The formation of multicellular structures from proliferating single cells is associated with cell volume reduction, cell stiffness increases and delayed cell cycle progression

Vaibhav Mahajan[1], Keshav Gajendra Babu[1], Antje Garside[1], Vinita Ajit Kini[1], Timon Beck[1,2], Byung Ho Lee[4], Carsten Werner[3], Raimund Schlüßler[1], Anna Taubenberger[1,3]

1 TU Dresden, Center for Molecular and Cellular Bioengineering (CMCB), Dresden, Germany
2 Max Planck Institute for the Science of Light & Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany
3 *Leibniz Institute of Polymer Research Dresden, Max Bergmann Center, Dresden, Germany*
4 Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany

Contact: anna.taubenberger@tu-dresden.de

Tumours are mechanically altered across multiple spatial scales, from the cellular level to complex tissues and these changes are thought to contribute to cancer progression. Effects of mechanically altered microenvironments on tumour cells can be studied in a systematic manner using bioengineered 3D in-vitro models. Previous studies indicate that tumour spheroids adapt their proliferation and mechanical properties when growing under 3D confinement. Still, the temporal dynamics and molecular basis of this mechanical adaption remain poorly understood. Here we studied single cancer cells forming tumour spheroids within mechanically well-defined biohybrid hydrogels. Confocal Brillouin microscopy revealed for several cell types consistent increases in the Brillouin frequency shift when single cells gave rise to small cell clusters and later larger tumour spheroids. These changes coincided with a drastic decrease in the mean nuclear volume of up to 60%, together with overall cell volume decreases. The volume changes were not explained by growth-induced compressive stresses but primarily by cell cycle changes that became evident in cells expressing the FUCCI cell cycle reporter. Specifically, smaller cells that were in the G1 cell cycle phase accumulated in the growing spheroids over time. In addition to these cell cycle delays, disaggregating multicellular structures rescued some of the seen cell volume decreases, suggesting that also water efflux from cells partially contributed to the cell volume reductions seen in the multicellular structures. This was further corroborated by optical diffraction tomography measurements showing higher optical density of cells within clusters compared to single cells. In turn, invasive cells lines, undergoing the reverse process by breaking away as single cells from denser cell clusters showed cell volume increases. Taken together, our study provides insights into how tumour cells adapt their cellular and nuclear volumes and mechanical properties when forming multicellular structures in 3D, which is relevant to tumour formation and progression.