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Electrospun cellulose acetate composites containing supported metal nanoparticles for antifungal membranes

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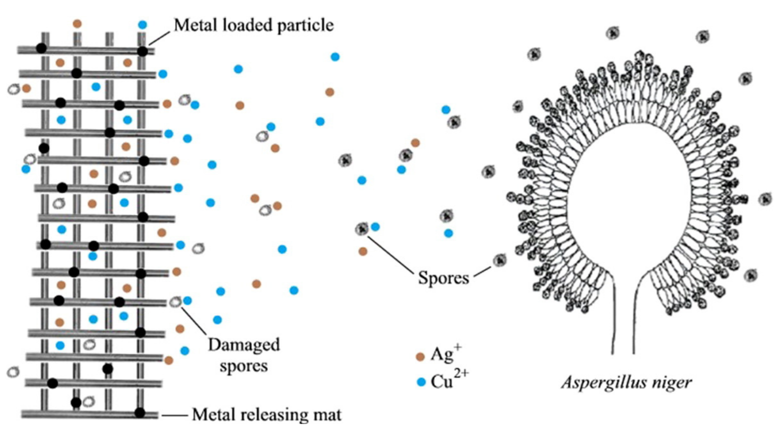
HIGHLIGHTS

- Electrospun composites containing supported silver and copper nanometals
- Non-nano particles acting as reservoirs for the controlled release of metals
- Fungistatic effect against *Aspergillus niger* for exposure during germination
- Significant growth reduction and metabolic impairment

GRAPHICAL ABSTRACT



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ABSTRACT

Electrospun cellulose acetate composites containing silver and copper nanoparticles supported in sepiolite and mesoporous silica were prepared and tested as fungistatic membranes against the fungus *Aspergillus niger*. The nanoparticles were in the 3–50 nm range for sepiolite supported materials and limited by the size of mesopores (5–8 nm) in the case of mesoporous silica. Sepiolite and silica were well dispersed within the fibers, with larger aggregates in the micrometer range, and allowed a controlled release of metals to create a fungistatic environment. The effect was assessed using digital image analysis to evaluate fungal growth rate and fluorescence readings using a viability stain. The results showed that silver and copper nanomaterials significantly impaired the growth of fungi when the spores were incubated either in direct contact with particles or included in cellulose acetate composite membranes. The fungistatic effect took place on germinating spores before hyphae growth conidiophore formation. After 24 h the cultures were separated from fungistatic materials and showed growth impairment only due to the prior exposure. Growth reduction was important for all the particles and membranes with respect to non-exposed controls. The effect of copper and silver loaded materials was not significantly different from each other with average reductions around 70% for bare particles and 50% for membranes. Copper on sepiolite was particularly efficient with a decrease of metabolic activity of up to 80% with respect to controls. Copper materials induced rapid maturation and conidiation with fungi splitting in sets of subcolonies. Metal-loaded nanomaterials acted as reservoirs for the controlled release of metals. The amount of silver or copper released

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daily by composite membranes represented roughly 1% of their total load of metals. Supported nanomaterials encapsulated in nanofibers allow formulating active membranes with high antifungal performance at the same time minimizing the risk of nanoparticle release into the environment.

1. Introduction

Many microorganisms have developed adaptive mechanisms for colonizing surfaces causing major problems in a number of man-made materials. The biofouling is particularly important for membrane filtration processes and determines their practical application in many water and wastewater treatment processes. Electrospun nanofibers represent an emerging category of membranes that offer low weight, high permeability and tunable pore size together with unique functionalization possibilities and the potential to incorporate nanoscale chemistry (Tabe, 2014). Electrospinning is the only technique available for the production of nanofibrous membranes, which is applicable to a wide variety of dissolved or melted polymers (Greiner and Wendorff, 2007). The development of antimicrobial surfaces is a field of intense research in order to reduce or eliminate microbial attachment and biofilm formation (Krishnan et al., 2008; Hasan et al., 2013). Antibiofouling behavior is obtained by manipulating surface chemistry or topography to create unfavorable environments for cellular attachment (Campoccia et al., 2013).

Membrane bacterial biofilms have been extensively studied, but other microorganisms including fungi have been largely ignored even if the presence of a high number of different fungi strains have been identified in membrane biofilms (Baker and Dudley, 1998; Al-Ashhab et al., 2014). Fungal infections significantly contribute to morbidity and mortality (Pfaller et al., 2006; Pfaller, 2012). *Aspergillus niger* is a generally non-pathogenic fungus widely distributed in nature, but during colonization it may produce the mycotoxins ochratoxin A and fumonisins B2 and B4, which have proven carcinogenic to humans and laboratory animals (Palma et al., 2007; Mogensen et al., 2010). Abundant in man-made environments, *A. niger* is responsible for the postharvest decay of a number of crops causing high economic impact (Pitt and Hocking, 2009; Flannigan et al., 2011).

