Statement of Purpose: Metastasis is the leading cause of breast cancer related mortality.\(^1,2\) Targeting metastatic disease is challenging due to the presence of disseminated tumor cells (DTCs) that can lie dormant in secondary organs for long time periods before reverting to an actively proliferative state (metastatic relapse).\(^3\) Pre-emptively targeting dormant DTCs is difficult owing to their quiescent behavior, poorly understood biological characteristics, and limits of clinical detection.\(^4,5\) DTC dormancy can be classified as cellular dormancy (G0/G1 arrest) or dormant micrometastasis (balanced proliferation and death). We demonstrate that highly metastatic breast cancer cells (MDA-MB-231s; 231s) can be induced to adopt a cellular dormancy, dormant micrometastasis, or invasive phenotype by modulating the crosslink density, degradability, stiffness, and adhesivity of the hydrogel they are cultured in. We also present the ability to reactivate dormant cells via dynamically changing matrix adhesivity. This hydrogel platform can be used to induce desired dormant and invasive phenotypes in metastatic breast cancer and may provide useful pre-clinical information for future drug development applications.

Methods: Poly(ethylene glycol) diacrylate (PEG)-based hydrogels containing the proteolytically-degradable peptide GGGPQGIWGQGK (PEG-PQ, 5% w/v), integrin-ligating peptide RGDS (0, 1, 5, 10 mM) and co-monomer n-vinyl pyrrolidone (NVP) (0.0, 4.7, 9.4, 18.7 mM) were used for encapsulation and 3D culture of 231s over 15 days. Hydrogel biochemical (ligand density, degradability) and physical (pore size, elastic modulus) properties were characterized for each formulation. Cell viability, proliferation, early apoptosis, metabolic activity and single cell and cell cluster morphology were quantified as a function of time and matrix properties.

Results: We hypothesized that biochemical and physical properties of the hydrogel could be tuned to control the fate of metastatic breast cancer cells with respect to dormancy or growth. Our results indicate that hydrogel crosslink density and adhesivity can be tuned to induce four different phenotypes (invasive growth, single cell dormancy, balanced single cell dormancy, and balanced micrometastasis) quantified by changes in cell viability, viable cell density, proliferation, early apoptosis, metabolic activity, and 3D cell/cluster morphology. Invasive growth was observed when the viable cell density at day 15 was statistically significantly greater than day 0 and the cell population contained clusters with filopodial projections (Figs. 1,3). This behavior was induced by softer, permissive hydrogels that supported adhesion (1-10 mM RGDS) and were highly degradable (0-4.7 mM NVP) (Fig. 2).

Single cell dormancy was observed when cell viability and viable cell density at day 15 were statistically significantly less than day 0 and by the dominant presence of single cells (Figs. 1,3). This behavior was induced by restrictive hydrogels with low adhesivity (0 mM RGDS) regardless of other properties (Fig. 1). These cells were able to be reactivated through a dynamic increase in matrix adhesivity after 40 days in culture (Fig. 3).

Balanced single cell dormancy was observed when cell viability and viable cell density at day 15 were not statistically different from day 0 and single cells composed greater than 80% of the cell population. This phenotype was induced by hydrogels that supported moderate cell adhesion (1 mM RGDS) and moderate degradability (9.4 mM NVP) resulting in tight cell confinement.

Balanced micrometastasis was observed when the cell viability and viable cell density at day 15 were not statistically different from day 0 and small cell clusters composed greater than 20% of the cell population. This phenotype was induced by hydrogels that supported moderate to high cell adhesion (1-10 mM RGDS) and had the lowest degradability (18.7 mM NVP).

Conclusions: We developed a hydrogel platform that allows modulation of breast cancer phenotype, invasive growth or various dormant states, via tuning of matrix adhesivity and crosslink density. We also demonstrate the ability to reactivate dormant single cells via a dynamic increase in matrix adhesivity. This platform may prove useful in determining mechanisms of cancer dormancy and for future drug development applications.