



Encapsulation of *Candida albicans* in Alginate Polymer

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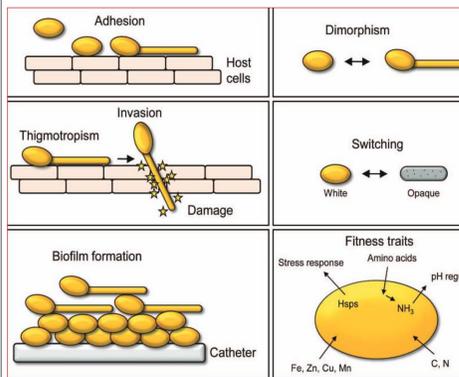
Background and Motivation

Candida albicans is the most common fungal pathogen in humans and are the fourth leading cause of hospital-acquired bloodstream infections in the United States.¹ While treatments exist, some species have shown increased resistance to antifungal agents, making rapid identification of disease-causing agents important for facilitating proper treatment. *Candida albicans* is a commensal opportunistic fungal pathogen. It is a polymorphic organism that exists in pseudohyphal and yeast forms in human hosts.² *C. albicans* causes superficial and systemic infections, including oral thrush, vaginal yeast infections and systemic bloodstream infections. Systemic candidiasis can be deadly in immunocompromised patients such as transplant recipients and patients that have HIV, cancer, and diabetes mellitus.³ Due to the toxicity of antifungal therapies to human host cells, the resistance of *C. albicans* to antifungal therapies, and *Candida's* ability to escape the white blood cells, newer approaches to better study *C. albicans* are needed. Encapsulation of yeast cells will allow observation of cell signaling, growth patterns, and, ultimately, enable development of better alternatives to prevent biofilm formation and *C. albicans* hyphal growth, thereby limiting virulence. Anionic alginate polymers were used to mimic human host cells for *Candida* encapsulation observation, and *C. albicans* strain SC5134 was embedded in the capsules.

Methods

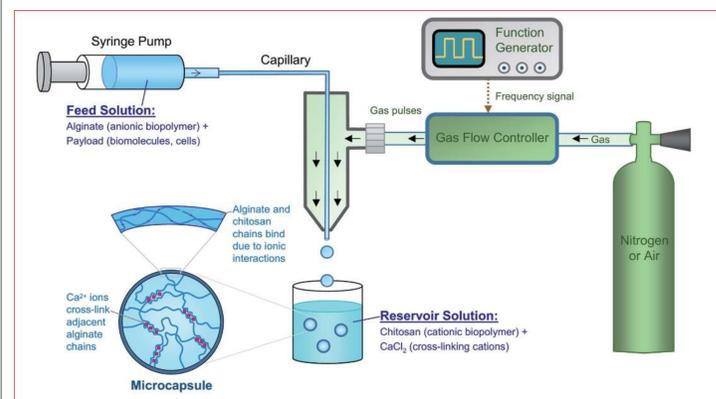
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Pathogenic Mechanism of *C. albicans*.²



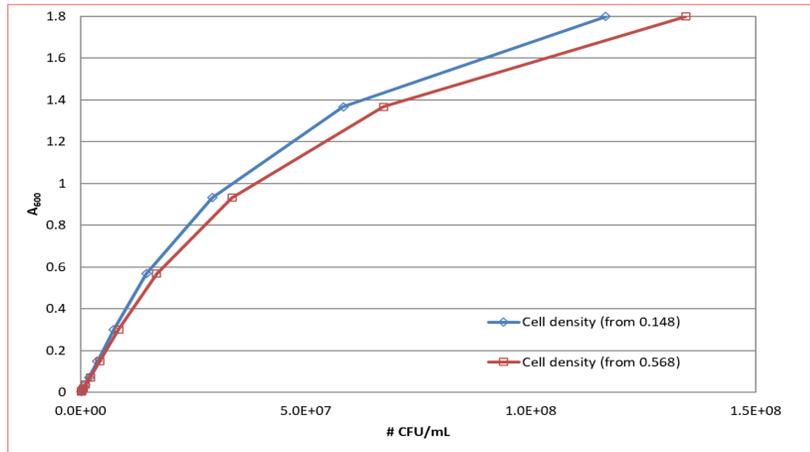
A better understanding of *C. albicans* characteristics was observed by incubating the cells in YPD liquid and solid media at 35 °C and 37 °C, followed by encapsulation in alginate polymer. The characteristics to be observed include cell adhesion, dimorphism, phenotypic switching, thigmotropism and biofilm formation.

