12

Role of Nanotechnology in the Management of Indoor Fungi

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12.1 Introduction

Concern over indoor fungal growth has increased over the last decades. Research that relates human health issues to the deterioration of indoor air quality due to microbiological pollutants have been accumulated (Rath et al. 2011; Weber 2012; Täubel and Hyvärinen 2015). In addition, since the 1980s, it has been registered that there is an increase in the occurrence of natural disasters such as floods and extreme rainfall, on a global scale, which has enhanced the problems related to biodeterioration in indoor buildings (Bloom et al. 2009; Chow et al. 2019; EASAC 2018). It is worth mentioning that these kinds of events are directly related to fungal growth since water is a key factor in the development of these microorganisms (Johansson et al. 2013; Møller et al. 2017). Considering the strong impact of indoor fungi on public health, several guidelines have been proposed from different European and North American institutions. These institutions include the University of Connecticut Health Center, which proposed Guidance for Clinicians on the Recognition and Management of Health Effects Related to Mold Exposure and Moisture Indoors (Storey et al. 2004); the United States Environmental Protection Agency (US EPA), which proposed Mold Remediation in Schools and Commercial Buildings (EPA 2008); the World Health Organization (WHO), which proposed guidelines for indoor air quality, dampness, and mold (WHO 2009); the Canadian National Collaborating Centre for Environmental Health Mould Remediation Recommendations (Palaty 2014); and the US Centers for Disease Control and Prevention (CDC), proposed Mold Clean-Up After Disasters (2018), among others (US CDC 2018). These guidelines proposed that there is a dose-effect relationship, where more visible mold showed more symptoms. The improvement in molecular techniques enhances the interest in the field of mycology because due to these techniques it becomes possible to understand the mechanisms by which fungi affect the health of exposed human being.

Considering these facts, a great deal of attention has been given on the development of antimicrobial materials, especially those based on nanotechnology (Mittal et al. 2013; Singh 2016a; Soliman 2017). Moreover, different strategies focusing on efficient association of nanoscale materials in the development of bioactive surfaces which can prevent biofilm formation in indoor environments

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have been intensively studied in the last two decades (Bellotti et al. 2015; Manjumeena 2017; Ghorbani et al. 2018; Han et al. 2019; Barberia-Roque et al. 2019).

The main aim of this chapter is to integrate current knowledge about indoor fungal deterioration and its control through the use of nanotechnology. Moreover, nanomaterials used as effective antifungal agents and possible mechanisms involved in the inhibition of fungal growth have been also described.

12.2 Indoor Fungal Deterioration

12.2.1 Indoor Mycobiota

The mycobiota of indoor environments contains about 150 species and it is considered that a high level of exposure for a building indoor occupant is one greater than 1000 CFU m^{-3} (Sedlbauer 2002; Crook and Burton 2010). Most species belong to the so-called anamorphic fungi, which include deuteromycetes, hyphomycetes, or fungi imperfecti (Yang and Heinsohn 2007). But most are in the ascomycota phylum. In addition to these micro-fungi, a number of basidiomycetes are also found in indoor environments, growing on wood in buildings, and are considered important degraders of wooden building material (Adan and Samson 2011). The important sources of indoor fungal spores include wood products, foodstuffs, vegetables, carpet dust, and fruits. It is important to point out that some fungi may come from more than one source. The fungal genera most frequently found in indoor environments are *Cladosporium*, *Penicillium*, *Aspergillus,* and *Stachybotrys* (Verdier et al. 2014).

After germination, the spores produce a mycelium which covers diverse materials such as textile, paper, wood, coatings, wall paper, and gypsum, among others, depending on the moisture availability (Grant et al. 1989; Annila et al. 2018). Many fungi can produce numerous spores or other propagules, and this explains why there can be high concentrations in the air. The spore of anamorphic fungi is called a conidium. The structure bearing conidia is known as a conidiophore. The formation of conidia varies between the different genera and their efficiency to produce airborne propagules is mainly determined by the mode of conidium formation (Cole and Samson 1979). For example, species of *Aspergillus* and *Penicillium* produce numerous dry conidia which easily become airborne, and this explains the presence of these fungi in an indoor environment (Guerra et al. 2019; Kavkler et al. 2015). In contrast, with blastic arthric conidiogenesis, *Cladosporium* species are among the most abundant fungi in outdoor and indoor air (Anaya et al. 2016; Bensch et al. 2018; Asif et al. 2019).

12.2.2 Factors Influencing Indoor Fungal Growth

Many environmental parameters can influence the growth of indoor fungi. Among some are biotic, and others are physical and chemical, or abiotic factors. Biotic factors include the presence of fungal propagules or spores, viability of spores, the nature of the fungal species, and competing fungi and other organisms. Abiotic factors include nutrients, temperature, moisture, pH, oxygen and carbon dioxide, relative humidity, and light (Crawford et al. 2015; Liu et al. 2018). The viability of fungal spores is associated with several factors, such as age, UV light, and extreme conditions (Johansson et al. 2005; Chen et al. 2017). The dead spores present may be allergenic and contain secondary metabolites, but they cannot germinate and grow and hence are unable to cause infections. Moreover, some viable spores may remain in dormancy. Dormant fungal spores are usually either physically or

chemically restricted from germination. A physical barrier, such as a thickened spore wall, restricts the absorption of water required for spore germination (Madelin 1994; Carlile et al. 2001).

Indoor fungi adapt and grow in environments that are not favorable for bacteria with low water activity and nutrients (Webster and Weber 2007). Different species of fungi have different abilities to access and utilize simple or complex forms of carbohydrate, organics, and mineral nutrients. Decomposition and degradation of a substrate is due to enzyme activities. The types of enzymes required depend on the substrates. The primary food source for indoor fungi is cellulosic matter (Yang and Heinsohn 2007). Fungi usually grow in a wide range of temperatures. In this context, range, including minimum, optimum, and maximum temperatures, can be defined as a temperature profile. Each species has its own profile. Some have narrow and some have wide temperature profiles. Fungi that can grow in a wider temperature range may also have a competitive edge (Griffin 1994). The relative humidity is critically important in indoor fungal growth; it has a secondary effect on condensation and the hygroscopicity of materials (Lattab et al. 2012). In fact, most indoor fungal growth occurs as a result of dampness, but not just due to high relative humidity and condensation on indoor surfaces (Crook and Burton 2010; Täubel and Hyvärinen 2015).

Vegetative and reproductive functions of fungi from indoor environments, together with biotic and abiotic factors, are responsible for deteriorating materials and affecting the health of people who are in these environments (WHO 2009; Hurraß et al. 2017). For example, Ponizovskaya et al. (2019) described the complex of microfungi colonizing mineral building materials, limestone and plaster, in interiors of cultural heritage (Ponizovskaya et al. 2019). These species can actively develop in materials, penetrating for years into the substrates and causing their deterioration in conditions of considerably more moisture content. In this group, *Acremonium charticola* and *Lecanicillium* sp. were able to solubilize calcium carbonate ($CaCO_3$). Moreover, the identification and quantification of filamentous fungi in samples from different indoor and outdoor environments based on traditional microculture methods, DNA extraction, and molecular analyses have been also proposed (Guerra et al. 2019).

