Targeting Fibronectin To Disrupt *In Vivo* *Candida albicans* Biofilms

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New drug targets are of great interest for the treatment of fungal biofilms, which are routinely resistant to antifungal therapies. We theorized that the interaction of *Candida albicans* with matricellular host proteins would provide a novel target. Here, we show that an inhibitory protein (FUD) targeting *Candida*-fibronectin interactions disrupts biofilm formation *in vitro* and *in vivo* in a rat venous catheter model. The peptide appears to act by blocking the surface adhesion of *Candida*, halting biofilm formation.

*Candida albicans*, the most common nosocomial fungal pathogen, adheres to medical devices and proliferates as a biofilm (1). Vascular catheters, urinary catheters, and dentures are commonly infected devices. Biofilms are routinely resistant to antifungal therapies, often withstanding drug concentrations 1,000-fold higher than those needed to kill free-floating cells (2, 3). Given the multidrug-resistant phenotype of biofilm infections, new drugs with antibiofilm activity are desperately needed.

Recent investigation of the host-biofilm interface showed that the vast majority of the extracellular matrix components surrounding *in vivo* biofilms are actually of host origin (4). For example, in a rat venous catheter biofilm, >95% of proteins identified by mass spectrometry were of rat origin. One of the intriguing categories of biofilm-associated proteins is a group of host matricellular proteins. Fibronectin, vitronectin, and fibrinogen were found to deposit on a variety of infected and uninfected medical devices, including a vascular catheter and urinary catheter, and denture devices (4). This finding prompted examination of the potential of disrupting these interactions for development of a novel antibiofilm therapeutic agent.

We adapted an XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] assay to examine the impact of matricellular proteins on biofilm formation (5). Fibronectin, vitronectin, and fibrinogen were selected for study based on their estimated concentrations in *Candida* biofilms with albumin as a control (4). Human fibrinogen and albumin purchased from Sigma and purified human fibronectin and vitronectin were used at concentrations based on their estimated concentrations in 6.25 to 12.5% plasma (6–9). *C. albicans* (strain K1) was used for all studies and was propagated overnight in yeast-peptone-dextrose with uridine at 30°C at 200 rpm and resuspended in RPMI medium-MOPS (morpholinepropanesulfonic acid) at 5 × 10^5 (10).

For adherence assays, *C. albicans* was seeded in the wells of microtiter plates with host components diluted in phosphate-buffered saline (PBS). After 1 h (37°C), nonadherent cells were removed, and an XTT assay was performed to estimate adherent *Candida*. For maturation assays, *C. albicans* was incubated for 1 h (37°C), nonadherent cells were removed, and host proteins in RPMI medium-MOPS were added for 18 h prior to the XTT assay. For the blocking experiments, FUD was included at 500 nM (11, 12). This peptide sequence is based on a *Streptococcus pyogenes* adhesin domain, which competitively binds the fibronectin type 1 module, inhibiting fibrillogenesis (11, 12).

A rat venous catheter model was used for *in vivo* *C. albicans* biofilm studies (13, 14). Animals were maintained in accordance with the American Association for Accreditation of Laboratory Care criteria, and all studies were approved by the institutional animal care committee. Briefly, 24 h after implantation, the jugular venous catheters were inoculated (10^6 cells/ml), flushed after 6 h, and collected after 24 h for microbiological enumeration or imaging by scanning election microscopy (SEM) (13). FUD peptide was included at 500 nM (11, 15).

The results of the adhesion experiments demonstrated that plasma augmented *C. albicans* adhesion to plastic by approximately 2-fold (Fig. 1A). However, among the host matricellular proteins selected for study, only fibronectin significantly promoted *C. albicans* adhesion to a similar degree. Although increased adherence was observed with fibrinogen, this did not reach statistical significance. In this model system, the influence of fibronectin was limited to the initial adhesion phase with no effect during maturation (Fig. 1B). However, plasma did augment biofilm maturation, indicating that alternative plasma factors were responsible for this phenomenon. We examined the impact of combining the matricellular proteins but did not identify synergistic enhancement of biofilm maturation (data not shown).

