Mechanically Tunable PEG-Collagen Composite Hydrogels for Probing Stiffness-Dependent Effects

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Statement of Purpose: Mechanical cues from the tissue microenvironment have been shown to impact cell differentiation, migration, and proliferation. These cues can become dysregulated in diseases such as breast cancer (1), affecting the cell behaviors mentioned above. Unfortunately, current research into this phenomenon is limited by the lack of suitable materials in which cells can be cultured in 3D under a wide range of mechanical conditions. Conventional two- and three-dimensional models frequently alter the concentration of extracellular matrix (ECM) components to achieve different substrate rigidities; however, this varies the amount of bioactive molecules and adhesion ligands presented to the cells. More recent research has focused on altering gel mechanics by chemically modifying the ECM, through glycation or increased cross-linking with materials such as collagen I (2,3). However, the range of stiffnesses that can be achieved with these methods (0.4 - 2.5 kPa) is too narrow to effectively model many physiological conditions, such as the changes that occur in breast cancer progression. Thus, our group has focused on creating a composite hydrogel of collagen I and polyethylene glycol dimethacrylate (PEG-DM), capable of being fabricated at a wide range of stiffnesses (1-30 kPa) while maintaining a constant collagen concentration. The current study characterizes this novel material and examines the impact of stiffness – independent of changes in collagen density – on MDA-MB-231 breast cancer spheroid invasion.

Methods: Primary amines on collagen I were covalently coupled to 4-armed thiol-PEG and retention of collagen structure confirmed via circular dichroism. Thiolated-PEG-collagen was then mixed with a photoinitiator and varying concentrations of PEG-DM (MW 8K; 5-12%). The pre-gel solution was polymerized via 365 nm UV exposure, yielding a PEG/collagen network crosslinked via acrylate and thiol-ene reactions. Constant collagen I content across conditions was confirmed by quantifying the hydroxyproline content of base-digested hydrogels. Distribution and organization of collagen was visualized via immunofluorescent staining of collagen I. The elastic modulus of each gel was recorded from the linear viscoelastic region of dynamic strain experiments performed on an ARES rheometer. MDA-MB-231 spheroids were formed in poly-HEMA-coated 96-well v-bottom plates and supplemented with 5% matrigel to aid cell compaction. Spheroids were placed into the center of the pre-gel solution directly before UV crosslinking. Gels were fed with serum-free media supplemented with 50 ng/mL TGF-α. Cell invasion and viability were imaged via Live/Dead staining 48 hours post-encapsulation. The percent of spheroids which invaded into the surrounding matrix as well as the total cell body area after invasion were measured and quantified with ImageJ. Invading spheroids were defined as having >10 cells separated from the central spheroid body.

Results: Over the range of PEG-DM concentrations used (5-12%), the elastic moduli of the hydrogels was increased approximately 30-fold (from 1.2 kPa to 33 kPa) while maintaining a final collagen concentration of 1 mg/mL (Table 1). Moreover, crosslinking with collagen enabled the creation of significantly stiffer gels than use of PEG-DM alone. The collagen was also shown to distribute evenly throughout the hydrogel and organize as randomly oriented fibers, a structure that did not vary across the different conditions (not shown). Both individual cells and spheroids were viable when embedded in the PEG/collagen hydrogels (Figure 1A). The percentage of spheroids that were invasive within 48 hours varied depending on gel stiffness (Figure 1B). Once initiated, however, the extent of invasion did not seem to be strongly impacted by the stiffness of the PEG/collagen gels (not shown).

Table 1: Properties of PEG/Collagen Hydrogels

PEG-DM Concentration	Elastic Modulus (kPa)	Collagen Content (mg/mL)
5%	1.2 ± 0.3	1.16 ± 0.04
7%	4.8 ± 0.6	1.07 ± 0.02
8.5%	9.6 ± 1.5	1.05 ± 0.05
12%	34.2 ± 0.7	1.08 ± 0.06

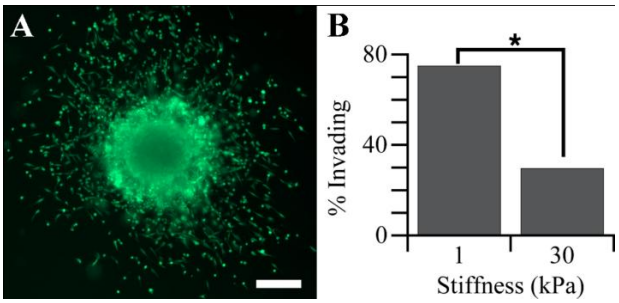

Figure 1: *(A) MDA-MB-231 spheroids were viable in PEG/collagen hydrogels and were able to invade. Scale bar is 250 μm. (B) Embedded tumor spheroids showed stiffness-dependent changes in the percent invasive spheroids after 48 hours.*

Conclusions: Utilizing thiolated-collagen and PEG-DM, we have created a composite hydrogel that enables variation of substrate stiffness over a 30-fold range while maintaining a constant concentration of collagen. We have also demonstrated cell viability of single cell and spheroid populations embedded within the material as well as stiffness-dependent effects on tumor spheroid invasion.

References:

1.Paszek et al. Cancer Cell. 2005; 8: 241-254
2.Lee et al. Acta Biomaterials. 2013; 9: 7178-7190
3.Mason et al. Acta Biomaterials. 2013; 9: 4635-44

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