# Chapter 10
# Models Hosts for the Study of Oral Candidiasis

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**Abstract** Oral candidiasis is an opportunistic infection caused by yeast of the *Candida* genus, primarily *Candida albicans*. It is generally associated with predisposing factors such as the use of immunosuppressive agents, antibiotics, prostheses, and xerostomia. The development of research in animal models is extremely important for understanding the nature of the fungal pathogenicity, host interactions, and treatment of oral mucosal *Candida* infections. Many oral candidiasis models in rats and mice have been developed with antibiotic administration, induction of xerostomia, treatment with immunosuppressive agents, or the use of germ-free animals, and all these models has both benefits and limitations. Over the past decade, invertebrate model hosts, including *Galleria mellonella, Caenorhabditis elegans*, and *Drosophila melanogaster*, have been used for the study of *Candida* pathogenesis. These invertebrate systems offer a number of advantages over mammalian vertebrate models, predominantly because they allow the study of strain collections without the ethical considerations associated with studies in mammals. Thus, the invertebrate models may be useful to understanding of pathogenicity of *Candida* isolates from the oral cavity, interactions of oral microorganisms, and study of new antifungal compounds for oral candidiasis.

## Oral Candidiasis

*Candida* species is an innocuous commensal of the microbial communities of the human oral cavity. Its primary locations are the posterior tongue and other oral sites, such as the mucosa, whereas the film that covers the dental surfaces is colonized secondarily. Frequently, when the host defense system is compromised, *C. albicans* becomes virulent, resulting in the disease candidiasis, which may spread to multiple oral sites, or infect the entire oral cavity (Webb et al. 1998; Salerno et al. 2010). The presentations of oral candidiasis can be classified in pseudomembranous, erythematous, plaque-like (nodular) and *Candida*-associated lesions: angular cheilitis and median-rhomboid glossitis (Niimi et al. 2010).

Of the many pathogenic *Candida* species, *C. albicans* is the major fungal pathogen of humans. In the oral cavity, a niche they frequently inhabit as commensals, these yeasts exist predominantly

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within biofilms: are spatially organized heterogeneous communities of fungal cells encased in a matrix of extracellular polymeric substances (Jin et al. 2004). The developing biofilms are constantly exposed and bathed in saliva. The effect of saliva on *Candida* adhesion and subsequent biofilm formation is controversial. Some studies have shown that saliva reduces the adhesion of *C. albicans* to oral surfaces. Indeed, saliva possesses defensive molecules, including lysozyme, lactoferrin, calprotectin and IgA, that decrease the adhesion of *Candida* to the oral surfaces. In other studies, salivary proteins such as the mucins and the statherins have been shown to act as adhesion receptors used by the mannoproteins present in the *Candida* species (Salerno et al. 2010).

The incidence of superficial and deep-seated fungal infections have increased markedly over the last 20 years. Several reasons have been proposed for the rise in incidence of fungal infections, including HIV infection, increasing use of immunosuppressive drugs, use of broad-spectrum antibiotics, prosthetic devices and grafts (Donelly et al. 2007). *Candida* strains have been isolated from 93% of patients with denture stomatitis, which is now considered to be the most common form of oral candidiasis (Noumi et al. 2010). Deng et al. (2010) verified that the incidence of oral candidiasis during radiation therapy in patients with head and neck cancer was significantly higher (55.2%) than in a nonirradiated control group (11.8%).

Oropharyngeal candidiasis (OPC) is the most frequent opportunistic infection encountered in HIV-infected individuals. OPC occurs in up to 90% of HIV-infected patients at some point during the course of HIV disease. The occurrence of OPC is associated with CD4 T-lymphocyte counts below 200 cells/mm$^3$, high viral loads, and disease progression (Hamza et al. 2008).

Several known virulence factors contribute to the pathogenicity of *C. albicans*, including the ability to adhere to epithelial cells, including the ability to form hyphae, and the secretion of extracellular enzymes (Noumi et al. 2010; Jayatilake et al. 2005; Pukkila-Worley et al. 2009). During the initial stages of superficial mucosal infection, *C. albicans* forms filamentous hyphae that show thigmotropism, a phenomenon also known as contact guidance, and release hydrolytic enzymes, such as extracellular phospholipases and secretory aspartyl proteinases (Jayatilake et al. 2005).

The widespread use of topical and systemic antifungal agents as the conventional treatment for oral candidiasis has resulted in the development of resistance in *C. albicans*. Although resistance of *C. albicans* to polyenes is rare, several mechanisms of azole resistance have been reported, including changes in the cell wall or plasma membrane, which decrease azole uptake, overexpression of or mutations in the target enzyme of azoles, and the efflux of azole drugs mediated by membrane transport proteins (Mima et al. 2010).

The versatility of the pathogenic mechanisms of fungi and their development of resistance to antifungal drugs indicate the importance of understanding the nature of these host-pathogen interactions in experimental systems, and warrant development of vertebrate and invertebrate models.

