Candida biofilms and Echinocandins

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Disclosures

• Research support and honoraria from
  – Astellas
  – MSD
  – Novartis
  – Pfizer
Fungi and biofilm

• Increased prevalence of fungi due to transplantation, immunosuppression, use of chronic indwelling devices and prolonged intensive care unit stays.

• *Candida* spp are now the 4\(^{th}\) most commonly isolated bloodstream pathogens with a crude mortality of 40%.

• At least 35 million devices are implanted yearly in the United States.
  – > 50% of nosocomial infections are associated with medical devices (biofilms)
  – Increasing proportion of device associated infections are caused by *Candida* spp.
Typical biofilm infections

**Device-related infections**
- Ventricular derivations
- Contact lenses
- Endotracheal tubes
- Vascular central catheters
- Prosthetic cardiac valves, pacemakers and vascular grafts
- Peripheral vascular catheters
- Tissue fillers, breast implants
- Urinary catheters
- Orthopedic implants and prosthetic joints

**Tissue infections**
- Chronic otitis media, chronic sinusitis
- Chronic tonsillitis, dental plaque, chronic laryngitis
- Endocarditis
- Lung infection in cystic fibrosis
- Kidney stones
- Biliary tract infections
- Urinary tract infections
- Vaginosis
- Osteomyelitis
- Chronic wounds

TABLE 1  Medically important fungi forming biofilms

<p>| |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Aspergillus</td>
</tr>
<tr>
<td>Blastoschizomyces</td>
</tr>
<tr>
<td>Candida</td>
</tr>
<tr>
<td>Cryptococcus</td>
</tr>
<tr>
<td>Fusarium</td>
</tr>
<tr>
<td>Malassezia</td>
</tr>
<tr>
<td>Pneumocystis</td>
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<tr>
<td>Trichosporon</td>
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<tr>
<td>Zygomycetes</td>
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</tbody>
</table>
What is a biofilm?

- A biofilm is a complex organization of bacteria or fungi
  - Attached to a surface
  - Embedded in a slimelike matrix composed of polysaccharides, nucleic acids, and proteins known as extracellular polymeric substances (EPS)
  - Live together in organized structures and communicate with one another in a cooperative manner.

Five stages of biofilm development

1. Initial reversible attachment of free swimming micro-organisms to surface
2. Permanent chemical attachment, single layer, bugs begin making slime
3. Early vertical development
4. Multiple towers with channels between, maturing biofilm
5. Mature biofilm with seeding / dispersal of more free swimming micro-organisms
Candida and biofilm-related infections

• *Candida albicans* is the most common fungal species associated with biofilm-related infections.

• In patients with candidemia, biofilm-producing strains of *Candida* species have been associated with increased morbidity and mortality compared to non-biofilm-producing strains.
The biofilm contains a mixture of

- Yeasts
- Germ tubes
- Pseudohyphae
- Hyphae
- Polysaccharide network of mannans and glucans known as extracellular polymeric material

Scanning electron micrograph of a C. albicans biofilm that has formed in vitro on the surface of a vascular catheter
Biofilm development

• Early (0-11h)
  – 0 to 2h: *C. albicans* adhere to the surface as blastospores (yeast forms)
  – 3 to 4h distinct microcolonies

• Intermediate (12-30h)
  – *C. albicans* communities appear as a thick tracks of fungal growth due to cell growth and aggregations
  – 12 to 14h predominate the noncellular material covering fungal microcolonies

• Mature (38-72h)
  – The amount of extracellular material is increasing, until *C. albicans* communities to be completely encased within this material.

