Fungi are chemoorganotroph eukaryotic microorganisms that can take part in endodontic infections and thereby may participate in the etiology of periradicular diseases. They possess virulence attributes—including adaptability to a variety of environmental conditions, adhesion to a variety of surfaces, the production of hydrolytic enzymes, morphologic transition, biofilm formation, and evasion and immunomodulation of the host defense—that may play a role in the pathogenesis of periradicular diseases. Fungi have occasionally been found in primary root canal infections, but they seem to occur more often in the root canals of obturated teeth in which treatment has failed. Candida albicans is by far the fungal species most commonly isolated from infected root canals, and this species has been considered a dentinophilic microorganism because of its invasive affinity to dentin. C. albicans has also been discovered to be resistant to some intracanal medicaments, such as calcium hydroxide. Its ability to invade dentinal tubules and resistance to commonly used intracanal medicaments may help to explain why C. albicans has been associated with cases of persistent root canal infections. Some medicaments, such as chlorhexidine digluconate, calcium hydroxide combinations (with camphorated paramonochlorophenol or chlorhexidine), and EDTA, have the potential to be used as effective intracanal medications for patients in whom fungal infection is suspected. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-41)

Of the approximately 50,000 species of fungi, more than 200 are known to cause diseases in vertebrate animals and human beings. Most of the pathogenic fungi fall into the groups Ascomycetes, Basidiomycetes, Zygomy- cetes, and Deuteromycetes. The most regularly encountered species of opportunistic fungal pathogens are members of the genera Candida and Aspergillus, both belonging to the Deuteromycetes group. Candida species can cause a wide variety of infections in human beings, ranging from superficial disease to life-threatening disseminated mycoses. Most pathogenic fungi are exogenous, yet the mycoses with the highest incidence (eg, candidosis) are caused by fungi that are part of the normal microbiota of the human body. Candida species are carried in the oral cavity, gastrointestinal tract, anus, groin, vaginal canal, and vulva of healthy people. Most of these endogenous fungi are opportunistic pathogens, and infections caused by them usually arise from an imbalance of normal microbiota induced by the administration of broad-spectrum antibiotics, immunosuppression, and the disruption of protective barriers. Fungal infections are usually “diseases of the diseased,” and some predisposition has to be present for the host to be affected.

Fungi constitute a small part of the oral microbiota. The largest proportion of the fungal microbiota is made up of Candida species. Candida albicans is the fungal species most commonly detected in the oral cavity of both healthy and medically compromised individuals. The incidence of C. albicans in the oral cavity has been reported to be 30% to 45% in healthy adults and 95% in patients infected with human immunodeficiency virus. The dorsum of the tongue is deemed to be the primary oral habitat of C. albicans, whereas other sites may be colonized secondarily. Such sites include the mucosa and supragingivae, the dentin, the root, the subgingivae, and the periodontal pockets. A large number of other yeasts have also been isolated from the oral cavity, including Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida krusei, Candida inopsica, Candida dubliniensis, Candida tropicalis, and Saccharomyces species.

A handful of studies showing the occurrence of fungi in endodontic infections have sparked a renewed interest in the role of these microorganisms in the etiology of periradicular diseases. The purpose of this review article was to outline the involvement of fungi in different types of endodontic infections, focusing on their prevalence in such infections and on their putative mechanisms of pathogenicity that may play a role in the pathogenesis of periradicular lesions. Furthermore, fungal susceptibility to intracanal antimicrobial substances is also discussed.
MORPHOLOGIC CHARACTERISTICS OF FUNGI

Fungi are chemoorganotroph eukaryotic microorganisms that may be found in 2 basic forms: molds and yeasts. Molds are multicellular filamentous fungi consisting of branching cylindrical tubes. A single filament is called a hypha. Hyphae are either septate (divided by partitions) or coenocytic (multinucleate without cross walls) and usually grow along a surface, then branch and form compact tufts, collectively called mycelium. There is extensive cytoplasmic streaming within a hypha, usually directed toward the hypha tip, and the older portions of the hypha usually become vacuolated and virtually devoid of cytoplasm. Even if a hypha possesses septum, cytoplasmic movement can occur because there is usually a pore in the center of the septum.

