

# Advances and Emerging Techniques in the Identification, Diagnosis and Treatment of Oral Candidiasis

Celeste M. Abraham\*

Baylor College of Dentistry, The Texas A & M University Health Science Center, 3302 Gaston Avenue, Dallas, TX 75246, USA

**Abstract:** Oral candidiasis is a significant, frequent and familiar infection. *Candida albicans* is the most common fungal pathogen. It presents in both pathogenic and nonpathogenic states and has been able to develop a myriad of powerful mechanisms that allows for its thriving colonization and subsequent infection of the host under appropriate conditions. The objective of this paper is to evaluate advances in the cellular and molecular mechanisms involved in the diagnosis and identification of oral candidiasis and assess diverse novel treatment modalities for oral candidiasis in the clinical setting. Various cellular and molecular mechanisms have been employed to help elucidate the interactions between *Candida* organisms and their environment. Polymerase chain reaction analyses, *Candida* biofilm communication, and recombinant antigen techniques have been found to facilitate explanations of the endurance and continued existence of oral candidiasis, specifically *C. albicans*. Various modes of treatment, both traditional and innovative, have been utilized to manage oral candidiasis. These include, but are not limited, to the novel use of mucobuccal adhesives, denture liners, mouth rinse and toothpaste combinations as well as probiotic cheese.

**Keywords:** Oral candidiasis, emerging techniques, identification, treatment, diagnosis, cellular mechanism.

## INTRODUCTION

*Candida albicans* is an important fungal pathogen that is not only capable of causing a localized intraoral *Candida* infection, but one that can permeate and initiate systemic effects as well. It is considered the most important of the *Candida* species and can be found in all areas of the mouth as well as on the surface of dentures [1-3]. The fungi that cause oral candidiasis are considered part of the normal flora of the mouth [4]. Intraoral candidiasis, particularly the prevalence of oropharyngeal candidiasis continues to escalate because of the increasing numbers of patients who are immunocompromised [5-7].

Oral candidiasis is caused by the fungal organism *C. Albicans*; it typically presents itself in a non-pathogenic state [8]. It is normally present in the mouth as the yeast form but under certain conditions can transform itself into the hyphal form which is pathogenic. There are certain environments which tend to set up this pathogenic state. These circumstances may include xerostomia, the use of antibiotics, systemic diseases, the presence of removable prostheses or the use of medications which suppress the immune system. Studies have suggested that in cases of denture stomatitis, *Candidal* proliferation occurs within denture plaque rather than within the mucosa; consequently, antifungal treatment should be targeted to both the denture in addition to the mucosa [8-10]. In a retrospective study of *Candida* colonization (count and species) in the presence and absence of removable dental prostheses, it was shown that a similar *Candidal* count and colonization pattern occurs on

both oral tissues as well as on dental prostheses [11]. It was also noted that the treatment of oral candidiasis in denture wearers should also include the denture prostheses as well as the oral mucosa [11].

The diagnosis of oral candidiasis can be based on the clinical recognition of a particular form of *Candida*. Various clinical manifestations of oral candidiasis exist including pseudomembranous candidiasis, hyperplastic candidiasis (Candidal leukoplakia), atrophic candidiasis -acute and chronic (erythematous) and angular cheilitis [12, 13]. Pseudomembranous candidiasis may present as white plaques of hyphal aggregation. The underlying mucosa may be erythematous [12, 13] (Fig. 4). Hyperplastic candidiasis has been also referred to as "Candidal leukoplakia". It clinically appears as white plaques that cannot be wiped away [12, 13] (Figs. 1, 2). Atrophic (Erythematous) candidiasis is a form where the lesions appear erythematous and often accompanied by dry mucosa. Chronic atrophic candidiasis is a form that is common to see in the hard palate and attached mucosa under a denture or removable partial denture [11-13]. This form is also known as denture stomatitis. Acute atrophic candidiasis commonly involves the dorsal tongue. It has been known by the term "median rhomboid glossitis" [12, 13]. Angular cheilitis is typically seen as red fissures at the corners of the mouth. It can be manifested as cracking and peeling and is often seen with other forms of candidiasis [12, 13] (Fig. 3). In addition to the clinical forms of *Candida* for diagnosis, there are also various species of oral *Candida*. Some of the species commonly seen intraorally are *C. Albicans*, *C. glabrata*, *C. guilliermondii*, *C. Krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, and *C. tropicalis* [12, 13].

