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Investigation of Mechanical Regulation on STAT3 Activity and MMP Production

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Transcription factor, STAT3, is inappropriately expressed in cancer cells, and has contrasting activation in 2D versus 3D microenvironments. 2D plates are often used for drug screening and do not always recapitulate *in vivo* responses. To combat inaccurate 2D drug studies, a 3D hydrogel was created to support the growth of cancer cells into a tumor-like environment. The hydrogel consists of a biocompatible dextran homopolysaccharide, cell adhesion RGD sequences, and crosslinker MMP labile peptides. A pH dependent reaction couples the RGD sequences to dextran then the polymers are crosslinked into a gel. Crosslinking is accomplished using terminal cysteine peptide sequences, allowing for gel stiffness tunability. Some of the breast cancer cell lines used do not readily form spheroids. Therefore, the cells were embedded in the hydrogel, then incubated to allow for spheroid formation from single cells. OVCAR-8 cancer cells acted as a control cell line that forms spheroids without 3D extracellular matrix (ECM) support. The dextran hydrogels provided mechanical regulation and promoted tumor spheroids formation. Antibody staining of KI67, a proliferation protein, showed cellular growth and not only aggregation of cells. Compounds that inhibit STAT3 were then tested to compare 3D to 2D viability responses. A difference was seen in each of the compounds tested suggesting the 3D environment has an effect on the cell signaling not seen in 2D. An array of treatments was done to test mechanical stimulus effect on the cells at various time points. The effect of 3D growth on STAT3 activation is also being studied. The results show that 3D microenvironments can change the survival and proliferative signals in cells to promote spheroid formation which modifies their response to treatment. Thus, it is important to screen drugs in a 3D environment whenever possible.