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Biofilm disruption with rotating microrods enhances antimicrobial efficacy

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ABSTRACT

Biofilms are a common and persistent cause of numerous illnesses. Compared to planktonic microbes, biofilm residing cells often demonstrate significant resistance to antimicrobial agents. Thus, methods for dislodging cells from the biofilm may increase the antimicrobial susceptibility of such cells, and serve as a mechanical means of increasing antimicrobial efficacy. Using *Aspergillus fumigatus* as a model microbe, we magnetically rotate microrods in and around biofilm. We show that such rods can improve the efficacy of antimicrobial Amphotericin B treatments *in vitro*. This work represents a first step in using kinetic magnetic particle therapy for disrupting fungal biofilms.

1. Introduction

Microbial biofilms are believed to be associated with a broad variety of infectious diseases (e.g., sinusitis, skin ulcers). New methods of preventing and combating these tenacious microbial havens are becoming increasingly important [1]. One characteristic of biofilms that makes them particularly difficult to treat is their increased resistance to antimicrobial therapy [2]. While free-floating microbes may be susceptible to a specific drug or therapy, biofilms of the same microbe often prove drug resistant due to a confluence of factors. The dense meshwork of polysaccharides that mechanically buttresses the biofilm [3] and the evolution of persister cells capable of resisting antimicrobial attack [4] are among the many defenses biofilms may evolve [2]. New approaches, such as dispersal agents and microbial interference, are currently being developed for treating biofilm-associated infections [1, 5, 6].

Aspergillus fumigatus (*A. fumigatus*) is a globally pervasive saprophytic mold that has been implicated in numerous respiratory diseases [7–9]. The spores of *A. fumigatus* are typically spherical in shape, with diameters between 2 μm and 3 μm [10]. *A. fumigatus* has been implicated in numerous respiratory tract diseases, including aspergillosis, invasive pulmonary aspergilloma, immunoglobulin mediated allergic rhinitis, and chronic necrotizing pneumonia [11]. Additionally, *A. fumigatus* produces cytotoxic and immunosuppressive proteins that allow it to live for long periods in the respiratory tract [7]. Proteases produced by *A. fumigatus* have been shown to damage epithelial tissue, and toxins produced by the mold can inhibit ciliary activity, making clearance of the invading microbes difficult [12]. *A. fumigatus* is known to form biofilms [13,14], and these biofilms have demonstrated reduced antifugal drug susceptibility [9].

Using *A. fumigatus* as a model biofilm, we attempted to determine whether biofilms could be disrupted mechanically using rotating magnetic microrods (under control of a magnetic field applied by electromagnet arrays). Previous research has implemented such rod motion for cell alignment [15], cell manipulation [16–18], and high frequency micromotors [19,20].

2. Experimental procedure

2.1. Magnetic microrods

Microrod dimensions were chosen such that the rods were similar in size to the fungal spores. Gold-iron-gold microrods were electroplated into the pores of anodized aluminum oxide membranes (AAO, Whatman Anodisc) with ~250 nm diameter pores. AAO membranes were first sealed on one side by thermal evaporation of a silver working electrode. After sealing one side, a gold layer (~10 nm) was deposited from commercial gold plating solution (Technic Inc.); iron was

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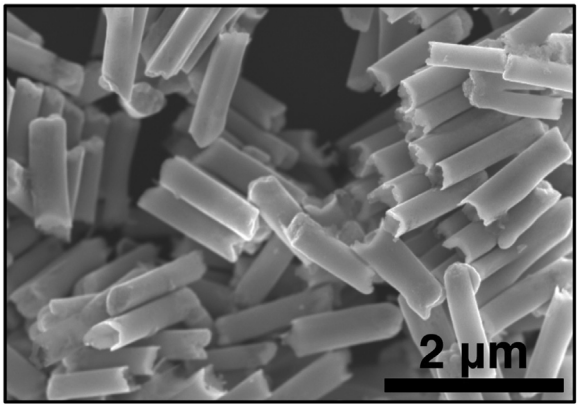

Fig. 1. SEM image of Au-Fe-Au microrods.

electroplated from a solution containing 140 g/l $FeSO_4$-$7H_2O$, 5 g/l $FeCl_3$-$6H_2O$, 45 g/l H_3BO_3, and 2 g/l ascorbic acid, using DC voltage [21]. Our rods were deposited using 1.2 V DC at a current of 0.4 mA. A capping layer of gold (~10 nm) was plated. Rods were approximately 1.2 μm long (Fig. 1). After growth, the silver working electrode was etched in dilute nitric acid and the AAO template was etched in 1 M sodium hydroxide. Detailed synthesis procedures have been well documented in the literature [22–27].

2.2. *Aspergillus fumigatus* culture

Aspergillus fumigatus cells (American Type Cell Culture #10894) were grown on glass cover slips (#1 thickness, 22 mm×22 mm) coated with potato dextrose agar. Cultures were grown for 10 days at 25 °C prior to treatments.