Physicochemical characterization analysis was performed to evaluate biological growth; the isolated species produce acids and metabolites capable of causing chemical alterations in mortar substrates and physical damages due to the growth of filamentous structures. Kavkler et al. (2015) studied the presence of indoor fungi on historical textiles, including the canvases of easel paintings stored in museums and religious institutions (churches and cloisters) in Slovenia. Initial observations revealed that such paintings contain relatively widespread fungal contamination (Kavkler et al. 2015). Moreover, examination of the structural and physical changes to the fibers on contaminated and non-contaminated objects showed the most pronounced structural changes on flax and other cellulosic fibers, while proteinaceous fibers (wool and silk) were generally not affected (Kavkler et al. 2015). Surfaces of building materials (plasterboard, mortar, bricks, etc.) are generally highly porous and rough. In damp environments, these materials can provide favorable conditions for the proliferation and growth of microorganisms. Sampling of microbial communities on building materials and in the air is necessary to evaluate its presence and proliferation in indoor environments (Verdier et al. 2014).

As discussed earlier, the most commonly found fungal genera on indoor building materials include *Cladosporium*, *Penicillium*, *Aspergillus,* and *Stachybotrys* (Gutarowska and Czyżowska 2009; Andersen et al. 2011), and various factors such as moisture content, chemical composition, pH, and the physical properties of surfaces play important roles in influencing microbial growth on such surfaces or materials (Adan and Samson 2011). The particular behavior of porous materials in terms of water sorption and the effect of water on microbial proliferation are of prime importance (Nielsen et al. 2004; Verdier et al. 2014).

As mentioned, not only building surfaces or materials, but also quality of air in indoor environments, especially in food production plants and where heritage documents are kept, also needs to be studied. In this context, De Clercq et al. (2014) studied whether the production environment and common ingredients of chocolate confectioneries could be potential sources of contamination with xerophilic fungal species. In this sense, the relevance of fungal spores for food microbiology has been discussed. The function of spores is to disperse fungi to new areas and to get them through difficult periods. A number of fungal species form sexual spores, which are exceptionally stress-resistant and survive pasteurization and other treatments (Dijksterhuis 2019).

Fungi play a considerable role in the deterioration of cultural heritage. Due to their enormous enzymatic activity and their ability to grow at low water activity (a_w) values, fungi are able to inhabit and decay paintings, textiles, paper, parchment, leather, oil, casein, glue, and other materials used for historical art objects (Allsopp et al. 2004). The weathering of stone monuments is significantly increased by epi- and endolithic fungi. In museums and their storage rooms, climate control, regular cleaning, and microbiological monitoring are essential in order to prevent fungal contamination (Sterflinger 2010; Paiva de Carvalho et al. 2018; Melo et al. 2019). Immunosuppressed people exposed to fungi with pathogenic potentials of indoor environments may suffer from mycosis, allergies, and asthma (Perez-Nadales et al. 2014; Sardi et al. 2014). The fungi produce mycotoxins and other biologically active metabolites when growing in buildings, so influence of environmental conditions on the production of these metabolites is intensely investigated (Nielsen 2003; Täubel and Hyvärinen 2015). It was shown that *Stachybotrys chartarum* produced a number of mycotoxins when growing in buildings; *Aspergillus versicolor* produced high quantities of the carcinogenic mycotoxin, sterigmatocystin; *Chaetomium globosum* produced high quantities of chaetoglobosins; whereas *Trichoderma* species did not produce detectable quantities of trichothecenes when growing on materials (Nielsen 2002, 2003).

12.3 Conventional Approach Used for the Control of Indoor Fungi

Various antimicrobial compounds such as disinfectants and biocides are commonly applied to control growth of indoor fungi and bacteria. The most commonly used indoor bioactive compounds can be classified according to their mechanisms of action into two major groups: electrophiles and membrane-active (Chapman 2003). The electrophiles react with nucleophilic groups from biomolecules such as enzymes and nucleic acids present in microbial cells. Some of the most commercially used biocides (electrophiles) in antimicrobial paints are: formaldehydes, formaldehyde releasers, isothiazolinones, carbamates, and metal salts of silver and cooper (Falkiewicz-Dulik et al. 2015a). Among electrophiles, oxidizers like sodium hypochlorite (bleach) have been used for a long time due to their low cost and efficiency (Pereira et al. 2015). These kind of compounds oxidize organic matter in general, but they are being questioned and limited in use due to their toxicity.

On the other hand, the membrane-actives react with the cell membrane leading to its disruption or have ability to change cytoplasmic conditions (Chapman 2003). This group generally includes compounds like alcohols, phenolic derivatives, quaternary ammonium salts and pH actives (e.g. organic acids, parabens, and pyrithiones). Moreover, the control of the indoor fungi can be achieved by maintaining the hygiene by the periodic cleaning with disinfectants and controlling the indoor conditions (temperature, humidity, ventilation, and water leaks) (Adan and Samson 2011; Weber and Rutala 2013). On the other hand, antimicrobial or hygienic coatings having active antifungal ingredients can be used to control indoor fungi and bacteria growth, and therefore biofilm development (Johns 2003; Stobie et al. 2010).

In recent decades, attention has increasingly been paid to the environmental effect of the biocides used, leading to the emergence of new legislation that seeks to restrict their use, especially in North America and Europe (Ribeiro et al. 2018). Some additives are no longer allowed to be used, such as phenylmercurials, however, some others have been restricted in relation to their concentration in formulations (Paulus 2004; Falkiewicz-Dulik et al. 2015a). Some examples of these are aromatic and halogenated derivatives. Conventional biocides commonly added in commercial formulations include 5-Chloro-2-(2,4-dichlorophenoxy) phenol (Triclosan), 2-octyl-4-isothiazolin-3-one (OIT), dichlorooctylisothiazolone (DCOIT), methyl-2-benzimidazolecarbamate (Carbebendazim), and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron) (Allsopp et al. 2004).

Usually, combinations of biocides are used to improve the spectrum of antifungal activity. However, the efficacy of such combinations, which are commonly determined in terms of minimum inhibition concentration (MIC) varies depending on the type and concentration of combination used. Therefore, more effective ("booster") compositions of biocides have been investigated in order to reduce the concentration of the active ingredients that would be used separately (Bellotti et al. 2012; Falkiewicz-Dulik et al. 2015a). For example, 2-methyl-4-isothiazolin-3-one (MIT) has relatively low antimicrobial activity, but it has shown a significant synergistic activity when combined with 1,2-benzisothiazolin-3-one (BIT) (Karsa and Ashworth 2002).

