Micro-/nano-scale platforms for the controlled *ex-vivo* mechano-stimulation of cells

Mark Schvartzman

[1]Department of Materials Engineering, [2]Ilse Katz Institute for the Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer-Sheva, Israel

It is becoming progressively clear that living cells sense physical cues of their environment, such as the spatial arrangement of signaling molecules, mechanical stiffness, and topography. Yet, understating the role of each cue on the cell function is challenged by the fact that *in-vivo* these cues are intermixed, and their effects on cells are thus often indistinguishable from each other. This challenge can be overcome by *ex-vivo* platforms for cell stimulation, which are designed to controllable mimic individual physical cues identical to those existing *in-vivo*. Recent advances in nanofabrication enable to structure and place these cues with a precision that reaches the molecular regime. In my talk, I will review three types of recently developed platforms for the study and regulation of cells, as described herein:

The first platform comes to explore the role of the ligand arrangement in the immune function of Natural Killer (NK) cells, which are the sentinels of our innate immune systems. Its first generation was based on arrays of nanoimprinted metallic nanodots functionalized with activating ligands, and it was used to discover the minimal ligand arrangement for the activation of NK cells[[1]. The next, more advanced platform was used to study how the segregation between activating and inhibitory ligands affects the inhibitory signaling in NK cells. It was based on binary arrays of metallic nanodots orthogonally functionalized with activating and inhibitory ligands, whose spacing was controllably varied at the nanometric scale.

The second type of platform was designed to study the role of environmental elasticity and topography in the function of cytotoxic lymphocytes. Here, we engineered a stimulating platform based on ligand-functionalized nanowires. The nanowires deliver chemical, nano-topographical, and mechanical cues, whose combination produces an enhanced immune response of NK cells [3]. While patterned in microdomains, these nanowires spatially guide the cytotoxic activity of NK cells [4]. To separately reveal the effect of each cue, we recently stimulated NK cells and CD8+ T cells on nanowires with varied length and bending moduli and found that these physical parameters of nanowires greatly affect the signaling and the immune function of the lymphocytes [5],[6]. Recently, we fabricated a functionally identical mechanostimulating platform using a scalable approach based on PDSM casting. We demonstrated its applicability for the highly effective activation and expansion of T cells for the immunotherapeutic application.

Finally, the third type of platform was designed to reveal whether the cell motility can be guided by the directed heterogeneity of environmental stiffness. To that end, we fabricated the first of its type stiffness micropattern by lithographic patterning of Silicon Dioxide based rigid lines, followed by their embedment within a soft matrix made of polydimethylsiloxane (PDMS) by pattern transfer, and unform functionalization of the resulted in stiffness pattern with cell adhesion molecules. The unique feature of such a pattern is that it has no topographic or chemical cues that can affect cell motility. We found that cancer cells positioned on such patterns exhibited pronounced elongation along the lines by forming protrusions on the stiff lines, which was, however, dependent on the pattern geometry. In this way, we isolated, for the first time to the best of our knowledge, purely mechanical contact guidance of cells, which is important for critical *in-vivo* processes involving cell motility, such as wound healing and cancer metastasis.

[1] Y. Keydar, ... M. Schvartzman *Nanoscale* 2018

[2] E. Toledo, G. Le Saux, ... M. Schvartzman *Science Adv*. 2021.

[3] G. Le Saux, N. Bar-Hanin,..., M. Schvartzman *Adv. Mater.* 2019

[4] V. Bhingardive..., M, Schvartzman *Small* 2021

[5] V. Bhingardive..., M, Schvartzman *Adv. Funct. Mater* 2021

[6] V. Bhingardive, A. Kossover.., M, Schvartzman *Nano Lett.* 2021