Recent mouse and rat methods for the study of experimental oral candidiasis

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The *Candida* genus expresses virulence factors that, when combined with immunosuppression and other risk factors, can cause different manifestations of oral candidiasis. The treatment of mucosal infections caused by *Candida* and the elucidation of the disease process have proven challenging. Therefore, the study of experimentally induced oral candidiasis in rats and mice is useful to clarify the etiopathology of this condition, improve diagnosis, and search for new therapeutic options because the disease process in these animals is similar to that of human candidiasis lesions. Here, we describe and discuss new studies involving rat and mouse models of oral candidiasis with respect to methods for inducing experimental infection, methods for evaluating the development of experimental candidiasis, and new treatment strategies for oral candidiasis.

Introduction

Oral candidiasis is caused by *Candida* yeasts, which are present in the oral cavities of approximately 50% of healthy individuals. *Candida albicans* is the most virulent and prevalent species, followed by the non-*albicans* species *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. dubliniensis*, and others.[1-7] *C. albicans* colonizes the oral surface and can cause damage through the expression of its virulence factors, including adherence to host cells, morphological transition, hydrophobicity and secretion of hydrolytic enzymes.[8-10] A major virulence factor of *C. albicans* is its ability to adapt to a variety of different habitats and the consequent formation of surface-attached microbial communities known as biofilms.[11] Initially, the biofilm is established by the adherence of yeast to host cells or abiotic surfaces, e.g., prostheses. These yeast form colonies, produce germ tubes and filaments, and secret extracellular polymeric substance (EPS) that

contribute to the three-dimensional structure of the biofilm.[12,13] The organization of the biofilm protects the *C. albicans* cells from host immune mechanisms and antifungal agents.[12,14,15]

C. albicans causes disease mainly in individuals with local or systemic risk factors,[16] including denture use,[17] steroid inhaler use,[18,19] reduced salivary flow,[20] a high-sugar diet,[21] extreme age or youth,[22] endocrine disorders,[23] nutritional deficiencies,[24] receipt of broad-spectrum antibiotics,[25] and immunosuppression.[5,7,26] In fact, oropharyngeal candidiasis (OPC) is the most frequent opportunistic infection encountered in HIV positive individuals. The disease occurs in up to 90% of patients during the course of HIV infection.[27,28]

Candida infections of the oral mucosa manifest in various guises and are subdivided into pseudomembranous, erythematous, and hyperplastic. The last form is chronic, whereas the first two are classified as acute lesions. Hyperplastic candidiasis is the least common of the triad of major clinical variants. Pseudomembranous candidiasis or thrush, which is characterized by white patches on the surface of the buccal mucosa, tongue, and the soft palate, occurs in patients using corticosteroids topically or by aerosol, in HIV-positive patients, and in other types of immunocompromised patients. Erythematous candidiasis is characterized by localized erythema of the oral mucosa that commonly occurs on the tongue and the palate and is associated with broad-spectrum antibiotics, corticosteroids, and HIV infection. In the tongue dorsum, erythematous candidiasis presents depapillated areas caused by the loss of filiform papillae. In addition to the three major variants of oral candidiasis, there are other clinical manifestations that are called "*Candida*-associated lesions", since the yeast is not the sole etiologic agent. These lesions include denture stomatitis, angular cheilitis, and median rhomboid glossitis.[29-34]

The recognition that *C. albicans* is an important pathogen, especially in immunocompromised patients, has led to the development of a suitable experimental model that supplies a standard and easy tool to control and manipulate the assays required to obtain knowledge of the disease process.[35,36] Thus, various animal models have been used to explore the host–*Candida* interaction during oral infections to assess the etiopathology, diagnose and manage the disease, and test therapeutic approaches.[37-43]