Yeast are unicellular fungi, and cells are spherical or oval in shape. Cell division usually takes place by budding, in which a new cell forms as a small outgrowth of the mother cell. The formation of the septum is preceded by the appearance of a filament ring containing a large amount of chitin. The nucleus migrates into the neck region between the mother and the daughter cell before dividing. The bud gradually enlarges and then separates from the old cell, leaving behind a bud scar. The buds that are formed are called blastoconidia. Pseudothymephae are elongated yeast cells that appear as filamentous cell chains.15

C. albicans, one of the most well-studied fungal species, can reproduce by budding, which results in the formation of yeast cells (also called blastospores or blastoconidia). The production of germ tubes results in the conversion to a hypha. The formation of pseudohyphae occurs by polarized cell division when yeast cells growing by budding have elongated without detaching from adjacent cells. In addition, under certain conditions, C. albicans can undergo the formation of chlamydocones, which are round, refractile spores with a thick cell wall. These morphologic transitions commonly represent a response of the fungus to changing environmental conditions and may permit the fungus to adapt to different sites.16

Fungi have a defined nucleus enclosed by a nuclear membrane. Their cell membrane contains lipids (including sterols) and glycoproteins. They also have mitochondria, Golgi apparatus, ribosomes, endoplasmic reticulum, and a cell wall. Fungal cell walls resemble plant cell walls architecturally but not chemically. Chitin, a polymer of N-acetylglucosamine, is a common constituent of fungal cell walls. Certain fungi contain cellulose in their cell walls. Microfibrils of chitin or cellulose are intertwined and embedded in an amorphous matrix that cements them together. Other polysaccharides, such as glucans, mannans, galactosans, and chitosans, replace chitin or cellulose in some fungal walls. Cell walls of fungi are usually 80% to 90% polysaccharide, with proteins, lipids, polyphosphates, and inorganic ions composing the cementing matrix of the cell wall.16

Approximately 80% to 90% of the cell wall of C. albicans is composed of carbohydrates, which are represented basically by 3 major polysaccharides: β-glucans (20%-40% of the wall’s dry weight), chitin (1%-2%), and mannoproteins (35%-40%), which consist of mannann associated with proteins.16,17 The cell wall of C. albicans also contains proteins (6%-25%) and minor amounts of lipids (1%-7%). In addition to providing rigidity and protection to the cell, the fungal cell wall plays an essential role in practically every aspect of the biology and pathogenicity of C. albicans.16

MECHANISMS OF FUNGAL PATHOGENICITY

Fungi have been demonstrated to possess virulence attributes that may play a role in disease causation. The mechanisms believed to be involved in pathogenesis are (1) adaptability to a variety of environmental conditions, (2) adhesion to a variety of surfaces, (3) production of hydrolytic enzymes, (4) morphologic transition, (5) biofilm formation, and (6) evasion and immunomodulation of the host defense.

Adaptability to a variety of environmental conditions

Candida species, particularly C. albicans, are versatile pathogens. An essential component of such versatility is related to their ability to survive as commensals in diverse, anatomically distinct body sites, each with its specific environmental conditions. For instance, C. albicans is able to adapt to a range of physiologic extremes, such as pH. This feature allows this species to survive and grow in the neutral pH of the bloodstream or in most tissues, as well as in the acidic pH of the vaginal canal.18 Such adaptability is attributable to the switching of gene expression dictated by environmental changes.

