Progress report for project “Engineered surface platform for immobilization of microorganisms”. March – May 2021 (Month 31 – 33).

With the easing of some COVID-19-related restrictions it was possible for more project team members to be on the premises and to continue involving student volunteers on a more regular basis. During this period the following activities were performed: cell deposition onto second-batch immobilization platforms for serial imaging and microbiological analysis, serial imaging of the immobilization platforms with attached cells, microbiological analysis of attached cells, real time cell attachment analysis using the previously described flow cell and time lapse microscopy, and further development of the LiSt-KPFS technique for studying the effects of illumination on surface electrical potential.

Cell deposition was performed onto the surfaces of immobilization platforms before and after exposure to UV light from the following groups – V-46, V-47, V-48, V-49, V-50, K-76, K-77, as well as several platforms from previously studied groups. Serial imaging and image processing was performed for all samples. Representative images before and after processing for representative samples from group V-46 are given in Figures 1 and 2.

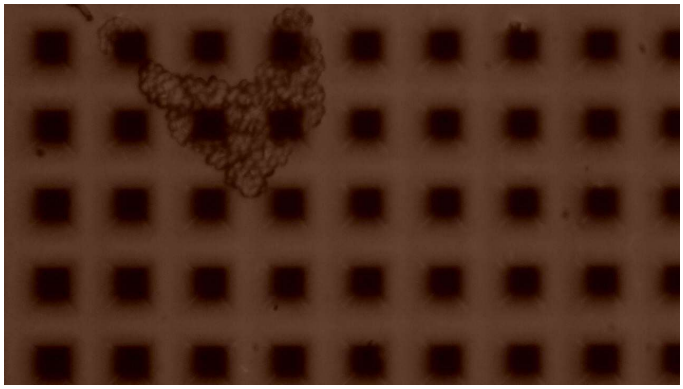

Figure 1. Image of cells immobilized on the surface of an immobilization platform from group V-46 before being processed via cell area estimation app.

Figure 2. Image of cells immobilized on the surface of an immobilization platform from group V-46 after being processed via cell area estimation app.

Microbiological studies, specifically cell growth rate and ability to produce ethanol, have also been performed for all mentioned platform groups. Figures 3, 4 and 5 show the platform surface area fraction populated by yeast cells, cell growth during a period of 48 hours, and changes in ethanol concentration during a period of 48 hours for cells deposited onto platforms from groups V-36 and V-

37. The main difference between the two groups of platforms were the distances between adjacent microscale structures those being about 4.5 micron and about 3.6 micron for V-36 and V-37, respectively.

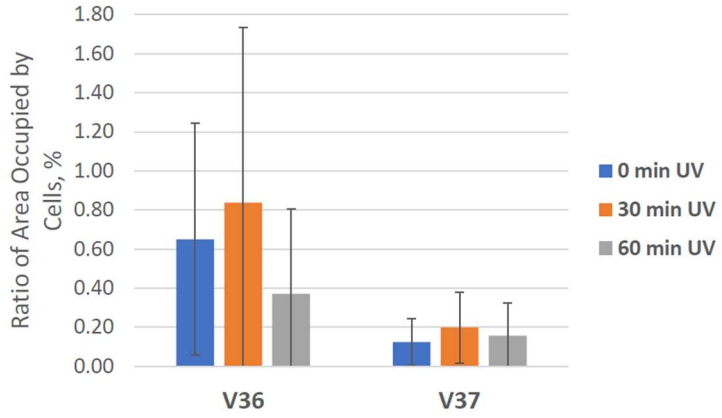

Figure 3. Average ratio of the area occupied by cells on the surfaces of UV exposed and non-exposed immobilization platforms from groups V-36 and V-37.

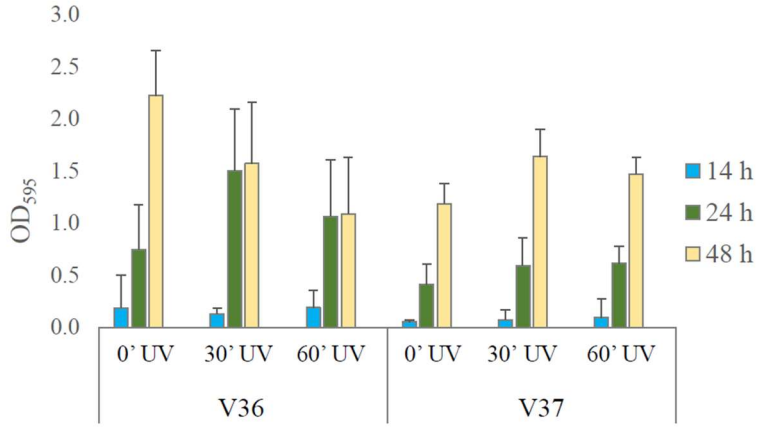

Figure 4. Biomass yield of *S. cerevisiae-77* cells deposited onto the surfaces of UV exposed and non-exposed immobilization platforms from groups V-36 and V-37.