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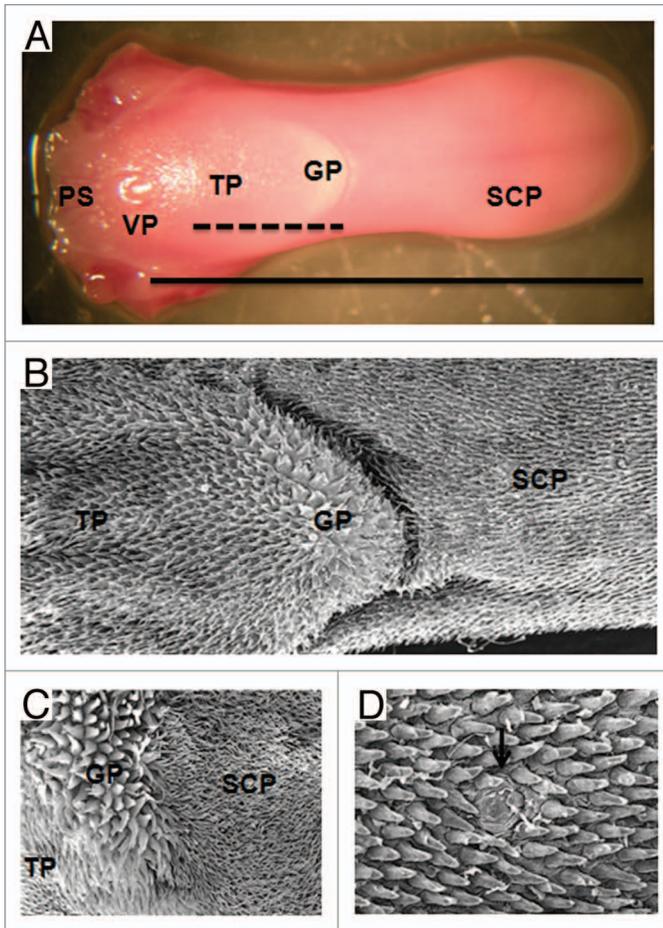
Figure 1. Morphological features of the murine tongue dorsum. (**A**) Macroscopic aspect of the mouse tongue dorsum. The vallate papilla (VP) shared the pharyngeal section (PS) from the buccal (___). In the buccal section, we can see the area of simple conic papillae (SCP), true papillae (TP), giant papillae area (GP) and the intermolar tubercle (---). (**B**) Distribution of the simple conic papillae (SCP), giant papillae (GP) and true papillae (TP) on the tongue dorsum of the mouse, SEM: 35×. (**C**) Distribution of the SCP, GP and TP on the tongue dorsum of the rat, SEM: 75×. (**D**) Fungiform papilla (↓) among the simple conic papillae of the mouse, SEM: 200×.

Therefore, the purpose of this review is to discuss new studies in the literature that describe experimental oral candidiasis in rats and mice and to compare different methods for the induction, development and treatment of oral candidiasis.

Structure of the Tongue Dorsum of Rats and Mice (Normal Aspects)

Since the tongue dorsum is the primary habitat of the yeast in the oral cavity of healthy and immunocompromised individuals,[44] most studies of experimental oral candidiasis in rodent models were performed on the tongue dorsum. Therefore, the general morphological features observed in the tongue dorsum of rats and mice are described below.

The rat or mouse tongue is a muscular organ coated with keratinized stratified squamous epithelium that forms an upper region, the dorsum, and a lower region, the ventral. Under the epithelium, the tongue is formed of conjunctive tissue, which is rich in blood vessels, and striated muscle, in which the fibers are grouped in bundles in three planes. Among the muscle fibers of the posterior region, there are serous and mucous salivary glands.

The tongue dorsum presents a buccal section, which extends from the tip to the vallate papilla, and a pharyngeal section, which extends from the vallate papilla to the most posterior area of the tongue dorsum. Similar to the human tongue dorsum, the buccal section of these rat and mouse models is formed by several projections, namely filiform and fungiform papillae (**Fig. 1**). In rats and mice, filiform papillae are classified as simple conic papillae (located on the anterior two thirds of the tongue dorsum), true papillae (located on the intermolar tubercle), and giant papillae (located between the simple conic papillae and the true papillae). Among the simple conic papillae, there are a lower number of fungiform papillae, which are short and broad with a rounded base that contains the taste corpuscle in the epithelium of the superior surface (**Fig. 2**).

Models of Experimental Oral Candidiasis in Rats

There are two varieties of rats, Sprague-Dawley and Wistar, that are commonly used to study experimental oral *Candida* infections. Rats have the advantages of a low maintenance cost, ease of breeding and handling, ready availability, and a sufficiently sized oral cavity that easily permits inoculation and sample collection.[35,36] Moreover, the tongues of the rats are easily colonized by *C. albicans,* eliciting disease conditions such as erythematous candidiasis and median rhomboid glossitis.[36,45] This model is useful for studying long-term candidal colonization and chronic infection.[35] For the establishment of persistent oral candidiasis in rats, *C. albicans* infection has been induced by exclusion of the protective effects of normal salivary flow, alteration or manipulation of the oral cavity by the administration of antibiotics and treatment with immunosuppressive agents.[35,46]

It has already been established that in humans alterations in salivary glands caused by Sjögren syndrome, radiotherapy, advanced age, and some medications lead to a reduction in salivary flow and xerostomia, favoring the development of oral candidiasis by decreasing the mechanical removal and action of antimicrobial peptides present in saliva.[47-49] This condition has been used by various researchers to induce oral candidiasis in rat models.[38,47-50] Green et al.[50] studied *C. albicans*' expression of *ALS* genes (encoding large cell-surface glycoproteins that function in the process of adhesion to host surfaces) in the hyposalivatory Sprague-Dawley rat model after surgical removal of the salivary glands. At 5 d post-infection, disease progression produced more severe lesions in a defined hierarchy of lesion severity (tongue > mandible > buccal mucosa > palatal and pharyngeal mucosa) and a greater number of different *ALS* transcripts, such as *ALS1–5* and *ALS9*. The expression of the ALS gene in the experimental candidiasis in rats were compared with clinical specimens collected from HIV-positive patients, demonstrating a similarity in expression patterns between the rat model and the human clinical specimens. This study suggests that the hyposalivatory rat

model can be useful for studying gene expression in oral candidiasis.

