Progress report for project “Engineered surface platform for immobilization of microorganisms”. December – February 2021 (Months 28 – 30).

Due to the harsh COVID-19-related restrictions which lasted from late December to early February access to the research facilities of RTU and LU was strictly limited. With only one person being allowed to work in a two-meter radius, cell immobilization procedures took longer then usual to perform. Since the facilities were shared with other research groups and since multiple crucial elements of research infrastructure are located in a rather cramped environment, access to this infrastructure had to be rationed as to not break any rules imposed by the restrictions. Therefore, it was decided to perform certain microbiological procedures starting March and in the meantime focus on estimating the amount of cells attached to the surfaces of immobilization platforms. Also, it was possible to make some improvements to the previously developed surface charge decay measurement approach and test out the newly developed flow cell.

Cell immobilization was performed on platforms from groups V-36, V-37, V-38, V-39, V-40, V-41, V-42, V-43, V-44, V-45 using the previously described rotation-based approach. Serial imaging and attached cell area estimation was performed for all of these platforms. This was only possible because of previously developed software that allowed the project team members to supervise the serial imaging process from home (one person would still be required to be in the lab to change the samples on the microscope stage) and perform image processing also without having to do that on the premises of either university. However, it was possible to perform cell metabolism studies only on representative platforms from groups V-36, V-37 and V-45 due to COVID-19-related constraints. The data so far suggests that cells prefer to attach in between structures and not in their tops, cells prefer to attach to smaller hexagonal structures with straight walls, with a side length of about 5-10 micrometers, with a height larger than that of the cell’s diameter, and with a gap in between the structures the size of about 2-4 cells. In terms of a response to surface charge, there is no clear trend at the moment, with some platforms exhibiting an increase in attached cell amount at 30 minutes of UV exposure, while in other cases its lower for the same exposure time, with other cases still having the most exposed cells after 60 minutes of exposure to UV light. Thus, for now, the results are inconclusive. As was mentioned previously, the shape of the water drops left on the surface during the removal of the cell deposition solution and during the washing procedure has a non-negligible impact on the attachment patterns of *S. cerevisiae-77* yeast cells.

To see how cells would attach to the surfaces of immobilization platforms while in liquid, the flow cell which was developed in the previous months and described in the previous report was used for real time cell deposition experiments. At this point, though, it was used to see how cells would attach to the surface of glass samples as to not waste the few remaining satellite samples. In this experiment, a distilled water / yeast cell solution with an OD@600nm of 0.12 was sent through the flow cell at a rate of 4 ml/h over the surface of glass samples that were non-exposed, exposed to UV light for 30 minutes and exposed to UV light for 60 minutes. Cell attachment was monitored for about 25 minutes via time-lapse photography at various spots on the sample surface. The cells for all acquired fields of view were counted and resulted in the cell attachment graph seen in Figure 1. The results show that the rate of cell attachment is dependent on glass exposure time with attachment trends for non-exposed samples differ from those of UV exposed samples.

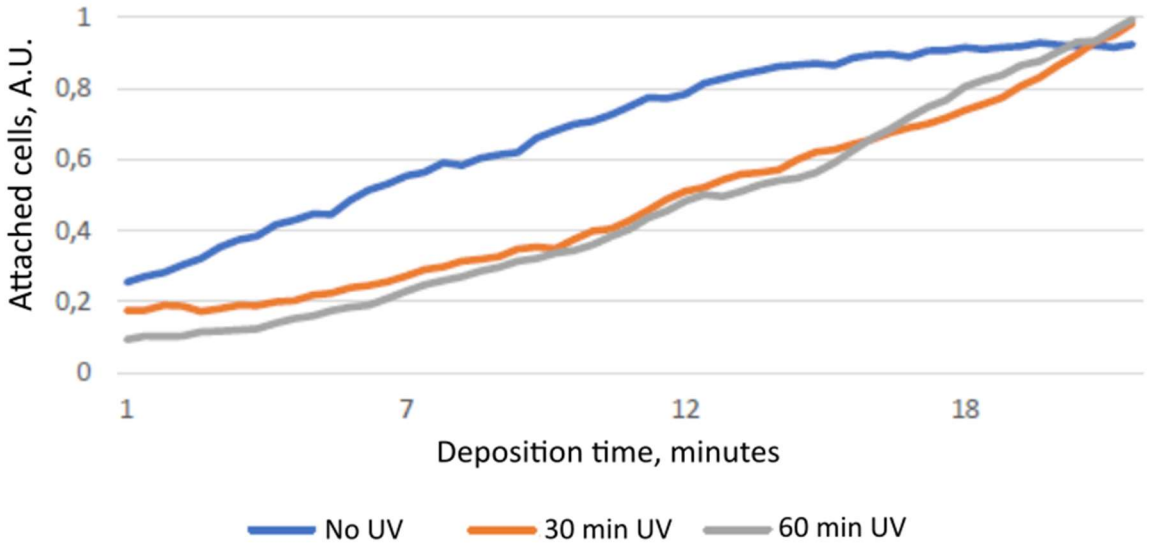

Figure 1. Cell attachment over 25 minutes for non-exposed and UV-exposed glass samples.