## Mammalian Animal Models for the Study of Oral Candidiasis

### *General Aspects of Animal Models*

Apart from the ethical dilemmas associated with experimentation on live humans, humans are notoriously different in terms of their dietary habits, social habits, immune status, and oral physiology, including salivary function. These factors, in addition to racial, ethnic, and cross-cultural variations in human demographics, influence the etiology and pathogenesis of diseases such as candidiasis. The development of an ideal animal model for oral candidiasis would provide a standardized tool which can be controlled and manipulated to derive universally comparable data on the etiopathology, diagnosis, and management of the disease process (Samaranayake and Samaranayake 2001).

Although many of the pioneering studies on mucosal candidiasis were performed in non-human primates, small mammals, including rats and mice, are the common choice for such studies for economical and ethical reasons and because of their relative anatomic and immunological similarity to humans (Samaranayake and Samaranayake 2001; Chamilos et al. 2007; Naglik et al. 2008).

Two species of rats, Sprague–Dawley and Wistar, have been widely used in experimental oral *Candida* infections. The main advantages of the rat model are the low maintenance cost and the sufficient size of the oral cavity, which easily permits *Candida* inoculation and sample collection. Furthermore, the tongue of this animal is fairly easily colonized by *Candida*, eliciting disease conditions such as median rhomboid glossitis and atrophic candidiasis (Samaranayake and Samaranayake 2001).

Mouse models are ideal for unraveling adaptive immune responses of the mucosal tissues to *Candida* infection because the immunobiology of the healthy murine oral mucosa has been fairly well characterized by a number of researchers. Furthermore, mice are easily obtained in large numbers and their maintenance is inexpensive (Samaranayake and Samaranayake 2001).

One caveat to studying *C. albicans*-host interactions in rodents is that *C. albicans* is not a natural colonizer of mucosal surfaces in these animals. The rodent equivalent of normal flora yeast is *Candida pintolopessi*, which can sometimes cause disease in immune-compromised rodents. This has both benefits and limitations. An advantage is that any host response to *C. albicans* is not affected by pre-existing adaptive immune responses to the fungus (Naglik et al. 2008). A disadvantage is that the establishment of mucosal colonization or infection usually requires intervention with antibiotics, induction of xerostomia, treatment with immunosuppressive agents, or the use of germ-free or transgenic animals.

## *Pros and Cons of Rat and Murine Models*

Many oral candidiasis models in rats have been developed with antibiotic administration followed by persistent infections. The most commonly examined drug has been tetracycline because of its broad spectrum of activity and its association with human candidal infection (Allen 1994). A variety of protocols have been evaluated with varying parameters, including the dose of the antibiotic and the schedule of its administration. For induction of experimental oral candidiasis in several studies, the animals were treated daily with a solution of 0.08–0.1% tetracycline hydrochloride in their drinking water. This treatment was initiated 7 days prior to inoculation with *C. albicans* suspension and was maintained throughout the experiment (Allen et al. 1982; Fisker et al. 1982; Junqueira et al. 2009).

Oral candidiasis can also be induced by xerostomia, either by means of pharmacologic agents or the surgical removal of the salivary glands. One of the first studies of oral candidiasis in an animal model used hyoscine hydrobromide in an attempt to induce a xerostomic state in Wistar rats to increase the likelihood of infection (Jones and Adams 1970). In this instance, the use of the xerostomic drug did not seem to have much impact on the incidence of infection compared with untreated controls (Allen 1994). Further studies that used the ligation or removal of major salivary glands (parotid, sublingual, and submandibular) in the rat have shown an increased severity of infection in the xerostomic animals compared with normal controls (Jorge et al. 1993; Totti et al. 1996; Green et al. 2006). Jorge et al. (1993) verified that after 32 weeks of *C. albicans* inoculation, 20% of normal and 70% of sialoadenectomized rats showed candidal infection on the tongue.

Because the broad-spectrum antibiotics used, such as tetracycline, eradicate the antagonistic population pressure of the commensal oral flora, the sialoadenectomy model appears preferable for the investigation of oral candidiasis because it maintains the normal oral flora with its competitive, colonization pressure akin to the clinical conditions in humans (Samaranayake and Samaranayake 2001).

The antibiotic therapy and hyposalivatory models are limited by the lack of local symptoms characteristic of oral candidiasis, particularly oral thrush, in humans. Takakura et al. (2003)

developed a murine model of thrush-type oral candidiasis that mimics the natural infection in humans by immunosuppression with injections of prednisolone and tetracycline hydrochoride in drinking water. These authors induced typical candidiaisis lesions consisting of white patches on the tongue dorsum comparable to the pseudomembrane observed in human thrush, with extensive colonization on the epithelium by numerous hyphae and destruction of several epithelial layers.

Candidiasis-related lesions observed in rats models employing antibiotic therapy and hyposalivatory conditions are characterized by areas of papillary atrophy in the tongue dorsum without white patches, because the rats in these models have an intact immune system. The basis of the rat model is the profound change in ecology of the oral cavity, whereas immunosuppression is the basis for disease in the mouse model. The rat model represents what would be expected in individuals with chronically irritated mouths like Sjogren's syndrome patients, while the murine model reflects the oral ecology for an AIDS patient (Green et al. 2006).

