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Photodynamic activity of nanostructured fabrics grafted with xanthene and thiazine dyes against opportunistic fungi

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ABSTRACT

Fungi are an important class of human pathogens for which considerable research has gone into defeating them. The photodynamic effects of rose bengal (RB), phloxine B (PB), azure A (AA), and toluidine blue O (TBO) dyes to inhibit *Aspergillus fumigatus*, *Aspergillus niger*, Trichoderma *viride*, Penicillium *funiculosum*, and Chaetomium *globosum* were investigated grafted to nano- and micro-structured fabrics. Three antifungal tests conducted: broth microdilution test of free dyes, zone of inhibition and quantitative antifungal assays on fabrics grafted with dyes. In the broth microdilution test, free RB displayed the lowest MIC at 32 μM to inhibit visible hyphal growth and germination but the antifungal ability of MIC for other photosensitizers below 63 μM was insignificant. RB and PB showed lower MIC than AA and TBO. In the inhibition zone tests, nanostructured fabrics grafted with RB and PB did not display fungal growth on the surface. Most microstructured fabrics grafted with AA and TBO showed little inhibition. In quantitative antifungal assay, nanostructured fabrics grafted with RB has the largest inhibition rate on *T. viride* and the lowest inhibition rate on *P. funiculosum* and the results showed the increasing inhibition rate in the order of AA < TBO < PB < RB.

1. Introduction

Aspergillus fumigatus, Aspergillus niger, Trichoderma viride, Penicillium funiculosum, and Chaetomium globosum are opportunistic human pathogens [1,2]. Life-threatening symptoms can occur when these fungi penetrate more deeply into luminal cells in human lung, especially in immune suppressed persons. The number of immuno suppressed patients is increasing annually and they are highly susceptible to opportunistic fungi, resulting in the risk of aspergillosis and pneumocytosis [3]. Furthermore, they are strong air pollutants, which are ubiquitous in soil and are frequently reported from indoor environments where the high incidence of their spores causes respiratory allergic reactions via inhalation such as asthma and allergic rhinitis [4]. Hence, it is highly desirable for upholsteries, home furnishings, and medical textiles to impart antifungal properties and protect the public from biological threats. Surface grafting provides the textile industry with a means of imparting finishes and properties to textiles that produce antimicrobial activities by embedding various active agents. Nano fibers have advantages particularly in biomaterials due to their large surface area to volume ratio and high porosity to give favorable cell adhesion [5]. The most popular methods to produce nano fibers, electrospinning, can control the thickness, composition, and porosity in the diameter in the range of 50–1000 nm depending on application [6].

One class of dyes used in photodynamic therapy (PDT) is the xanthene dyes such as rose bengal and phloxine B, and thiazine dyes such as azure A and toluidine blue O. They are commonly used in the drug, food, cosmetic, dental and textile industries [7]. Several xanthene dyes and thiazine dyes are used as color additives in the USA, the European Union (EU) and Japan [8,9]. The $^1O_2$ produced by these photochemicals reacts with biological cell membranes, particularly with lipids in microorganisms to induce photooxidation, which leads to the cell damage, resulting in an increase in membrane permeability [10,11].

Numerous studies have shown photodynamic therapy to be highly effective in the *in vitro* destruction of fungi [12–14]. In particular, the effect has been studied against the dermatophyte *Trichophyton rubrum* or *Candida* species using thiazine dyes such as methylene blue and toluidine blue O [15–17]. Xanthene dyes have been reported to induce photoinactivation of pathogenic bacteria species such as *Staphylococcus aureus* due to the formation of reactive singlet oxygen, which oxidizes biological molecules [18–21].

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Many experiments have been performed to develop antimicrobial agents using photochemical dyes due to their production of singlet oxygen; however, there are fewer studies about antifungal fabrics using photochemicals against opportunistic fungi such as *A. fumigatus, A. niger*, *T. viride*, *P. funiculosum*, and *C. globosum*. These limitations have affected the utilization of photodynamic actions in textile applications. The work below focuses on the antifungal activity of nanostructured antifungal fabrics grafted to photochemicals using xanthene and thiazine dyes.

2. Materials and methods

2.1. Fungi

A. fumigatus (ATCC 13073), *A. niger* (ATCC 6275), *T. viride* (ATCC 28020), *P. funiculosum* (ATCC 10509), and *C. globosum* (ATCC 6205) were donated by the United States Department of Agriculture, Agricultural Research Service, Peoria, IL, USA.

2.2. Xanthene and thiazine dyes and other materials

Rose bengal (RB) and the meltspun microstructured fabric, Cerex Spectramax® nylon 6.6 were donated by LaamScience (Cary, NC, USA). Phloxine B (PB), Azure A (AA), Toluidine blue O (TBO), potato dextrose agar (PDA), phosphate buffer saline (pH 7.0), Tween® 20, RPMI 1640 medium, sterile water, 3-(*N*-morpholino)-propanesulfonic acid (MOPS), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and nylon 6,6 pellets and formic acid (reagent grade >95%) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) while 0.5 McFarland standard was purchased from Fisher Scientific Co. (Pittsburgh, PA, USA).