Concurrently with real time cell deposition observations the method for studying surface charge decay using Kelvin probe force spectroscopy, both with and without simultaneous light stimulation, was being further developed. During its development, a charge decay curve has been recorded for glass that was exposed to UV light for 30 and 60 minutes. These results were compared with cell attachment rates for both types of UV-exposed glass samples which resulted in the graph, seen in Figure 2. The graph shows a possible relationship between charge decay and cell attachment rate, which suggests a possible avenue for future research.

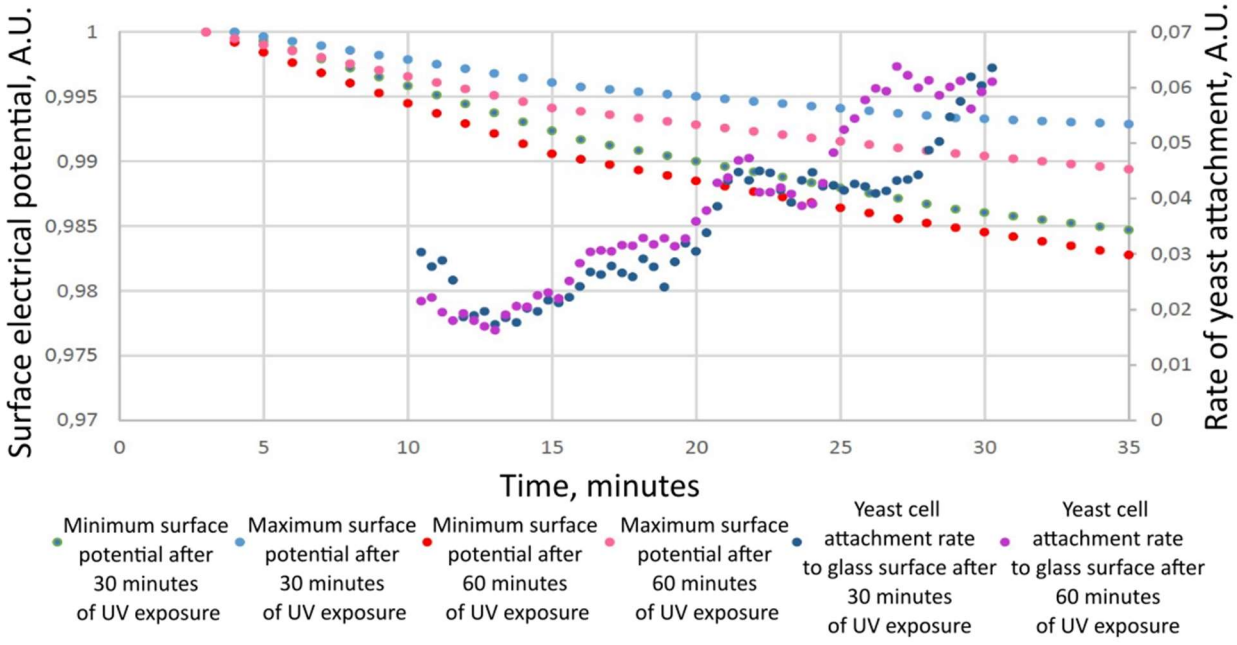

Figure 2. Cell attachment rate versus surface electrical potential decay over time.

The light-stimulation part of the light stimulated Kelvin probe force spectroscopy (LiSt-KPFS) was also updated and now uses an upgraded laser carriage outfitted with six (405, 450, 505, 532, 650 and 685

nm) 5mW lasers, liquid crystal light latch serving as an electronically adjustable ND filter, and a sensitive photodiode serving as an intensity monitoring and feedback element. The carriage focuses the light into a quartz optic fiber the emitting end of which is located over the sample surface and positioned via a custom-made guide with two degrees of translational and one degree of rotational freedom. This new device will allow the project team to study light-induced charging phenomena in dielectric materials with electrons trapped in levels with energies that range from 1.8 eV to 3 eV.

As for the future uses for the developed flow cell, it is planned to perform real time cell attachment studies on several second-batch immobilization platforms in the coming months as long as the currently eased COVID-19 restrictions aren’t made harsher once again.