Germ-free or gnotobiotic mice appear uniquely suited for studies of mucosal candidiasis, because mucosal surfaces can be naturally and chronically colonized by *C. albicans* without the need for sialoadenectomy, antimicrobial therapy, or immunosuppression. In addition, colonization can persist for the lifetime of the animal. However, the germ-free/gnotobiotic mouse is not ideal for the study of fungal dissemination from mucosal sites or natural host-pathogen interactions, because the absence of the normal microbiota is likely to have a major effect on both fungal pathogenicity and the host response (Naglik et al. 2008).

Transgenic mice are also extremely useful for experimental studies of sex-linked anemia, metabolic disease resembling diabetes mellitus in humans, and severe combined immune deficiency (SCID syndrome). The utilization of transgenic or congenitally immunodeficient mice has been instrumental in advancing our understanding of the critical roles of $CD4^+$(helper) T cells, $CD8^+$ (cytotoxic) T cells, polymorphonuclear leukocytes, macrophages, and cytokines in host defense against oral candidiasis (Naglik et al. 2008; He et al. 2010).

## *Microscopic Aspects of Experimental Oral Candidiasis*

The microscopic features of experimental oral candidiasis in the tongue dorsum of rats with intact immune systems have been described by Junqueira et al. (2005). These authors observed yeasts and hyphae agglomerates in the keratin layer of epithelium by optical microscopy (MO) 6 h after inoculation with *Candida* yeasts. Many polymorphonuclear leucocytes in the prickle and basal cell layers were also observed. In scanning electron microscopy (SEM), only yeasts were found among the filiform papillae of the tongue dorsum. The hyphae could not be observed by SEM because they were present in the interior of the keratin.

After 24 h of *C. albicans* inoculation, the MO analysis showed high levels of hyphae and keratin desquamation. *Candida* infection increased the number of mitotic cells per unit length of the basal layer of the epithelium and the amount of desquamation. These changes were defense mechanisms of the rat against fungal invasion. The keratin desquamation allowed the observation of the large quantity of hyphae by SEM.

Seven days after *C. albicans* inoculation, the authors observed the presence of a small quantity of hyphae and of tissue lesions characterized by loss of filiform papillae, acanthosis and epithelium hyperplasia by MO. Although infection by *Candida* was restricted to the keratin layer in the epithelial surface, tissue changes occurred in the deepest layers of the epithelium. These effects can likely be attributed to defense mechanisms and extracellular enzyme production, such as proteinase and phospholipase. In the SEM examination, areas of atrophy and destruction of the filiform papillae and increased interpapillar surface area were observed.

The normal morphology of the tongue dorsum of rat and the development of experimental candidiasis can be observed in Figs. 10.1–10.6.

