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LS-6-P-2897 Comparison of freeze fracture images of mixed bacterial/yeast biofilm in cryo-SEM with high pressure freezing fixation

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Microscopic organisms include bacteria and yeasts have been studied in this project. Besides the planktonic way of living, microbes are able to adhere to surfaces or interfaces and to form organized communities, a so-called biofilm, which are embedded in a matrix of extracellular polymeric substances that they produce; visualization and quantification of this microscopic formation is the main goal of this study. In medicine the biofilm formation allows microorganisms to colonize the surface of implants and it also protects the microbial cells from attacks by the immunity system as well as from the effect of antibiotics. Therefore, the biofilm is considered to be important virulence factor in these microorganisms. The characteristic features of the biofilm infections, especially high resistance to antifungal agents, complicate therapy [1]. Understanding of the biofilm structure can contribute to understanding the biofilm formation and basic biochemical mechanisms underlying this process. It may help to develop more efficient treatment strategy for biofilm infection.

Yeast like Candida albicans and bacteria like Staphylococcus epidermidis have been recently recognized as an important cause of serious biofilm infections associated with implanted medical devices. The multi-layered biofilms formed by these microorganisms were observed by cryo-scanning electron microscope (cryo-SEM) using freeze-fracturing technique [2] and high pressure freezing (HPF) as a fixation method. The freeze-fracture technique consists of physical breaking apart (fracturing) a rapidly frozen biological sample; structural details exposed by the fracture plane may be then visualized by cryo-SEM. Our samples (mixed cultures of C. albicans and S. epidermidis) were cultivated in BHI medium at 37°C for two days on sapphire discs; fractured after high pressure freezing (EM PACT2, Leica Microsystems), then followed short sublimation of ice contamination (Alto 2500, Gatan) in two steps; at first, the sublimation takes 1 minute at -96°C (Figure 1 A, B); in the second phase, the sublimation was 7 minutes at the same temperature settings (Figure 1 C, D). In both cases, the same place of the sample was imaged at low temperature in the field emission SEM JSM 7401F (JEOL). The sublimation time reveals substances in our sample with different chemical composition that is related with the ability of sublimation; we are able to recognize water, cultivation medium, extracellular matrix and cells of our microbes.

References:

[1] R. M. Donlan and J. W. Costerton, Clin. Microbiol. Rev. 15 (2002), p. 167.
[2] K. Dobranska in “Characterisation of bacterial/yeast biofilms by scanning electron microscopy”, et al., (EMC 2012 Proceedings, Manchester) (2012), p. 671.

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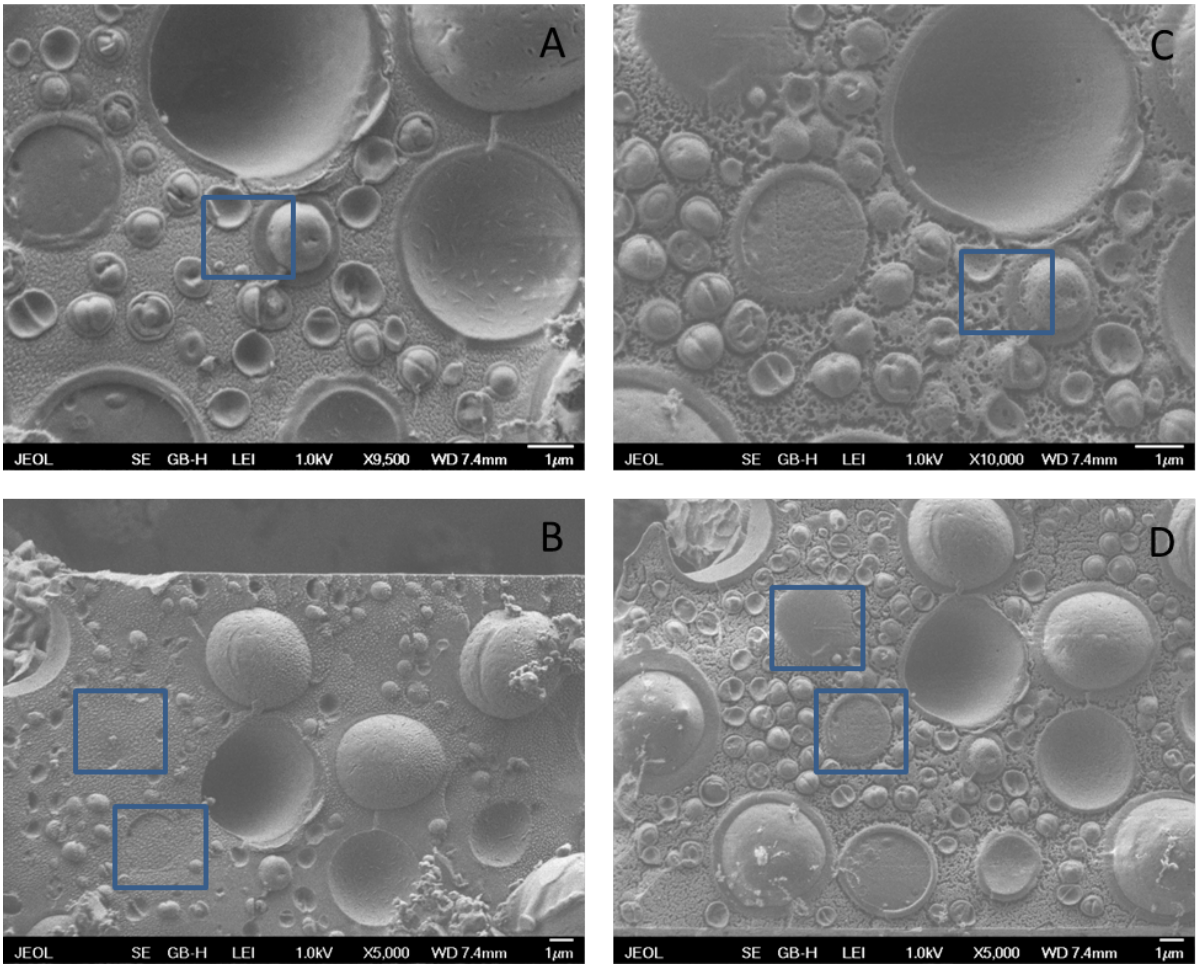

Fig. 1: Figure 1 A, B: Cryo-SEM image of biofilm of C. albicans and S. epidermidis after short 1 minute sublimation at -96˚; C, D: images after longer 7 minutes sublimation at -96˚C; there are compared fields of interest (the presence of extracellular matrix) after short and longer sublimation time in blue boxes.