A progress report for the Latvian Council of Science project conducted within the Fundamental and applied research projects framework "*Engineered surface platform for immobilization of microorganisms*" (lzp-2018/1-0460) on the work done during the 01.12.2018. – 01.03.2020. time frame

The report includes progress information on the following tasks:

- New approaches for the studies of immobilized yeast cells
- Cell viability studies

Achieved results

Sample surfaces with seeded cells were analyzed using SEM. Representative images of cells attached to microstructures is given in Figure 1 and 2. This activity has only just begun; therefore, images have not yet been taken from samples that were exposed to UV radiation before being seeded with cells.

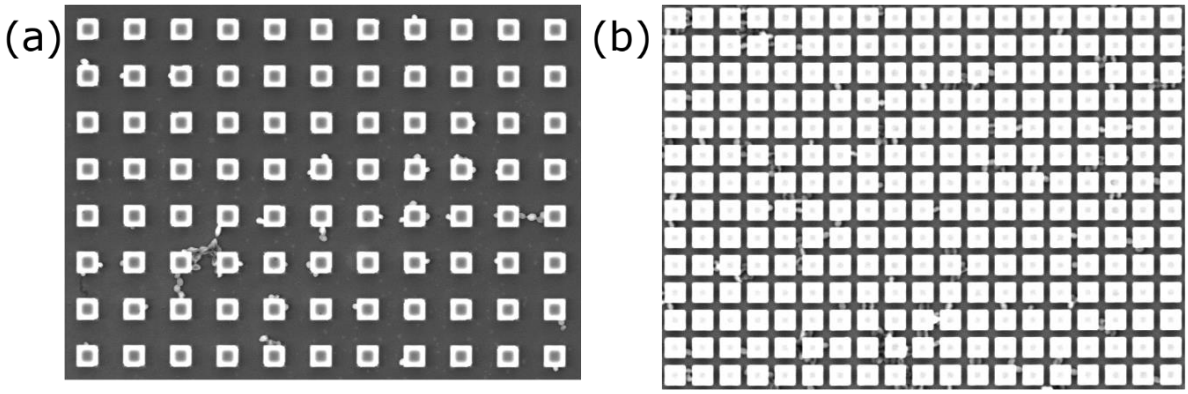


Figure 1. *Top-view scans of the samples after cell deposition acquired through SEM: (a) 39-1-6, (b) 39-1-10.*

As can be seen in Figure 1 single yeast cells tend to attach to the sides of microstructures close to the structures center. This has been observed on all structures with a side length of ~10 mkm. For larger structures the cells become attached along most of the length of the structures side excluding the corners. Larger cell agglomerates, however, attach more to the corners with "string"-type agglomerates preferring the center of the microstructures side. Very few cells become attached to the plateaus with most cells residing in the valleys. Figure 2 shows how cells tend to position themselves along the sides of the microstructures. Mostly they align vertically along the side which could be because of the capillary force pulling on the cells when the liquid medium dries out.

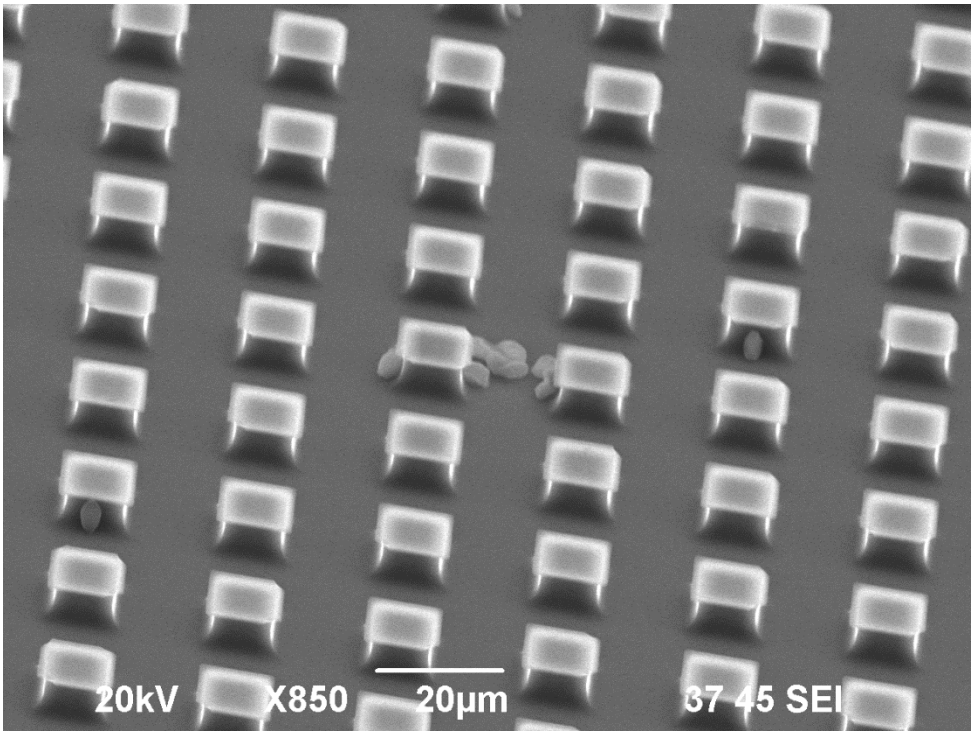
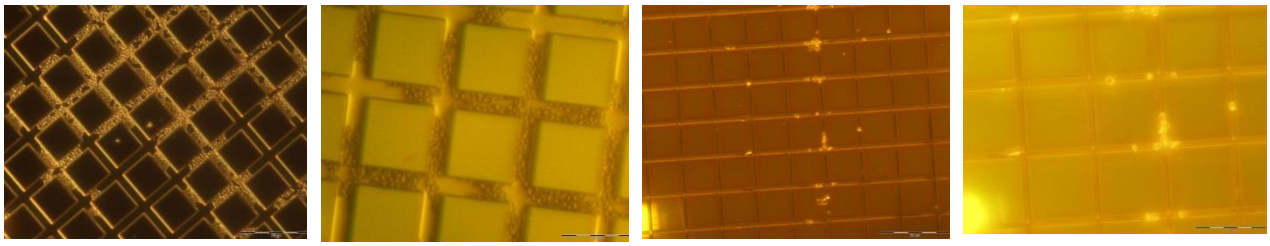