Adhesion to a variety of surfaces

Candida species have surface molecules that mediate adherence to host tissues. These molecules include a receptor homologous to the human CR3 integrin, which binds RGD (arginine-glycine-aspartic acid) groups on iC3b, fibrinogen, fibronectin, laminin, and vitronectin; a lectin that binds sugars on epithelial cells; and mannose-containing proteins that bind to lectinlike molecules on host cells and tissues.19,20 Candida species are also able to bind to collagen types I and IV.16,21

Some Candida species have been demonstrated to coaggregate with certain oral bacteria.22-28 For instance, C. dubliniensis coaggregates with Fusobacterium
nucleatum and Candida tropicalis coaggregates with Streptococcus gordonii. Grimaudo et al observed that strains of oral Actinomyces coaggregated to various degrees with C albicans strains. Coaggregations possibly involved a protein on the Candida surface that may interact with carbohydrates or carbohydrate-containing molecules on the surface of Actinomyces. The same researchers revealed that F nucleatum strains, Fusobacterium periodonticum, and Eubacterium sulci also coaggregated to various degrees with all C albicans strains tested. Other authors have reported that C albicans showed coaggregation with strains of Streptococcus sanguinis, S gordonii, Streptococcus oralis, and Streptococcus anginosus, but not with Streptococcus mutans and Enterococcus faecalis. These interactions may favor survival in mixed microbial communities. Coaggregation (or coagglutination) reactions with other microorganisms can play an important role in the colonization of oral mucosal and hard tissues, and the presence of C albicans causes a significant increase in total plaque formation in vitro.

Production of hydrolytic enzymes
C albicans produces hydrolytic enzymes that may be involved in the damage to the periodontal tissues. Enzymes include secreted aspartyl proteinase, collagenase, aminopeptidases, glucosaminidases, acid and alkaline phosphatases, hyaluronidase, and chondroitin sulfatase, all of which have some effect in the degradation of extracellular matrix proteins. It has been shown that a collagenolitic enzyme produced by this fungal species may degrade the human dentin collagen. Phospholipases can be associated with membrane damage of the host cells caused by the cleavage of phospholipids, which destabilizes the membrane and results in cell lysis. Most phospholipase activity has been detected at hyphal tips during tissue invasion.

Morphologic transition
C albicans is often described as a dimorphic fungus that exists in blastospore and hyphal forms. Nonetheless, it is in fact a polymorphic fungus because it has been reported to grow in a number of morphologic forms such as blastospores, germ tubes, true hyphae, pseudo hyphae, and chlamydospores, depending on the environmental conditions. Exception for chlamydospores, interconversion to each form can occur. It has been consistently suggested that the transition from the blastospore to the hyphal form represents a change from a commensal to a pathogenic state, but this concept is no longer considered to be true. The properties of a growing hypha may confer the ability to invade host tissues and escape phagocytosis by macrophages. However, although transformation from yeast to hyphal form may be important, it is not always a prerequisite for infection to occur. Indeed, most infections caused by C albicans are populated by both yeast and filamentous morphologic forms, suggesting that both have a role in the development and progression of the disease. This assumption is supported by the fact that yeast cells have a number of proteins that are involved in host cell recognition and are thought to be the colonizing form of this microorganism.

Biofilm formation
C albicans has the ability to form biofilms on different surfaces, and this property may be one of the reasons why this species is considered more pathogenic than species that are less able to form biofilms, such as C glabrata, C tropicalis, and C parapsilosis. According to Donlan and Costerton, a biofilm can be defined as a community of microorganisms irreversibly attached to a surface, containing extrapolymeric matrix and exhibiting distinctive phenotypic properties. In addition to favoring establishment in a given site, biofilm formation
can provide the community with other advantages, such as protection against potential hazards. For instance, cells in a sessile state (such as in biofilms) can be much more resistant to antimicrobial agents than those in a nomadic state (eg, planktonic cells). C albicans cells growing in a biofilm can be 100-fold or more resistant to the antifungal fluconazole and 20-30-fold more resistant to the antifungal amphotericin B than are planktonic cells.41