The addition of biocides in paints during the dispersion process is often not satisfactory, due to the fact that biocidal activity can be lost before the end of life of the coating (Sørensen et al. 2010; Mardones et al. 2019). It has been recorded that paint films in buildings, which should maintain their biocidal functionality for more than 10 years, actually do it for less than two years in extreme conditions (Eversdijk et al. 2012). The reasons that limit the efficiency of antimicrobial paints and coatings are: loss of biocidal efficacy of the film on the surface due to leaching or engaged in reactions with resin, pigment, and additives; degradation of the bioactive component by environmental factors; incompatibility between biocides within the paint; and commercial organic biocides could be used as a nutrient source by some microorganism (Edge et al. 2001; Falkiewicz-Dulik et al. 2015a; Andersson-Trojer et al. 2015; Kakakhel et al. 2019). The architecture of the paint films must be considered for a better understanding of this issue. Waterborne acrylic paints, most commonly used in buildings, are constituted by aqueous dispersion of polymer lattices, which after drying leads to the emergence of macroscopic pores. Therefore, this porosity favors the release of the biocides which reside in the pores or are adsorbed on particles (Andersson-Trojer et al. 2015).

Considering the facts mentioned here, several efforts are currently seeking to improve the bioactive ingredients used in formulation to prolong their useful life, to replace the toxic conventional ones, to decrease the concentrations used, and prevent their loss from the film. In this context, the emergence of nanotechnology presents an extensive field of study for new functional materials with size-dependent properties. Some strategies are based on finding free nanoparticles with antimicrobial activity, while others are based in associate antimicrobial agents to nanomaterials to protect them and control their release.

12.4 Nanotechnology for the Control of Fungal Growth

Nanotechnology has developed as one of the most groundbreaking scientific fields in the last few decades, since it exploits the enhanced reactivity of materials at a nanoscale. Currently, most scientists believe that nanomaterials are one of the mainstays of developing science and technology in the twenty-first century.

Materials with at least one dimension lower than 100 nm are considered "nano," and these materials have different physical and chemical properties from those in the microscale or bulk form

(Haupert and Wetzel 2005; Singh 2016b). They have surface/volume ratio higher than bulk ones, which is reflected in the fact that the majority of the atoms are located on the surface (Morones et al. 2005; Mathiazhagan and Joseph 2011). A large number of public domain investigations proposed that properties of nanoparticles (NPs) fundamentally depend on their size and shape. Similarly, the antimicrobial activity of NPs can be changed if they are modified (Morones et al. 2005; Raza et al. 2016).

NPs can be prepared using two major approaches: top-down (reduce in size to a suitable material) and bottom-up (build them from elemental entities like atoms and molecules) (Mittal et al. 2013). The top-down approach uses physical or chemical methods, which frequently have high-energy demand and produce NPs with surface imperfections; milling is a typical example of this approach (Landge et al. 2017; Thakkar et al. 2010). Bottom-up approaches are based on chemical or biological synthesis and usually produce colloidal dispersions of the NPs with fewer defects and more homogeneous chemical composition (Cao and Sun 2009; Singh et al. 2016).

Antifungal activity of nanomaterials depends on their properties, such as surface charge, composition, size, shape, and partial oxidation capacity (Kumar et al. 2013; Mittal et al. 2013; Singh et al. 2016). Studies that attempt to explain the mechanism of action of NPs are focused on assays that intrude the inhibition of spore germination, radial mycelial growth, and aflatoxin synthesis (Kasprowicz et al. 2010; Kairyte et al. 2013; Mitra et al. 2017). Taking into account the chemical nature of the NPs that were probed to be active against fungal strains, they can be classified into three main groups: metal, non-metal, and hybrid (metal/non-metal). Table 12.1 shows various nanomaterials which can be effectively used in the management of different fungi. Figure 12.1 shows NPs adhered to silica filler after the green synthesis process (Figure 12.1a,b). Visible differences between the surfaces of the siliceous material decorated with NPs and the original are indicated in Figure 12.1 c1 and c2.

12.4.1 Metal Nanoparticles

Metal (and metal oxide) NPs can be fine-tuned with several chemicophysical properties, size, surface to volume ratio, structural stability, and target affinity, for better efficiency and to facilitate their application in different fields (Elbourne et al. 2017). The most widely used synthesis method for metal NPs is wet-chemical, where NPs are formed from respective metal ions using a liquid system which contains reducing (e.g. sodium borohydride, methoxy polyethylene glycol, or hydrazine) and stabilizing agents (e.g. sodium dodecyl benzyl sulfate, polyvinyl pyrrolidone, or citrate) (Badawy et al. 2010; Singh 2016b). The chemical methods have been questioned due to the use of toxic solvents and the generation of hazardous by-products, which has increased interest in eco-friendly alternatives framed in green syntheses like bioreduction or biological methods (Singh et al. 2016).

The most studied metal NPs with antimicrobial activity are: silver (Ag), copper (Cu), zinc oxide (ZnO), and titanium dioxide (TiO_2) (Ruffolo et al. 2010; El Saeed et al. 2016; Nguyen et al. 2019). Without a doubt, AgNPs are the best known for their antimicrobial activity and their synthesis typically occurs by reduction of soluble silver salts in the presence of reducing agents such as citrate, glucose, ethylene glycol, or sodium borohydride (Badawy et al. 2010; Singh et al. 2016). AgNPs with different shapes have been produced besides the most common spherical ones, including pyramids, rods, triangular prisms, and cubes (Pal et al. 2007; Raza et al. 2016). Silver is able to control various pathogens with relative safety, if compared to synthetic fungicides, and it displays multiple modes of inhibitory action to microorganisms (Ogar et al. 2015). Hitherto, AgNPs have been shown to be effective biocides against *Aspergillus niger*, *Alternaria alternata, Alternaria bras-*

Table 12.1 Details about antifungal efficacy of different nanoparticles against a variety of fungi (MIC: minimum inhibitory concentration).

Nanoparticles	Synthesis method	Size of NPs (nm)	Test method	Fungi tested	MIC	References
Metal-based nanoparticles						
AuNPs	Chemical: using $SnCl_2$ and $NaBH_4$	7–15	Broth dilution (CLSI/M27-A3)	*Candida albicans, Candida tropicalis,* and *Candida glabrata*	4–16 μg ml^{-1}	Ahmad et al. (2013)
ZnONPs	Biological: using *Artocarpus gomezianus* fruit extract	30–40	Agar diffusion	*Aspergillus niger*	—	Anitha et al. (2018)
SiO_2 NPs doped Fe_2O_3	Chemical: coprecipitation method	6–20	Agar diffusion with dilutions	*Candida parapsilosis* and *Aspergillus niger*	0.18–0.24 mg l^{-1}	(Arshad et al. 2019)
ZnNPs-doped SiO_2	Chemical: deposition precipitation	7	Disc diffusion	*C. parapsilosis* and *A. niger*	—	Arshad et al. (2018)
AgNPs, CuNPs, and FeNPs	Biological: using tea leaf extracts	10–20, 26–40 and 42–60, respectively	Oxford cup diffusion And broth dilution (CLSI, 2012)	*Aspergillus flavus* and *A. parasiticus*	8 (Ag)-32(Cu)-128(Fe) μg ml^{-1}	Asghar et al. (2018)
AgNPs, CuNPs, ZnONPs, and AuNPs	Biological: extracellular products from *Enterococcus faecalis*	9–130, 20–90, 16–96 and 20–70, respectively	Broth dilution (potato dextrose)	*C. albicans* MTCC 3017, *Candida neoformans* MTCC 1347, *A. niger* MTCC 282, and *Fusarium oxysporum* MTCC 284	8–64 (Ag) 8 to ≥128(Cu) 8 to ≥128 (ZnO) ≥128 (Au) μg ml^{-1}	Ashajyothi et al. (2016)
AgNPs	Biological: using *Cassia* spp. aqueous leaf extracts	15–30	Agar well diffusion	*A. niger*, *Aspergillus fumigatus*, *A. flavus, Penicillium* sp., *C. albicans, Rhizoctonia solani, F. oxysporum,* and *Curvularia* sp.	—	Balashanmugam et al. (2016)

