Microorganisms and Biofilms

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Adhesive properties of Aspergillus Fumigatus biofilms probed by atomic force microscopy and effects of Alginate Lyase enzyme.

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Aspergillus fumigatus (A. fumigatus) has become a leading cause of fungal morbidity and mortality, especially in immunocompromised patients [1]. This fungus is able to grow as a multicellular community and produce a hydrophobic extracellular matrix (ECM), mainly composed of galactomannan and α1,3 glucans, to protect itself from host defenses and antimicrobial drugs [2]. This matrix envelops the fungus hyphae, binding them into a contiguous sheath on the colony surface, forming the biofilm and increasing the fungal resistance to adverse environmental factors [3]. Adhere to host cells and resist physical removal play a key role in fungal colonization and invasion of the host and in a wide range of infections. In cases of pharmacological investigations, the efficiency of an antifungal agent can only be assessed by clinical symptoms since repeated biopsy and fungal cultures hinder continuous observation of treatment response [4].

Combining high resolution atomic force microscopy (AFM) and adhesion force spectroscopy we were able to detect simultaneously the pathophysiological conditions of ECM, hyphae and spores. We show that, by using AFM, is possible to exploit the peculiar hydrophobicity of the biofilm components (i.e. cell walls, ECM) to detect the biofilms spread, its growth and lysis on rough surfaces.

We tested our approach by means of several pharmacological strategies commonly used in clinic-treatment, moreover we tested a new approach based on Alginate Lyase (AlgL), an enzyme known to reduce negatively charged alginate levels in microbial biofilms [5]. We also mixed these with amphotericin B (AMB) deoxycholate and its lipid formulations (e.g., liposomal AMB [LAMB]). AFM analysis showed that when A. fumigatus biofilms were treated with AlgL or polyene alone, as well as with their combination, both a reduction of hyphal thicknesses and an increase of adhesive forces were observed compared to the findings for untreated controls, probably owing to the different action by the enzyme or the antifungal compounds. Our results suggest that a combination of AlgL and a polyene antifungal may prove to be a new therapeutic strategy for invasive aspergillosis, while reinforcing the EPS as a valuable antibiofilm drug target. Finally, an important fall out of our results is that AFM and adhesion force spectroscopy, it's possible to develop an effective diagnostic tool able to detect the pharmacological effects on biofilms fungus and thus to transfer advanced microscopy techniques to a clinical purpose.

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