Progress report for project “Engineered surface platform for immobilization of microorganisms”. March – May 2020 (Months 19 – 21).

During this period severe restrictions were placed on the ability of project team members to work at the premises of both Riga Technical University and University of Latvia. Participation of students in the research process was also stifled. Thus, the team focused on those tasks the performance of which was possible mainly from home. Nevertheless, significant progress was made in designing, manufacturing and micropattern shape characterization of a new batch of immobilization platforms, as well as some test runs of cell immobilization using the new platforms.

After working with the first batch of immobilization platforms during the first half of the project as well as noticing how over-etching of parallelopiped-shaped microscale features produces pyramid-like structures, it was decided to design a second batch of immobilization platforms with microscale features possessing the initially planned shapes. When discussing the results with the manufacturer of the first batch (A/S ALFA RPAR) they agreed to produce an experimental batch of immobilization platforms with over-etched microscale features of pyramid-like shape. They also agreed to produce several other experimental batches of immobilization platforms with the microscale features having much straighter walls then the first batch structures (produced via reactive ion etching (RIE)) and also several types of microscale structures with slightly slanted walls (produced via a combination of RIE and liquid solution etching). Additionally, the possibility of including a hexagon-shaped microfeature type (the previous ones were all square-shaped) into the microfeature shape pool was discussed. It was planned for the surfaces of the immobilization platforms to be composed of amorphous silicon dioxide no less then 100 microns in thickness thermally deposited on the surface of a silicon wafer. As this was planned to be a sorts of a test run to see whether the production of such structures is possible with the tools and methods available to the manufacturer, A/S ALFA RPAR agreed to produce the whole second batch of immobilization platforms free of charge as long as the project team would provide the manufacturer with both a set of technical drawings of the desired surface micropatterns and a set of design files for the production of photolithography masks. Although the manufacturer was kind enough to provide this service for free, the production of all possible versions of microscale features on individual substrates would be too costly for the manufacturer. Therefore, it was decided to design the individual platforms in such a way where each chip would have 4 different types of micropatterns on its surface with each platform having micropatterns comprised of small squares, large squares, small hexagons, and large hexagons. Thus, the project team developed technical drawings for a set of 27 immobilization platform groups each of which would have 4 types of microscale patterns on their surfaces. After discussing the designs with the manufacturer three immobilization platform groups were deemed too complex even for experimental production, therefore they were excluded from the batch. For the remaining 24 groups design files needed to produce photolithography masks were made under the supervision of A/S ALFA RPAR technologists and were later used to fabricate the second batch of immobilization platforms.

Characterization of microscale roughness using scanning electron microscopy. After receiving the second batch of immobilization platforms scanning electron microscopy to determine the actual shape and dimensions of the resultant microscale features was performed on several randomly selected platforms from each of the 24 groups. While access to the electron microscope was limited by COVID-19 preventive measures at the University of Latvia (where the microscope was located) it was nonetheless possible to perform imaging of the samples as long as only one person was present on

the premises during the whole process. Several representative images of the new microscale structures is given in Figures 1, 2 and 3.

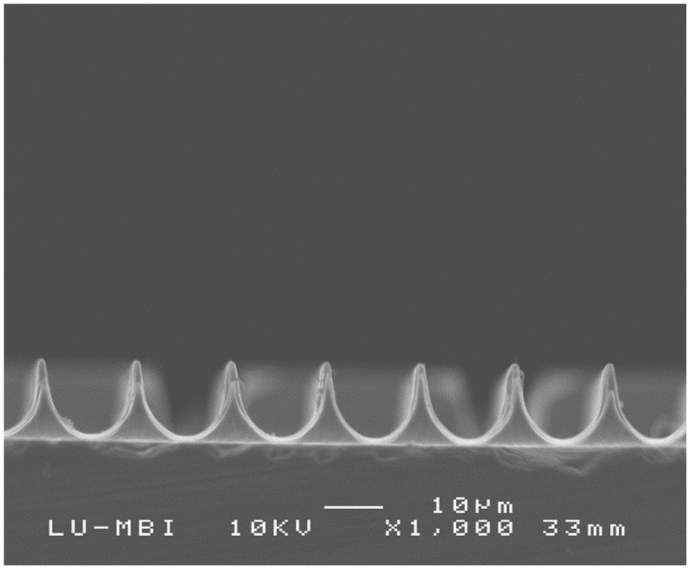

Figure 1. Pyramid-like microscale structures present on the surface of group K-79 immobilization platforms.

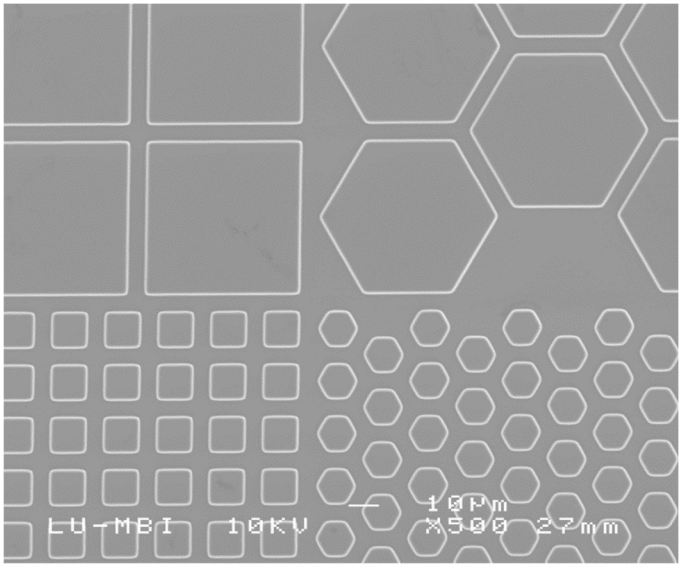

Figure 2. Four types of microscale structures present on the surface of group V-39 immobilization platforms.