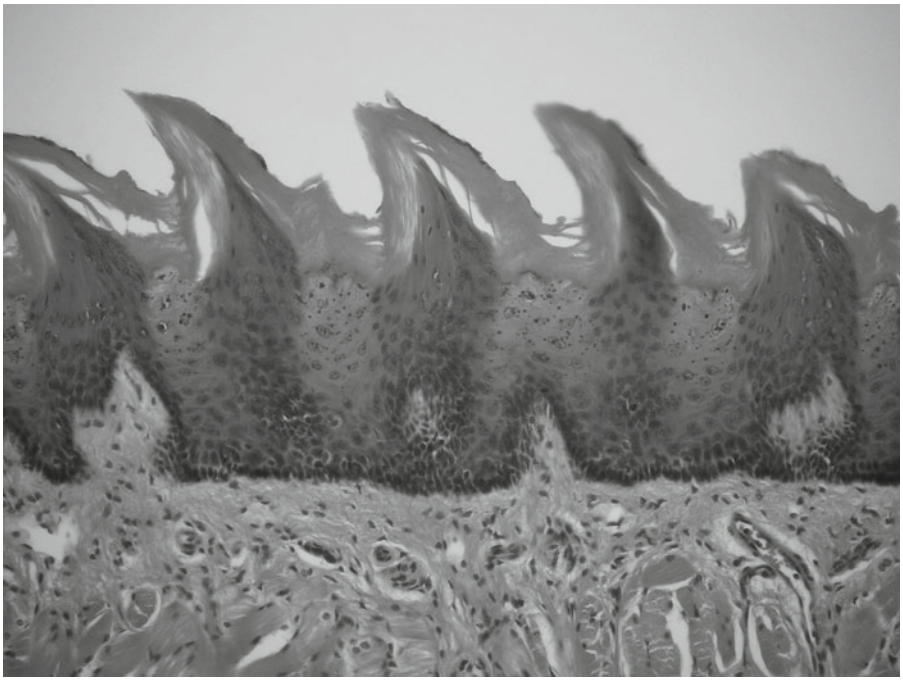

**Fig. 10.1** Normal morphology of the tongue dorsum of rat. Optical microscopy: 200×

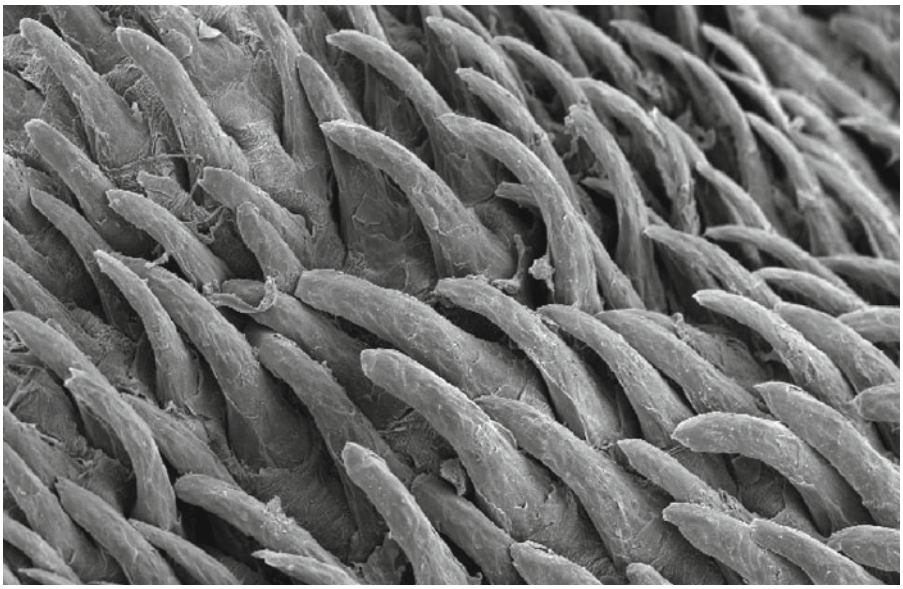

**Fig. 10.2** Normal morphology of the tongue dorsum of rat. Scanning electron microscopy: 180×

## *Current Studies of Rat and Murine Models*

Animal models of oral candidiasis have provided a wealth of information with respect to this disease process. Currently, they are used in the investigation of the pathogenicity, host interactions, and treatment of oral mucosal *Candida* infections.

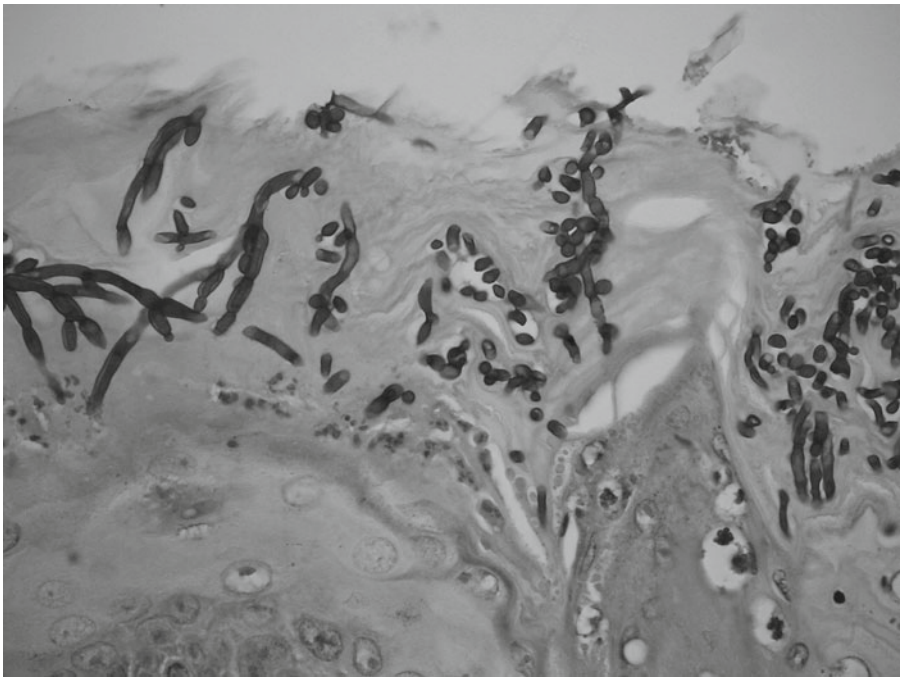

**Fig. 10.3** Sagittal cut of the tongue dorsum of rat 6 h after C. albicans inoculation. Yeast, hyphae and polymorphonuclear leucocytes can be observed. Optical microscopy: 630×