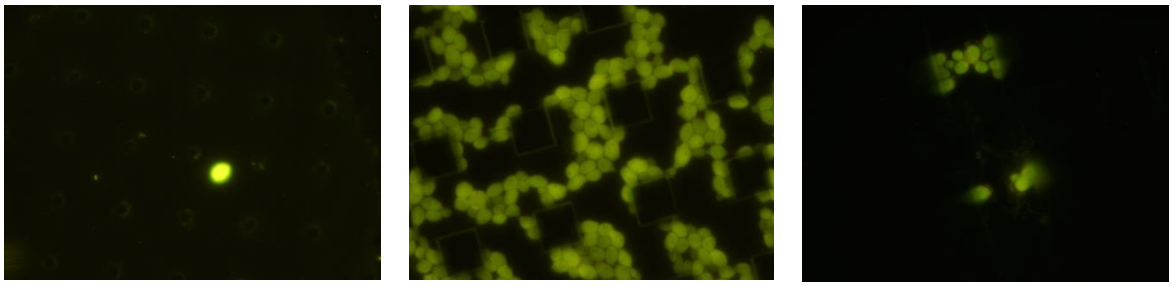
Figure 2. *Semi-isometric view of cells attached to structures of a 39-1-6 type sample.*



(a) **(b)** **(c)** **(d)**

Figure 3. *Phase-contrast microscopy images of yeast cells immobilized on platform surface structures when the rotation speed of the suspension 50 rpm was used. Microstructure type 39-1-9: (a) objective 20x, (b) objective 40x. Microstructure type 39-1-7: (c) objective 20x, (d) objective 40x. Scale bars: 20 µm.*

Due to these goals' fluorescence microscopy was used for the evaluation of the viability of yeast cells after their immobilization. This method is based on the use of fluorochrome primulin (mol. wt. 475.5). In the case of viable cells this fluorochrome binds only to the cell wall which when studied using fluorescence microscopy manifests as a dimly lit green cell with a slightly brighter ring around it. In the case of severely damaged dead cells this fluorochrome binds to the cytoplasm because of its changed physicochemical characteristics which leads to the bright yellow-green fluorescence of the whole cell. This method is widely used by the LU MBI team during studies of various treatments of yeast cells. At the same time, it was necessary to confirm the applicability of this method in the case of the cells immobilized on an opaque carrier. The corresponding experiments demonstrated that it is possible to use this method for the determination of viability of immobilized yeast using a fluorescence microscope Olympus BX51 (Olympus, Tokio, Japan).



