Extracellular Matrix Hydrogel Composition Drives 4T1 Tumor Spheroid Invasion

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Statement of Purpose: Local invasion and metastasis are hallmarks of cancer that are driven by cancer cells intrinsically and their interactions with the tumor microenvironment. The extracellular matrix (ECM) is a critical component of this environment that provides mechanical and biochemical signals to cancer cells. Decellularized tissues provide ECM scaffolds with more physiological relevant features than current in vitro ECM models that are generally overly simplified since they contain only a few ECM components (Type 1 collagen alone) or are not specific to the relevant tissue of interest (Matrigel). The purpose of this study was to determine how ECM composition influences tumor spheroid invasion through native ECM isolated via tissue decellularization compared to purified ECM components: Type I collagen or laminin/Type IV collagen (Matrigel).
Methods: Small intestinal submucosa (SIS) was decellularized to preserve the proteomic complexity of the native matrix. The SIS was lyophilized and digested with pepsin for use as a hydrogel or cryomilled to form ECM particles. Multicellular cancer cell spheroids were fabricated with an aggregation method from established murine cancer cell lines (colon – MC38 & CT26; breast 4T1, **Fig. 1a**). The spheroids were embedded in 3D ECM hydrogels: 1) Matrigel, 2) Type 1 collagen, or 3) SIS ECM digest (**Fig. 1b-d**). SIS and Type I collagen hydrogels had similar soluble collagen concentration via the Sircol assay. The spheroids were cultured for three days and imaged daily to evaluate cellular migration. SIS ECM was used to model the intestinal microenvironment and the 4T1 breast cancer cell line was used as an invasive control. In addition to cells-only spheroids, 4T1 cells were seeded with SIS particles during the spheroid formation and then embedded in hydrogels as described above to determine whether "normal" SIS ECM within the spheroids could reprogram tumor invasiveness.
Results: Cancer cell migration away from spheroids was both cell line and ECM dependent and with the greatest differences observed with the 4T1 cells. The 4T1 cell line is documented to be highly invasive, but there was minimal 4T1 migration in Matrigel (**Fig. 1b, e**, 79.6 & 106 um on days 1 and 3, respectively). Conversely, 4T1 spheroids completely dissociated and spread out to the surrounding Type 1 collagen hydrogel (**Fig. 1c**). 4T1 spheroid dissociation and cellular migration were moderate within the SIS hydrogel (**Fig. 1d**) with the average distance from the center of a spheroid to the outer most edge increasing from 232 um on day 1 to 429 um on day 3 (**Fig. 1e**). The incorporation of SIS particles within the 4T1 spheroids reduced the degree of cellular migration away from the spheroid within the Type 1 collagen and SIS digest hydrogels. MC38 and CT26 cells have lower metastatic potential than 4T1 cells, but their invasion characteristics were also ECM-dependent. CT26

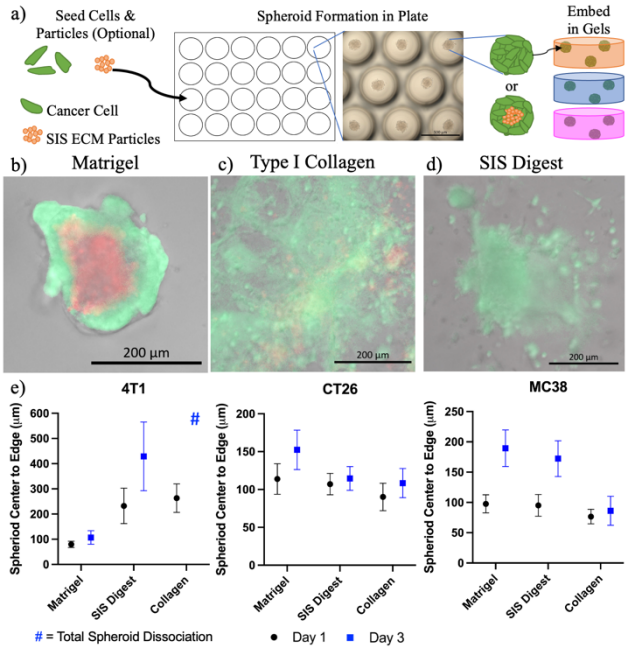

Figure 1: Cancer cells were seeded alone or with SIS ECM particles in **a)** plates with microcavities to form spheroids over 24 hours. The spheroids were then embedded in hydrogels. 4T1 spheroids are shown after 3 days of culture with live (green)/dead (red) staining in **b)** Matrigel, **c)** Type I Collagen, and **d)** SIS Digest hydrogels, which resulted in varying degrees of cellular migration within the hydrogels. **e)** Quantification of distance from the center of a spheroid to outer edge or protrusion within the hydrogels.

spheroids had a clearly defined outer edge in SIS hydrogels, whereas cells were observed migrating away from the edges of the spheroids in the other hydrogels. The protrusions appeared greatest in length in Matrigel compared to collagen (**Fig. 1e**, 189.5 & 86.2 um at day 3, respectively). MC38 spheroids in Matrigel had many protrusions that appeared to be sprouting away from the dense spheroid core with the protrusion length increasing each day of culture in SIS Digest and Matrigel (**Fig. 1e**). The dense core was also observed in collagen and SIS, but there was a less dense perimeter. These spheroids were surrounded by many single cells that increased in number over the course of the experiment.
Conclusions: Cell-matrix affinity is not captured in cells-only spheroid models and our results highlight the importance of modeling these interactions to study cancer cell invasion and migration. We found 3D cancer spheroid invasion was dependent on cell and ECM type. Spheroids incorporating ECM from decellularized tissues can be used to model invasion dynamics that are not found in traditional hydrogels composed of purified collagen or Matrigel. Future studies will investigate cancer spheroid migration in decellularized tissues typical of metastasis such as lung and liver.