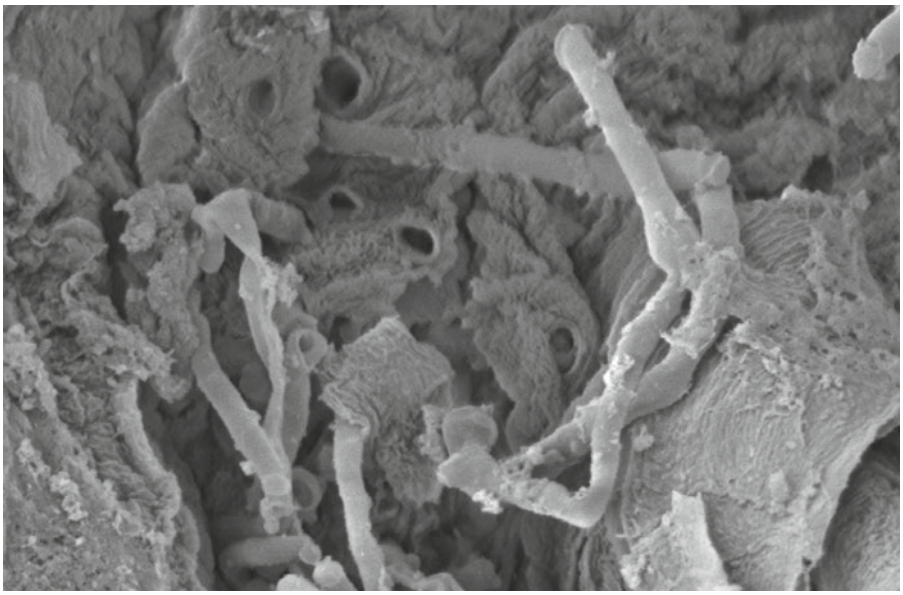

**Fig. 10.4** Tongue dorsum of rat 24 h after C. albicans inoculation. Hyphae and tissue degradation are observed. Scanning electron microscopy: 2,500×

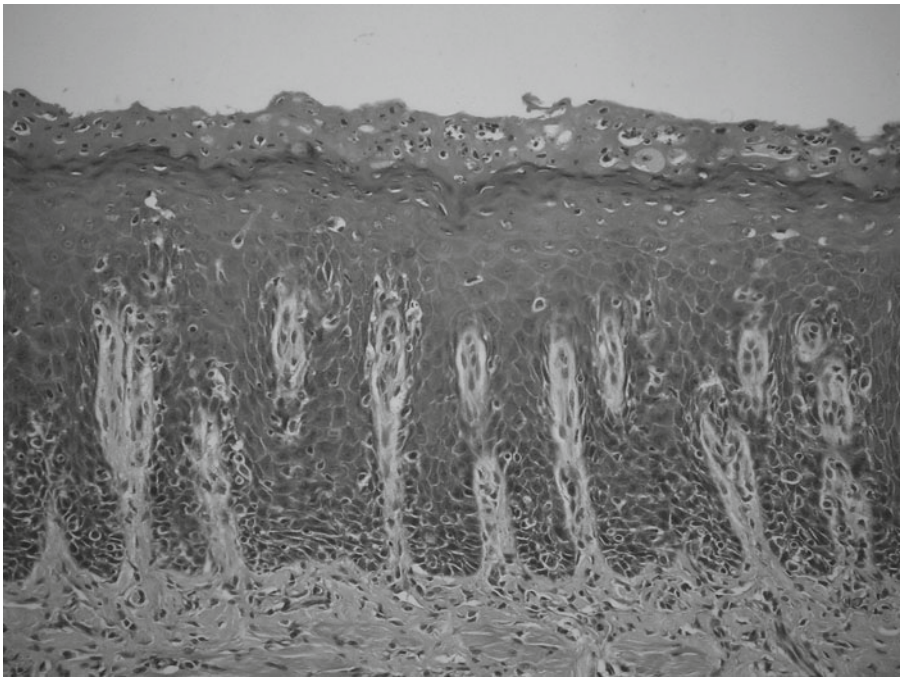

**Fig. 10.5** Sagittal cut of the tongue dorsum of rat 7 days after C. albicans inoculation. Tissue lesion characterized by loss of filiform papillae, hyperparakeratosis and epithelium hyperplasia are observed. Optical microscopy: 200×

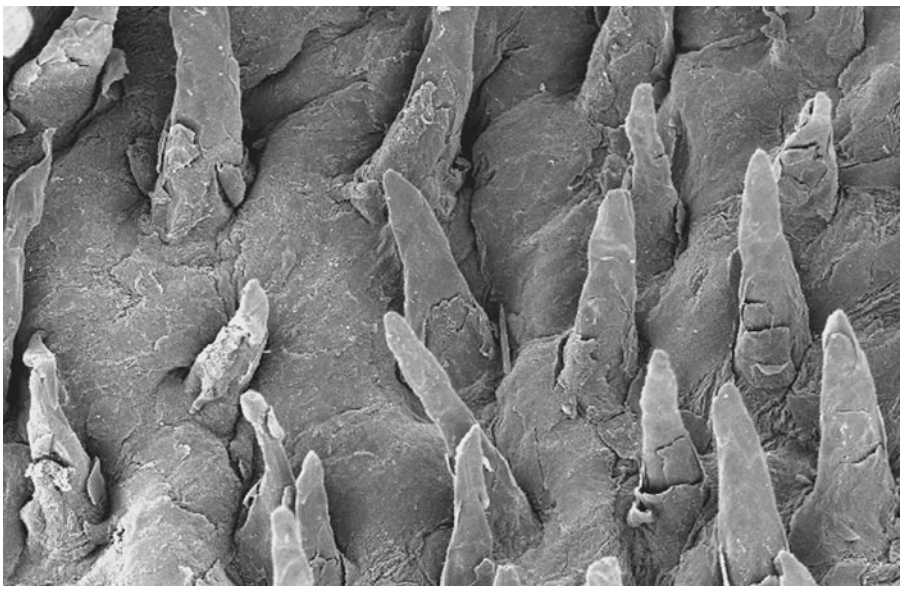

**Fig. 10.6** Tongue dorsum of rat 7 days after C. albicans inoculation. Atrophy of filiform papillae and increase of interpapillar surface can be observed. Scanning electron microscopy: 300×

