3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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Comparison of biofilm formation of mixed yeast/bacterial cultures by FIB-SEM tomography

K. Dobranska[1], J. Nebesarova[2], F. Ruzicka[3], J. Dluhos[4], O. Samek[1], V. Krzyzanek[1]

[1]ISI ASCR, Brno, Czech Republic
[2]Biology center of the ASCR, Ceske Budejovice, Czech Republic
[3]St. Anne´s hospital, Brno, Czech Republic
[4]Tescan, Brno, Czech Republic

dobranska@isibrno.cz
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Yeasts and bacteria are considered as microscopic organisms. We focus our research on microbial strains, namely on yeast *Candida albicans* and bacteria *Staphylococcus epidermidis*, which are widely studied organisms in many medical and microbiological laboratories. These studies are of high interest because microbes can cause serious complications associated with infections after surgery attacks, although they are a part of normal microflora of human body in physiological conditions.

The complications start with the uncontrolled growth of biofilm. Because besides the planktonic way of living, microbes are able to adhere to surfaces or interfaces and to form organized communities. These communities are covered by extracellular polymer matrix composed mostly of polysaccharides, that structure creates so-called biofilm. 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. The characteristic features of the biofilm infections, especially high resistance to antifungal agents could complicate therapy [1]. The structure of the biofilm layer and proportion and composition of extracellular matrix differs by type of organism and environmental conditions - where bacterial/yeast cultures are evolving and growing. Knowledge 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 *Staphylococcus epidermidis* have been recently recognized as an important cause of serious biofilm infections associated with implanted medical devices. In our experiments the multi-layered biofilms formed by these microorganisms were investigated by Focused Ion Beam Scanning Electron Microscope (FIB-SEM) tomography that allows the 3D reconstruction of large volumes with high resolution.

Here we present comparison of two mixed yeast-bacterial cultures grown on sapphire disks. The first sample contains one-day cultivation of *C. albicans* followed by additional one-day cultivation of bacteria *S. epidermidis* on the same disk (two-steps cultivation) [Fig 1]. The second sample represents a two-day cultivation of mixture of the two above mentioned microbes [Fig 2]. After the cultivation sample preparation for FIB-SEM investigations was performed to preserve the biofilm with its extracellular polymer substance which is very soft and sensitive. Both samples were fixed by high pressure freezing (HPF Leica EM Pact II), followed by freeze-substitution procedure (Leica EM AFS). To increase the contrast in BSE imaging samples were stained with an osmium-thiocarbohydrazide-osmium (OTO) method. The preparation procedure was finished by embedding into Epon resin.

A focused ion beam technique was used for both precise cross section preparations as well as for FIB-SEM tomographical acquisition of 3D dataset using the signal of backscattered electrons (BSE) [3]. The microscope LYRA 3 FIB-SEM (Tescan, Czech Republic) was used in our experiments. The resulting 3D reconstruction was processed with the software ORS visual.

Our results show that the FIB-SEM tomography is suitable method for structural investigation of microbial biofilms. We present that final 3D reconstructions well demonstrate the disparity between above mentioned mixed microbial cultures. In the first sample with the two-step cultivation, separated colonies of yeast-bacteria are clearly visible. On the other hand, the second sample which contains the mixture shows quite uniform structure without visible separation. We believe that the described methodology of biofilm preparation combined with FIB-SEM tomography will be helpful in clinical research of microbial infection.