Immunosuppression and administration of broad-spectrum antibiotics are also important risk factors for developing of oral candidiasis in humans.[29,30] Thus, these predisposing factors have been widely used to induce oral candidiasis in rats.[45,51,52] Chami et al.[53] compared the effect of substances from a vegetal source (carvacrol and eugenol) to nystatin on experimental oral candidiasis induced in Wistar rats that were immunosuppressed with dexamethasone and treated with tetracycline in the drinking water. Microbiological analysis showed that treatment with carvacrol, eugenol, and nystatin reduced 94.5%, 76.9%, and 91.54% of the CFUs of *C. albicans*, respectively, compared with a control group (infected and untreated). Histologically, the control group showed extensive colonization of the epithelium of the tongue dorsum by numerous hyphae. For the group treated with carvacrol, no *C. albicans* were observed in the folds of the tongue mucosa. However, under eugenol and nystatin treatment, hyphae were found in some folds of the tongue mucosa. The authors reported that this model of experimental oral candidiasis in rats was a simple and highly reproducible method of studying the efficacy of antifungal agents.

Junqueira et al.[52] induced oral candidiasis in Wistar rats while treating the animals with tetracycline only in the drinking water. A *C. albicans* suspension was dripped into the mouth and spread on the tongue with a swab that was previously soaked in the suspension. This study verified that the majority of the rats no longer showed *C. albicans* colonization after 8 d of infection, suggesting that during the development of experimental candidiasis, the yeast and hyphae are eliminated from the organism by the host's immune system. However, all animals showed typical candidiasis lesions, which were characterized by numerous epithelial lesions, including epithelial hyperplasia, disorganization of the basal layer, exocytosis, spongiosis, and loss of filiform papillae. After the establishment of candidiasis lesions, the rats were treated with photodynamic therapy (PDT) using methylene blue and a laser. Subsequently, the action of PDT on oral candidiasis was evaluated by the quantification of the epithelial lesions and by the intensity of inflammatory infiltrate in the conjunctive tissue. The rats treated with photodynamic therapy exhibited fewer epithelial alterations and had a lower inflammatory response than the untreated rats, suggesting that PDT was effective for the treatment of oral candidiasis. Furthermore, the experimental oral candidiasis in rats was a useful model to assess the effects of in vivo PDT.

In addition to the studies relating to genes expression and new antifungal countermeasures, such as substances from a vegetal
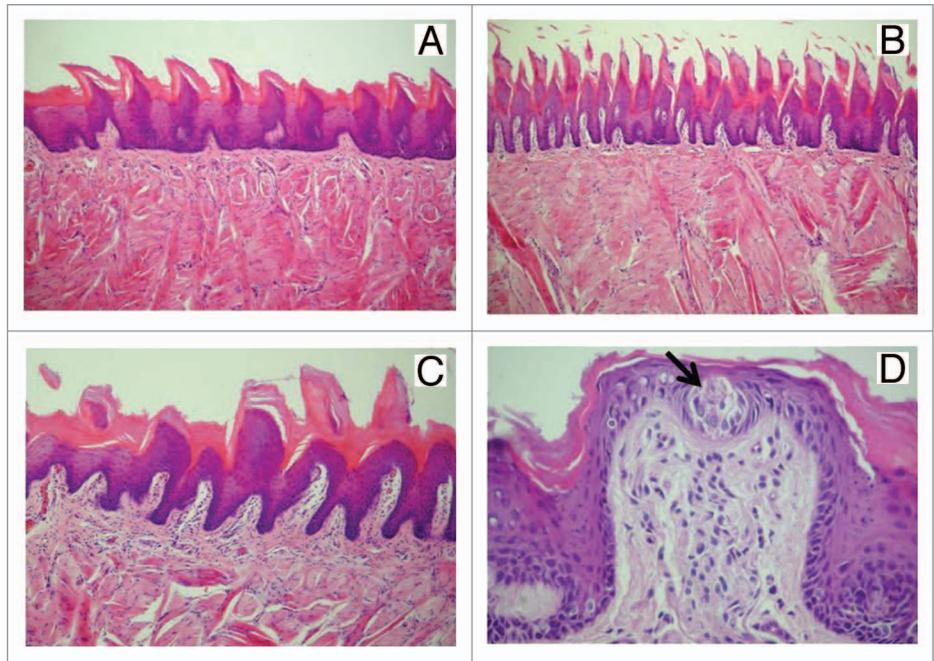
Figure 2. Sagittal cut of the tongue dorsum of the rat. (**A**) The simple conic papillae are short and distant from each other with many interpapillar surfaces, HE: 200×. (**B**) The true papillae are more elongated and closer to each other, with few interpapillar surfaces, HE: 200×. (**C**) The giant papillae are larger, are present in fewer places and are more visible than the other types of papillae, 200×. (**D**) Fungiform papilla with taste corpuscle (↓), HE: 400×.

source and PDT, rat models have also been used to study the influence of physiological factors on oral candidiasis. The influence of ovarian hormone on the development of oral candidiasis was demonstrated in the literature by Junqueira et al.[39] These authors inoculated the oral cavity of ovariectomized Wistar rats and sham-ovariectomized rats with *C. albicans* suspensions. The ovariectomized rats presented fewer occurrences of candidiasis than sham-ovariectomized rats (33% vs. 75%) and exhibited a faster recovery time (22 d vs. 67 d), suggesting that ovarian hormones have a significant influence on oral candidiasis.

