Candida albicans - *Staphylococcus aureus* interactions altered by pH-induced changes to biofilm architecture

Helen M. Kurkjian and Babak Momeni

Boston College, Biology Dept
Chestnut Hill, MA

BACKGROUND

Candida albicans

- Fungal opportunistic human pathogen carried commensally by ~30-60% of humans[1]
- Can become pathogenic, resulting in serious complications and/or death especially in immunocompromised persons
- Forms biofilms on organs and medical devices
- Several cell phenotypes, including yeast (ovoid) and hyphae (filamentous)[2]
- Transitions between cell phenotypes triggered environmental cues, including changes in temperature, pH, and nutrient availability

Staphylococcus aureus

- Bacteria carried commensally by ~30% of humans[3]
- Can become pathogenic causing a variety of diseases including endocarditis and bacteremia
- Typically moves passively via simple diffusion

C. albicans and *S. aureus* interactions

- *C. albicans* and *S. aureus* co-occur in the human nasal cavity more often than would be expected by chance[4]
- *S. aureus* can bind to the Als3p adhesin on *C. albicans* hyphae and *C. albicans* penetration of mucosal epithelium can be a point of entry for *S. aureus* to the bloodstream[5]

Experimental Motivation

- While it has been demonstrated that *C. albicans* and *S. aureus* can interact it is not well understood how those interactions might be affected by environmental context.
- We hypothesized that changes in environmental pH would cause architectural changes to *C. albicans* biofilms that might reduce or prevent *S. aureus* penetration to the biofilm interior

METHODS

C. albicans biofilm architecture

- We first cultured a *C. albicans* strain constitutively expressing YFP in yeast phase (YPD medium, 30°C, unshaken) for 24 hours
- We transferred the *C. albicans* to filamentation medium (Roswell Park Memorial Institute 1640) at one of 8 pHs (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5)
- We added *C. albicans* to an 8-well chambered coverglass and incubated it at 37°C and 60 rpm for 90 minutes. We washed the wells to remove unattached cells, replaced the media with fresh media of the same pH, and allowed the biofilms to grow for 24 hours at 37°C and 60 rpm.
- We imaged each biofilm (Fig 1) on a scanning confocal microscope at 400x in 1um slices
- We used the Biofilm Architecture Inference Tool[6] to calculate 6 metrics of biofilm architecture for each biofilm (Fig 2) and compared the z-standardized metrics across pHs using polynomial regression

S. aureus penetration into biofilm

- We added *S. aureus* constitutively expressing GFP to each corner of each 24-hour-old *C. albicans* biofilm
- We imaged the center of each biofilm every ~17 minutes on a scanning confocal microscope at 200x
- We calculated the z-standardized mean number of fluorescing pixels per image for each z-stack and compared them across pH values at a single time point using polynomial regression (Fig 4)
- We compared z-standardized *S. aureus* fluorescence with each z-standardized biofilm architecture variable using simple linear regression (Fig 5)

Changes to *Candida albicans* biofilm architecture can be induced by altering environmental pH