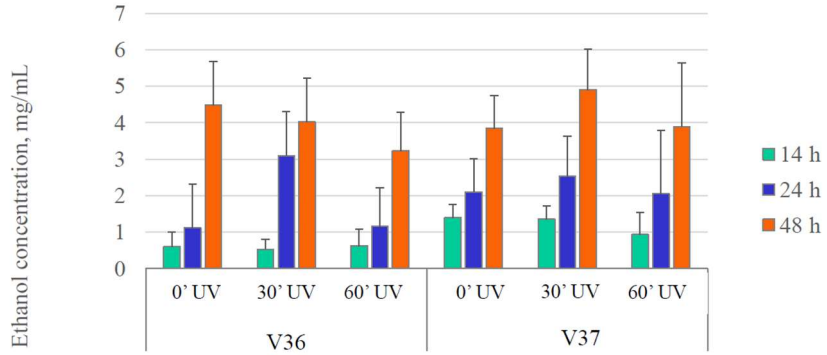

Figure 5. Dynamics of ethanol synthesis by the *S. cerevisiae-77* cells deposited onto the surfaces of UV exposed and non-exposed immobilization platforms from groups V-36 and V-37.

As can be seen in Figure 3 the average deviation in the amount of attached cells is large for both immobilization platform types. However, in general, there was a larger number of cells on the surfaces of platforms from group V-36. There is also some difference in the amount of attached cells when comparing UV exposed and non-exposed platforms with representative from both groups exhibiting a maximum at 30 minutes of UV exposure and then a decrease in the amount of retained cells after 60 minutes of exposure.

Biomass yield test data seen in Figure 4 doesn't seem to indicate a unified trend in the growth pattern of the attached cells, while Figure 5 which depicts the dynamics of ethanol synthesis by the attached cells suggests that yeast cells attached to surfaces with smaller gaps are more active in producing ethanol, at least in the initial 14 hours. Gathered data for this and all the other sample groups will be further analyzed at a later date. Meanwhile, it is planned to present these results at the upcoming "European Biotechnology Congress 2021".

Real time yeast cell attachment analysis using the developed flow cell was performed on UV exposed and non-exposed platforms from groups V-35 and V-38. This time, the main difference between the microscale structures was their height with it being about 6.2 microns for group V-35 platforms and 2.8 micron for group V-38 platforms. The deposition parameters were kept the same as for the glass samples in the previously mentioned experiment. In the case of the platforms, serial imaging was performed for all four surface types present on the surfaces of platforms from each f the two groups. Along with non-exposed platforms, UV-exposed platforms were also studied. Figures 6 and 7 show the amount of attached cells after 32 minutes of deposition.

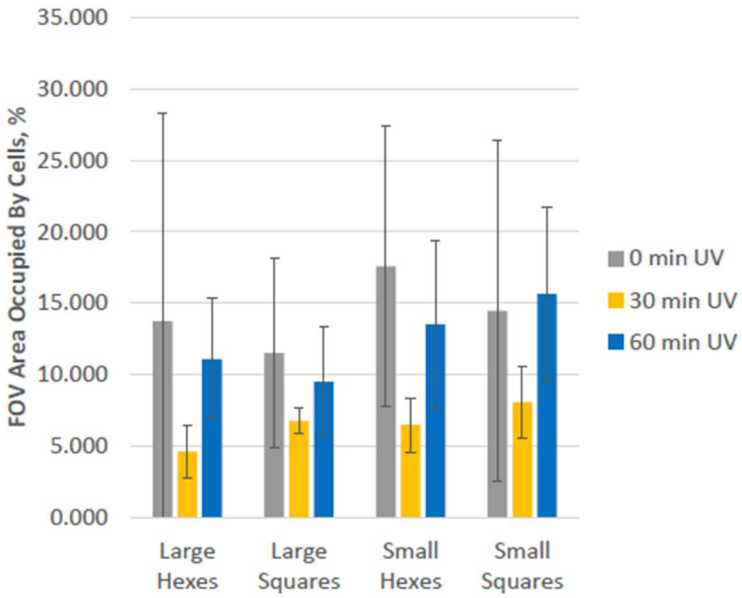

Figure 6. Amount of cells on the four types of patterns present on the surfaces of group V-35 immobilization platforms.

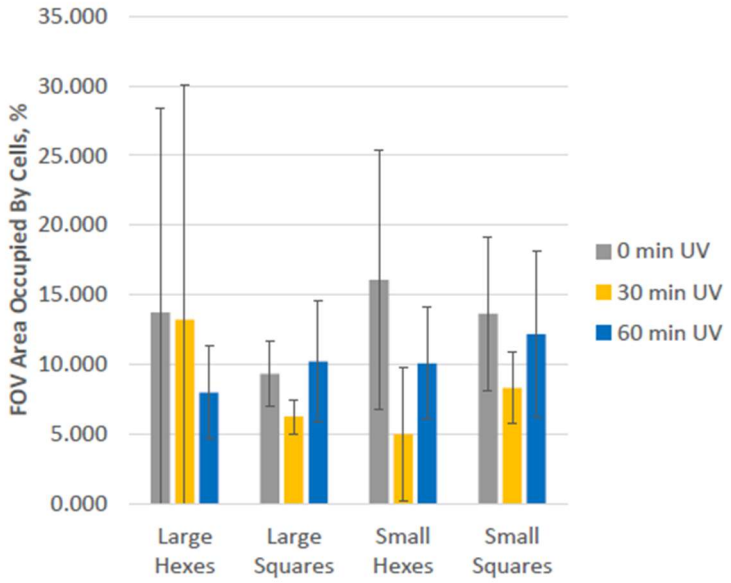

Figure 7. Amount of cells on the four types of patterns present on the surfaces of group V-38 immobilization platforms.