Two distinct morphological layers
A thin basal yeast layer of blastospores
A thicker but more open hyphal layer of a matrix consisted of extracellular material and hyphal elements

Chandra et al. J. Bacteriol. 2001a;183:5385
Interstrain variability in biofilm production between *Candida* spp

- *C. albicans* is more prone to biofilm formation than other pathogenic *Candida* species such as
  - *C. parapsilosis*
  - *C. glabrata*
  - *C. tropicalis*


- Biofilm formation is species dependent, being
  - Highest in *C. lusitaniae*
  - Intermediate in *C. albicans* and *C. guilliermondii*
  - Lowest in *C. parapsilosis* and *C. krusei*

• Infections due to fungal biofilms are characterized by
  • Protection from host defenses
  • Increased resistance to antifungal therapy
    – The implant often has to be removed
Biofilm and Resistance mechanisms

1. Limited diffusion of molecules through the extracellular matrix
2. The presence of persisters in the biofilm, which are able to tolerate high concentrations of antimycotics.
   - These persisters are not mutants but rather phenotypical variants of wild type cells.
3. Amplified expression of efflux pumps
4. A changing sterol composition in the membrane

A. Bink et al Te Open Mycology Journal, 2011, 5, 29-38
Antifungal drug susceptibility

- *C. albicans* in biofilms (on polyvinyl chloride disks) has been reported to be
  - 30 to 2,000 times more resistant to fluconazole, amphotericin B, flucytosine, itraconazole, and ketoconazole than planktonic cells
  - The biofilm structure remained intact at an amphotericin B concentration of 11 times the MIC.
- Non-*albicans Candida* species were also resistant.
- The newer triazoles were also found to be ineffective against *C. albicans* and *C. parapsilosis* biofilms
- Echinocandins have been shown to be effective against *C. albicans* biofilms
  - Glucan synthesis may be an effective target for biofilms.

Mechanism of action of echinocandins

- Echinocandins inhibit the synthesis of glucan in the cell wall, via inhibition of the enzyme 1,3-β glucan synthase.
- Immunomodulatory effect
  - it was found that subinhibitory doses of echinocandins “unmask” the underlying (1→3)-β-D-glucan in the cell wall of *C. albicans*, causing the exposed fungi to elicit a strong immune response.
Susceptibility testing of AMB, NYT, Chlor, TRB, FLC, VRC, Ravu revealed resistance in all Candida isolates examined when grown as biofilms, compared to planktonic forms.

In contrast, lipid formulations of AMB and echinocandins showed activity against Candida biofilms.
The growth rate was calculated by measuring the change of biofilm thickness every hour.

5-h-old biofilms of *C. albicans* were treated with either micafungin (MCFG) or fluconazole (FLCZ).

The untreated biofilms exhibited linear growth.

MCFG began to suppress biofilm growth only minutes after the start of the treatment.

In contrast, FLCZ suppressed biofilm growth at a lower rate.

Kaneko et al. AAC 2013;57:2226
The thickness and the growth rate of *Candida parapsilosis* biofilms

- MCFG also showed fast antifungal activity against *Candida parapsilosis* biofilms.
- Untreated biofilms exhibited linear growth.
- MCFG began to suppress biofilm growth only minutes after initiation of the treatment.

Kaneko et al. AAC 2013;57:2226
Real-Time Microscopic Observation of *Candida* Biofilm Development and Effects Due to Micafungin and Fluconazole

Kaneko et al. AAC 2013;57:2226
Species-Specific Differences in the Susceptibilities of Biofilms Formed by *Candida* Bloodstream Isolates to Echinocandin Antifungals

Micafungin and caspofungin were active against biofilms formed by *Candida albicans* or *C. glabrata* but not those formed by *C. tropicalis* or *C. parapsilosis*.
Species-Specific and Drug-Specific Differences in Susceptibility of *Candida* Biofilms to Echinocandins: Characterization of Less Common Bloodstream Isolates

### TABLE 1 MICs of echinocandins for planktonically grown cells and biofilms of different bloodstream *Candida* isolates determined by the XTT assay

