TITLE: COHESIVE GEOMETRY OF C*RYPTOCOCCUS NEOFORMANS* DISTRIBUTION MEDIATES FLOWER-LIKE BIOFILM DEVELOPMENT

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ABSTRACT:

Microbial biofilms are highly structured and dynamic communities, in which phenotypic diversification allows microorganisms to adapt to different environments under distinct conditions. Biofilms are ubiquitous in nature and colonize many niches of the human body and implanted medical devices, being an important factor in *Cryptococcus neoformans* infections. A new approach was used to characterize the underlying geometrical distribution of *C. neoformans* cells during the adhesion stage of biofilm formation. Geometrical aspects of adhered cells were calculated from the Delaunay triangulations and Voronoi diagrams obtained from scanning electron microscopy images (SEM). A correlation between increased biofilm formation and higher ordering of the underlying cell distribution was found. Mature biofilm aggregates were analyzed by applying a novel protocol developed for ultrastructure visualization of cryptococcal cells by SEM. Flower-like clusters consisting of cells embedded in a dense layer of extracellular matrix were observed as well as morphotype switches related to biofilm formation and distinct levels of spatial organization: adhered cells, clusters of cells and community of clusters. The results add insights into yeast motility during the dispersion stage of biofilm formation. This work emphasizes the importance of cellular organization for biofilm growth and may represent novel approaches to establish potential targets for the inhibition and disruption of biofilms with clinical relevance.

Keywords: biolfilms, *Cryptococcus neoformans*, scanning electron microscopy

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