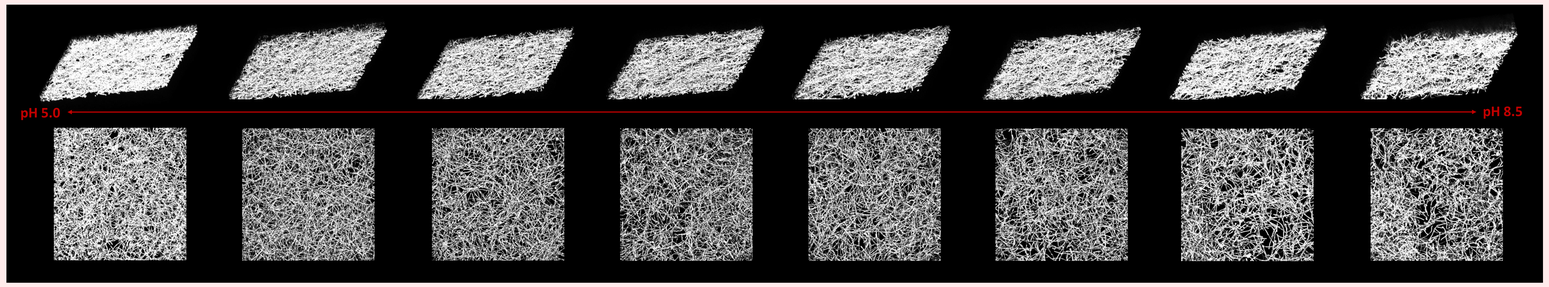


Figure 1. *Candida albicans* biofilms grown for 24 hours in Roswell Park Memorial Institute 1640 medium at pH (left to right): 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5. Top: Oblique view, 400x, 3D projection. Bottom: View from above, 400x, 3D projection.

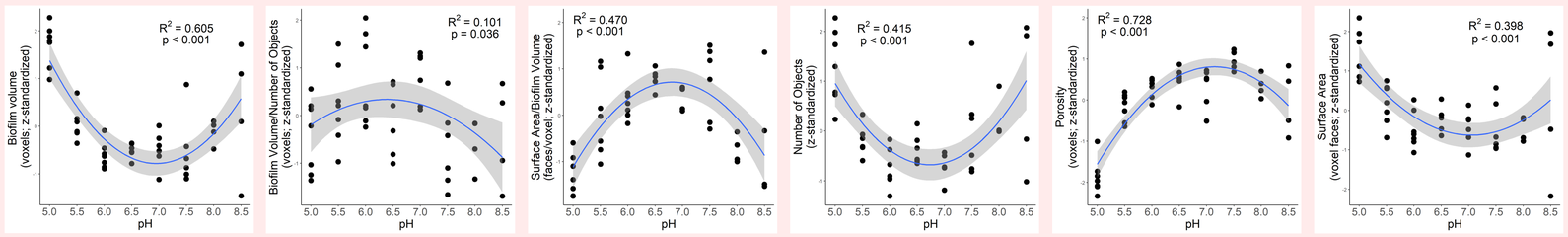


Figure 2. Changes in pH can alter *Candida albicans* biofilm architecture. Each panel shows a polynomial regression using pH as the explanatory variable. The response variables are z-scores standardized by experimental replicate (of (left to right): biofilm volume (voxels), biofilm volume/number of objects (voxels), surface area/biofilm volume (faces/voxel), number of objects, porosity (voxels), surface area (faces). All biofilm architecture metrics were calculated using the Biofilm Architecture Inference Tool.[6]

Changes in pH also affect the ability of *Staphylococcus aureus* to penetrate *Candida albicans* biofilms

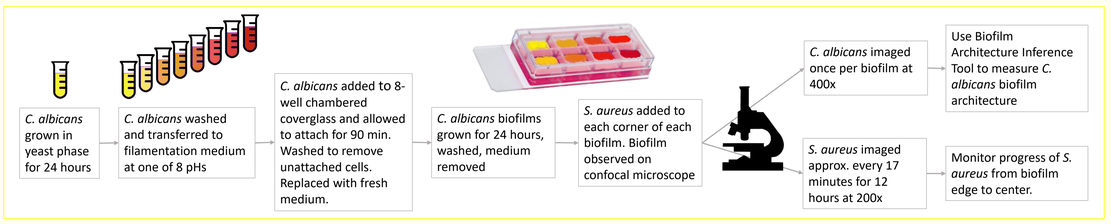
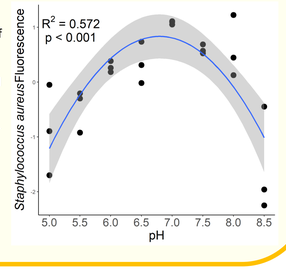


Figure 3. Experimental design to test effects of *C. albicans* biofilm architecture on *S. aureus* penetration into biofilm

Figure 4. The *S. aureus* population size at the center of *C. albicans* biofilms varies with pH. Polynomial regression using the z-score (standardized by experimental replicate) of the mean number of green fluorescent pixels in the center of a *C. albicans* biofilm approximately two hours after gfp-marked *S. aureus* was added at the edge of the biofilm as the response variable and pH as the explanatory variable.