(Continued)

Table 12.1 (Continued)

Nanoparticles	Synthesis method	Size of NPs (nm)	Test method	Fungi tested	MIC	References
AgNPs	Biological: using *Selaginella Bryopteris* leaf extracts	15–30	Agar well diffusion	*A. niger*	1 mg/100 µL	Dakshayani et al. (2019)
AgNPs	Biological: using extracellular products from *Fusarium oxysporum*	93	Agar diffusion (M51-A2) and broth dilution (M38-A2) of CLSI	*A. flavus* MCT 00335, *Aspergillus nomius* MCT 00328, *A. parasiticus* MCT 00336, *Aspergillus melleus* MCT 00144, and *Aspergillus ochraceus* MCT 00435.	MIC_{50}: 4–8 µg ml^{-1}	Bocate et al. (2019)
FeNPs doped ZnO	Biological: using *Hibiscus Rosa* leaf extracts	15–170	Agar diffusion	*C. albicans*	—	Chai et al. (2019)
ZrO_2NPs	Chemical: calcination	17	Mycelium growth	*R. solani*	>100 µg l^{-1}	Derbalah et al. (2019)
AgNPs and AgClNPs	Biological: using *Malva sylvestris* leaf extracts	10–50	Broth microdilution according to CLSI (M7-A7)	*C. glabrata* CCUG 35267, *Candida orthopsilosis* ATCC 20503, *C. parapsilosis* ATCC 22019, and *C. tropicalis* CCUG 34274	7.8–62 µg ml^{-1}	Feizi et al. (2018)
AgNPs	Biological: using the culture supernatants of *Cryptococcus laurentii* and *Rhodotorula glutinis*	35–400 and 15–220 respectively	Broth microdilution	*B. cinerea*, *P. expansum*, *A.niger*, *Alternaria* sp. and *Rhizopus* sp.	2–4 mg l^{-1}	Fernández et al. (2016)

AgNPs	Chemical: coated with dodecanethiol		Antibiofilm resistance	*C. albicans* ATCC 18804	—	Ferreira et al. (2019)
CuONPs	Chemical: precipitation and autoclaved at 120 °C for 2 h	20–100	Agar diffusion	*Penicillium* sp.	—	Ghorbani et al. (2018)
TiO_2NPs and ZnONPs	Chemical synthesis	50–100	Agar diffusion	*C. albicans* ATCC 10231	—	Haghighi et al. (2011)
MgNPs doped ZnONPs	Chemical: coprecipitation	43–63	Agar diffusion Broth dilution	*C. albicans* ATCC 10231	2000 $\mu g\,ml^{-1}$	Haja Hameed et al. (2015)
ZnONPs	Commercial	~20	Agar dilution	*Botrytis cinerea* *Penicillium expansum*	>12 $mmol\,l^{-1}$ (>9.8 $\mu g\,ml^{-1}$)	He et al. (2011)
CuONPs and Fe_2O_3NPs	Biological: using *Euphorbia helioscopia* leaf extracts	7–10	Agar well diffusion	*Cladosporium herbarum*	—	Henam et al. (2019)
MnNPs doped ZnS	Chemical synthesis	~2.7	Agar dilution	*A. niger, A. fumigatus, A. flavus, Aspergillus terreus, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton gypsum, Microsporum audouinii, Microsporum canis, F. oxysporum*	>30% p/v	Ibrahim et al. (2017)
AuNPs	Biological: using *Abelmoschus esculentus* aqueous extract	62	Agar well diffusion	*Puccinia graminis tritci*, *A. flavus*, *A. niger* and *C. albicans*	—	Jayaseelan et al. (2013)
ZnONPs	Commercial aqueous dispersion	195	Agar dilution with light exposition (400 nm lamp)	*Botrytis cinerea*	$>5\times10^{-3}$ M	Kairyte et al. (2013)

(Continued)

Table 12.1 (Continued)

Nanoparticles	Synthesis method	Size of NPs (nm)	Test method	Fungi tested	MIC	References
CuNPs	Chemical synthesis: with isopropyl alcohol	3–10	Agar disc diffusion	*Phoma destructiva* DBT-66, *Curvularia lunata* MTCC 2030, *Alternaria alternata* MTCC 6572, and *F. oxysporum* MTCC 1755	—	Kanhed et al. (2014)
AgNPs	Biological: using the culture filtrate of *Fusarium chlamydosporum* NG30 and *P. chrysogenum*	6–26 and 9–17 respectively	Broth microdilution (germination inhibitory effect)	*A. flavus* NRRL 3145 and *Aspergillus ochraceus* ATCC 22947	45–51 $\mu g\,ml^{-1}$	Khalil et al. (2019)
AgNPs, TiO_2NPs, ZnONPs, and SiO_2 NPs	Chenical synthesis	5, 11, 25, and 35 respectively	Broth microdilution	*C. albicans* ATCC 10145	—	Khan et al. (2018)
ZnONPs	Biological: using *Medicago sativa* aqueous extract	~14	Broth microdilution according to CLSI	*C. albicans* ATCC10231	9.31 $\mu g\,ml^{-1}$	Król et al. (2019)
AuNPs	Biological: using *Croton Caudatus Geisel* aqueous extract	20–50	Agar diffusion with dilutions	*A. niger* MTCC 281, *A. flavus* MTCC 277, *A. terreus* MTCC 1782, *F. oxysporum* MTCC 284, and *C. albicans* MTCC 227.	50–150 µg/well	Vijaya Kumar et al. (2019)
PtNPs	Biological: using *Xanthium strumarium* aqueous extract	22	Agar well diffusion with dilutions	*A. niger* MTCC 281, *A. flavus* MTCC 277, *C. tropicalis, C. parapsilosis,* and *C. albicans* (MTCC 227)	50 µg/well	P. V. Kumar et al. (2019a)
AgNPs	Biological: using the culture filtrate of *Trichoderma viride* MTCC 5661		Agar dilution	*Alternaria brassicicola* and *F. oxysporum*	5 $\mu g\,ml^{-1}$	Kumari et al. (2019)