The antifungal effect of silver nanoparticles has been the subject topic of several studies with emphasis on strains of *Candida* spp. (Falletta et al., 2008; Roe et al., 2008; Panáček et al., 2009). Magnetic silver nanocomposites prepared with maghemite and magnetite have been tested for antimycotic activity using several strains of *Candida* spp. (Prucek et al., 2011). ZnO nanoparticles have been proposed based on their capacity of inducing oxidative damage (Sharma et al., 2010; Lipovsky et al., 2011). Antifungal silica nanoparticles have been prepared containing Amphotericin B covalently immobilized on silica nanoparticles or coated with a quaternary ammonium cationic surfactant (Paulo et al., 2010; Botequim et al., 2012). Gold nanoparticles were also studied against various strains of *Candida* spp. (Wani and Ahmad, 2013), but in general much less attention has been paid to antifungal nanoparticles in comparison to antibacterial applications. The applications with free nanoparticles must tackle the problem of their possible release into the environment, which is a major concern in light of the potential risk of nanoparticles (Grieger et al., 2012). Alternatively, the attachment of nanometals to non-nano supports offers advantages regarding regulatory restraints for nanomaterials or at least reducing the probability of environmental leaching of nanoparticles (Pasricha et al., 2012). The EU definition of nanomaterial (2011/696/EU) indicates that a material containing particles with one or more external dimensions is in the size range 1–100 nm. Therefore, supporting nanoparticles in a non-nano material and embedding them into fibers circumvents possible regulatory constraints.

Electrospun nanofibers based on high molecular weight chitosan were tested against *A. niger* and *Candida albicans* (Nada et al., 2014). The grafting of styrene/maleic anhydride copolymers with poly(propylene glycol) monoamine and 5-amino-8-hydroxyquinoline was also explored as covalent post-processing for producing nanofibrous membranes against *C. albicans* (Ignatova et al., 2010). The preparation of antifungal nanofibers has been undertaken by blend electrospinning using a number of encapsulated drugs. Kim and Michielsen (2015) included and tested several antifungal photochemical dyes active due to the production of singlet oxygen grafted into electrospun nylon. Karthikeyan et al. (2015) used acrylic electrospun polymers to prepare a topical gel containing fluconazole for the treatment of infections produced by *C. albicans*. Liu et al. (2012) used coelectrospinning or blend-electrospinning of cellulose acetate and polyester urethane with inclusion of the antimicrobial agent, polyhexamethylene biguanide. Metronidazole blended into poly(vinyl alcohol)/poly(ethylene oxide) fibers produced using Nanospider technology were tested against *A. niger*, *Penicillium notatum* and *Aspergillus flavus*, with good results in terms of inhibition zone (El-Newehy et al., 2012). Other nanofibers have been developed including antifungal compounds such as clotrimazole, amidoxime and others (Sirelkhatim et al., 2015; Lakshminarayanan et al., 2014; Tonglairoum et al., 2015).

The incorporation of metals is a well-known way of enhancing the antibiofouling properties of membranes (Knetsch and Koole, 2011). Sun et al. (2011) reported the use of copper ions to increase the antimicrobial activity of epigallocatechin-3-gallate by limiting its oxidation in electrospun polyvinyl alcohol nanofibers tested against *C. albicans*. Aqueous poly(vinyl alcohol-co-vinyl acetate)/octadecyl amine-montmorillonite electrospun nanocomposites were prepared with in situ generated silver nanoparticles displaying antifungal activity against several species of the genus *Candida* (Rzayev et al., 2014). Electrospun polyacrylonitrile nanofibers externally loaded with silver nanoparticles showed excellent results for inhibiting the growth of the fungus *Monilia albicans* (Shi et al., 2015). Composite polycaprolactone nanofibers with silver particles precipitated onto their surface inhibited >99% of the growth of *C. albicans* (Dobrzański et al., 2014). The immobilization of nanoparticles in electrospun fibers also offers a way of overcoming the tendency of nanoparticles to agglomerate in complex natural media (Xiao et al., 2009). Additionally, the polymer creates a barrier between particles and the environment allowing a controlled metal release that takes place through diffusion to the surface of fibers (Quirós et al., 2015a).

In this work, we studied the use of cellulose acetate composite fibers loaded with silver and copper nanometals supported on particles of sepiolite and mesoporous silica. The rationale was to prepare stable materials thanks to the encapsulation of metals within nanofibers by avoiding loosely attached nanoparticles that could be easily dispersed into the environment. The efficiency of metal-loaded membranes against the fungus *A. niger* was quantitatively assessed by tracking its growth after the exposure of spores and by using a fluorescent viability stain.

2. Materials and methods

2.1. Materials

Pluronic P123 (Aldrich $EO_{20}PO_{70}EO_{20}$, EO ethylene oxide, PO propylene oxide, MW = 5800) and tetraethoxysilane (TEOS 98% GC Aldrich) were used as received. Cellulose acetate (CA) was purchased from Sigma Aldrich with molecular weight ~50,000 g/mol and 39.7 wt.% acetyl. Acetone (≥99.5%) was reagent grade, obtained from Sigma Aldrich

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