STUDY CELLULAR RESPONSES AT THE MICROSCALE BY CREATING HETEROGENEITY IN CULTURED CELLS USING A MICROFLUIDIC PROBE

Nadia E. Casimiro[1,2], Gema V. Gonzalez[1,3], Aditya Kashyap[1,3], Lena V. von Voithenberg[1], Govind V. Kaigala[1]
[1]IBM Research – Zurich, CH; [2]University of Basel, CH; [3]ETH Zurich, CH

Key Words: *cell lysis, gene expression, microfluidics, heterogeneity in cancer, drug resistance*

We introduce a new approach to study cellular responses in different cell subpopulations while not disrupting the microenvironments. We believe this might become a useful tool to investigate resistance-related cellular responses in cancer cells.

Drug resistance of cancers is currently one of the defining issues in providing effective treatment to patients. The heterogeneity in cell responses to a therapeutic agent is challenging to deconvolve not only owing to neo-antigens that arise in a tumor, but also due to complexity of interconnected molecular events that occur in a cell, making it difficult to decipher by which mechanism(s) they survived the treatment. There is therefore a need to develop new *in-vitro* models taking into account cancer heterogeneity and sub-population cross talk. Spatial localization of stimuli/insult and study of spatial cues provide one such avenue that may allow to study the effect of them on cancer cell microenvironments and provide meaningful information to help understand cellular behavior.In the last years, the microfluidics community has introduced different *in-vitro* models with organs-on-a-chip research and new analytical methods for such applications. However, the transfer of cancer *in vitro* models into microfluidics has faced some limitations to reproduce the complex microenvironment and subpopulations of cells found in a cancer niche. Interestingly, a new class of microfluidic devices called 'open space microfluidics' - in contrast to traditional microfluidic devices - enables probing cells without disrupting the biological surface, thereby preserving the natural microenvironment of the cells/tissue.The microfluidic probe (MFP) is one implementation of open space microfluidics that consists of a microfabricated probe head that locally confines and shapes nanoliter volumes of multiple reagents on a biological surface. Liquid localization is achieved by simultaneous injection and aspiration of the reagent from proximate apertures while the apex of the MFP maintains a fixed distance from an immersed surface (hydrodynamic flow confinement - HFC). These confined reagents can be stimulants, insults or biochemicals in molecular biology assays.We are developing assays to create heterogeneity on cultured surfaces by means of local treatment of cells. We are combining spatial lysis sequentially within the same assay to study gene expression signatures of the treated and untreated populations on the same biological substrate. While studying the uptake of small molecules such as Calcein-AM, we observed faster uptake (3.5×) of Calcein in MCF-7 cells when compared to bulk assays, which we will use to our advantage for shortening treatment times to create heterogeneity. In addition, we have formulated different lysis solutions locally confined with the MFP for RNA isolation. Using a Triton-X detergent and proteinase K lysing solution, we are able to obtain RNA containing lysates in less than 1 min at room temperature. We are translating the Calcein-AM assay for EGF loading to obtain gene expression signatures of proliferation indices such as *c-fos*

Spatial localization on biological substrates is an attractive approach to apply in cancer research. The advantages to create heterogeneity and supply more the one chemical at a time in shorter times make it suitable to think that we can perform synergistic and antagonistic treatments while studying their toxic effects on cancer cells.

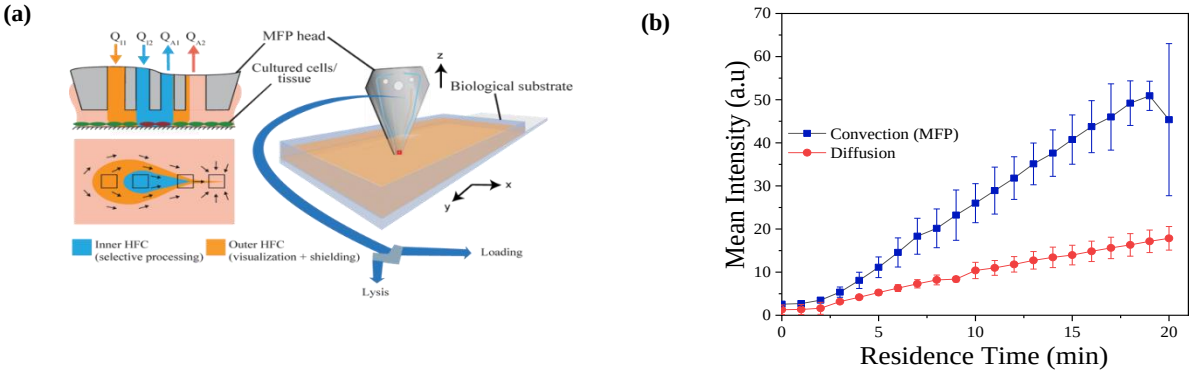

Figure 1 (a) Working principle of the MFP (b) Comparison of Calcein-AM loading with MFP versus bulk assays in MCF-7 cells (c) Spatial lyse for local RNA isolation from of cells tracked with CMFDA Dyes.