Microfluidic Device for Encapsulation.⁴



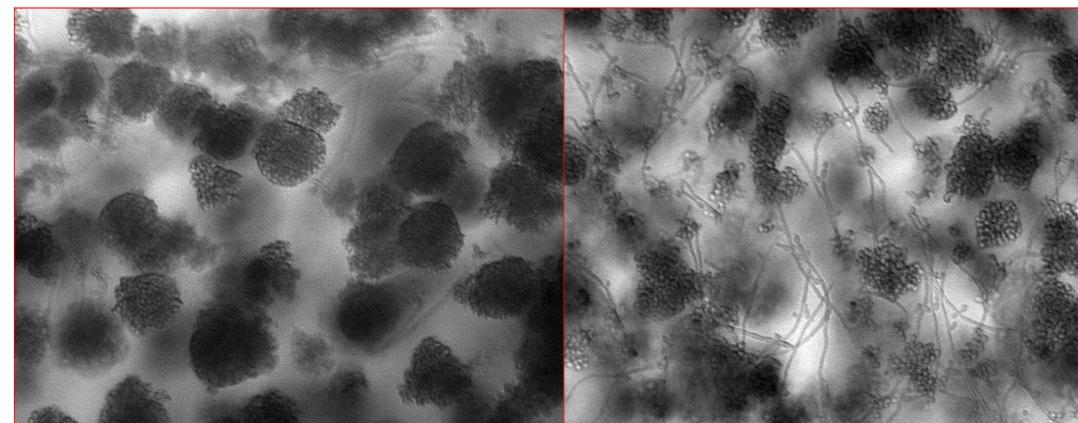
C. Albicans strain SC5134 was centrifuged out from YPD media solution, washed with PBS buffer and stirred into already sterilized alginate solution. A syringe is then used to introduce the alginate + cells mixture into the receiving 0.1 M calcium chloride solution. The calcium chloride solution aids in crosslinking and capsule formation. This procedure is sufficient to produce macro-capsules. However, to produce microcapsules, a gas flow controller is often needed.

Growth Curve of *C. albicans*



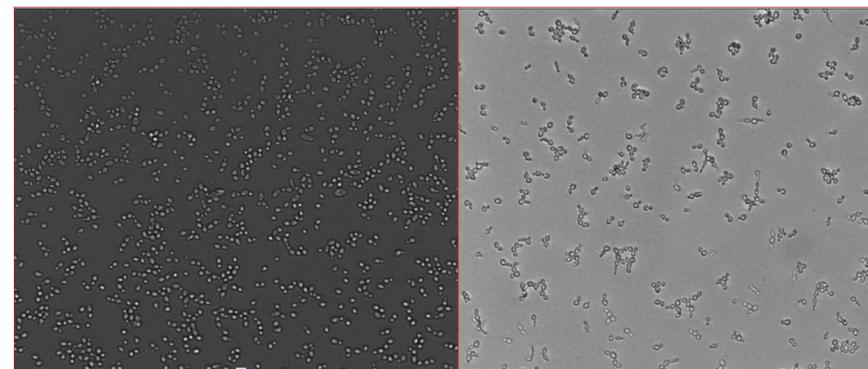
The absorbance at 600 nm has been graphed against the colony forming unit per mL. This graph will be used to vary the cell density of *C. albicans* within each capsule to test for difference in hyphae and biofilm formation, as well as cell signaling in future experiments.

Morphology of *C. albicans* Tested at 35 °C and 37 °C in Alginate Capsules



The polymorphic forms of *C. albicans*: Yeast and Hyphae were observed at 35 °C and 37 °C. The hyphal forms are more prevalent in the capsules incubated at 37 °C, and the cells incubated in capsules at 35 °C show predominantly yeast growth.

Morphology of *C. albicans* Tested at 35 °C and 37 °C in YPD Media



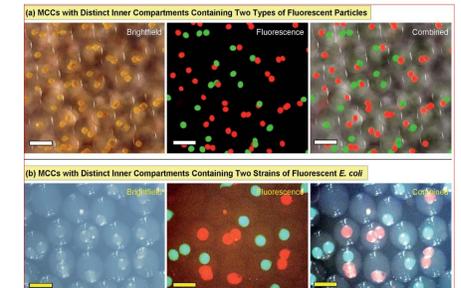
The polymorphic forms of *C. albicans*: Yeast and pseudohyphae were observed at 35 °C and 37 °C in media. However, the hyphal forms are more prevalent in the 37 °C Cell culture as was expected.

Conclusions

- C. albicans* characteristics such as cell adhesion, polymorphism, thigmotropism (directional growth) has been observed on both YPD solid media and within the alginate capsules.
- Yeast form of *C. albicans* has been observed to be predominant at 35 °C both on YPD solid media, and within the alginate capsules.
- Hyphal and pseudohyphal forms of *C. albicans* has been observed to be predominant at 37 °C both on YPD solid media, and within the alginate capsules.

Future Work

- Explore different biopolymer conditions, such as the alginate polymer modulus/ stiffness.
- Test encapsulation procedure at different environmental conditions within the alginate capsule (such as pH) and cell density, to observe biofilm formation or/and hyphal growth.
- By testing and varying these conditions, a better understanding of *C. albicans* pathogenic mechanisms can be understood, which can be used to reduce virulence and biofilm formation.
- Another possible future work involves multicellular compartments (MCCs) with *C. albicans*, to study cellular signaling, as is being done with *E. coli* cells in the Raghavan lab.



References

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Acknowledgements

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