Since psychological stress has been found to suppress cell-mediated immune responses that are important for limiting the proliferation of *C. albicans*, Núñez et al.[54] evaluated the effects of stress on oral candidiasis in rats exposed to a repeated auditory stressor. The results showed that stress exacerbated *C. albicans* infection in the tongue dorsum of the rats. Significant increases in *C. albicans* counts, the percentage of the tongue's surface covered with clinical lesions, the percentage of abnormal papillae, and the colonization of the epithelium by hyphae were found in stressed rats compared with the nonstressed rats.

Another advantage of oral candidiasis model in rats is that this model allows the placement of a prosthetic device in the palate to mimic human *Candida* denture stomatitis, the most common form of oral candidiasis in the elderly population denture wearers. Unlike mice, rats have a larger oral cavity that facilitates denture placement. Previous models of denture stomatitis in rats were pioneered in the 1970s and 1980s. However, research since then has been scarce, presumably due to the HIV epidemic and

the concomitant rise in the incidence of oropharyngeal candidiasis.[55] Recently, new studies using rat models for denture stomatits were developed with a focus on *Candida* biofilm formation.[55,56] Nett et al.[56] induced denture stomatitis in Sprague-Dawley rats by using acrylic denture material applied over a palate around the cheek teeth (8 by 10 mm), immunosuppression with cortisone, and subsequently administering *C. albicans* (10^8 cells/mL) on the palate. The analysis of the images revealed a biofilm composed of adherent cells, yeasts, hyphae, host cells encased in extracellular matrix, and associated bacteria. The mucosal histopathology was consistent with that of acute human denture stomatitis, as it exhibited fungal invasion and neutrophil infiltration. In this study, the authors demonstrated that the oral denture model in rats can be very useful for testing the impact of gene disruption on biofilm formation, studying anti-infectives agents, examining the biology of mixed *Candida*-oral bacterial flora biofilm infections, and characterizing the host immunologic response to the disease process.

Models of Experimental Oral Candidiasis in Mice

The major advantage of using mice instead of rats for experimental oral candidiasis is that the immunobiology of the healthy mouse mucosa has also been fairly well characterized, enabling the suitable evaluation of the humoral and cellular immune response in oral candidiasis.[35,45,46] Additionally, mice are widely available, easy to handle and to inoculate, and inexpensive to maintain.[35,46] The availability and ease of production of genetically modified animals is another advantage of mice.[46,57] The development of transgenic and knockout mice with targeted immune defects has prompted a more closely focused assessment of the role of specific components of the immune response to *C. albicans*.[58] According to Samaranayake and Samaranayake,[35] the variants of the mouse model include gnotobiotes, athymic (*nu/nu*), euthymic (*nu/+*), beige (*bg/bg*), black (*bg/+*), beige athymic (*bg/bg nu/nu*), beige euthymic (*bg/bg nu/+*), severe combined immune deficiency (SCID), and murine acquired immune deficiency syndrome (MAIDS). Nevertheless, the mouse model has the disadvantage of a small oral cavity that interferes with the examination of the mucosa by the naked eye.

As in rat models, mice models require the use of immunosuppressive agents or other predisposing factors to establish persistent infection since these animals are not naturally colonized by *C. albicans*.[35,38,46,59-61] The effect of xerostomia in sialoadenectomized mice prolonged oral colonization by *C. albicans* to 75 d post-infection vs. 30 d of colonization for the control animals.[38] With 1 or 4 inoculations with a *C. albicans* suspension, the last procedure increased the permanence of *C. albicans* in the oral cavity of sialoadenectomized mice and produced lesions in the tongue that contained pseudohyphae inside the epithelium, acanthosis, and neutrophilic infiltrate forming intraepithelial microabscesses, with a higher prevalence of lesions in the true and simple conic papillae.[59] This model favored the permanence of *C. albicans* in the oral cavity, and higher frequencies of yeast inoculation influenced the presence and extension of candidiasis lesions.

Rhaman et al.[60] reported a new murine model of oral candidiasis using estrogen administration. BALB/c mice received a weekly intramuscular and subcutaneous 5 µg dose of estrogen, of which the intramuscular administration was more powerful for inducing oral colonization. After 2 and 3 weeks of *C. albicans* infection, fungal cells and hyphae were detected in the keratinized and upper prickle-cells layer of the epithelium at varying sites, namely, the tongue dorsum, the buccal mucosa, the mouth floor, the palate, and the lingual alveolar mucosa. By week 5, fungal cells and hyphae were still clearly present in the oral epithelium. In this study, the authors also evaluated immunization for *C. albicans*. The animals were immunized intranasally on two consecutive days with 5 µg purified Sap2 (secreted aspartyl proteinase 2) that is a putative virulence factor of *C. albicans* thought to contribute to human mucosal infections). On days 14 and 15 after the second immunization, mice were inoculated orally with *C. albicans* and exhibited a significant reduction of the fungal burden compared with the control group. According to the authors, this model of mucosal colonization can be used to assess potential vaccine candidates and permits the detailed analysis of host-fungal interactions during the natural state of *Candida* colonization.