2.3. Preparation of inoculum

A. fumigatus, A. niger, *T. viride*, *P. funiculosum*, and *C. globosum* were cultured at 35 °C on PDA media for seven days. After inoculation, a RPMI-1640 with 0.2% glucose and buffered to a pH of 7.0 with 0.165 mol L^{-1} MOPS (3-*N*-morpholino propanesulfonic acid) was used as medium. The medium with 0.01% wetting agent Tween® 20 was added to fungi cultures. The cultures were scraped while immersed in this solution to separate the spores. The scrapping solution was filtered through Millipore® polytetrafluoroethylene film with 5 μm pore size.

2.4. Antifungal assay

CLSI M38-A standard describes a broth microdilution method for testing antifungal susceptibility of filamentous fungi as molds that cause invasive infections [22]. In the broth microdilution method, the inoculum preparation of spores was adjusted using 0.5 McFarland standard and a hemocytometer (Hausser Bright-Line and Hylite Counting Chambers, Horsham, PA, USA) in the range 2×10^6 colony forming units per mL (CFU mL^{-1}). The 100 μL RB, PB, AA, and TBO solutions at 500 μM were transferred to the first well and the second well and then diluted by two-fold in the 96-well plates and 100 μL put into each well. Then the 100 μL inoculum of fungal spores was deposited into each well. The tray was placed under the lamp equipped with a Pyrex glass dish above the tray and filled with water to absorb infrared light from a photoflood lamp (Smith Victor, Griffith, IN, USA) to avoid heating the tray. The lamp was placed 35 cm above the glass dish and the vials were placed below the glass dish. The light intensity was measured using a digital illuminance meter (Model LX1330B, Union City, CA, USA) at 16,000 Lux. After illumination for 5 h, the plate was scored for turbidity and growth of fungi, and the results were compared to growth control and sterile control in the last two wells.

2.5. Electrospinning of nanostructured nylon 6, 6

Nylon 6,6 pellets were dissolved into formic acid at a concentration of 18% wt and then stirred for 8 h at 70 °C. The solution was put into a 10 mL syringe. In the electrospinning, a high voltage power supply (Gamma) supplied the high voltage of 20 kV. The syringe feeding rate used was 1 mL h^{-1} and the syringe needle tip to collector distance was 11 cm. The interconnected nanofibers were deposited on one side as shown in Fig. 1.

2.6. Polymerization of photochemical dye polymers

The xanthene dyes were incorporated into poly(acrylic acid) via polymerization.

This polymerization enabled xanthene dyes to be grafted to fabric surfaces producing permanent chemical bonds. Amine groups of nylon fabric can react with carboxylic acid groups of the polymerized dye solutions thus producing amide linkages. The procedure described by Zhang was followed [23]. Briefly, xanthene dye such as RB or PB and vinyl benzyl chloride were stirred in the mixture of distilled water and acetone at 65 °C for 3 h. Then the precipitate (vinyl benzyl-xanthene dye) was filtered and washed. Next vinyl benzyl-xanthene dye was dissolved in water and acetone and polymerized with 4-styrene sulfonic acid and acrylic acid to increase water solubility to polymers and confer functional groups to graft onto fabrics [24,25]. After copolymerization, poly(acrylic acid-co-styrene sulfonic acid-co-vinyl benzyl rose bengal or phloxine B) was obtained.

For thiazine dyes grafted with poly(acrylic acid) (PAA), a 5% PAA solution in distilled water was prepared at room temperature. 100 μM of azure A (AA) or toluidine blue O (TBO) was added to the PAA solution. After stirring for 1 h, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was added to the mixture [25]. After stirring for another 3 h, 0.3 g more of DMTMM was added. DMTMM is a condensing reagent to promote chemical reactions between –COOH groups of PAA to NH_2 groups of thiazine dye. Thus the thiazine dye-grafted-PAA solution was prepared [26].

2.7. Grafting of photosensitizers to nylon 6, 6

The polymerized solutions above were prepared on flat glass plate covered with aluminum foil. Electrospun nano-structured fabrics and meltspun microstructured fabrics were immersed into the solution bath. The 0.3 g of condensation agent, DMTMM, was dissolved in the solution bath. After 12 h, the fabric was removed from the solution and placed into an oven (Werner Mathis AG LTF 134489, Concord, NC, USA) for 1 min at the temperature of 170 °C. Amine groups of nylon 6,6 can react with carboxylic acid group of polymerized dye solutions producing amide linkage by chemical reactions as shown in Fig. 2. The polymerization enables

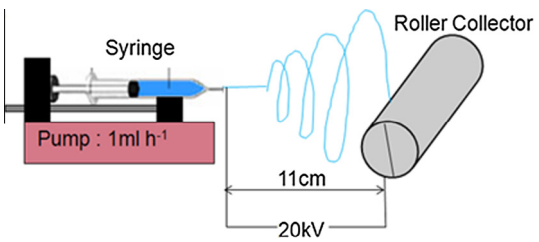

Fig. 1. Set-up for producing nanostructure nylon 6,6 using electrospinning.