The objective of this paper is to review advances in the cellular and molecular mechanisms in the diagnosis and

\*Address correspondence to this author at the Department of Periodontics, Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, USA; Tel: 214-828-8467; E-mail: CABraham@bcd.tamhsc.edu

identification of oral candidiasis and assess various novel ways of treating oral candidiasis in a clinical setting.



**Fig. (1).** Hyperplastic Oral candidiasis: Left buccal mucosa



**Fig. (2).** Hyperplastic candidiasis: Dorsal tongue



**Fig. (3).** Angular Cheilitis. Candidiasis at the right commissure.

#### CELLULAR AND MOLECULAR MECHANISMS UTILIZED IN ORAL CANDIDIASIS IN THE DIAGNOSIS AND IDENTIFICATION OF VARIOUS *CANDIDA* SPECIES

Oral *Candida* organisms have a manner in which they adhere to the oral mucosa as well as to non-oral surfaces (such as the denture) *via* the use of biofilms. *Candida* continues to exist intraorally because they are able to form biofilms that allow them to grow and flourish [14]. In the mouth, *Candida* exists in mixed biofilms, where they can

live together with bacteria and successfully thrive in those environments [14]. It has been noted that it is the structure of the cell wall of *Candida* that displays properties that promote the formation of the biofilms, thereby allowing it to infiltrate the tissue and ultimately inhabit various organs in the body, including the oral mucosa [14, 15]. *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) [14, 15]. 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 [14]. This propensity to adhere to host cells of the epithelium is a virulence factor of species of oral *Candida*.



**Fig. (4).** Pseudomembranous candidiasis: Left side of the palate, left maxillary alveolar ridge and left buccal mucosa.

Additionally, it has been found, that *Candida* species and bacteria communicate with one another *via* a mechanism known as “quorum-sensing.” [14, 16]. *Candida* and bacteria utilize signaling mechanisms where they have a direct effect on each other. *C. albicans* employs two major quorum-sensing molecules known as tyrosol and farnesol. Tyrosol has the ability to stimulate the formation of hyphae in *C. albicans* while farnesol inhibits the shift from the yeast form to the hyphal form and regulates the morphology of the cell [16, 17]. Quorum-sensing is a phenomenon where the accrual of signaling molecules enables a single cell to sense the number of bacteria/fungi that are present; this allows bacteria/fungi to manage and organize the way they behave so that they are able to respond quickly to their changing environments in order to sustain themselves.

#### POLYMERASE CHAIN REACTION (PCR) FINGER-PRINTING

Polymerase Chain Reaction (PCR) fingerprinting has been utilized to differentiate between various strains of *C. Albicans* and *C. dubliniensis*. This is a challenging problem because of the high degree of similarity in the phenotype between *C. dubliniensis* and *C. Albicans* [18]. Multiplex PCR has been used to identify various *Candida* species, particularly *C. Albicans*, *C. glabrata*, and *C. tropicalis* in a single sample [19]. Furthermore, PCR has been successfully used to identify *C. dubliniensis* in gingival fluid of healthy individuals with periodontal disease [20]. Tests were identified using conventional sampling methods as well as

specific PCR assays. Results showed that *C. dubliensis* was isolated and identified in subgingival fluid of periodontal pockets of persons with healthy immune systems [20]. Real-time PCR and lucigenin-based chemiluminescence have demonstrated that *Candida* biofilms can exert resistance to many commonly employed antifungals in the clinical setting [21]. Real-time PCR chemistries allow for the detection of PCR amplification during the early phases of the reaction; measuring the kinetics during the early phases provides an advantage over the traditional PCR where agarose gels are used for the detection of PCR amplification at the final end-point phase of the reaction [21]. *C. Albicans* genes have also been identified that regulate the communication between oral epithelial cells involved in oropharyngeal candidiasis and vascular endothelial cells [22]. These genes increase their interaction as they come into contact with epithelial or endothelial cells and as they endure the stresses that they likely encounter as they grow in the oropharynx and in the blood [22].