Although predisposing factors, such as xerostomia and estrogen administration, have been used for inducing oral candidiasis in mice, most studies have been performed in immunosuppressed mice models.[37,41,43,62-68] Takakura et al.[37] developed a murine model for experimental candidiasis that combines immunosuppression and treatment with tetracycline for successful disease progression, allowing the study of fungal pathogenesis and new therapeutic options. These authors observed symptoms characteristic of oral thrush in the oral cavities of mice treated with prednisolone at a concentration of 100 mg/kg body weight and 0.83 mg/mL tetracycline in the drinking water 1 d prior to infection. At 3 and 7 d post-infection, 10^5–10^6 CFU were recovered from each mouse, and whitish, curd-like patches were observed on most of the tongues. Animals with symptoms of pseudomembranous candidiasis showed extensive colonization on the epithelium of the dorsal surface by numerous hyphae, as well as the severe destruction of the epithelial layers. Furthermore, this model was useful for verifying the therapeutic activity of fluconazole and amphotericin B, both of which reduced *C. albicans* and cured the lesions.

This murine model of experimental candidiasis, developed by Takakura et al.,[37] inspired many other studies, which are described in **Table 1**.[37,41,43,62-68] The advantage of this model is the development of thrush-type oral candidiasis that mimics the natural infection in humans and is useful for both symptomatological and mycological evaluation of the responsiveness to antifungal treatments. Therefore, several treatments for oral candidiasis were tested in this model including: salivary factors,[62,63] quorum-sensing molecules,[64] photodynamic therapy,[43,66] natural plant extracts,[41,67] and inhibitors of efflux pump.[68]

In addition to the murine model developed by Takakura et al.,[37] other models of experimental candidiasis in immunosuppressed mice have been published in the literature and are described below. Teichert et al.[69] induced oral candidiasis in

Table 1. Studies of experimental candidiasis using the methodology developed by Takakura et al. (2003)[26] for establishment of a mice model with local symptoms characteristic of oral thrush that mimics the natural infection in humans

Study	Agents tested for the control of oral candidiasis	Results
Takakura et al.[62] (2004)	Bovine lactoferrin	Lactoferrin, that is an iron-binding glycoprotein, present in saliva and milk, showed a direct antifungal activity and induced the enhancement of Th1-type cytokine production in the mice model. Therefore, lactoferrin from cows' milk could be used as a dietary supplement with action for antifungal treatment.
Kamagata-Kyoura et al.[63] (2004)	Human saliva	The administration of human saliva inhibited the colonization of the oral cavity by *C. albicans* in mice and the subsequent onset of oral candidiasis. The human salivary factors, such as lactoferrin, transferrin, histatin, cystatin, and lysozyme, should be studied as new therapeutic agents against oral candidiasis.
Hisajima et al.[64] (2008)	Farnesol	Farnesol, known as a quorum-sensing molecule for *C. albicans*, showed a protective effect against oral candidiasis in a dose-dependent manner. Farnesol suppressed mycelial growth of *C. albicans* on the surface of tongues, but did not prevent the change of CFU of *C. albicans* cells, suggesting that farnesol has very characteristic roles in protection against mucosal candidiasis.
Mima et al.[66] (2010)	PDT mediated by Photogen® and LED	PDT promoted significant reduction in the viability of *C. albicans* biofilm, but there was no significant reduction in macroscopic or microscopic lesions, indicating that further in vivo studies are still necessary to investigate the parameters of PDT required for complete inactivation of *C. albicans*.
Taguchi et al.[41] (2010)	Spices and herbs	The cassia (*Cinnamomum cassia*) preparation significantly reduced the lesions on the tongue and the hyphae and inflammatory infiltrate in the epithelium. On the other hand, preparations of lemongrass (*Cymbopogon citratus*) and green tea (*Camellia sinensis*) reduced neither symptoms nor the viable *C. albicans* cells. These finds suggested that cassia may possibly be a candidate for a prophylactic or therapeutic tool against oral *Candida* infection.
Hayama et al.[68] (2012)	Association of inhibitor of Cdr1p efflux pump with azoles	High-level azole resistance is often caused by the overexpression of *C. albicans* efflux pump Cdr1p. The co-application of the d-octapeptide derivate RC21v3, an inhibitor of efflux pump Cdr1p, potentiated the therapeutic performance of fluconazole for mice infected with a fluconazole-resistant *C. albicans* clinical isolate. These results indicated that RC21v3 in combination with azoles has potential as a therapy against azole-resistant oral candidiasis.
Ninomiya et al.[67] (2012)	Tea tree oil (TTO) (Essential oil of *M. alternifolia*)	TTO and its major component, terpinen-4-ol, caused a decrease in the symptom of oral candidiasis of tongues and in the viable *Candida* cell number in the oral cavity of mice infected by azole-resistant *C. albicans*. These results suggested that TTO and terpinen-4-ol may have the potential of therapeutic ability for oral candidiasis.
Costa et al.[43] (2012)	PDT mediated by erythrosine and green LED	PDT showed a significant reduction of viable *C. albicans* cells without damaging adjacent tissues, but there was no difference among the groups in macroscopic and microscopic analysis.