Green et al. (2006) 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 rat model after surgical removal of the salivary glands. These authors verified that patterns of ALS gene expression were similar between the rat model and human clinical specimens, suggesting that the model could be used to study the phenotypes of *als/als* mutant strains.

He et al. (2010) reviewed several studies silencing or disrupting specific genes in knockout mice and concluded that the regulatory pathways governing *C. albicans* virulence are different during oropharyngeal candidiasis (OPC) and hematogenously disseminated candidiasis (HDC). For example, the *C. albicans* TPK2 (Takashi's protein kinase 2) gene product is one of two catalytic subunits in protein kinase A. The Ras-protein kinase A pathway regulates multiple processes in *C. albicans*, including hyphal formation, phenotypic switching, and clamydospore formation. Park et al. (2005) found that a tpk2D mutant of *C. albicans* had significantly reduced virulence in the mouse model of OPC, but normal virulence in the mouse model of HDC was maintained. According He et al. (2010) future studies are needed to determine whether there are differences in the specific virulence factors required for these distinct disease states.

Animal models have also been used to develop new therapies for oral candidiasis. Junqueira et al. (2009) and Mima et al. (2010) verified that photodynamic therapy promoted significant reduction of oral candidiasis in both the rat model with intact immune system and in an immunosuppressed mouse model. Taguchi et al. (2010) examined the therapeutic activity of spices and herbs for oral candidiasis in a murine model and suggested that oral intake of *Cinnamomum cassia* preparation is a clinical candidate for a therapeutic tool against oral *Candida* infection. The development of research with new therapeutic approaches in mammalian animal models is extremely important because the action of therapeutic agents on microorganisms could be affected by the environmental conditions of the oral cavity, including the presence of saliva, pH variations, mucosa characteristics and the action of the immunological system (Kömerik et al. 2003).

## Invertebrate Models for the Study of Oral Candidiasis

Over the past decade, invertebrate mini-host models with well-characterized genetics and simple immunity have been effectively used to explore several aspects of both fungal pathogenicity and host immune response. Several factors sparked the development of these models. First, the mammalian animal models remain logistical barriers to large-scale studies. Second, the realization that innate immune mechanisms are evolutionarily conserved between invertebrates and mammals and that several common virulence factors are involved in fungal pathogenesis in phylogenetically disparate hosts – e.g., fruit flies, nematodes, and mammals – further expanded the field. Third, invertebrate organisms have been increasingly used as in-vivo assays for antifungal drug efficacy studies because of their low cost and simplicity (Chamilos et al. 2007).

Furthermore, because the genome sequences of medically important fungi such as *Aspergillus*, *Candida,* and *Cryptococcus* have been completed, the increase in genetic information has created an increasing need for simple innovative ways to screen for virulence mechanisms and assess the contribution of individual genes to fungal pathogenesis (Chamilos et al. 2007).

Model hosts, including the grater wax moth *Galleria mellonella*, the roundworm *Caenorhabditis elegans*, and the fruit fly *Drosophila melanogaster,* have been used for the study of *Candida* pathogenesis.

*Galleria mellonella* have been successfully used as models of *Candida* pathogenesis because of their relatively large size (about 5 cm long), which allows for the injection of standardized fungal inocula and studies of drug pharmacodynamics. In addition, *G. mellonella* can be maintained under

various temperature conditions ranging from 25°C to 37°C. The ability to study pathogens at 37°C enables the study of temperature-related virulence traits. *Galleria* presents haemolymph with six types of hemocytes (prohemocytes, coagulocytes, spherulocytes, oenocytoids, plasmatocytes and granulocytes), which play a role in fungal-pathogen defense. The major disadvantage of these insects is the absence of methods for genetic analysis and the lack of a full genome sequence for this model (Chamilos et al. 2007; Fuchs and Mylonakis 2006; Mylonakis 2008).

Fuchs et al. (2010) evaluated the roles of five genes BCR1, FLO8, KEM1, SUV3 and TEC1, in *C. albicans* filamentation using a *G. mellonella* model. Among the five mutant strains tested, the authors observed that only the flo8/flo8 mutant strain did not form filaments within *G. mellonella*. This strain also exhibited reduced virulence in *G. mellonella* larvae. Another strain that exhibited reduced pathogenicity in the *G. mellonella* model was tec1/tec1 but, by contrast, the tec1/tec1 strain retained the ability to form filaments, suggesting that filamentation alone is not sufficient to kill *G. mellonella* and that other virulence factors may be associated with genes that regulate filamentation.