AgNPs/Zeolitet	Chemical: ion exchange and thermal treatmen	2–34	Agar diffusion	*Trichoderma harzianum* Rifai	—	Machado et al. (2019)
ZnONPs	Biological: using *Pithecellobium dulce* peel extract	30	Broth dilution (biomass weighing)	*A. flavus* and *A. niger*	>1000 ppm	Madhumitha et al. (2019)
AgNPs	Biological: using lignin	10–15	Agar diffusion with dilutions	*A. niger*	0.3 μg ml^{-1}	Marulasiddeshwara et al. (2017)
NiONPs coupled CdO	Chemical: presipitation with ammonia and calcined at 200 °C		Agar well diffusion	*A. terreus*	—	Nallendran et al. (2019)
AgNPs	Commercial	~55	Agar dilution	*Penicillium brevicompactum, Aspergillus fumigatus, Cladosporium cladosporoides, Chaetomium globosum, Stachybotrys chartarum, and Mortierella alpina*	>100 ppm	Ogar et al. (2015)
Fe_2O_3NPs	Chemical: using tannic acid solution	10–30	Agar diffusion Broth dilution	*Trichothecium roseum, C. herbarum, P. chrysogenum, A. alternata* and *A. niger*	0.016–0.063 mg ml^{-1}	Parveen et al. (2018)
AuNPs	Chemical: with carbosilane–synthesis with $NaBH_4$	~3	Broth microdilution according to CLSI (M7-A7)	*C. albicans* ATCC 10231 and *C. glabrata* ZMF40	47.6 ppm	Peña-González et al. (2017)
Ag-TiO_2NPs	Chemical: electrochemically deposited of Ag on commercial TiO_2 NPs	20	Agar diffusion	*A. niger, Aspergilus terreus, A. flavus, Chaetominum globusum, Mirothecium verrucaria, Paecilomyces varioti, Aureobasidium pullulans, Penicilium cyclopium, Penicilium funiculosum, Penicilium glaucum, T. viride.*	—	Petica et al. (2019)

(Continued)

Table 12.1 (Continued)

Nanoparticles	Synthesis method	Size of NPs (nm)	Test method	Fungi tested	MIC	References
Non-metal and metal/non-metal (hybrid) nanoparticles						
β-d-glucan	—	60	Agar diffusion	*Pythium aphanidermatum*	—	Anusuya and Sathiyabama (2014)
PLGA loaded with Amphotericin B	Chemical: emulsion solvent evaporation	˂200	Broth microdilution (M27-A2) of CLSI.	*C. albicans* ATCC 64548 and *C. neoformans* ATCC 90112.	0.05–0.5 μg ml^{-1}	Moraes Moreira Carraro et al. (2017)
Chitosan loaded with clove essential oil	Chemical: emulsionic gelation technique	40–100	Agar dilution	*A. niger*	1.5 mg ml^{-1}	Hasheminejad et al. (2019)
Luliconazole	Nanocrystal	263–611	Agar well diffusion	*C. albicans* MTCC No 183	—	M. Kumar et al. (2019b)
Chitosan-gellan gum loaded with Ketoconazole	Synthesis by nanosuspension	~156	Mycelium growth inhibition	*A. niger* NICM 590	>100 μg ml^{-1}	Kumar et al. (2016)
Chitosan loaded with *Schinus molle* L. essential oil	Chemical: ionotropic gelation	~500	Broth microdilution	*A. parasiticus* ATCC 16992	>500 μg ml^{-1}	López-Meneses et al. (2018)
Chitosan/Ag	Chemical synthesis	373	Broth dilution Mycelium growth	*F. oxysporumin*	100 μg ml^{-1} >1000 μg ml^{-1}	Dananjaya et al. (2017)
Chitin nanofibers/Ag	Physical: UV light reduction	10	Antibiofilm resistance	*A. alternata*, *Alternaria brassicae*, *Alternaria brassicicola*, *Bipolaris oryzae*, *Botrytis cinerea*, and *Penicillium digitatum*	—	Ifuku et al. (2015)
Chitosan	Chemical: by emulsion	~173	Broth microdilution	*C. albicans*	>75 μg ml^{-1}	Pan et al. (2019)
Sodium hyaluronate/TiO_2	Chemical: sol-gel method and calcined at 650 °C	23	Agar diffusion	*A. niger*	—	Safaei and Taran (2017)

CuO/C	Chemical: precipitation with sucrose and calcined at 500 °C	25–30	Agar dilution	*A. niger* and *A. flavus*	>1000 ppm	Roopan et al. (2019)
Alginate/CuO	precipitation, mixed, and dried	37	Agar diffusion	*A. niger*	—	Safaei et al. (2019)
Chitosan/Ag loaded with fluconazole	Mixed	30–50	Agar diffusion	*C. albicans* MTCC 4748 and *T. rubrum* MTCC 7859	—	Samrat et al. (2016)
Chitosan loaded with anionic proteins from *Penicillium oxalicum*	Mixed	10–30	Agar diffusion	*F. oxysporum, Pyricularia grisea,* and *Alternaria solani*	—	Sathiyabama and Parthasarathy (2016)
Clotrimazole/Ca $(OH)_2$	Mixed	294	Agar dilution	*A. niger* and *P. corylophilum*	4%	Sequeira et al. (2017)
Pectin/Ag	Mixed	5–50	Agar diffusion	*Aspergillus japonicus*	—	Su et al. (2019)
5-amino-2-mercapto benzimidazole/Ag_3O_4	Chemical: presipitation, mixed, and dried	18–29	Agar diffusion with dilution	*A. niger*	20 µl	Suresh et al. (2016)
Thyme and dill oil/Cu	Chemical synthesis and mixed	100–250	Conidia germination Mycelium growth	*Colletotrichum nymphaeae*	90–600 ppm	Weisany et al. (2019)
Chitosan loaded with *Origanum vulgare* oil	Electrospraying synthesis	290–483	Agar dilution	*A. alternata*	0.02–0.005% (w/v)	Yilmaz et al. (2019)
16-mercaptohexadecanoic acid/amino silano/magnetic particles	Chemical synthesis	—	Fungal cell viability in planktonic form	*C. albicans*	—	Wilczewska et al. (2019)