The figures show that for all surface types barring one the amount of first decreases for platforms exposed to UV light for 30 minutes and then increases for platforms exposed to UV light for 60 minutes. For non-exposed platforms in both groups cells tend to prefer to attach to surfaces patterned with small hexagonal structures. In some cases this trend is reversed after 30 minutes of exposure, with the same surfaces having on average fewer attached cells after 32 minutes of deposition. At 60 minutes of exposure surfaces covered in small squares tend to attach the most cells, but the resulting amount of attached cells is still lower than that of unexposed small hexagons. Cell attachment dynamics to surfaces patterned with small hexagonal structures can be seen in Figures 8 and 9.

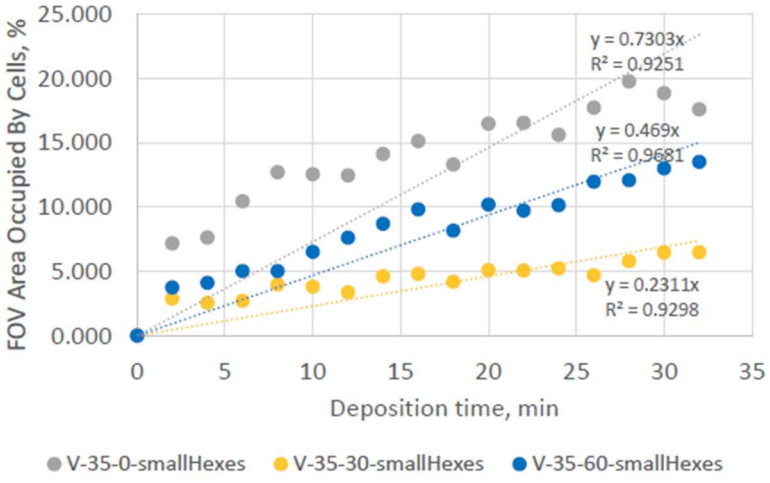

Figure 8. Cell deposition dynamics over a period of 32 minutes for small hexagonal surface micropatterns of group V-35 platforms.

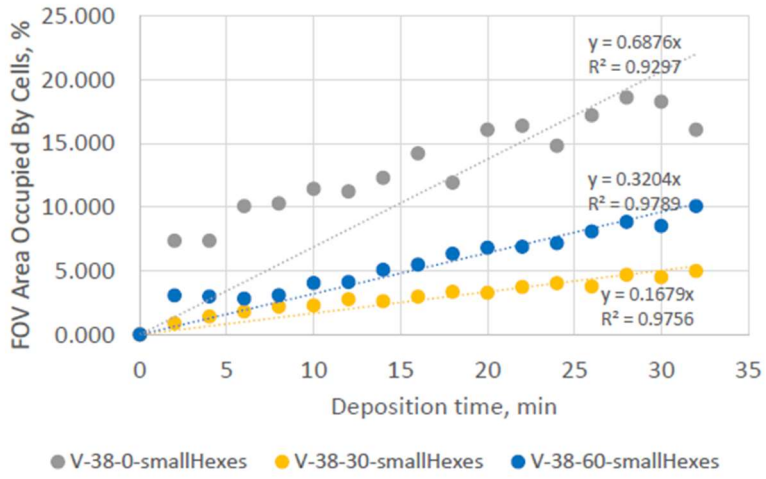

Figure 9. Cell deposition dynamics over a period of 32 minutes for small hexagonal surface micropatterns of group V-38 platforms.

The data show that cell attachment rate seems to slow down at 30 minutes of UV exposure and then increase at 60 minutes of UV exposure for samples from both platform groups. Judging by the graphs, cell attachment rate can be approximated by a straight line which changes its slope with UV exposure time but remains linear. This could indicate that the discharge rate for the exposed immobilization platforms is low enough to be undetectable at such time frames. Possibly, the discharge rate for the amorphous silicon dioxide surfaces exposed to polychromatic UV light might be as long as days or weeks if not longer. This correlates neatly with data acquired during charge decay measurements using Kelvin probe force spectroscopy of non-exposed and UV-exposed amorphous silicon dioxide surfaces. For non-exposed amorphous SiO_2 samples surface electrical potential changes from 0.574 V to 0.579 V over the course of 40 minutes. For amorphous SiO_2 samples exposed to UV light for 60 minutes the potential changes from -0.557 V to -0.533 V within the same time frame. In both cases the process starts off being slightly non-linear and then assumes linear behavior roughly at the 20-minute mark. Unfortunately, this trend also appears for amorphous silicon dioxide samples that were exposed for 30 minutes with the potential decay starting and ending at similar negative voltage values.