Rowan et al. (2009) employed larvae of *G. mellonella* to assess the in vivo antifungal efficacy of ($[Ag_2(mal)(phen)_3]$), $AgNO_3$, and 1,10-phenanthroline. Larvae pre-treated with these compounds were protected from a subsequent lethal infection by the yeast *C. albicans,* and larvae treated 1 and 4 h post-infection showed significantly increased survival compared to control larvae. Administration of these compounds resulted in an increase over 48 h in the density of insect hemocytes. These results demonstrate an increase in hemocyte numbers, which could contribute to the ability of the insect to kill *C. albicans;* this may function in combination with the antifungal properties of the compounds.

*Caenorhabditis elegans* is much smaller (about 1 mm long) than all other mini-host models. Nevertheless, *C. elegans* has a sequenced genome and fully developed genetic tools. Additionally, its hermaphroditic lifestyle and short lifespan (2–3 weeks) facilitate genetic experiments with this organism. *C. elegans* is maintained in Petri dishes and infected by ingesting the pathogen as a substitute for the usual laboratory food source, an auxotrophic strain of *Escherichia coli*. The simplicity of experimental infection in *C. elegans* allows for individual screening of thousands of virulence genes and candidate antimicrobial compounds (Pukkila-Worley et al. 2009; Chamilos et al. 2007, 2009; Fuchs and Mylonakis 2006).

Coleman et al. (2010) performed a compound screen to identify potential antifungal natural products using *C. elegans* model. Of the 12 antifungal saponins identified, two saponins (A7 and A20) were as effective as amphotericin B in promoting *C. elegans* survival. According to the authors, these compounds represent an opportunity to expand the current classes of antifungal agents in use and to improve available antifungal drugs by exploiting these new chemical scaffolds.

*Drosophila melanogaster* is larger (about 3 mm long) than *C. elegans* and is substantially smaller than *G. mellonella.* In the laboratory, *D. melanogaster* can be infected with fungal pathogens using various methods such as injection, direct spraying of fungal spores onto the flies, and ingestion. The genetic tractability and well-characterized immune system of Drosophila is a major advantage. The Drosophila genome sequence was one of the first to be completed and is one of the most fully annotated eukaryotic genomes available (Chamilos et al. 2007, 2009; Fuchs and Mylonakis 2006).

Chamilos et al. (2006) hypothesized that *C. albicans* has developed evolutionary conserved mechanisms to invade disparate hosts and tested whether *Toll* mutant flies of *D. melanogaster* could serve as a model host for high-throughput screening of *C. albicans* virulence genes. Of the 34 *C. albicans* mutants tested, only the prototrophic *cas*2 , mutant exhibited attenuated virulence in *To*ll mutant flies. Similarly, BALB/c mice infected intravenously with the *cas*2 , mutant had significantly better survival and a lower fungal burden in kidneys and spleen than did those infected with the isogenic wild-type strain. *CAS*5 encodes a key transcriptional regulator of genes involved in cell wall integrity. These findings support the notion that *D. melanogaster* is a promising model for large-scale studies of genes involved in the pathogenesis of *C. albicans* infection in mammals.

## Future Directions and Conclusion

Experimental mammalian animal models for the study of oral candidiasis have been much explored, and they are important tools in assessing fungal pathogenicity, host immune defenses and treatment of oral mucosal *Candida* infections. However, there are no studies relating oral candidiasis and invertebrate models. Future research of oral candidiasis using invertebrate models are required to gain a better understanding of pathogenicity of *Candida* isolates from the oral cavity, interactions of oral microorganisms, and study of new antifungal compounds, since invertebrate models allow screening of virulence genes and antimicrobial compounds with low cost and simplicity.

## References

Allen CM (1994) Animal models of oral candidiasis. A review. Oral Surg Oral Med Oral Pathol 78:216–221

Allen CM, Blozis GG, Rosen S, Bright RS (1982) Chronic candidiasis of the rat tongue: a possible model for human Median Rhomboid Glossitis. J Dent Res 61:287–291

Chamilos G et al (2006) *Drosophila melanogaster* as a facile model for large-scale studies of virulence mechanisms and antifungal drug efficacy in *Candida* species. J Infect Dis 193:1014–1022

Chamilos G, Lionakis MS, Lewis RE, Kontoyiannis DP (2007) Role of mini-host models in the study of medically important fungi. Lancet Infect Dis 7:42–55

Chamilos G, Nobile CJ, Bruno VM, Lewis RE, Mitchell AP, Kontoyiannis DP (2009) *Candida albicans* Cas5, a Regulator of cell wall integrity, is required for virulence in munrine and *Toll* mutant fly models. J Infect Dis 200:152–157

Coleman JJ et al (2010) Characterization of plant-derived saponin natural products against *Candida albicans*. ACS Chem Biol 5:321–332

Deng Z, Kiyuna A, Hasegawa M, Nakasome I, Hosokawa A, Suzuki M (2010) Oral candidiasis in patients receiving radiation therapy for head and neck cancer. Otolaryngol Head Neck Surg 143:242–247

