LS-8-P-3502 An extended analysis of the extracellular matrix of Candida albicans biofilms extracted during sample preparation process

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Candida albicans biofilms are formed by yeasts and hyphas adhered to a surface and enclosed in an extracellular polymeric matrix (ECM) secreted by the biofilm cells. ECM is mainly composed by polysaccharides and its water-soluble nature hampers its observation by electron microscopy. Thus, the aim of this work was to evaluate if ECM is extracted from C. albicans biofilms during routine SEM preparation techniques. Biofilms were formed in vitro on the surface of central venous catheters (CVCs) and prepared for Scanning Electron Microscopy (SEM). Samples were chemically fixed, dehydrated in an ethanol series, critical point dried in CO2 and coated with gold for observation in a scanning electron microscope. After each step of sample preparation, an aliquot of the supernatant liquid was collected for further analysis. ECM physically extracted from biofilms was used as a control sample. Negative staining (ammonium molybdate, 1%) and gas chromatography followed by mass spectrometry (GC-MS) were used to evaluate the presence of ECM in the supernatant collected. Environmental Scanning Electron Microscopy (ESEM) and Confocal Laser Scanning Microscopy (CLSM) were used to confirm that ECM was being produced during in vitro biofilm growth. SEM images confirmed that no ECM could be observed after routine preparation methodologies (Figure 1). Negative staining confirmed that ECM fibers are being released from the biofilms during sample preparation. Supernatants from all SEM preparation steps presented large amounts of fibers similar to control sample (Figure 2) and all fractions exhibited similar thickness (22-28 nm). GC-MS analysis corroborates with this hypothesis and shows that all supernatants have the same carbohydrate content as the control sample, being mannose and glucose the main monosaccharide, present in every step of sample preparation. Additionally, analysis using ESEM mode confirmed that yeast cells were completely covered by ECM before sample preparation and the mannose/glucose nature of the ECM around the cells were demonstrated by the intense labeling of biofilms with Concanavalin-A, as shown in CLSM images (Figure 3). Here we demonstrate that chemical fixation followed by dehydration of biofilms samples lead to the total extraction of biofilm ECM. This result shows that utilization of only routine preparation methods for SEM leads to a reduced or mistaken analysis of biofilm structure. Comparing results from ESEM, CLSM and SEM we also showed that each microscopy technique has some information to offer and that the combination of several methodologies would lead to a better understanding of the whole biofilm structure.

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Fig. 1: Candida albicans biofilms formed in vitro on central venous catheters and prepared for SEM using chemical fixation and dehydration methodology. No extracellular matrix can be observed in the biofilm.

Fig. 2: Negative staining of the supernatants collected after each sample preparation step. (A) An aliquot of physically extracted extracellular matrix (ECM) was used as control; (B) sample from fixation step; (C) sample from Ethanol 50% step; (D) sample from ethanol 70% step.

Fig. 3: Candida albicans biofilms labelled with concanavalin-A and visualized using a confocal scanning microscopy. (A) ECM can be observed involving biofilm cells (white arrow); (B) Biofilm distribution.