PhD thesis, title:

"Controlling the cytoskeletal structure and dynamics using chemically micropatterned substrates".

PhD THESIS ABSTRACT

Goal: To reveal cancer cell motility mechanisms by using micropatterning

Cell motility is one of the key areas of research in cancer cell biology. This is so because directional and highly persistent motility is one of the hallmarks of metastasis leading to the seeding of secondary cancers and, ultimately, to over 90% of cancer-related deaths.

Micropatterning techniques have recently emerged as a useful family of tools allowing scientists to obtain quantitative data on cancer cell behaviors (and motility in particular) in geometrically well defined environments. In my work, I combined cell biological techniques with various micropatterning techniques with which I controlled cell shape both in two and three dimensions, deconstructed and manipulated cytoskeletal structures inside of living cells, and controlled their motility patterns. These and other background techniques will be reviewed in the first chapter of the proposed Thesis (this material is concurrently being prepared for an invited review to *Advanced Material Interfaces* journal). Subsequent chapters of my Thesis will deal with system specific applications of the microfabrication/micropatterning toolkit to study cancer cell morphology and motility.

The second chapter will narrate the surface chemistry functionalization schemes allowign for the patterning of cell-confining micropatterns. This chapter will be based on a *Langmuir* paper I co-authored and entitled **"Carboxybetaine Methacrylate Polymers Offer Robust, Long-Term Protection against Cell Adhesion".** In this work, my colleagues and I compared and contrasted two types of molecules forming self-assembled monolayers resisting cell adsorption: a novel poly(carboxybetaine methacrylate) vs. more traditional hexa(ethylene glycol). alkanethiolates. Experiments revealed that p(CBMA) films can be stored in dry or wet states without a marked loss of their bio-protective properties. These characteristics suggest that p(CBMA) substrates can become more practically applicable in cell studies than EG6 SAMs.

Capitalizing on the ability to limit cell adhesion onto micropatterned islands alone, the next step of my work was to investigate how the imposed cell shape can control the internal organization of the cytoskeleton and cytoskeletal dynamics in micropatterned, living cells. These results were recently described in the *J. Cell. Sci.* paper entitled **in "Microtubule guidance tested through controlled cell geometry"** and will be described in the third chapter of my Thesis. Specifically, using cells constrained to micropatterned triangles, I was able to selectively position focal-adhesion structures at the vertices of the patterned cells. This selective localization then allowed me to study the trajectories of microtubules emanating from the centrosome and targeting the adhesions. This study answered an outstanding question of cell biology: namely, whether microtubule-adhesion targeting is a random or a guided process. Rigorous analysis of the microtubule trajectories I recorded substantiated the second scenario whereby the microtubules are guided along the underlying actin structures. In this process, Myosin II was found to be a key factor underlying the microtubule/actin interactions; its knockdown resulted in unguided/random microtubule growth.

With the understanding of how the cytoskeletal structures work in live but constrained cells, I then proceeded to study cytoskeletal dynamics in motile and unconstrained cells. The fourth

chapter of my Thesis will thus focus on the work recently published in *Integrative Biology* **"Motility efficiency and spatiotemporal synchronization in non-metastatic vs. metastatic breast cancer cells".** This work provides the first convincing proof that invasive cancerous cells move more efficiently – in terms of chemical energy used – than their non-invasive variants. In doing so, the metastatic cells synchronize the protrusions at the cell front and retractions at cell rear. In this way, their dynamics is converted into net motion in an energetically most optimal fashion. This "energy saving" and synchronization are previously unknown hallmarks of metastatic behavior.

Finally in the fifth chapter of my Thesis I will describe results that have just been submitted for publication in *Nature*. (**"Predatory, Lévy walks of metastatic cancer cells and their reprogramming into benign, diffusive migrations"**). Here, I worked as a part of a large and international team that showed that as cells metastasize, they develop a fundamentally different motility strategy called Levy walking. Levy walks are often employed by animal predators and are known to represent an optimal strategy of searching space for scare resources. In the context of our cancer studies, this means that as cancer cells become invasive, they search our bodies in a deadly but optimal fashion. While this is a concerning finding, our team has also shown how these Levy walks can be reverted to normal, diffusive walks characterizing non-invasive cells.

To summarize, my thesis will narrate my four-year-long journey that took me from the basics of modern surface engineering and microfabrication to the forefront of cancer cell biology research. I hope this research will be a worthy contribution to our ongoing struggle to eradicate cancer and, before this lofty goal is achieved, will provide a basis for a doctoral thesis meeting the high academic standards of your Department.

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