<table>
<thead>
<tr>
<th>Species (n = 54)</th>
<th>ANID</th>
<th>CAS</th>
<th>MFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (n = 15)</td>
<td>Planktonic cells</td>
<td>Biofilms</td>
<td>Planktonic cells</td>
</tr>
<tr>
<td>≤0.007</td>
<td>0.06&lt;sup&gt;a&lt;/sup&gt; (0.015–0.06)</td>
<td>0.5 (0.25–0.5)</td>
<td>0.5 (0.25–4)</td>
</tr>
<tr>
<td>C. parapsilosis (n = 6)</td>
<td>0.06 (0.03–0.125)</td>
<td>32&lt;sup&gt;a,b&lt;/sup&gt; (2–64)</td>
<td>1 (0.25–1)</td>
</tr>
<tr>
<td>C. krusei (n = 12)</td>
<td>0.125 (0.06–0.125)</td>
<td>0.125&lt;sup&gt;b&lt;/sup&gt; (0.06–0.125)</td>
<td>1 (0.5–1)</td>
</tr>
<tr>
<td>C. lusitaniae (n = 16)</td>
<td>0.125 (0.03–0.125)</td>
<td>&gt;256&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (0.25–1)</td>
</tr>
<tr>
<td>C. guilliermondii (n = 5)</td>
<td>1 (0.5–1)</td>
<td>32&lt;sup&gt;a&lt;/sup&gt; (8–128)</td>
<td>1 (0.5–1)</td>
</tr>
</tbody>
</table>

*C. lusitaniae* and *C. guilliermondii* biofilms were highly recalcitrant to all echinocandins.

While echinocandins exhibited generally high MICs against *C. parapsilosis* biofilms, MFG exhibited the lowest MICs against these isolates.

Micafungin and Caspofungin activity (used as lock solution) against *Candida albicans* biofilms on intravascular catheters

- An in vitro model of a *C. albicans* biofilm associated with silicone catheters was used.
- Micafungin and caspofungin showed excellent activity against both intermediate- and mature-phase biofilms of two separate *Candida albicans* strains.
- The reduction in the metabolic activity of biofilm yeasts was maintained, even after 48 h.

![Graphs showing biofilm growth inhibition percentages for different strains and incubation times.](image-url)
Possible role of azole and echinocandin lock solutions in the control of *C. albicans* and *C. glabrata* biofilms in vitro

- Efficacy of the antifungal lock was tested against biofilms aged 12 h and 5 days following exposure to caspofungin (5 mg/L and 25 mg/L), micafungin (5 mg/L and 15 mg/L) and posaconazole (10 mg/L) for 12 h.
- Antifungal lock was considered effective in the event of a significant decrease (P < 0.001) in the metabolic activity of the biofilm yeast.
- Both echinocandins in lock solutions in vitro showed an excellent activity against *C. albicans* and *C. glabrata* young (5h) Candida biofilms

### Table 2
Reductions in the metabolic activity of young Candida biofilm yeasts induced by antifungal lock solutions.

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Post-lock interval (h)</th>
<th>Decrease in metabolic activity (%)a</th>
<th>Caspofungin</th>
<th>Micafungin</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 (mg/L)</td>
<td>25 (mg/L)</td>
<td>5 (mg/L)</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>24</td>
<td>81.3 ± 8.8</td>
<td>76.9 ± 12</td>
<td></td>
<td>75.1 ± 14.1</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>76.8 ± 12.4</td>
<td>78.6 ± 11.1</td>
<td></td>
<td>77.7 ± 14.4</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>78.6 ± 17.4</td>
<td>79.5 ± 8.3</td>
<td></td>
<td>81.2 ± 9.3</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>24</td>
<td>76.7 ± 10.1</td>
<td>N/S</td>
<td></td>
<td>92.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>55.8 ± 16.7</td>
<td>N/S</td>
<td></td>
<td>91.6 ± 20.7</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>42.8 ± 19.1</td>
<td>N/S</td>
<td></td>
<td>92.4 ± 0.8</td>
</tr>
</tbody>
</table>

Possible role of azole and echinocandin lock solutions in the control of *Candida albicans* and *C. glabrata* biofilms in vitro

Micafungin had significant inhibitory effectiveness against young and mature *C. albicans* and *C. glabrata* biofilms. Moreover, this activity appeared to persist for up to 3 days.

Caspofungin displayed similar activity against all *C. albicans* biofilms, but the activity was less persistent for *C. glabrata* biofilms.