Evasion and immunomodulation of the host defenses

C albicans can evade host defenses as a result of different mechanisms. The polymorphonuclear neutrophil seems to be the most important inflammatory cell involved in the defense against C albicans.42 This species has been demonstrated to block polymorphonuclear neutrophil functions such as oxygen radical production, and degranulation, in addition to killing monocytes.43 Candida species can also evade host defense molecules through the production of proteinases that degrade complement factors and immunoglobulins IgG1, IgA1, and IgA2.16,44

Cell wall constituents, such as glucan, chitin, and mannoproteins, have immunomodulatory (activation or depression) effects that can be involved in the pathogenesis of periradicular lesions through an indirect mechanism. C albicans has been demonstrated to be capable of stimulating proinflammatory cytokine synthesis and release by macrophages, endothelial cells, and fibroblasts.42,45,46

Fungi can activate the complement system, which may have biological consequences in that the cleavage of complement factors during cascade activation yields soluble fragments that are proinflammatory. The products of complement activation can induce changes in vascular permeability (C3a, C4a, and C5a), be chemotactic to leukocytes (C5a), or act as opsonins, enhancing phagocytosis (C3b). There are at least 2 possible mechanisms involved in the activation of the complement system in response to C albicans infection.47 One involves IgG antibody that is reactive with C albicans mannan and can lead to complement activation by the classical pathway. The other may directly involve mannan, which is a significant component of the C albicans surface. Serum mannan-binding protein is a lectin that is found in a wide range of concentrations in the sera of healthy adults. Serum mannan-binding protein is structurally similar to C1q, a subcomponent of C1 that recognizes and binds to the Fc region of IgG or IgM antibody bound to an appropriate antigen, triggering the activation of the complement cascade by the classical pathway. Binding of serum mannan-binding protein to carbohydrate structures, including mannan, leads to complement activation by the lectin pathway.

Fungi in primary endodontic infections

Fungi have not been reported to be common members of the microbiota associated with primary endodontic infections.48 Even so, their occurrence has been reported by some researchers using culturing, molecular genetic methods, and in situ electron microscopy (Table). Möller49 isolated a Candida species from 1 of 29 samples from necrotic pulps of teeth with intact crowns that showed positive growth. Debelian et al50 reported the isolation of Saccharomyces cerevisiae from 1 of 26 root canals associated with asymptomatic periradicular lesions. They also detected this fungal species in the blood of a patient undergoing endodontic therapy. Lana et al51 isolated C tropicalis from 2 patients, and S cerevisiae from 1 of 27 patients with initially infected root canals. Baumgartner et al52 detected C albicans in 5 of 24 root canal samples by means of a polymerase chain reaction assay. In contrast, Siqueira et al,53 who also used the polymerase chain reaction, detected fungi in only 1 of 50 infected root canals. Sen et al54 found yeasts heavily invading the root canals of 4 of 10 extracted teeth associated with periradicular lesions. In 1 specimen, hyphal structures were also observed. Siqueira et al55 investigated the patterns of microbial colonization in primary root canal infections through scanning electron microscopy and found yeastlike cells in 1 of 15 examined teeth. They were forming a large colony with some cells in the process of budding (Fig 2). In addition,
the presence of yeast cells was shown in the resorption lacunae of periapical root surfaces and also in periradicular granuloma.