### RECOMBINANT DNA

Use of recombinant antigens for the diagnosis of invasive candidiasis has been recently studied [23]. The detection of antibodies directed against antigens which are expressed on the *C. albicans* germ tube surface by indirect immunofluorescence has been shown to be valuable for diagnosis [23]. Among the *Candida* enzymes detected in sera of patients who have systemic candidiasis, enolase has been found to be highly diagnostic. Advances in molecular biological techniques have also allowed for the completion of the *Candida albicans* genome sequence. Overall, these molecular biology techniques have allowed the production of recombinant antigens which are useful for the discovery and identification of antibodies against them. It has been displayed that the detection of antibodies against purified and well-defined recombinant antigens allows for the diagnosis of *Candidal* organisms which are invasive in nature [23].

### RECONSTITUTED HUMAN ORAL EPITHELIUM (RHE)

Host-fungus interactions were analyzed using a combination of microscopic, cellular and molecular techniques as well as an *in vitro* RHE (reconstituted human oral epithelium) model of oral candidiasis so as to scrutinize the *Candida* infection process [24]. It was found from this research analysis that morphology and adaptive response of *Candida* organisms play important roles in the way the host and the fungus respond to one another in the presence of an infection. *Candida* has also been recognized by the study of structural mucosal and innate immune cells [25]. The activation of mechanisms which are protective in nature in both immune and non-immune effector cells depends on appropriate recognition of pathogens. Pattern-recognition receptors expressed on host cells allow for the rapid recognition of pathogens [26]. *C. Albicans* has a assortment of pathogen-associated molecular patterns; recognizing this array of patterns on the cell wall of *C. Albicans* is mediated through many pattern-recognition receptors such as the Toll-like receptors [27]. These Toll-like receptors are a separate family of pattern-recognition receptors that help to recognize microbes and eventually activate phagocytosis and inflammatory responses.

Various techniques have been used to identify *Candida* species. Multiplex PCR was used to quickly identify *Candida* species in samples of oral rinses without having to use an additional step of extraction in the process; ten species were identified in just a few hours [28]. Species that were identified included *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. famata*, *C. dubliniensis*, *C. kefyr*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, and *C. lusitanae*.

### IMMUNOLOGICAL METHODS

Denture stomatitis is a very real and common problem encountered within the dental setting; a significant emphasis has been placed in this review in both the immunology as well as in the treatment because of the practical implications that this topic carries for the general as well as specialty dental practitioner.

In a denture-related stomatitis study, the main yeast strain isolated by far was *C. Albicans*. In the denture-related stomatitis group (versus the non-denture related stomatitis group), there were a greater variety of strains of *Candida* which were identified *via* microbiological sampling [29].

*Candida* mannan antigen detection was found to be a reliable as well as a sensitive method for the serologic diagnosis of systemic candidiasis [30]. The kit used in the study utilized an ELISA (Enzyme Linked Immunosorbent Assay) using polyclonal antibodies against *Candida albicans* mannan; the results revealed that the mannan assay had high positive results for *Candida* in comparison to the standard of the culture medium.

Denture stomatitis is a very real and common problem within the dental setting and a significant portion of this has been reviewed here with regards to immunologic aspects. It was noted in another investigation, that *C. Albicans* is the predominant fungal species isolated from those who wear dentures and that *C. dubliniensis* was isolated from the underlying mucosa but not from the prosthetic surface [31]. *C. glabrata* has been found to be an opportunistic pathogen in individuals who use long term immunosuppressants and broad-spectrum antibiotics-especially in the elderly [32]. This may be due to several factors, including decreased salivary gland hypofunction as well as pH changes 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 [32].

### MICROBIAL CULTURE

Identification of *Candida* species has been examined using CHROMagar [33]. CHROMagar® can differentiate *C. albicans* as green colonies, *C. krusei* as pink colonies and *C. tropicalis* as blue colonies. *Candida* species were isolated and identified using this agar from HIV patients with oropharyngeal candidiasis. *C. albicans* and *C. krusei* were identified with a few strains of *C. tropicalis*. A few strains of *C. tropicalis* generated colonies with a trace of a greenish color making it complicated to differentiate it from *C. albicans*.

