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A pilot study of antimicrobial photodynamic therapy of encapsulated *Aspergillus fumigatus* in a rabbit maxillary sinus model

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online

INTRODUCTION

Aspergillus fumigatus is a commonly isolated agent in invasive aspergillosis and chronic invasive fungal rhinosinusitis. These conditions are often associated with immunosuppression. Standard management involves surgical debridement and long-term antifungal treatment (3 – 15+ months, follow-up <5 yr.), along with tight glycemic control and discontinuation of corticosteroids. Prompt diagnosis and initiation of appropriate therapy for relapse are essential to avoid a protracted or fatal outcome. This study was undertaken to demonstrate the potential for antimicrobial photodynamic disinfection (PDD) in the treatment of aspergillosis.

METHODS

Agar bead preparation (Fig. 1). *A. fumigatus* (ATCC 32820) conidia were harvested, added to warm YDP media with agar and dispersed in 52°C mineral oil, forming microbeads. After cooling, the solution was added to PBS, centrifuged and washed 3 times, and filtered through a 250-µm mesh filter prior to use.

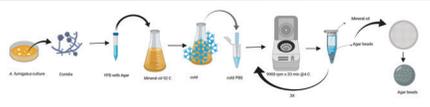


Fig 1. Agar beads preparation

PDD, planktonic model. 20 µL of 10⁶ conidia/ml was added to a stock solution of photosensitizer containing 0.03% methylene blue chloride (PS) in USP water. 670 nm illumination was conducted for 60 s at an intensity of 150 mW/cm² while stirring. Samples were removed for PDA plate enumeration.

PDD, biofilm model (Fig. 2). 200 µL of 10⁶ conidia/mL were added to each well of a 96-well plate and incubated while shaking at 125 rpm at 37°C for 48 hr. The resulting biofilm was washed 3X with PBS and 200 µL of PS added to each well for 4 min. Residual PS was removed and illumination conducted for 8 min at an intensity of 150 mW/cm². Swab samples were taken for PDA plate enumeration.

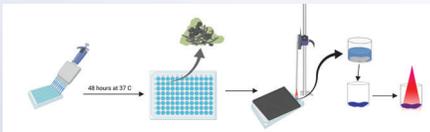


Fig 2. PDD, biofilm

In vivo PDD (Fig. 3). Bead-encapsulated *A. fumigatus* was inoculated into the NZW rabbit maxillary sinus. No immunosuppression was required. After 48 hr, PS was applied to the affected sinus followed by 670 nm illumination at 150 mW/cm² for 8 min via a custom diffuser.



Fig 3. A. Sagittal incision through dorsum and periosteum. B. Opening of maxillary sinus and agar bead application. C. Closure. D. Application of PS solution after 48 hours. E. 670 nm illumination 150 mW/cm², 8 min

IN VITRO RESULTS

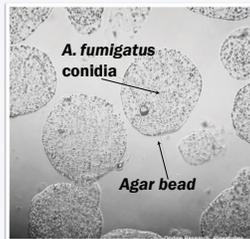


Fig 4. In vitro 40X encapsulated *A. fumigatus* conidia in ~250 µm beads

In vitro PDD against *A. fumigatus* encapsulated in agar beads, planktonic model

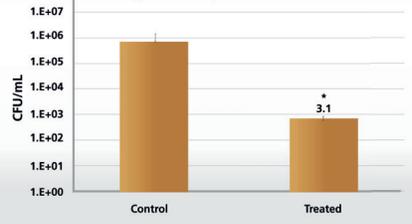


Fig 5. >3 log₁₀ (99.9+%) kill of *A. fumigatus* encapsulated in agar beads. Values are mean (n=3) ± SD CFU/mL. Asterisk indicates statistically significant difference from control (p<0.05)

In vitro PDD against *A. fumigatus*, biofilm model

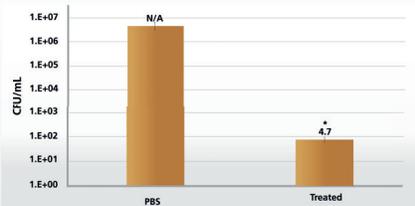


Fig 6. >4 log₁₀ (99.99+%) kill of *A. fumigatus* in a biofilm model. Values are expressed in CFU/mL as the mean (n=3) with SD. Asterisk indicates statistically significant reduction from control (p<0.05).

IN VIVO RESULTS

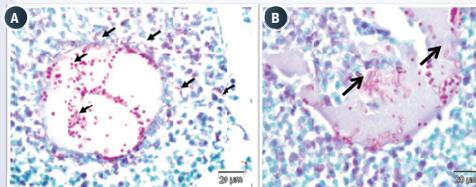


Fig 7. Histopathology of PAS-stained maxillary sinus tissue (100X). Arrows indicate PAS-positive hyphae of *A. fumigatus*. A. Sinus tissue infected with conidia B. Hyphae of *A. fumigatus* within disrupted agar bead.

IN VIVO RESULTS

In vivo PDD against *A. fumigatus*, NZW rabbit model

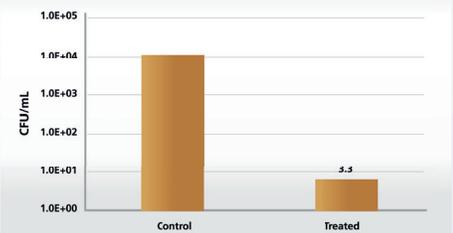


Fig 8. >3 log₁₀ (99.9+%) kill of bead-encapsulated *A. fumigatus* in vivo, rabbit maxillary sinus model. Values are CFU/mL. Controls were saline treated only. PDD conducted via custom diffuser catheter, 150 mW/cm², 8 min. Culture samples were taken by saline-Tween 80[®] lavage to ensure thorough mucosal surface assessment. Treatment eradicated 3.3 log₁₀ of *A. fumigatus* compared with controls.

CONCLUSIONS

Because fungi are present throughout the environment, human exposure is inevitable and normal respiration will routinely deposit fungal elements within the nose, paranasal sinuses, and in the remainder of the airway. In cases of immunosuppression or otherwise poor health, invasive aspergillosis may result in lethal outcomes. Current approaches to treatment using azoles may result in severe hepatotoxicity and steroid treatment can induce diabetes and other negative sequelae. This pilot study demonstrated that topical in vivo antimicrobial photodynamic therapy was capable of eradicating >3 log₁₀ (>99.9%) of *A. fumigatus* inoculated into the NZW rabbit maxillary sinus in a recognized agar bead model of aspergillosis. This represents a >4,000-fold reduction in the number of viable conidia per unit area. These in vivo results closely matched in vitro experimental data in planktonic and biofilm cultures. This pilot study will be replicated in a larger sample size and with polymicrobial cultures of important human fungal pathogens including *A. niger*, *A. flavus* and *M. indicus*.

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