significant reduction in oral yeast counts as well as decrease of *Candida* spp. prevalence in biofilms formed on voice prostheses were observed after consumption of probiotic bacteria, the potential benefits of probiotics use in the management of yeast biofilms grown on denture acrylic surfaces may also be taken into consideration (Thein *et al.*, 2009; van der Mei *et al.* 2000).

In a conclusion, development of *C. albicans* biofilm on a denture surface constitutes a difficult and hard to resolve problem which may concern every single prosthesis-wearer. Thus, careful oral and denture hygiene is highly recommended for all population with artificial teeth.

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