Donelly RF, Mcarrin PA, Tunney MM, Woolfson AD (2007) Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. J Photochem Photobiol B 86:59–69

Fisker AV, Rindon-Schiott C, Philipsen HP (1982) Long-term oral candidosis in rats. Acta Pathol Microbiol Immunol Scand 90:221–227

Fuchs BB, Mylonakis E (2006) Using non-mammalian hosts to study fungal virulence and host defense. Curr Opin Microbiol 9:346–351

Fuchs BB, Eby J, Nobile CJ, El Khoury JB, Mitchell AP, Mylonakis E (2010) Role of filamentation in Galleria mellonella killing by Candida albicans. Microbes Infect 12:488–496

Green CB, Marretta SM, Cheng G, Faddoul FF, Ehrharts EJ, Hoyer LL (2006) RT-PCR analysis of *Candida albicans* ALS gene expression in a hyposalivatory rat model of oral candidiasis and in HIV-positive human patients. Med Mycol 44:103–111

Hamza OJM et al (2008) Species distribuition and *in vitro* antifungal susceptibility of oral yeast isolates from Tanzanian HIV infected patients with primary and recurrent oropharyngeal candidiasis. BMC Microbiol 8:135

He H, Cong Y, Yang H, Dong Y (2010) Mutative expression in *Candida albicans* infection and cytokine signaling network in gene knockout mice. Eur J Clin Microbiol Infect Dis 9:913–916

Jayatilake JA, Samaranayake YH, Samaranayake LP (2005) An ultrastructural and a cytochemical study of candidal invasion of reconstituted human oral epithelium. J Oral Pathol Med 34:240–246

Jin Y, Samaranayake LP, Samaranayake Y, Yip HK (2004) Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. Arch Oral Biol 49:789–798

Jones JH, Adams D (1970) Experimentally induced acute oral candidosis in the rat. Br J Dermatol 83:670–673

Jorge AO, Totti MA, Almeida OP, Scully C (1993) Oral candidosis established in the sialoadenectomized rat. J Oral Pathol Med 22:54–56

Junqueira JC, Colombo CE, Martins JS, Koga-Ito CY, Carvalho YR, Jorge AOC (2005) Experimental candidosis and recovery of *Candida albicans* from the oral cavity of ovariectomized rats. Microbiol Immunol 49:199–207

Junqueira JC, Martins JS, Faria RL, Colombo CED, Jorge AOC (2009) Photodynamic therapy for the treatment of buccal candidiasis in rats. Lasers Med Sci 24:877–884

Kömerik N, Nakanishi H, MacRobert AJ, Henderson B, Speight P, Wilson M (2003) *In vivo* killing of *Porphyromonas gingivalis* by Toluidine Blue-mediated photosensitization in an animal model. Antimicrob Agents Chemother 47:932–940

Mima EG et al (2010) Susceptibility of *Candida albicans* to photodynamic therapy in a murine model of oral candidiasis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109:392–401

Mylonakis E (2008) *Galleria mellonella* and the study of fungal pathogenesis: making the case for another genetically tractable model host. Mycopathologia 165:1–3

Naglik JR et al (2008) Animal models of mucosal *Candida* infection. FEMS Microbiol Lett 283:129–139

Niimi MN, Firth NA, Cannon RD (2010) Antifungal drug resistance of oral fungi. Odontology 98:15–25

Noumi E et al (2010) Adhesive properties and hydrolytic enzymes of oral *Candida albicans* strains. Mycopathologia 169:269–278

Park H et al (2005) Role of the fungal Ras-protein kinase A pathway in governing epithelial cell interactions during oropharyngeal candidiasis. Cell Microbiol 7:499–510

Pukkila-Worley R, Peleg AY, Tampakakis E, Mylonakis E (2009) *Candida albicans* hyphal formation and virulence assesses using a *Caernorhabditis elegans* infection model. Eukaryot Cell 8:1750–1758

Rowan R, Moran C, McCann M, Kavanagh K (2009) Use of *Galleria mellonella* larvae to evaluate the *in vivo* antifungal activity of $[Ag_2(mal)(phen)_3]$. Biometals 22:461–467

Salerno C et al (2010) *Candida*-associated denture stomatitis. Med Oral Patol Oral Cir Bucal 16:139–143

Samaranayake YU, Samaranayake LP (2001) Experimental oral candidiasis in animal models. Clin Microbiol Rev 14:398–429

Taguchi Y et al (2010) Therapeutic effects on murine oral candidiasis by oral administration of Cassia (*Cinnamomum cassia*) preparation. J Med Mycol 51:13–21

Takakura N et al (2003) A novel murine model of oral candidiasis with local symptoms characteristic of oral thrush. Microbiol Immunol 47:321–326

Totti MAG, Santos EB, Almeida OP, Scully C (1996) Implantation of *Candida albicans* and other *Candida* species in the oral cavity of rats. J Oral Pathol Med 25:308–310

Webb BC, Thomas CJ, Willcox MH, Harty DW, Knox KW (1998) *Candida*-associated denture stomatitis. Aetiology and management: a review. Aust Dent J 43:45–50