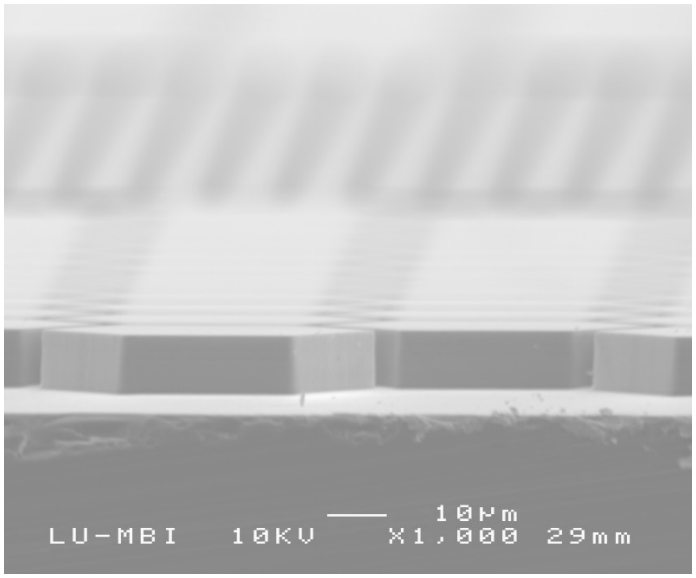

Figure 3. Large hexagonal structures on the surface of group V-34 immobilization platforms.

The acquired images were used to determine the heights, the lengths at the base and at the top, and the distances between the bases of individual structures for all structure types in all platform groups with the standard deviation of dimensions for the sampled structures being below 0.1 micron which coincidently correlates with the spatial resolution of the acquired images. Thus, it can be inferred that the dimensional distribution of microscale features is uniform with the standard deviation value lying below the microscale threshold.

Additionally, it was possible to perform cell immobilization test runs on a few second-batch immobilization platforms as well as on the satellite samples that accompanied the platforms to see how the *S. cerevisiae*-77 yeast cells attach to the new surfaces. A representative image of cells attached to the surface of an immobilization platform is given in Figure 4.

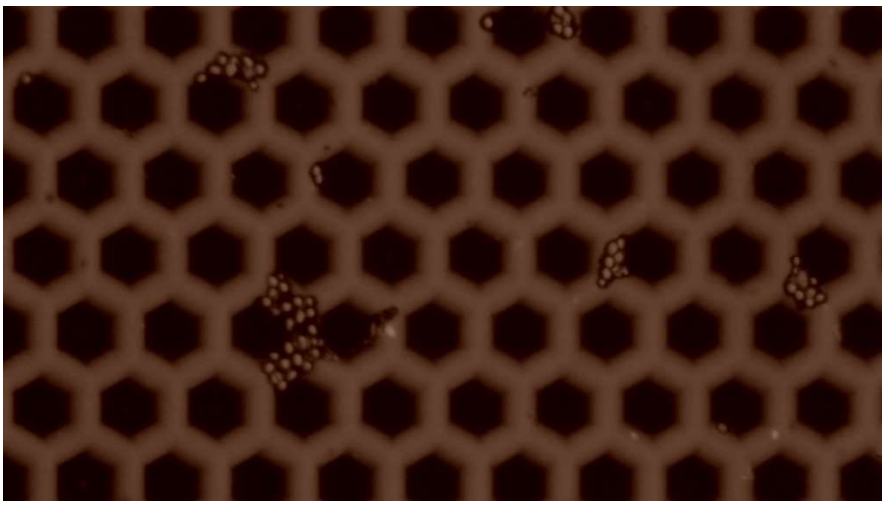

Figure 4. Cells attached to a region patterned with small hexagonal structures on the surface of an immobilization platform from group V-46.

Generally, it looks like the cells are distributed in a similar way as they were for first batch samples. First impressions suggest that (1) cells tend to attach to the centers of microstructure sides and (2) cells prefer to stay the structures and not on their surface, just as they did in the case of immobilization

platforms from the first batch. The amount of cells attached to the surfaces will be estimated at a later date when full scale immobilization activities will be performed.

Aside from the described activities and based on the uncertain nature of the current COVID-related restrictions, it was deemed necessary to upgrade some of the critical tools used during the project for remote use. Specifically, the tools for cell area estimation and serial imaging of immobilization platforms. For cell area estimation it was decided that a user-friendly application needs to be developed to be usable at home and easily accessible to both project team members and the students involved in the project. For serial imaging a PC-based application which can control the available motorized stage and perform semi-automatic serial imaging from home should also be developed to limit the need of the operator to be in the laboratory during the whole run of the procedure.