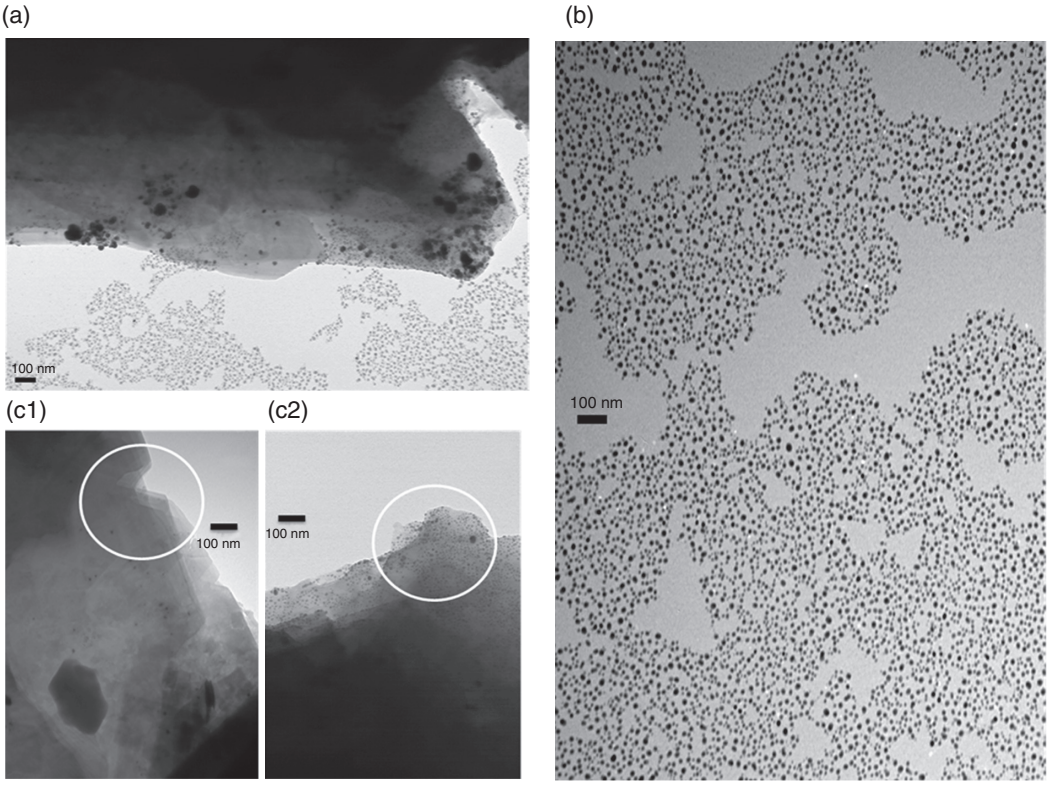

Figure 12.1 TEM micrographs of: (a) nanoparticles adhered to silica filler during the synthesis process; (b) free nanoparticles obtained by green synthesis; (c1) Siliceous filler before decorating with nanoparticles, and (c2) result of decorating with silver nanoparticles by green synthesis.

sicicola, *Alternaria solani, Botrytis cinerea, Fusarium oxysporum, Penicillium* spp., *Rhizoctonia solani*, and *Colletotrichum* spp. (Sardella et al. 2019).

AgNPs liberate silver ions in the fungal cells, which could attack the respiratory chain and cell division leading to cell death (Moritz and Geszke-Moritz 2013). In addition, they interact with the thiol groups of several enzymes, inactivating them and affecting processes such as nutrition (Chung and Toh 2014). Silver can also generate reactive oxygen species (ROS) that have high cytotoxic activity and can cause cell death (Morones et al. 2005; Singh et al. 2016). Silver ions can cause denaturation of proteins and DNA, which affects the replicative machinery in the fungal cell (Dananjaya et al. 2017). During the formation of the germ tube, the wall of the hypha is thinner and more fragile in the apical part; this may be the moment that allows the interaction of AgNPs in the cell wall, causing an increase in the permeability of the membrane, and with it an alteration in conidial viability (Jo et al. 2009; Mahmoud et al. 2014).

ZnONPs and their mechanism of action against two post-harvest pathogenic fungi *B. cinerea* and *Penicillium expansum*, were investigated (He et al. 2011). In this case, *P. expansum* was found to be more sensitive to treatment with ZnONPs than *B. cinerea*; NPs inhibited the growth by affecting cellular functions, which caused deformation in fungal hyphae, and prevented the development of conidiophores and conidia of *P. expansum* (He et al. 2011). In addition, Kairyte et al. (2013) obtained similar results against a *B. cinerea* strain when exposed to these NPs in suspension. Similarly, Sharma et al. (2010) studied the antifungal activity of ZnONPs against *Fusarium* sp. and

proposed the fungal growth inhibition was due to the rupture of the cell membrane, resulting in the possible decrease in fungal enzymatic activity.

Iron oxide NPs were evaluated for their antifungal activity against the following: *Trichothecium roseum*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *A. alternata,* and *A. niger*. The maximum inhibition in spore germination was caused against *T. roseum* (~88%) followed by *C. herbarum* (~85%) (Parveen et al. 2018). Iron oxide NPs cause oxidative stress through the generation of ROS and Fenton reaction. Since iron is a strong reducing agent, it induces the decomposition of functional groups in membrane proteins and lipopolysaccharides. Iron-based NPs also cause oxidation by intracellular oxygen, leading to oxidative damage via Fenton reaction. These NPs penetrate through disrupted membranes causing further damage and death of cells (Parveen et al. 2018).

Antimicrobial NPs obtained from aqueous plant extracts are reported to be very promising because these are accessible, effective, low cost, and eco-friendly (Mittal et al. 2013; Singh et al. 2016). AgNPs synthesized using aqueous extracts from different plants (*Schinus molle*, *Equisetum giganteum*, and *Ilex paraguariensis* Saint Hilaire) have been studied by Barberia et al. (2019) against fungal strains, i.e. *A. alternata* and *Chaetomium globosum*. These filamentous fungi are known to deteriorate indoor waterborne acrylic paints (Bellotti et al. 2013). Suspension with AgNPs from *E. giganteum* proved to be the most active, with a minimum inhibitory concentration of 3.3 and 67.5 $\mu g\,ml^{-1}$, respectively (Barberia-Roque et al. 2019). Biosynthesis of NPs is considered an economical and eco-friendly approach; moreover, it can be a novel substitute for NPs obtained by chemical synthesis (Xue 2016; Malkapur et al. 2017). The enhanced antifungal activity was reported for AgNPs biosynthesized by cell-free filtrate of *Trichoderma viride* (MTCC 5661) compared to chemically synthesized AgNPs of similar shape and size (Kumari et al. 2019). In this sense, biosynthesized AgNPs enhanced the reduction in dry weight by 20% and 48.8% of *F. oxysporum* and *A. brassicicola*, respectively, in comparison to their chemical counterparts; *A. brassicicola* revealed that osmotic imbalance and membrane disintegration are the major cause for fungal cell death after treatment with the biosynthesized AgNPs (Kumari et al. 2019).

12.4.2 Non-metal and Hybrid (Metal/Non-metal) Nanoparticles

Due to its structure, Kraft lignin formed by phenyl propanol and aryl-alkyl ether bonding can be a good source of polyols. The multiple hydroxyl groups present in the lignin's structure are essential raw materials for polyurethane production. Also, for polyolefins, polyethylene terephthalate (PET), and polycarbonate production, the plastics can be either replaced or enriched with bio-based components (Brodin et al. 2017). Considering sustainability concerns and the fact that petroleum products are commonly used in the polyurethane industry, bio-based polyols and lignopolyols could be an environmentally friendly solution (Mahmoud et al. 2014). Although a bioplastic is characterized as being produced from a renewable source, bioplastics are not necessarily biodegradable. As an example, biopolyethylene (BioPe) is similar to the fossil-based polyethylene and thus is not biodegradable. Hence, plastic biodegradability is determined by the chemical structure rather than origin (Brodin et al. 2017).