But changes in *C. albicans* biofilm architecture explain only some of the differences in *S. aureus* penetration

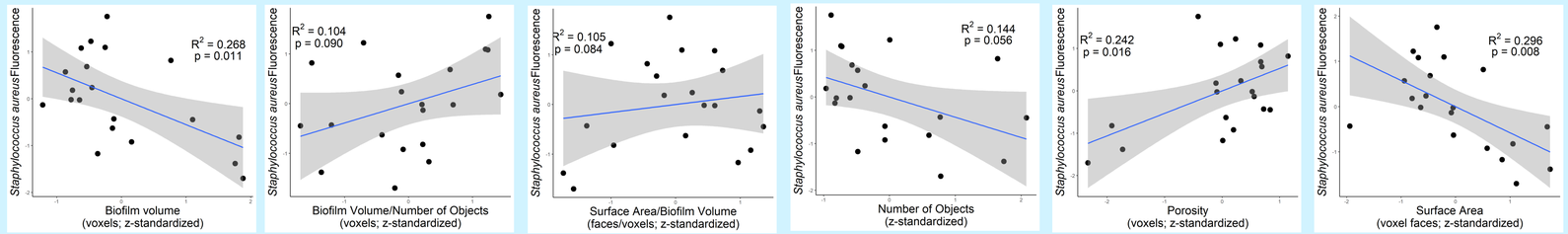


Figure 5. Changes in biofilm architecture can explain some, but not all, of the variability in the time required for *S. aureus* to penetrate to the center of a *C. albicans* biofilm. Each panel shows a simple linear regression using the z-score (standardized by experimental replicate) of the mean number of green fluorescent pixels in the center of a *C. albicans* biofilm approximately two hours after gfp-marked *S. aureus* was added at the edge of the biofilm as the response variable. The explanatory variables are z-scores (standardized by experimental replicate) of (left to right): biofilm volume (voxels), biofilm volume/number of objects (voxels), surface area/biofilm volume (faces/voxel), number of objects, porosity (voxels), surface area (faces).

DISCUSSION

Some metrics of *C. albicans* biofilm architecture showed relationships with pH, others did not

- Biofilm volume and porosity both had strong hump-shaped relationships with pH
- Some of the variance in surface area, the ratio surface area to biofilm volume, and the number of objects was also explained by pH
- There was only a very weak relationship between the ratio of biofilm volume to number of objects and pH

S. aureus was most able to penetrate to the center of *C. albicans* biofilms grown at pHs close to neutral

- In biofilms grown at high and low pH extremes, the *S. aureus* population size was lower in the biofilm center ~2 hrs after *S. aureus* was introduced to the biofilm edge
- In biofilms grown closer to neutral pH, *S. aureus* was able to move from biofilm edge to center faster

Changes to *C. albicans* biofilm architecture can explain some, but not all, of the variance *S. aureus* biofilm penetration

- Some of the variance *S. aureus* travel time from biofilm edge to center can be explained by biofilm architecture metrics, especially surface area, biofilm volume, and porosity
- However, these relationships are weak and only explain a small amount of variance in *S. aureus* travel time, despite the strong relationship with pH
- Other possible explanations for the relationship between *S. aureus* travel time and pH include direct effects of pH on *S. aureus* growth or movement (despite our attempts to remove media, some might have remained) or pH-induced changes to *C. albicans* molecular composition

Implications for *C. albicans*-*S. aureus* interactions and human health

- In order to interact directly, organisms must encounter each other. Here, we demonstrate the environmental context in which *C. albicans*-*S. aureus* interactions take place may cause spatial heterogeneity in the frequency with they encounter each other
- This could alter the outcomes of these interactions depending on their environmental context – for example, while the human oral cavity typically has a near neutral pH (6.7-7.3)[7], while the vagina is acidic (pH ~4.5)[8]

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