2.3. Treatments

A. fumigatus cultures were divided into four treatment groups (Groups A through D) and each group was challenged with one of four treatment regimens (Fig. 2). Treatments were delivered in 0.1 ml aliquots. All treatments were delivered in phosphate buffered saline (PBS). In Fig. 2, PBS (treatment carrier fluid) is shown in yellow. Group A received phosphate buffered saline only (control). Group B received the antifungal Amphotericin B (AmpB) only. The Amphotericin B dose was 750 ng (in 0.1 ml PBS), less than the intranasal doses given in clinical trials [28]. Group C received ~100,000 rotating microrods. Group D received combined antifungal (750 ng AmpB) and ~100,000 rotating microrods. Rods were rotated using a magnetic field of ~10 mT and a frequency of 10 Hz.

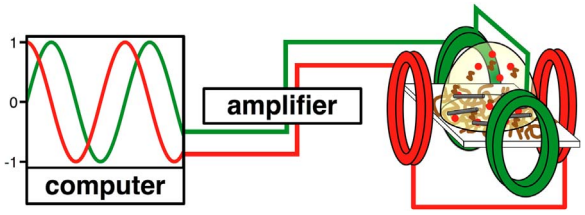

Fig. 3. Apparatus used to manipulate microrods. A computer-generated signal is amplified before reaching two orthogonally arranged Helmholtz coils. Magnetic fields rotate the particles in the plane of the sample.

The magnetic actuation apparatus consisted of two pairs of Helmholtz coils. Signals were generated using Matlab and amplified via audio amplifiers (Fig. 3). The electromagnets generated a rotating magnetic field in the plane of the sample, and microrods rotated in the plane of the fungal cell culture. As they are actuated, microrods interact with *A. fumigatus* cells, underlying potato dextrose agar growth surfaces, and extracellular matrix materials involved in adhering the hyphae of *A. fumigatus* to one another and to surfaces [8].

Following a 20 min treatment period, the supernatant from fluid treatments was extracted. Microrods were magnetically separated from the extracted supernatant fluids. Supernatant fluids were cultured on potato dextrose agar coated petri dishes. The method of counting colony-forming units (CFUs) was used to determine the number of viable cells contained in the supernatant of each treatment group [29].

3. Results

In samples containing microrods, we observed microrod rotation in phase with the applied magnetic field. Microrods formed chains as they rotated, and stirred the fluidic environment. Fig. 4 shows *A. fumigatus* biofilm in the presence of rotating microrods.

Culturing the extracted treatment fluid from Groups A, B, and C resulted in large quantities of viable colony-forming units in these groups ($78.3\times10^6 \pm 32.7\times10^6$, $84\times10^6 \pm 26.6\times10^6$, and $67.7\times10^6 \pm 12\times10^6$ CFU/ml, respectively). Significantly reduced CFUs ($5.33\times10^6 \pm 1.5\times10^6$ CFU/ml) were observed in the group treated with both microrods and antifungal AmpB. CFU results are shown in Fig. 5. Overall, combined microrod disruption and AmpB treatment demonstrated > 90% kill rate, as compared to control, AmpB, or magnetically rotated microrods alone. We observed collisions of the rods with *A. fumigatus* hyphae, leading to disruption of the biofilm integrity.

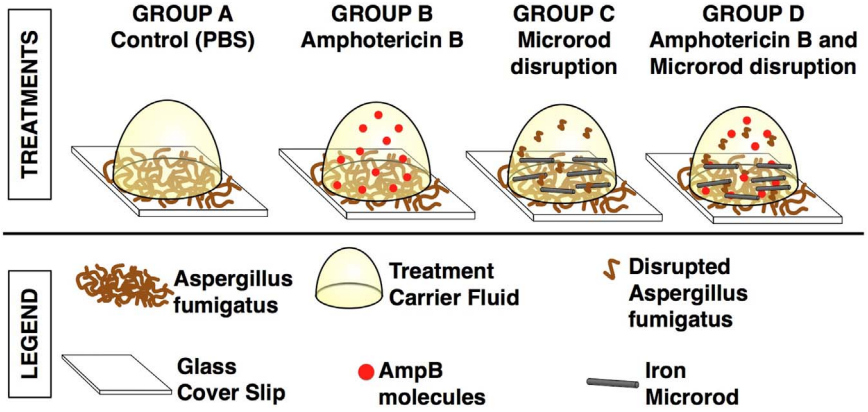

Fig. 2. Four treatments (Groups A through D) were tested on biofilms of *A. fumigatus*. Biofilms were grown on glass cover slips coated with potato dextrose agar. For all experiments, treatment carrier fluid was deposited onto the biofilm and recovered after 20 min of interaction. Cartoon of experiments shows treatment groups. From left to right, treatments group are: PBS only (Group A): Amphotericin B (Group B); microrod disruption (Group C); and combined Amphotericin B and microrod disruption (Group D). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)