There are several examples of organic NPs obtained by encapsulation with various polymers such as chitosan, alginate, and poly(lactic-co-glycolic acid) (Pan et al. 2019; Safaei et al. 2019; Yilmaz et al. 2019). Bioactive biogenic compounds obtained from plants as essential oils or some of their components have been used. For example, chitosan nanoparticles (ChNPs) loaded with clove essential oil (CEO) were developed with the emulsion ionic gelation technique to improve the antifungal efficacy of CEO (Hasheminejad et al. 2019). The ChNPs demonstrated a superior performance against *A. niger*, isolated from spoiled pomegranate, compared with free CEO being

active with a minimum concentration of 1.5 mg ml^{-1}. Similarly, López-Meneses et al. (2018) prepared polymeric ChNPs loaded with *S. molle* essential oil (SEO). These NPs have been studied as a possible substitute for fungicides against *Aspergillus parasiticus* and showed significant inhibition on spore germination (>80%) and aflatoxin production (>59%) at a concentration of 500 mg ml^{-1}. Antifungal agents such as ketoconazole and amphotericin B loaded in chitosan-gellan gum and poly(lactide-co-glycolide) NPs, respectively, were assessed against *A. niger* (Kumar et al. 2016; Moraes Moreira Carraro et al. 2017).

A large number of nanofiber fabrication methods have been reported, including template synthesis, molecular self-assembly, and hydrothermal methods. Spinning methods, including electrospinning, blow spinning, centrifugal spinning, and draw spinning, allow researchers to fabricate nanofibers from a precursor solution. A variety of polymeric nanofibers can be obtained by spinning methods. Compared with other techniques, spinning allows easier integration into industrial large-scale production (Huang et al. 2019). Polymer nanofibers fabricated via the facile electrospinning technique, mainly biopolymers, have ease of processing, excellent biocompatibility, and non-toxicity (Ambekar and Kandasubramanian 2019). Chitin nanofibers with AgNPs have been synthesized by Ifuku et al. (2015) and tested against 11 fungal strains. These hybrid AgNPs/chitin films showed an inhibition of spore germination >90% with 8 of the strains used.

Roopan et al. (2019) synthesized the bioactive hybrid CuO/C nanocomposite using sucrose as a capping agent. The antifungal activity of CuO/C nanocomposite was tested against *A. niger* and *A. flavus* at 1000 ppm and about 70% and 90% of inhibition, respectively, was reported. The authors proposed that this nano-complex causes interrupted transmembrane e^- transport, cell membrane disruption, mitochondria damage, and cytoplasm leakage.

Other examples are metal-organic framework (MOF) nanosheets which have attracted great attention due to their distinctive characteristics such as nanoscale and tunable thickness, high-aspect-ratio, large surface area, more exposed accessible active site, favorable mechanical flexibility, and optical transparency (Li et al. 2019). Recently, nanostructured MOFs, as a kind of crystalline material, were also constructed by the diversified interconnection of the organic linkers and metal nodes. These features endow MOF nanosheets with enhanced applications in gas separation, catalysts, sensing, energy storage and transfer, and enzyme inhibition.

12.4.3 Nanotechnological Management of Indoor Fungi

As mentioned, nanotechnology in general and NPs (nanomaterials) in particular play a key role in the control of growth of various fungi-causing infections in humans, plants, and other life forms. Colonization of fungi in indoor environments is considered a major concern because they have ability to cause many health-related issues. Therefore, management of indoor fungi is extremely important to avoid the ill effects caused by them. As discussed earlier, conventional approaches are available for the control of indoor fungi, but they have certain limitations. In this context, considering the antifungal potential of various NPs as discussed earlier, it is believed that such NPs can be effectively used in the management of indoor fungi. It is most unfortunate that very few reports are available on the management of indoor fungi using nanotechnological solutions. However, available reports revealed that NPs can be used as novel, effective, and eco-friendly alternative antifungal agents to chemical fungicides. Some of the nanotechnological applications that have been reported to the management of indoor fungi include the adoption of nano-enabled disinfectants, surface biocides, air filters, packaging, and rapid detection methods for contaminants (Vance et al. 2015; He and Hwang 2016; Chen et al. 2017).

Although the fate and potential toxicity of nanomaterials are not fully understood at this time and scientific risk assessments are required, it is evident that there have been significant advances

in their applications (Brincat et al. 2016; King et al. 2018; Jogee and Rai 2020). Peanuts are vulnerable to fungal infections during long-term storage. Fungi infecting peanuts are toxigenic and cause health hazards. Further, the antifungal potential of AgNPs was evaluated and showed ability to inhibit fungal growth. *Cymbopogon citratus* leaf extract-mediated AgNPs were found to have prominent antifungal potential against all test fungi and its MIC was found to be 20 $\mu g\,ml^{-1}$ (Jogee et al. 2017). Pokhum et al. presented a facile and cost-effective approach to remove airborne microbes from indoor air by employing silver (Ag) and zinc oxide (ZnO) to decorate fibrous air filters (Pokhum et al. 2018). This method successfully led to homogeneous coating of active nanomaterials on the filter's surface. The developed Ag/ZnO air filter reduced the airborne psychrotrophic germ concentrations by ~50% and its efficiency increased to ~70% when combined with UVA illumination. Based on these results, a simple and low-cost ZnO/Ag air filter was successfully introduced as an effective strategy for removal of psychrotrophic microbes from indoor air.

Household cleaning products have been incorporating antimicrobial agents for past several decades, and they have achieved overwhelming success in gaining the confidence of the consumers. The staggering customer demands have motivated the industrial sector to constantly look for new effective antimicrobial products. For example, Microban® is a combination of polyvinylidene difluoride coating matrix along with silver as an active ingredient. Microban® technology offers protection from the deterioration of the coating from mold and mildew. The silver-based particles, on contact with microbes, do not allow the reproduction of microbes by interfering with metabolism and disrupting/damaging the cell walls. Moreover, the active ingredient interferes with the conversion of nutrients into energy, thereby inhibiting the reproductive process. This product, when used in waterborne or solvent-borne paint or coating, provides excellent protection in both indoor and outdoor environments (Tiwari and Chaturvedi 2018).

12.5 Hygienic Coatings and Nanotechnology

In order to inhibit or prevent the growth of microorganisms, including fungi, on building materials, the disruption of their vital processes is required. Figure 12.2 shows microscopic pictures of three mold strains commonly found in indoor spoilage materials growing on coatings in controlled conditions.

Hygienic paints are important tools to avoid indoor biological colonization and prevent biodeterioration which creates health problems in people and pets (Stobie et al. 2010; Falkiewicz-Dulik et al. 2015b). These functional paints can be used for painting in dwellings and hospitals. They can be also used in the food industry because in this sector they must deal with microbial growth as one of the most critical issues affecting production, processing, transport, and storing. Several applications of metal NPs in food safety are currently available (e.g. packaging material, air filter coatings) (Souza and Fernando 2016). Nanotechnology applied for the design of antimicrobial surfaces can eliminate pathogens in close proximity to the surface, preventing biofilm formation (Kaiser et al. 2013). The precise biocidal mechanism arising from these materials is complex in nature and is dependent on both the microbe and nanomaterial used (Bapat et al. 2018).

