Progress report for project “Engineered surface platform for immobilization of microorganisms”. September – November 2020 (Months 25 – 27).

Although the project team members are allowed to perform their duties almost without hindrance, due to the COVID-19 pandemic prevention measures none of the volunteer students were allowed to participate in lab work as assistants. Nevertheless, during this three-month period it was possible to perform local electrical potential measurements and local nanoscale roughness measurements of UV-exposed and non-exposed glass samples, satellite samples and immobilization platforms from the second batch. Also, cell immobilization was performed on several types of second-batch immobilization platforms Furthermore, some work has been done in updating the previously developed cell area estimation app, and further microbiological studies were performed.

Local potential measurements of UV-exposed and non-exposed satellite samples, glass samples and immobilization platforms were performed using Kelvin probe force microscopy. For second-batch immobilization platforms local surface potential was measured both at the tops and at the bottoms of microscale surface features wherever such measurements were possible. This means, that after some initial measurements it was determined that the currently used atomic force microscope can’t accurately measure the surface potential if a microscale surface feature has a sharp peak. Same goes for nanoscale roughness. In any case, surface potential for both tops and bottoms was similar and uniform, measuring at about 0.5 V for non-exposed immobilization platforms, about -0.5 V for 30-minute UV exposed immobilization platforms and -0.6 V for 60-minute UV exposed immobilization platforms. The same trend repeated for second-batch satellite samples. For glass samples the potential change was different, with non-exposed samples having a potential value of about 0.1 V, samples exposed to UV light for 30 minutes having a potential of about 0.2 V and for samples exposed for 60 minutes having a potential value of about 0.3 V. This suggests different modes of surface charging for amorphous SiO2 and glass.

Local surface roughness measurements of UV-exposed and non-exposed satellite samples, glass samples and immobilization platforms were performed using atomic force microscopy in tapping mode parallel to Kelvin probe force microscopy measurements. For second-batch satellite samples nanoscale roughness (Ra) was measured to be around 5 nm, for all surfaces of immobilization platforms it was measured to be around 11 nanometers and for glass samples nanoscale roughness was measured as 15 nanometers. The low roughness value for satellite samples is the lowest since it was thermally grown on the surface of a silicon wafer the roughness of which is generally 2-5 nm. A higher value for immobilization platforms is related to etching which may increase the Ra value. Generally, the samples can be considered optically smooth. Exposure to UV light within 60 minutes of exposure in no way affects nanoscale surface roughness of any of the studied samples.

Cell deposition experiments for UV-exposed and non-exposed immobilization platforms from second-batch groups V-32, V-33, V-34, and V-35 of the second batch. Cells were immobilized for both microbiological studies and for attached cell amount estimation. Imaging was performed using the Zeiss Jena NU-2 microscope equipped with a digital camera and a motorized stage controlled by the previously mentioned custom-made software. For each platform the whole surface was imaged using serial microscopy at 200x magnification. Using these images, the previously mentioned cell area estimation software was tested, and its settings adjusted for best possible cell contour recognition. Representative images for acquired images before and after processing are given in Figures 1 and 2.