beige nude mice with severe combined immunodeficiency disease (SCID) by swabbing the oral cavity with *C. albicans* 3 times a week for a period of 4 weeks. The animals were treated with photodynamic therapy using methylene blue and diode laser light. The therapy exhibited antifungal activity, decreasing the number of fungal cells and increasing the inflammatory infiltrate and neutrophilic exocytosis, thereby demonstrating the capability of the SCID model to generate an immune response despite the absence of T cells and B cells. Solis and Filler[61] described a simple method to induce oropharyngeal candidiasis by injecting cortisone acetate (220 mg/kg) subcutaneously before infection with *C. albicans*. The mice were inoculated sublingually for 75 min with a swab saturated with the *C. albicans* suspension. On day 5 after inoculation, 10^5–10^6 CFU were recovered from the oral tissue, and the histopathology showed characteristics of oropharyngeal candidiasis.

In summary, multiple experimental models of oral candidiasis using immunosuppressed mice have been developed and, regardless to the type of immunosuppression used, the animals developed typical lesion of pseudomembranous candidiasis that is seen in humans. These models have become important tools to study the virulence of *C. albicans* mutants, the host response to mucosal infection, and the efficacy of new antifungal agents.

Methods for Analysis of Experimental Oral Candidiasis

To estimate colonization by *C. albicans* in the rat or mouse oral cavity, CFU counting is the most useful method. Samples from the entire oral cavity or only from a specific site, e.g., the surface of the tongue, are collected with the aid of a swab for posterior CFU counting onto Sabouraud dextrose agar (SDA) with antibiotics. This method assesses whether the animals were colonized by *C. albicans* and the antifungal effects of the therapeutic modality tested.[37,42,43,60,69] Additionally, the tongue and other oral tissue can be removed from the oral cavity, homogenized in saline
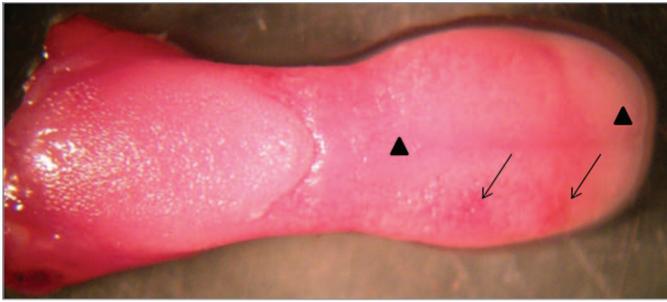
Figure 3. Macroscopic analysis of a mouse tongue with oral candidiasis presenting whitish regions (▲) and papillary atrophy (↓).
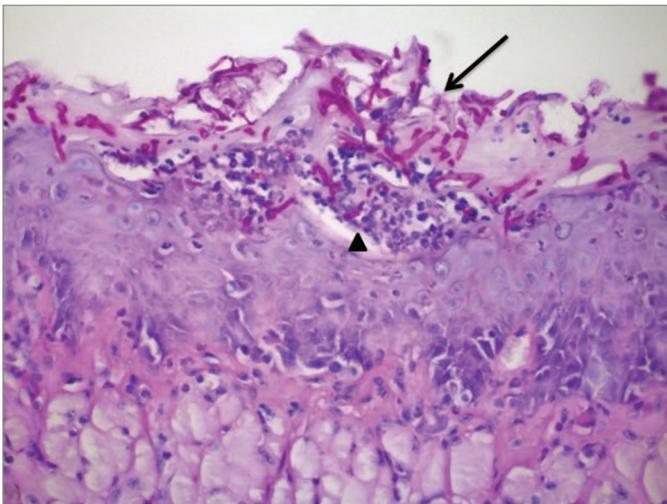
Figure 4. Sagittal section of mouse tongue dorsum showing yeasts and hyphae in keratin (↓) and intraepithelial microabscesses (▲). PAS: 400×.
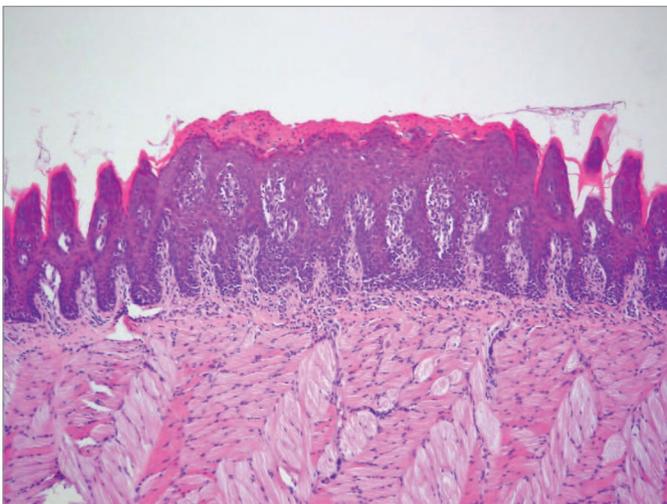
Figure 5. Sagittal section of rat tongue dorsum showing tissue lesion characterized by epithelial hyperplasia, disorganized basal layer, exocytosis, spongiosis, loss of filiform papillae, and hyperparakeratosis. HE: 100×.