Additive paints and coatings with antimicrobial NPs have been studied (Kumar et al. 2008; Jo et al. 2009; Zielecka et al. 2011; Holtz et al. 2012; Dominguez-Wong et al. 2014; Barberia-Roque et al. 2019; Machado et al. 2019). There are two possible ways to incorporate NPs into a paint formulation: free or supported in other material. The direct use of metal NPs such as Ag, Cu, and ZnO in waterborne paints (latex type) can result in the decrease of their antimicrobial activity due to their reactivity with other components present in the formulation or their agglomeration (Zielecka et al. 2011; Bellotti et al. 2015; Arreche et al. 2017). Taking this into account, there are several

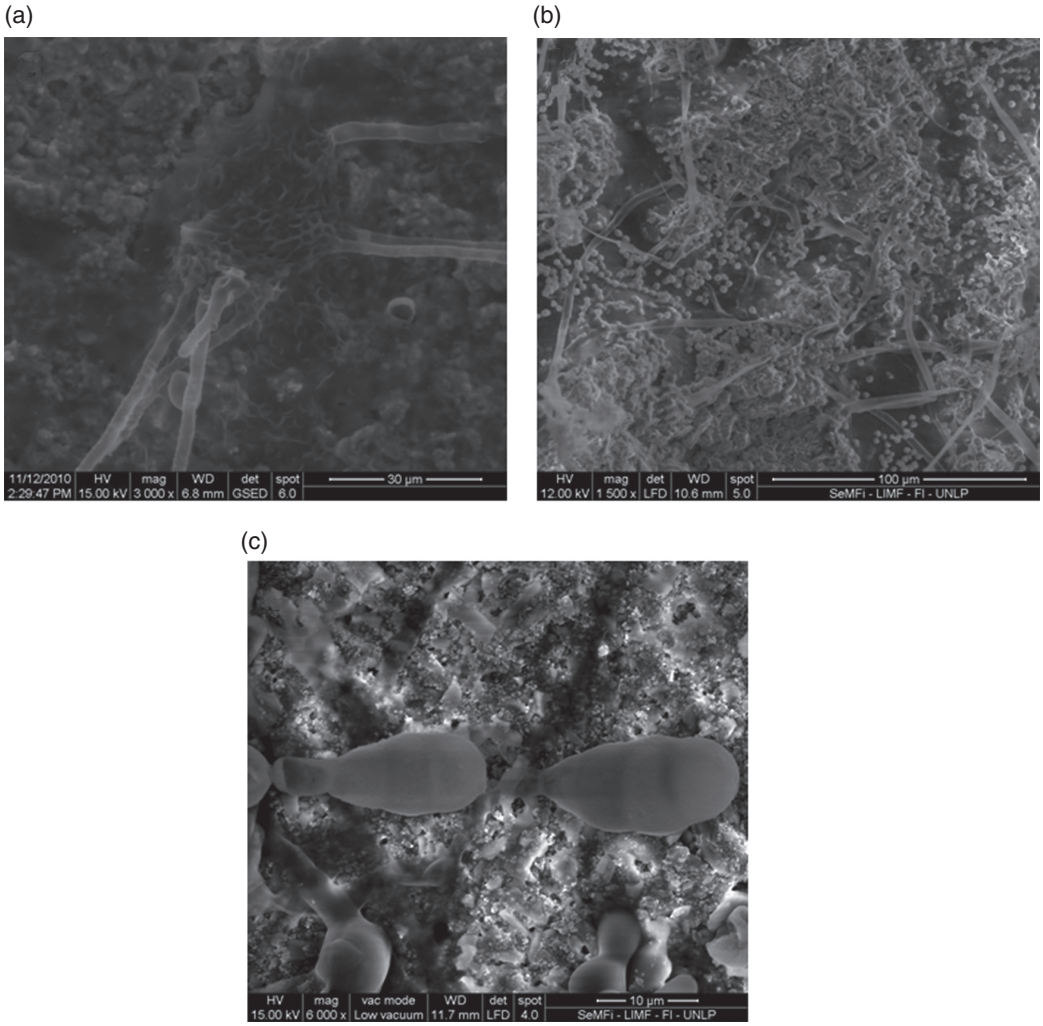

Figure 12.2 Active fungal growth on coatings: (a) *Chaetomium globosum* (KU936228), (b) *Aspergillus versicolor* (MG725821), and (c) *Alternaria alternata* (KU936229).

works performed that showed the efficient incorporation of bioactive nano-additives (in supported or immobilized form) in other materials to be applied in paints (Zielecka et al. 2011; Arreche et al. 2019; Machado et al. 2019).

Often the biocidal activity of the organic compounds ends long before the lifetime of the coating due to its low retention or degradation (Edge et al. 2001; Mardones et al. 2019). Therefore, usually they are loaded in natural or synthetic NPs that act as carriers (Hendessi et al. 2016; Kamtsikakis et al. 2017; Nguyen-Tri et al. 2018). However, some nanostructures have been developed which can be used as carriers in organic or inorganic matrix associated to the bioactive compound by electrostatic or covalent bonds (Hendessi et al. 2016; Kamtsikakis et al. 2017). For example, carvacrol, the active agent of essential thyme oil, has been loaded in halloysite nanotubes as a natural carrier to be applied in paints and coatings (Hendessi et al. 2016; Alkan Tas et al. 2019).

In the specific case of the paints, nanofunctionalized components commonly used, such as resins, pigments, fillers, and additives, have been reported (Kumar et al. 2008; Stobie et al. 2010; Dominguez-Wong et al. 2014; Fernández and Bellotti 2017; Machado et al. 2019). In this sense, conventional

pigments such as TiO_2 and $CaCO_3$ have been modified at nanoscale level to gain antimicrobial functionality (Ferreira et al. 2013; Dominguez-Wong et al. 2014). Another example of a paint nano-functionalized component is acrylic resin associated to ZnONPs which have both anti-electrostatic and antibacterial functionalities at a concentration of ~ 5 wt% (Xu and Xie 2003). Siliceous matrix has been used in coating technology by the application of natural clays such as halloysite nanotubes; other aluminosilicates intensively studied are zeolites, which were associated with Ag and Zn to incorporate in waterborne acrylic formulations and probed to be efficient against fungal growth (Pereyra et al. 2014; Machado et al. 2019). On the other hand, synthetic matrix based in sol-gel technology have been incorporated to architectural paints (Arreche et al. 2017; Arreche et al. 2019). The synthesized nano-spheres by sol-gel method with Ag and Cu NPs were assessed in controlled conditions, showing a broader spectrum of antimicrobial activity (Zielecka et al. 2011).

12.6 Conclusion

The search for alternatives to control fungi in indoor environments has been nourished by the great impulse that nanotechnology has shown in recent decades. Antifungal nanoparticles that seek to replace commercial active compounds have led to the production of a large number of published works, but fewer publications are found in relation to specific applications of these. In relation to articles that deal specifically with this topic, it can be observed that there is diversity in assessment methods (e.g. agar diffusion, dilution in solid or liquid cultured medium, antibiofilm test, resistance to biodeterioration of films) and fungal strains selected as target. Mostly, the tests performed are carried out in controlled laboratory conditions.

The development of "smart" surfaces in nanotechnology, capable of responding to microbial cell interaction and avoid the biofilm development, is still a challenge. Eco-friendly biogenic compounds are intensely investigated; their incorporation in paint formulation largely requires the application of nanotechnology to the design of the nanostructured carriers. It is worth mentioning that it would be interesting in the future to count research works that faced the application and the assessment of these materials in more realistic conditions.

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