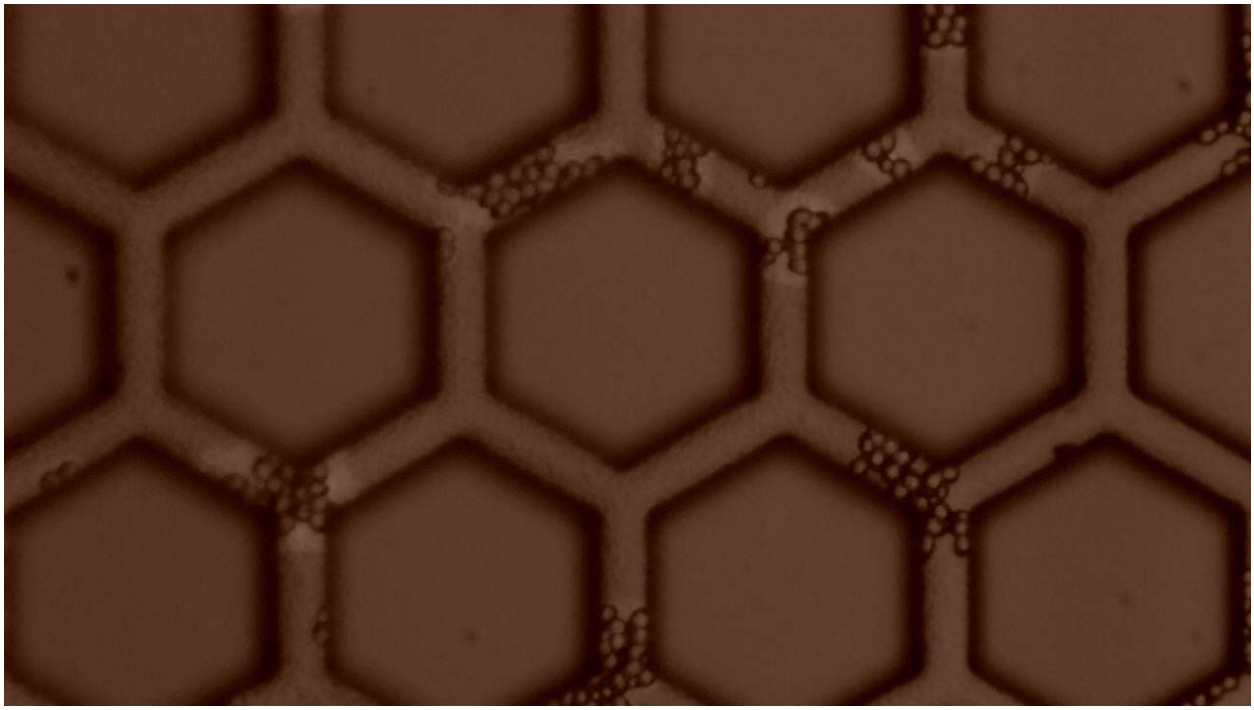

Figure 1. Image of cells immobilized on the surface of an immobilization platform from group V-32 before being processed via cell area estimation app.

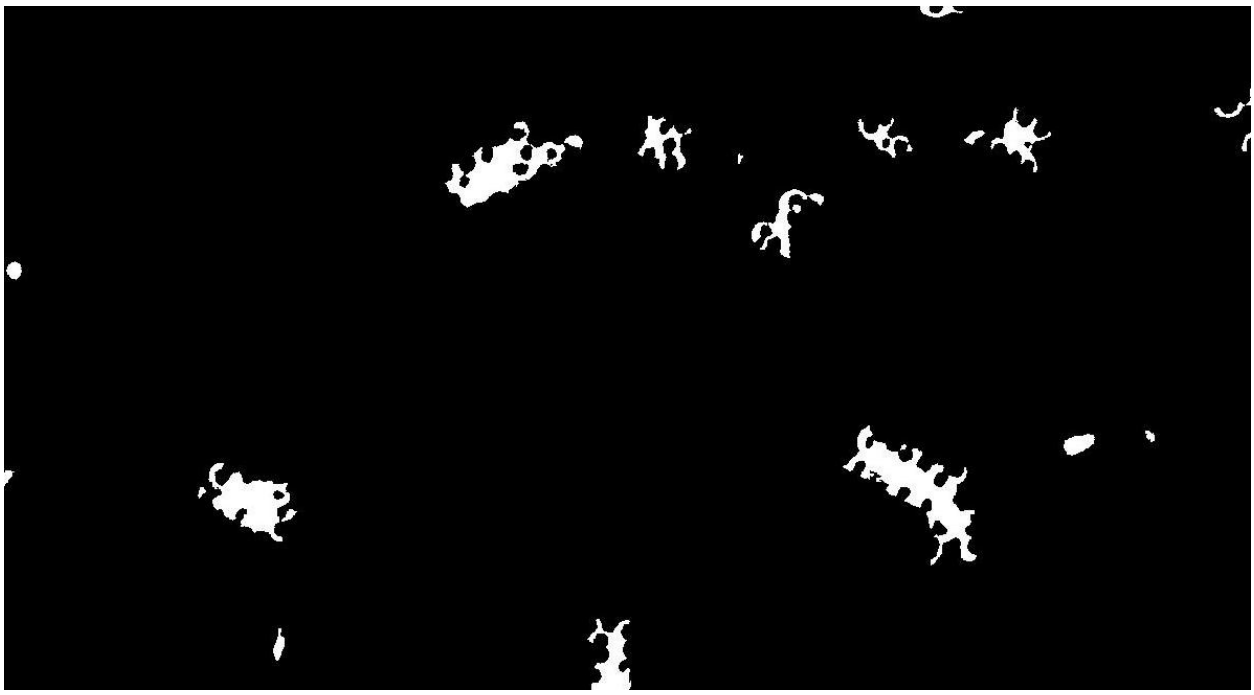

Figure 2. Image of cells immobilized on the surface of an immobilization platform from group V-32 after being processed via cell area estimation app.

During cell immobilization rounds different patterns of water drop formation were noticed on different types of immobilization platforms when performing cell deposition from a rotating solution with subsequent platform extraction from the solution, washing and drying steps. In some cases, this led to uneven cell deposition along the platform surface and the formation of “coffee rings”. This behavior might affect the amount of immobilized cells and obscure the effect of surface charge on cell attachment. Therefore, it was decided to develop a flow cell where cell deposition can be monitored

in real time while the solution is in contact with the platform and cell attachment can be ascertained without the effects of liquid surface tension. Figure 3 shows a parametric 3D model of the developed device.