solution, and later plated on SDA.[61] After plating, the Petri dishes are incubated at 35–37 °C for 24 or 48 h.

Acute oral candidiasis can present as pseudomembranous or erythematous forms. These manifestations cause macroscopic lesions (**Fig. 3**) that can be quantitatively analyzed. Takakura et al.[37] evaluated macroscopic lesions of pseudomembranous candidiasis by scoring lesions from 0 to 4 according to the extension and severity of whitish, curd-like patches on the tongue surface. The authors also described the appearance of lesions consisting of white patches on the tongue dorsum. This methodology has been used by many others authors to analyze the development of disease during their experiments.[41,43,62,63,67,68]

After euthanasia of the animals, the tissues are removed and prepared for histopathological study. The sections are stained with periodic acid-Schiff (PAS) reagent, which allows for the verification of the extent of fungal filamentation and the depth of tissue penetration.[61] Some authors proposed a quantification of the amount of yeasts/hyphae by attributing scores to histological fields in the anteroposterior direction (**Fig. 4**).[39] Through hematoxylin-eosin (HE) staining, many epithelial alterations and the presence of inflammatory cells are observed and can be quantified according to the methodology of Junqueira et al.,[52] such as epithelial hyperplasia, disorganization of the basal cell layer, exocytosis, spongiosis, loss of filiform papillae, hyperkeratosis, development of intraepithelial microabscesses, and extension of the inflammatory infiltrate (**Fig. 5**). Histopathological data are important because from a histopathological and diagnostic point of view, most of the lesions described in animal models faithfully reproduce human candidal lesions.[35]

In addition to the histopathological study, the tissue, which is usually the tongue dorsum, can be analyzed by scanning electron microscopy (SEM) and laser confocal scanning microscopy (LCSM). In the SEM analysis, alterations and fungal elements are observed on tongue dorsum tissue from rats and mice infected with *C. albicans*. Analysis reveals hyphae penetrating perpendicularly into the tissue on the anterior surface of the papillae, desquamation and degradation of tissues, atrophy and destruction of the filiform papillae, and increased interpapillar surfaces (**Fig. 6A and B**).[39]

The use of LCSM to analyze the oral candidiasis in mouse model was recently proposed by Dongari-Bagtzoglou et al.[70] Oral candidiasis was induced in mice immunosuppressed by inoculation of a GFP-expressing strain of *C. albicans*. Confocal images followed by 3D reconstruction of *C. albicans* biofilms formed on the dorsum tongue revealed microanatomical epithelial variations of the lingual papillae, including "valleys", higher "elevations" of stacked fungal cells, and dark areas suggestive of extracellular matrix. The presence of β-glucan was immunoaccessible throughout the biofilm mass and on the surfaces of both yeast and hyphal organisms. Neutrophils were visualized as forming "nests" within the biofilm mass. The biofilm formed on the tongue was a mixed biofilm composed of *C. albicans* and various bacteria that were identified as *Enterococcus* spp., *Lactobacillus* spp., and *Staphylococcus* spp., while some bacteria were observed invading the epithelium together with *C. albicans*. Therefore, LCSM analysis provided data about
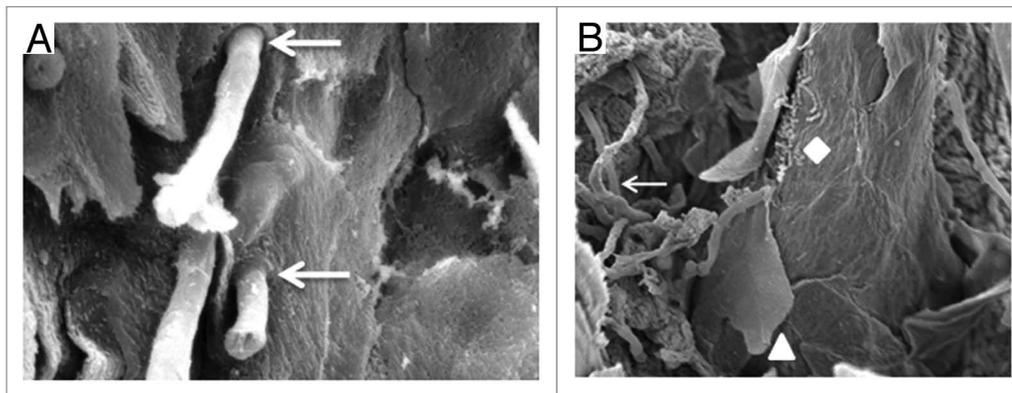
Figure 6. (**A**) SEM of the tongue dorsum of the mouse. The presence of hyphae penetrating perpendicularly into the tissue (↓) is verified. SEM: 3 700×. (**B**) SEM of the tongue dorsum of the rat. Hyphae (↓), keratin desquamation (▲), and bacteria (◆) can be observed. SEM: 1200×.