Fungi in persistent or secondary endodontic infections
Fungi have occasionally been found in primary root canal infections, but they seem to be more common in the root canals of obturated teeth in which the treatment has failed (Table). Nair et al. observed yeasts in 2 of 9 surgical block biopsy specimens from periradicular lesions refractory to the endodontic treatment. Waltimo et al. reported the occurrence of fungi in 47 of 692 cases of persistent endodontic infections either in pure culture or along with bacteria. C. albicans was the most common isolate. Other fungal species isolated were C. glabrata, C. guilliermondii, C. inconspicua, and Geotrichum candidum. Sundqvist et al. isolated C. albicans from 2 of 24 canals of teeth in which endodontic treatment had failed. Under similar conditions, Molander et al. found C. albicans in 3 of 68 samples and Peciuliene et al. in 6 of 33 culture-positive root-filled teeth associated with periradicular lesions. Hancock et al. recovered C. albicans from 1 of 34 root-filled teeth with chronic periradicular lesions that showed detectable microbial growth. Cheung and Ho. isolated this fungal species from 2 of 12 patients from southern China in whom treatment had failed. By examining samples from treatment failures in Brazil, Pinheiro et al. found Candida species in 2 of 51 patients by using culture, whereas Siqueira and Rôças. detected C. albicans in 2 of 22 patients by using polymerase chain reaction. Taken together, all these reports lend support to the assertion that fungi can gain access to the root canals through contamination during endodontic therapy and can be involved in the etiology of recalcitrant periradicular lesions.

Dentin colonization by fungi
Dentin colonization may be an important step during infection of the root canal system and may assume special ecological relevance in that colonizing species have more chances to compete for space and nutrients with the other members of the endodontic microbial community. In addition, the invasion of dentinal tubules can protect microbial cells from the effects of intracanal...
procedures and may play an important role in the establishment of persistent root canal infections. Yeasts are on average 1 to 6 μm in diameter, whereas hyphae are usually 1.9 to 2.6 μm in diameter. On the basis of these cell dimensions, one can surmise that fungi have the ability to penetrate into dentinal tubules. In fact, this assumption is supported by some studies.

Sen et al. investigated the growth patterns of \( \text{C. albicans} \) in relation to human radicular dentin and observed blastospores and hyphal structures on the root canal walls of all specimens. Most of these structures, particularly pseudohyphae, exhibited penetration into dentinal tubules. It was proposed that the contact-sensing (thigmotropism) ability of hyphal structures of \( \text{C. albicans} \) made dentinal invasion inevitable. Therefore, on the basis of this invasive affinity to dentin, they considered \( \text{C. albicans} \) a dentinophilic microorganism. In another study, Sen et al. revealed that enamel and cementum were readily colonized by \( \text{C. albicans} \). Hyphae were seen penetrating into cracks or growing over the ridges. When a smear layer was present on dentin, there was a thick biofilm consisting of different forms of \( \text{C. albicans} \). In contrast, in the absence of smear layer, the biofilm was not present and there were distinct—but separate—colonies. In a consecutive, colorimetric study, Sen et al. demonstrated that the presence of smear layer increased the adhesion of \( \text{C. albicans} \) to dentin. They hypothesized that this increased adhesion was attributable to the availability of the disintegrated organic structure of dentin and the availability of calcium ions as a source for growth and adhesion.

In an in vitro study, Waltimo et al. investigated the penetration of \( \text{C. albicans} \) cells into human dental tubules and reported slight penetration both by hyphae and yeast cells. A few tubules were invaded; the penetration depth was up to 60 μm. However, the in vivo penetration of \( \text{C. albicans} \) into dentinal tubules was revealed to be vigorous. The reason for less penetration in vitro may be the use of agents to remove smear layer. NaOCl and EDTA have generally been used to remove smear layer. This procedure, in turn, decreases organic (mainly collagen) and inorganic content (mainly \( \text{Ca}^{++} \)) of dentin. Thus, dentin no longer becomes a suitable substrate for \( \text{C. albicans} \) demonstrating less attachment and less penetration.

Siqueira et al. investigated the pattern of radicular dentin colonization by 5 fungal species—\( \text{C. albicans}, \text{C. glabrata}, \text{C. guilliermondii}, \text{C. parapsilosis}, \) and \( \text{S. cerevisiae} \)—using scanning electron microscopy. Regardless of the species, single or budding yeast cells were the only fungal forms observed. Whereas \( \text{C. albicans} \) colonized most of the specimens, the other 4 fungal species presented discrete or no colonization of the radicular dentin. \( \text{C. albicans} \) had different patterns of dentin infection. In some specimens, colonization of the dentinal surface was slight and no penetration within dentinal tubules was observed. In the other specimens, some areas of the root canal walls were covered with large colonies of yeast cells and some dentinal tubules were heavily infected.