Posaconazole was less effective against *C. albicans* biofilms.

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**Table 3**

Reductions in the metabolic activity of mature Candida biofilm yeasts induced by antifungal lock solutions.

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Post-lock interval (h)</th>
<th>Decrease in metabolic activity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Caspofungin</th>
<th>Micafungin</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 (mg/L)</td>
<td>25 (mg/L)</td>
<td>5 (mg/L)</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>24</td>
<td></td>
<td>84.1 ± 7</td>
<td>75.2 ± 15.3</td>
<td>71.7 ± 15.3</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>80.4 ± 11.1</td>
<td>81.5 ± 8.5</td>
<td>78.7 ± 16.1</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>24</td>
<td></td>
<td>77.7 ± 10.6</td>
<td>N/S</td>
<td>87.5 ± 11.9</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>44.4 ± 18.5</td>
<td>N/S</td>
<td>90.3 ± 4.3</td>
</tr>
</tbody>
</table>

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Paradoxical effect (PE) of echinocandins

- Paradoxical growth of Candida isolates may occur at concentrations above the MIC for echinocandins.

- A paradoxical effect (PE) of echinocandins against Candida spp biofilms treated with high concentrations of these drugs has been described.

- This effect appears to be species and echinocandin-specific.
In vitro assessment of the antifungal and paradoxical activity of different echinocandins against *Candida tropicalis* biofilms

A PE, (increased metabolic activity at concentrations above SMIC50), was seen in 3 out of 5 strains tested.

CFG produced a PE in two strains

AFG produced a PE in one strain

MFG did not exhibit a PE in any of the strains tested.

Table 1. SMIC<sub>50</sub>, SMIC<sub>80</sub> and PE of the three echinocandins for all strains tested

<table>
<thead>
<tr>
<th></th>
<th>SMIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>SMIC&lt;sub&gt;80&lt;/sub&gt;</th>
<th>PE (drug concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain</td>
<td>ATCC</td>
<td>ATCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44</td>
<td>750</td>
</tr>
<tr>
<td>AFG</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>CFG</td>
<td>1</td>
<td>0.5</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>MFG</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
</tbody>
</table>

Paradoxical effect (PE) of echinocandins

- Paradoxical growth effect was observed
  - With caspofungin and anidulafungin
    - but not with micafungin
  - Against both planktonic cells and biofilms of
    - *C. albicans* and *C. parapsilosis*
      - But not against *C. krusei, C. lusitaniae*, or *C. guilliermondii*
Current therapeutic options

- The current treatment options for fungal biofilm-related infections are very scarce.
- Patients with fungal biofilm-infected devices are rarely cured with mono-antifungal therapy and affected devices generally need to be removed.
  - Percutaneous vascular catheters may be removed quickly.
  - The removal of infected heart valves, joint prostheses, central nervous system shunts and other implanted medical devices is problematic because these implants generally have a life-supportive function.

Donlan CID 2001;33:1387-92
A. Bink et al The Open Mycology Journal, 2011, 5, 29-38
Approach to the treatment of a patient with a long-term central venous catheter (CVC) or a port (P)-related bloodstream infection.

2013 ECIL Guidelines

• When catheter cannot be removed
  – treatment with an echinocandin or
  – a lipid formulation of amphotericin B is preferred (BIII)
Guidelines recommend a combined medical and surgical approach for fungal endocarditis.¹
A recent summary of case reports and small case series reports that 53% of the *Candida* endocarditis cases were cured without surgery, being treated only with an echinocandin.²

Conclusions

- *Candida* biofilms are intrinsically resistance to most antifungal drugs.
- Echinocandins have been shown to be effective against *C. albicans* biofilms and this effect was independent of the state of maturation of the biofilm and fluconazole resistance.
- Differences in the susceptibilities have been described for non *albicans* *Candida* species.
- Echinocandins could be good candidates for lock therapy.
- A paradoxical effect of echinocandins has been described and this effect appears to be species and echinocandin-specific.