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 01.09.2018. – 01.12.2018. time frame

Immobilization onto a surface is very important for cell proliferation and development. One of the parameters that affects cell immobilization is surface roughness, although it is not yet fully understood what governs this interaction. A possible cause might be presence of surface charge on roughness peaks. Biological cell interactions with surfaces are facilitated primarily by electrostatic and van der Waals forces, so the presence of surface charge on peaks could affect cell immobilization. However, a definitive link between surface roughness, surface charge and cellular immobilization is yet to be established. The aim of this project is to determine existence of this link by creating a set of samples with strictly defined surface features of a controlled size and shape mimicking elements of surface roughness (immobilization platforms), then determine the charge distribution on the surface and then immobilize S. cerevisiae yeast cells to see which regions were attachment-favorable. Additionally, surface charge induction will be promoted by UV irradiation to further enhance the effects of surface charge on cell immobilization. Cell physiology after immobilization on the surfaces will be studied. The result of the project will be knowledge gained about the nature of surface roughness' effect on cell immobilization and its relation to surface charge.

The report includes progress information on the following tasks:

- Development of immobilization platform models with strictly defined surface features using CAD software.

Achieved results

The immobilization platforms for cell seeding studies that will be used during this project are surfaces covered with an array of identical microstructures. The shape and size of the microstructures as well as the spacing in between of them are the same for each type of immobilization platform. In essence, these structures simulate certain aspects of naturally occurring microscale surface roughness repeated over and over again on a single otherwise smooth surface. By isolating separate types of micro-scale roughness features it becomes possible to study cell attachment phenomena in a more precise way than it would be possible when using the chaotic roughness patterns that occur naturally. Knowing the shape of a microscale surface feature also allows to decouple the contribution of surface charge from the geometry-related phenomena and studying the effect of a substrates surface charge on the attachment of cells to a surface is one of the main goals of this project.

Since the size of the structures is in the microscale the best way to produce such structures would be by using microelectronics manufacturing techniques. Since silicon dioxide – a material frequently used in the production of microelectronic devices – is an electrical insulator, thus accumulates surface charge, it can be considered as a suitable material to produce immobilization platforms. Initially for this project, it was expected to use idealized microstructures shaped like cones and four-sided pyramids. However, the manufacturing of such shapes utilizing standard planar techniques used for manufacturing microscale semiconductor devices requires substantial modifications to be applied to the standard manufacturing process. Therefore, it was necessary - for the time being - to compromise on the shape and use parallelepipeds or truncated pyramids instead of cones and four-sided pyramids.

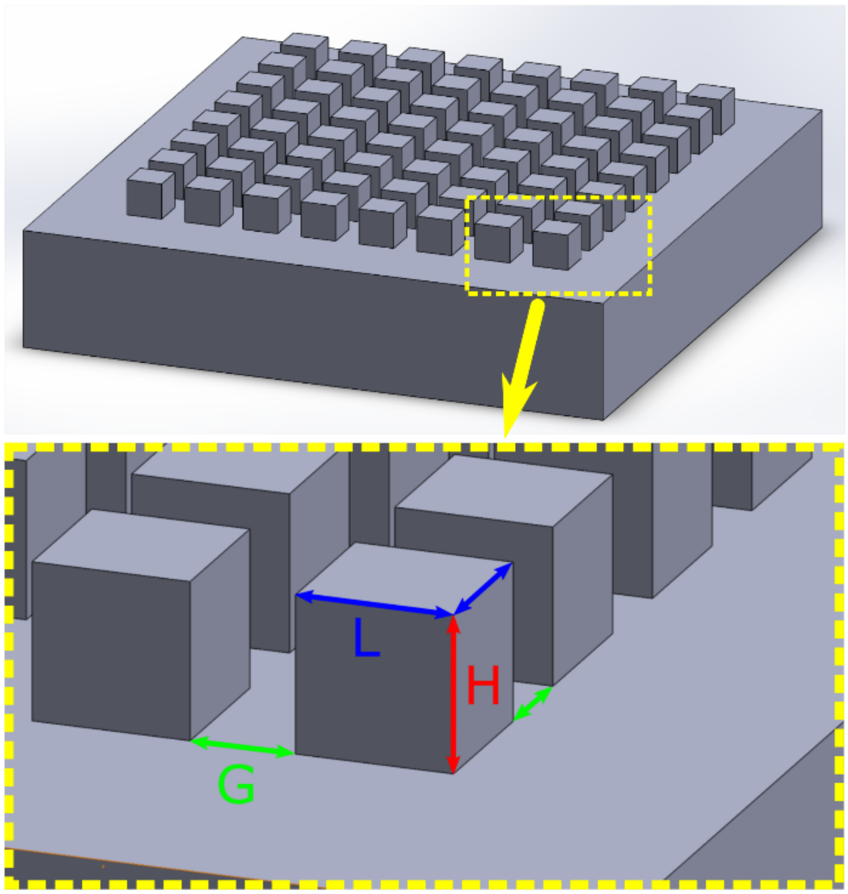

Figure 1. *A 3D representation of the immobilization platform model developed using SOLIDWORKS 2018. The arrows and letters on the magnified image depict the distance between the microstructures (G, green), the height (H, red) and the side length (L, blue).*

Based on the recommendations provided by the manufacturers a set of 7 immobilization platforms with micropatterned surfaces has been developed. The parallelepiped was selected as the microstructure shape of choice. Two side length values (12 and 48 mkm; both larger than the diameter of an average *S. cerevisiae* yeast cell), two height values (3 and 12 mkm; the first one is similar and the second one is larger than the diameter of an average *S. cerevisiae* yeast cell) and two values for the distances between microstructures (2 and 12 mkm; the first one is similar and the second one is larger than the diameter of an average *S. cerevisiae* yeast cell) Geometrical 3D models and technical drawings of the structures have been produced using SOLIDWORKS 2018. The height and lateral dimensions of the microstructures as well as the distances between them are given in Table 1. An image depicting the micropattern is given in Figure 1.

After preparing all the required technical documentation a tender was announced for the production of 7 types of immobilization platforms with 150 platforms of each type as well as a set of satellite samples made from the same material as the platforms but lacking any microscale features to be manufactured and delivered to the project team. The contract to produce the immobilization platforms was won by ALFA RPAR, a Latvian semiconductor device manufacturer.

Table 1. Immobilization platform types and the model dimensions of their structures

Designation of immobilization platform type	Microstructure height (H), mkm	Microstructure side length (L), mkm	Distance between microstructures (G), mkm
39-1-1	3	12	2
39-1-2	3	3	12
39-1-3	3	12	12
39-1-6	12	12	12
39-1-7	12	48	2
39-1-9	12	48	12
39-1-10	12	12	2

Conclusion

The immobilization platforms were developed and modeled, their technical parameters have been defined and technical drawings have been developed. The tender for the manufacturing of a set of 7 types of immobilization platforms has been initiated and concluded with ALFA PRAR acquiring the manufacturing contract.