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.
3. M. Zadražil in “The Step Towards an Ultimate Multifunctional Tool for Nanotechnology” et al., (3M Nano 2012 Proceedings, X'ien) (2012), p. 175
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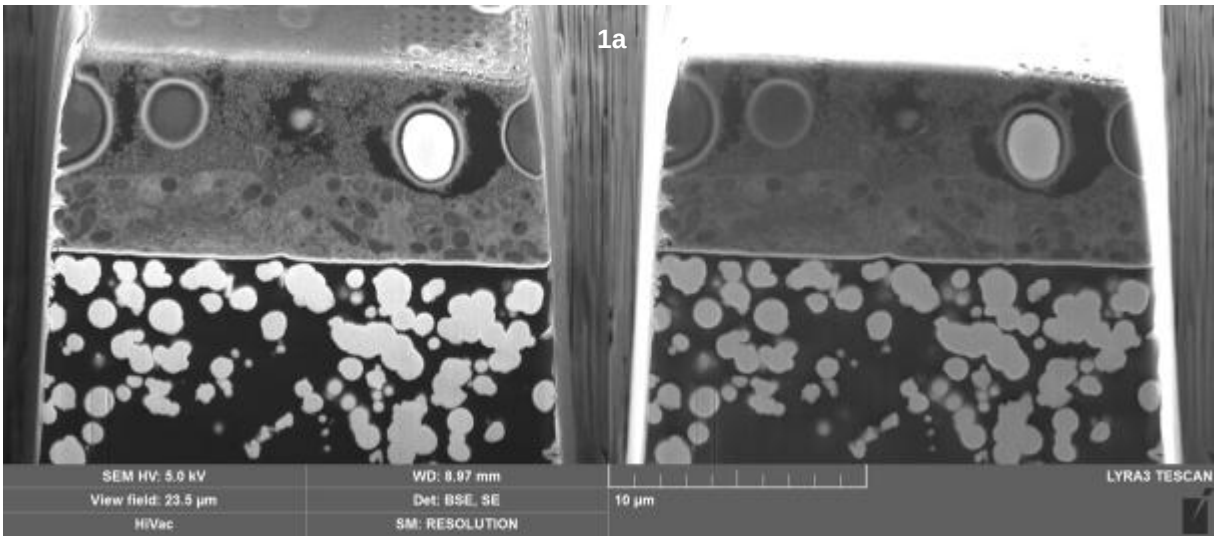

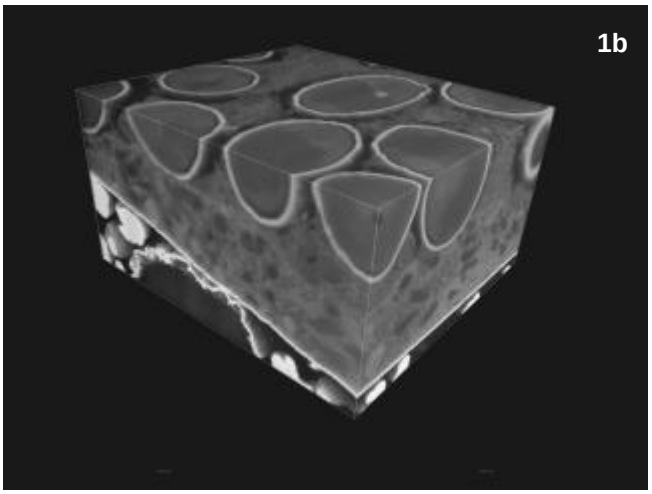

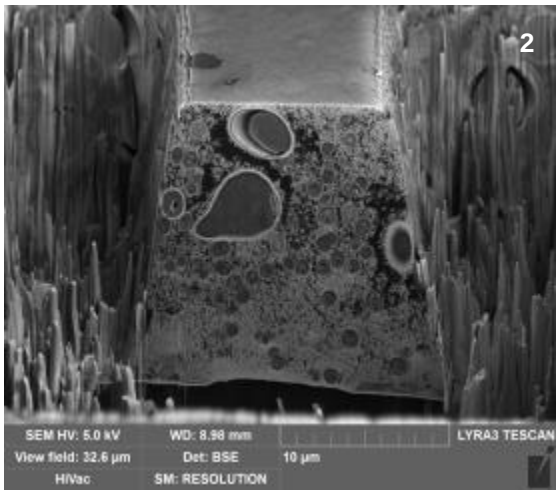

Figure 1a. Back-scattered electron (BSE) and secondary electron (SE) image of the two-steps cultivation of the two microbes culture.

Figure 1b. 3D reconstruction (FIB-SEM tomography) of the two-steps cultivation.

Figure 2. BSE image of two-day cultivation of the mixture *C. albicans* and *S. epidermidis.*