In another identification and treatment analysis, fifty-five patients with complete upper dentures were examined before and after the use of a ten percent vinegar solution. Vinegar solution was used to disinfect their denture. Before the use of the solution, *C. albicans*, *C. glabrata*, *C. krusei*, *C.*

*lusitaniae*, *C. guilliermondii*, *C. tropicalis*, and *C. parapsilosis* were identified [34]. After the use of the vinegar solution, *C. guilliermondii*, *C. lusitaniae*, and *C. parapsilosis* were not detected; all the other strains of *Candida* were still present. In a previous retrospective study of *Candidal* colonization and removable denture prostheses, forty-four patients exhibited various *Candidal* counts with respect to four groups of species: *C. albicans*, *C. krusei*, *C. tropicalis*, and unspecified *Candidal* species. The frequency of *C. albicans* was 87 percent with prostheses versus 90 percent without prosthesis; unspecified *Candidal* species was 58 percent versus 60 percent, *C. krusei* 14 percent with prosthesis versus 13 percent without prostheses, while the frequency of *C. tropicalis* was 6 percent for both [11].

### TREATMENT OF ORAL CANDIDIASIS

Various traditional modes of treatment have been used to manage oral candidiasis. Treatment can be accomplished with the use of appropriate antifungal medications and routine disinfection of removable intraoral prostheses [35]. Both topical and systemic medications have been used. Topical agents include Nystatin oral suspension and nystatin oral powder as well as clotrimazole troches. Systemic antifungals can be given by prescription and include itraconazole, ketoconazole, and fluconazole. Mycostatin® is a brand name for nystatin solution. Medications specifically for the treatment of angular cheilitis include nystatin ointment, miconazole cream (2%), and clotrimazole cream (1%). Disinfection of oral appliances can be accomplished via the use of Chlorhexidine and nystatin solution. (2% chlorhexidine gluconate, Peridex) [36].

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 [37]. They all have been found to be less toxic antifungal alternatives to Amphotericin B. Posaconazole was approved in 2006 for the treatment of invasive *Aspergillosis* and *Candida* infections. Ravucanazole is highly effective against a variety of fungi, including *Candida* pathogens and has been used to treat esophageal candidiasis in HIV (human immunodeficiency virus) patients. Caspofungin has inhibitory effects against various *Aspergillosis* and *Candida* species. Micafungin has been shown to be highly effective against esophageal candidiasis and has fewer side effects than Caspofungin. Anidulafungin is the newest echinocandin and appears to be very effective against many *Candida* species in vitro. Anidulafungin was found also to be more efficacious in one study than the traditional form of systemic fluconazole for the treatment of invasive candidiasis [38].

Recent developments in the treatment of oral candidiasis include the use of probiotic bacteria which are known to reduce the growth of pathogenic microbes [39]. 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 [39].

Another group of elderly individuals were evaluated for possible reduction of *Candida* overgrowth with the use of an amine fluoride-stannous fluoride mouth rinse and toothpaste combination [40]. The number of elderly with high *Candida albicans* counts decreased after use of the combination. Other *Candida* counts (non-*albicans*) were also low in the participants of the study.

In another novel application, fluconazole mucoadhesive buccal films were prepared and placed in the buccal mucosa where the drug was released over a five hour period and ultimately the disc eroded away. This topical method was used as another alternative to the traditional form of systemic fluconazole and seemed to reduce side effects of the medication that may have been encountered in the systemic form [41].

There has been current acceptance by the FDA for the miconazole Lauriad in a mucobuccal tablet form to treat oropharyngeal candidiasis in a once-a-day dosing format [42].

In a different treatment of denture stomatitis, denture-liner containing nystatin was placed for a period of one week. This was compared to a denture-liner filled with nystatin and left on the denture surface for four days. Dentures left with nystatin containing denture liner for four days had higher yeast counts than those that were left in place for one week [43]. Salivary yeast counts were much lower for individuals who had a denture liner that contained nystatin as the antifungal than individuals who did not use a denture liner containing an antifungal [43].

These and other recent advances have allowed the practitioner to treat various forms of *Candida* species in an effective manner in the clinical setting.

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