the architecture of the living tongue biofilm, epithelial micro-anatomical variations and the distribution of fungal cells in the structure of the biofilm, and gave an idea of the dispersion of the extracellular matrix. In addition, this study provides new insights related to potential synergistic relationship between *C. albicans* and the oral bacterial flora during the development of oral candidiasis.

Recently, a new bioluminescence mouse model for real-time monitoring of oral candidiasis was described by Mosci et al.[71] Experimental oral candidiasis was induced in immunosup-pressed mice by inoculation of a *C. albicans* strain that produces a genetically-engineered cell surface luciferase. At selected days post infection, mice were imaged in the IVIS-200TM Imaging system under s.c. anesthesia. The total photon emission from oral areas within the images of each mouse was quantified with Living ImageR software package. The authors obtained highly reproducible levels of infection, providing tools for longitudinal monitoring of the course of OPC in live animals. The advantages of this novel method compared with the conventional methods for analysis of experimental oral candidiasis include: (1) it gives an accurate estimation of the *C. albicans* load in the whole oral cavity, including the load of invasive hyphae; (2) it allows the longitudinal monitoring of oral candidiasis in individual ani-mals, providing a unique possibility to determine the progression or resolution of OPC in real-time and avoiding individual varia-tions when the time course of CFU is assessed; (3) it allows the monitoring of *C. albicans* dissemination from the oral cavity to the gastrointestinal tract; and (4) it decreases the need to sacrifice a large number of animals.

All of the methods described above are very important in analyzing the experimental oral candidiasis in rat or mouse and comparing results from different laboratories. In addition, a mon-itoring of the body weight of each animal can be performed to estimate the disease severity. The magnitude of weight loss caused by the infection and induction of predisposing factors indicate the time point to conclude the experiment, avoiding unneces-sary suffering of rats and mice.[61] Another important aspect to consider in the evaluation of experimental oral candidiasis is the use of a sedative agent in the animals for *C. albicans* inoculation.

The inoculation of *C. albicans* cells in the oral cavity without any anesthetic drug impairs the colonization by *Candida* and subsequently the development of disease. According to Takakura et al.,[37] the degree of severity of the infection is correlated with the length of the sedated period. The sedation avoids the intake of *Candida* by animals, decreasing the spread for esophagus and stomach, and promoting a better colonization and tissue invasion of *C. albicans* in the oral cavity.

Conclusions and Future Directions

The study of oral candidiasis in rats and mice constitutes an important tool for the understanding of the pathogenesis of *Candida* in the oral mucosa and the interference of predispos-ing factors in the disease process. These animal models have also been very useful in testing different therapeutic modalities for candidiasis lesions in the oral cavity.[42,43,66-68] Furthermore, the elucidation of the virulence mechanisms of *Candida* spp. and of the host–pathogen interaction in experimental animal models can provide important information for the discovery of possible targets of new antifungal agents. For any type of research to be conducted, it is worth noting that the choice of animal model must be guided by the aim of the study and must follow standard protocols to obtain reliable and reproducible results.

In summary, rats and mice models that employ antibiotic therapy and hyposalivatory conditions to induce oral candidia-sis develop lesions characterized by areas of papillary atrophy in the tongue dorsum without white patches, such as erythematous candidiasis and median rhomboid glossitis. The animals in these models have an intact immune system and represent what would be expected in individuals treated with broad-spectrum antibi-otics or patients with reduced salivary flow caused by Sjögren syndrome, advanced age, or radiotherapy. In the other hand, the immunosuppression models induce candidiasis lesions with white patches that are comparable to the pseudomembrane observed in human thrush and reflect the oral conditions of AIDS patients.[50] In addition, rat models have a sufficiently sized oral cavity for placement of a prosthetic device in the palate that induces candi-diasis lesions similar to human *Candida* denture stomatitis. The

advantages of using mice for experimental oral candidiasis is that the immunobiology of the healthy mouse mucosa has been well characterized and there are several genetically modified animals available to research.

According to the studies cited in this review, it is evident that the most studies of oral candidiasis in mice and rats were performed with *C. albicans*. However, other non-*C. albican*s species have arisen in recent years due to a growing population of immunosuppressed patients, and future studies must be conducted to investigate the interaction of *Candida* mixed biofilms in the oral cavity and their relation to recurrent episodes of oral candidiasis in these patients. Furthermore, the methods for the analysis of experimental oral candidiasis should be improved because we have new technologies available, such as the real-time monitoring of microbial infections using bioluminescent fungi described recently by Mosci et al.[71] This new technology and many more under development will be very useful for the advancement of oral experimental candidiasis.

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