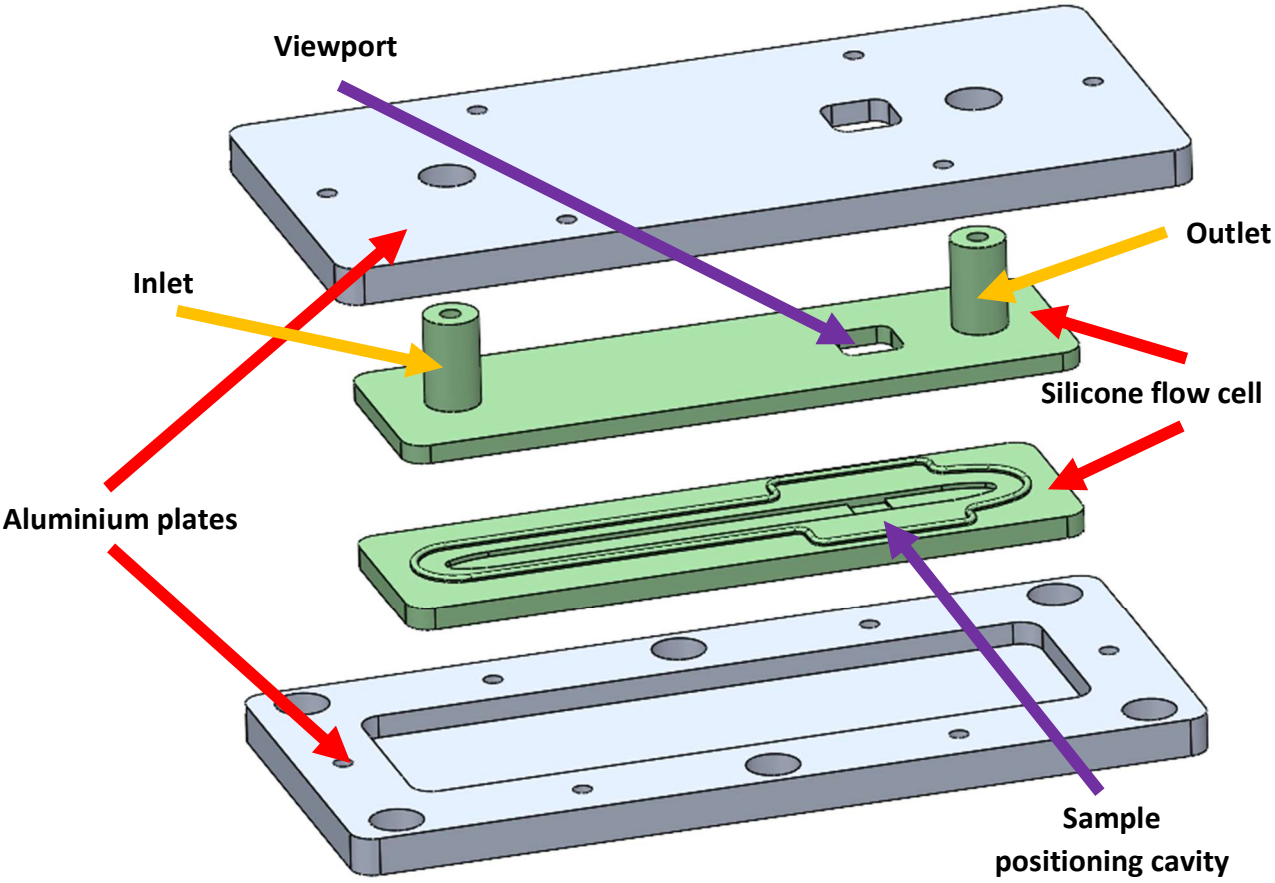

Figure 3. Parametric 3D model of the flow cell used for real time cell immobilization studies.

The flow cell consists of four parts – two silicone-cast parts through which liquid is flown and two aluminium plates which apply pressure to the silicone-cast parts to keep them sealed. The upper silicone-cast part has an inlet and an outlet for the liquid solution, a viewport with a cavity for placing a cover slip, and a hermetization groove (not visible in Figure 3). The lower silicone-cast part has a trench for liquid to flow through, a sample positioning cavity, and a hermetization seal. The two aluminium plates have screw holes for assembly and fixation onto the perforated surface of a microscope stage as well as holes to accommodate the protruding inlet/outlet and the viewport. After placing a sample into the sample positioning cavity, the flow cell is assembled, and liquid can be poured through. In this case the use of a syringe pump is planned. The device has already been fabricated and in the coming months will undergo testing and experimental use.

Microbiological tests to determine the metabolic activity of the immobilized yeast cells onto the previously mentioned platform groups have also been performed. At this point of the project, however, the team would like to refrain from mentioning the results of these tests.