Susceptibility to antimicrobial endodontic medicaments

Endodontists have been long aware of the need to use proper antimicrobial strategies that include fungi elimination from infected root canals. This can be attested to by the statement of Grossman: “One of the problems in endodontic treatment is the presence of \( \text{C. albicans} \) organisms in infected root canals; it is necessary to eliminate these organisms to maintain the periapical tissue in a normal state or to restore it to a state of health.”

He proposed the use of antifungal agents as intracanal medication.

From the 1950s to the 1970s, antibiotic preparations were extensively used in the treatment of infected root canals, commonly as an intracanal medication. Most antibiotic pastes contained an antifungal agent, mainly nystatin or sodium caprylate. This reflects the importance that was attributed to fungi in endodontic infections at that time. However, when the decline in the use of antibiotics in endodontic therapy ensued, because of the obvious risks of the selection of resistant microorganisms and host sensitization, the use of substances with antifungal effects in endodontic therapy was paid little attention.

Because fungi may be involved in cases of persistent and secondary infections associated with recalcitrant periradicular lesions, the spectrum of antimicrobial activity of endodontic medicaments and irrigants should include these microorganisms. Thus, strategies with medicaments that have antifungal effectiveness may assist in the successful management of persistent or secondary endodontic infections caused by fungi.

Smith and Wayman assessed the antifungal effects of citric acid and sodium hypochlorite and reported that \( \text{C. albicans} \) was more resistant than \( \text{E. faecalis} \) or \( \text{Bacillus} \) species. Moreover, citric acid was not as effective as NaOCl. Sen et al. investigated the antifungal properties of 0.12% chlorhexidine, 1% NaOCl, and 5% NaOCl and found \( \text{C. albicans} \) to be more resistant in the presence of smear layer than in the absence of smear layer. When smear layer was absent, NaOCl started to display antifungal activity after 30 minutes. In another study, Sen et al. evaluated the antifungal effect of EDTA on \( \text{C. albicans} \) as compared with other disinfectants and routine antifungal agents by using the agar diffusion test. They revealed that EDTA had the most effective antifungal activity. Nystatin, ketoconazole, and
1.5% chlorhexidine gluconate solution exhibited the next most effective activity. A 5% NaOCl solution generated large inhibition zones and followed those substances in effectiveness. The decreased concentration of NaOCl (2.5%) significantly reduced its antifungal activity. They have suggested that EDTA demonstrates its antifungal activity in 2 particular ways: anticolonization (ie, reducing adhesive properties of *C albicans*) and antigrowth (ie, decreasing the metabolic activity and pathogenicity of *C albicans* by extracting calcium ions both from the cell wall and the medium). In a subsequent study by the same group,

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**To be continued.**
with the medications for 1 hour, 2 days, and 7 days. The specimens treated with calcium hydroxide/CPMC/ glycerin paste or with chlorhexidine/zinc oxide paste were completely disinfected after 1 hour of exposure. Calcium hydroxide/glycerin paste only consistently eliminated *C. albicans* infection after 7 days of exposure. Calcium hydroxide mixed with chlorhexidine was ineffective in disinfecting dentin even after 1 week of medicament exposure. Of the medicaments tested, the calcium hydroxide/CPMC/glycerin paste and chlorhexidine digluconate mixed with zinc oxide were the most effective in eliminating *C. albicans* cells from dentinal specimens.

On the basis of these reports, it seems that some medicaments, such as chlorhexidine digluconate, calcium hydroxide combinations (with CPMC or chlorhexidine), and EDTA, have the potential to be used as effective intracanal medications for patients in whom fungal infection is suspected.

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Candida


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