ABSTRACT

Authors: Kássia de Carvalho Dias, Paula Aboud Barbugli, Carlos Eduardo Vergani.

Effect of biofilm toxins on apoptosis X necrosis of Human Keratinocyte Cell Line (HaCat)

The aim of the study was to investigate the effect of biofilm toxins from single and dual cultures of *C. albicans* and methicillin-sensitive *Staphylococcus aureus* (MSSA), after 36 hours of biofilm formation. The microorganisms used were *C. albicans* SC5314 and MSSA ATCC25923. Microbial cultures were used in the middle of the exponential phase. The optical densities of the suspensions were standardized to the concentration of $1x10^7$ CFU/mL for both microorganisms. The supernatant of the biofilm suspension was filtered in low protein binding filter SFCA (0.20 um). HaCat were cultured until reaching confluence (90%). For the experiments, cells were used between the 3nd and 8th pass. Cells were counted in a Neubauer chamber, and plated ($4,5x10^4$cells/well). Human Keratinocytes (HaCat) were treated with biofilms toxins and LDH enzyme release was measured to assess cell membrane damage (necrosis) caused by the supernatant from the microorganisms. Determination of apoptosis x necrosis was performed by labeling the apoptotic cells with Annexin V and the necrotic cells with propidium iodide (PI). The analysis and photographic documentation were performed in an Fluorescence Inverted Microscope (Leica DMI 3000B). Data were analyzed statistically by two-way ANOVA, followed by the Tukey's test, with a significance level of 5%. All studies were performed in triplicate. The maximum HaCat LDH released was achivied by dual biofilms toxins incubation suggesting the necrotic cell death mechanism. An increased amount of apoptotic HaCat cells stained with annexin V was observed after single *C. albicans* supernatant incubation while dual biofilms toxins was predominantly marked with PI confirming necrosis. Results suggest that the dual biofilm disrupt more the integrity of the cell membranes. From the bright field microscopy images of HaCat cells stressed by the supernatants of *C. albicans* and MSSA biofilms for 2 to 24 hours, it was observed that cell damage was directly proportional to the time of exposure to metabolites. Our data suggest that metabolites, from the supernatants after 36 hours of biofilm formation are harmful to epithelial cells,

and the supernatant of MSSA biofilm was less cytotoxic. Metabolites from dual biofilms of *C. albicans* and MSSA are more harmful to HaCat that those single species biofilms.

Keywords: Biofilm, *Candida albicans*, *Staphylococcus aureus*, Keratinocytes, Toxicity.