Introduction: Cell migration is one of the fundamental elements of many physiological and path-physiological processes in the body. Normally, migratory cells of the body are confined by the extracellular matrix (ECM), which is a complex 3D terrain of structural proteins and polysaccharides that encapsulates most tissue cells. Therefore, the ECM plays a central role in the ability of migratory cells to move and remodel their microenvironment as part of the normal cellular remodeling events. At the individual cell level, different cells migrate and invade the ECM using either an amoeboid (stiffness-dependent) or a mesenchymal (proteolysis-dependent) migration mechanism. By altering the mechanical properties, degradation rate or structural motifs of the ECM, for example, one can control the invasion and migration of different cell types in 3D microenvironments of biomaterials that mimic the natural ECM. Towards this goal, using a structural modifications approach, we developed a biomimetic material that can harness the bioactive properties of fibrinogen but still retain full control over structural features of the material based on a synthetic polymer conjugate. In this work we sought to specifically decouple the matrix stiffness and biodegradation rate in the protein–polymer materials for studying cell invasion and migration. The matrix stiffness is governed by the cross-linking density, whereas the rate of proteolysis is a function of both cross-linking density and molecular shielding by the synthetic polymer. In order to decouple between the two, the cross-linking should be independent of the molecular shielding. In order to accomplish this decoupling, we conjugated fibrinogen to three different synthetic polymers having different molecular shielding effects: PEG, Pluronic and Tetronic.

Materials and Methods: Fibrinogen was conjugated to the PEG, Pluronic or Tetronic polymers to create a biosynthetic precursor by Michael-type addition of PEG-acrylate, Pluronic-acrylate or Tetronic-acrylate to denatured fibrinogen under reducing conditions for 3 h in the dark [1]. A hydrogel matrix was formed from the biocompatible fibrinogen-polymer adducts by free-radical polymerization using light-activated photochemistry (photo-polymerization). A non-toxic reaction condition was chosen using Irgacure2959 initiator and long-wave UV light (365 nm, 4-5 mW/cm²). The mechanical properties of the hydrogels (shear modulus, G’) were measured using a strain-rate controlled shear rheometer with an in situ UV curing attachment. The protease susceptibility of this P/T-fibrinogen hydrogel biomaterial was measured by fluorescently labeling the fibrinogen constituent and measuring its release in the presence of a 0.1 mg/ml trypsin PBS solution. The G’ and protease degradability of the hydrogels was adjusted by changing the molecular weight of the pendant polymer or by adding additional polymer cross-linker. In order to quantify the cell-scaffold interactions following these adjustments to the hydrogel properties, we used 3-D cell-seeded constructs with human dermal fibroblasts (HDF) and HeLa cells cultured for up to 6 days in standard culture conditions. Cell viability and morphogenesis, including cell spreading, migration, and matrix invasion, were documented by fluorescence microscopy.

Results and Discussion: The protein-based materials convey inductive signals to cells through bioactive sites on the fibrinogen backbone, as evidenced by high viability of the HDFs compared to synthetic polymer controls. The PEG, P/T fibrinogen adducts (made with PEG-12KDa, Pluronic-F127 or Tetronic-T1307) exhibited proteolytic break-down in the presence of trypsin, as well as in the presence of cells. In this context, the F127 and T1307 elicited fewer steric interactions that shielded the protein backbone from proteolysis, thus providing independent control over modulus via the cross-linking, without affecting the biodegradation rate of the hydrogel. An additional advantage of using F127 and T1307 was the ability to cause a phase transition in the hydrogel during the light-induced chemical cross-linking (at physiological temperatures), based on the fact that Pluronic and Tetronic are both synthetic block co-polymers that exhibit reverse thermal gelation (RTG). We successfully used the RTG properties to create structural features in the materials in order to manipulate migration and invasion as evidenced by time-lapse microscopy of cellularized constructs. The precise mode of control over migration was primarily through the structural properties such as matrix compliance, porosity, and mesh size of the cell-laden hydrogel.

Conclusion: The P/T-fibrinogen hydrogels retain the biocompatibility of their fibrinogen constituent, and the conjugation reaction does not alter the unique RTG self-assembly properties of the P/T. These RTG features endow the P/T-fibrinogen adducts with unique structural features. Using a combination of photo-polymerization crosslinking and temperature, we were able to demonstrate superior control over physical properties of the P/T-fibrinogen and this independent control of modulus was afforded by a combination of physical and chemical cross-linking in the P/T-fibrinogen polymeric matrix. Moreover, our ability to demonstrate the importance of matrix modulus in controlling cell spreading and invasion in a 3-D hydrogel milieu (independently of proteolysis) was revealed using the P/T-fibrinogen as an encapsulating cell culture matrix.

References:  

Disclosure: The authors have nothing to disclose.