(a) **(b)** **(c)**

Figure 4. *Fluorescent microscopy images of immobilized yeast cells dehydrated during 24 hours at 30^{O}C. Microstructures: (a) 39-1-2, (b) 39-1-3, (c) 39-1-9. Objective 100x.*

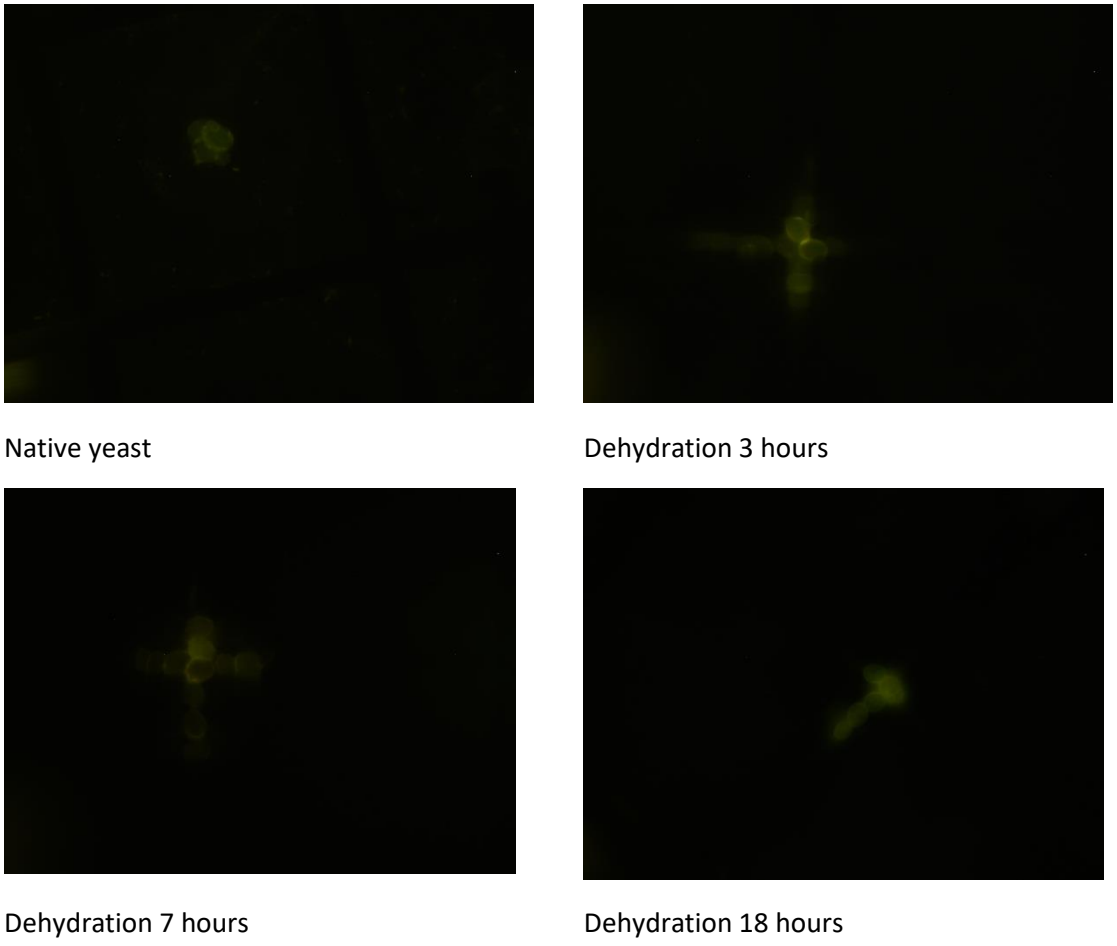


Figure 5. *Viability of immobilized yeast cells dehydrated at 30^{O}C in dynamics. Fluorescent microscopy images. Microstructure 39-1-7. Objective 100x.*

A series of experiments was devoted to the understanding of the localization of immobilized yeast cells on the used platforms. Results obtained using phase contrast microscopy and the one using reflected light mode of optical microscope Zeiss Jena NU-2 were compared. The comparison of these results demonstrates that application of phase contrast method allows to reach better visualization of the platform surface and the distribution of immobilized cells on this platform. Analysis of these images gives

us the information that yeast cells were immobilized on the surface of carrier in one layer strictly in valleys between plateaus.

Another series of experiments was devoted to probing the viability of deposited yeast cells after different periods of drying (Figure 5). The obtained results show that after 18 h of drying at 30°C immobilized cells still maintain their viability.

Conclusion

Not only do yeast cells attach to the sides of the microstructures, they become aligned vertically along the sides of the microstructures. This kind of behavior can't be visualized using conventional light microscopy. Thus, SEM studies of attached cells should be performed more frequently during all immobilization experiments.

Different modalities of light microscopy have been compared. The best method for imaging the deposition of cells seems to be phase contrast microscopy. However, since the motorized stage that is being used in this study can be mounted only on the reflected light microscope large throughput imaging will be performed using the Zeiss NU-2 and in certain cases phase contrast microscopy will be used.

Viability studies show that yeast cells remain viable even after 18 hours after being immobilized.