Hematopoietic stem cells (HSCs) produce and maintain the body’s entire complement of blood and immune cells. HSCs are a vital tool for development of artificial stem cell models as they have well-defined isolation and functionality assays. To elucidate the role of biophysical and cellular cues in a synthetic niche, mesenchymal stromal cells (MSCs) and HSCs were co-cultured in a library of gelatin-based hydrogels with tunable poroelasticity (Fig A). After 7 days of culture, HSCs in the presence of MSC-secreted factors had improved maintenance and quiescence. The secretome was analyzed via microarray, and a Projected Latent Structure (PLS) iterative filter method was used to relate cytokine concentration to HSC response (Fig B). The output of the model was validated in single and co-cultures of HSCs and MSCs, and TGFβ-1 was shown to increase HSC maintenance (Fig C) and quiescence compared to a non-stimulated control. To examine the single-cell response to cell-cell soluble interactions, a microfluidic device (Garcia Lab) was used to produce single and multi-cellular gelatin-based microdroplets (D≈145µm, Fig D). This technology enables high-throughput generation of microenvironments for rapid screening of culture conditions that lead to HSC maintenance. Stemness was measured as a function of number of nearest neighbors to examine HSC response to autocrine vs. paracrine signaling. These efforts will be integrated with the statistical approach outlined above (TGFβ-1) to build-up a feeder-free hematopoietic stem cell niche.

Figure. A. Overview of initial culture system B. Output of PLS, showing cytokines positively (green) and negatively (red) correlated to HSC response C. Validation of PLS model, with TGFβ-1 stimulating a higher HSC response compared to the control (p<0.05) D. Overview of microfluidic device and encapsulation of stem cells within gelatin microdroplets (Live/Dead)