To further examine the interaction of fibronectin and *Candida* biofilms, we utilized a peptide (FUD) which inhibits fibronectin assembly by competitively binding the fibronectin type 1 module that has been shown to interact with Gram-positive bacteria (11, 15). In *vivo*, addition of this peptide counteracted the positive effect of fibronectin on adherence (Fig. 2A). It also inhibited the increased plasma-promoting effects on *Candida* adherence. The inhibitory effect of the FUD peptide was significantly more pronounced *in vivo*. Administration of the peptide along with *C. albicans* decreased the viable catheter burden by nearly an entire order of magnitude (1 log) of growth (Fig. 2B). Visualization of the catheters by SEM qualitatively confirmed a sub-

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stantial decrease in biofilm formation in the presence of FUD (Fig. 2C). We suspect that much of the inhibitory activity of FUD may occur during the initial adherence period. Although the safety and tolerability of FUD have not been extensively studied, the animals tolerated the peptide well. The finding that inhibition of the fibronectin- *Candida* interaction disrupts biofilm formation suggests that targeting the biofilm-host interaction may be a viable option for preventing or treating biofilm infections.

FIG 1 Impact of various host factors on *Candida* adherence and biofilm formation. (A) When added during the 1-h adhesion phase, plasma and fibronectin significantly promoted *C. albicans* adhesion. (B) When added during the 18-h biofilm maturation phase, only plasma promoted biofilm formation. Concentrations were 100 μg/ml fibrinogen, 2 μg/ml vitronectin, 100 μg/ml albumin, 6.25% plasma, and 25 μg/ml fibronectin. Viable burdens were estimated by an XTT assay following washing to remove nonadherent cells. Error bars indicate standard deviations about the means. Experiments were performed in triplicate on two occasions. An analysis of variance (ANOVA) with pairwise comparisons using the Holm-Sidak method was used for statistical analysis. *, *P* < 0.05.

FIG 2 Fibronectin peptide inhibitor FUD impairs *C. albicans* biofilm formation *in vitro* and *in vivo*. *C. albicans* was incubated with fibronectin (25 μg/ml) or human plasma (12.5%) with or without FUD peptide for 1 h. (A) Viable burdens were estimated by an XTT assay following washing to remove nonadherent cells. Error bars indicate standard deviations about the means. Experiments were performed in triplicate on two occasions. *C. albicans* was inoculated into a jugular venous rat catheter with or without FUD peptide. Catheters were collected at 24 h and analyzed for viable burdens following biofilm disruption (B) or imaged by SEM (C). Microbiological counts were performed in triplicate. For SEM, images were obtained at ×75 magnification, and measurement bars represent 500 μm. The addition of FUD impaired biofilm formation. The Student *t* test was used for comparison. *, *P* < 0.05.
Matricellular proteins, including vitronectin, fibronectin, and fibrinogen, have previously been shown to bind to *C. albicans*, likely augmenting invasion of various tissues (16–23). These proteins consistently associate with biofilms of medical devices and have been studied as host-conditioning proteins prior to biofilm initiation (24–29). In this study, we demonstrate that fibronectin is influential for adhesion to plastic and that targeting this interaction reduces biofilm formation.

Interestingly, the impact of the fibronectin inhibitory peptide was far more pronounced *in vivo*. There are several factors which may account for this phenomenon. First, *Candida* cells adherent to a rat vascular catheter are likely subjected to higher shear stress in the form of the flushing and blood flow; thus, poorly adhesive cells may be more easily removed. Second, *C. albicans* has been shown to express multiple receptors for fibronectin with differing binding affinities, including Als1p, Als5p, and two inducible receptors (30–33). One of these receptors, associated with fibronectin with spreading factor (vitronectin) is present at the cell surface and in tissues. Proc Natl Acad Sci U S A 80:4003–4007. http://dx.doi.org/10.1073/pnas.80.13.4003.


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