Micropatterned substrates for dissecting heterogeneity in reprogramming human somatic cells

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Reprogramming of patient-derived somatic cells to induced pluripotent stem cells (iPSCs) has great promise for disease modeling, drug discovery, and personalized cell therapy. However, this process can take weeks, be stochastic, be inefficient and be partial, resulting in highly heterogeneous populations of cells. Much of the work to identify, evaluate, and enrich for high quality iPSCs has relied on methods which require cells be fixed (Immunocytochemistry), destroyed during the preparation of cell extracts (PluriTest), differentiated (Embryoid Body Formation, Teratoma Assays), or single cell studies which disrupts the cell’s microenviornment. However, cell microenvironment has been shown to play an important role in stem cell biology and there has been a lack of understanding of microenvironmental-cell interactions during the middle of reprogramming, precisely when reprogramming cultures are becoming heterogeneous. To address these shortcomings, we developed a micropatterned substrate that allows for dynamic live-cell microscopy of thousands of cell subpopulations undergoing reprogramming. On this substrate, we were able to both watch and physically confine cells into discrete islands during reprogramming while preserving many of the biophysical and biochemical cues within the cells' microenvironment. We are trying to develop regression models based on the nuclear characteristics and the metabolic state of reprogramming somatic cells to accurately predict their reprogramming status. To this end, we are visually and nondestructively tracking the nuclear characteristics of reprogramming cells within the islands using fluorescence microscopy and we have seen that a combination of eight nuclear characteristics can potentially distinguish partially reprogrammed cells from those that were fully reprogrammed (Figure 1). Additionally, we are tracking the metabolic state of reprogramming cells using a nondestructive technique called Optical Metabolic Imaging (OMI). OMI detects autofluorescence intensity and lifetime of the metabolic co-enzymes NAD(P)H and FAD to probe their relative proportion and binding, thereby quantitating the redox state of the cell. Our preliminary data suggests that OMI is sensitive to the changes in the metabolism of cells during the progression of reprogramming (Figure 2) and can potentially be used as a reliable indicator to predict the reprogramming status of cells. Overall, this study will help in the identification of homogeneous populations of full reprogrammed iPSCs in a nondestructive, cost-effective, and rapid manner.