Biocompatible and Tunable Elastic Hyaluronic Acid Hydrogel for Adult Stem Cell Differentiation

Florian Rehfeldt, Adam J. Engler, and Dennis E. Discher
Biophysical Engineering Lab, University of Pennsylvania, Philadelphia

Statement of Purpose:
In the past years, many studies revealed the significant impact of matrix elasticity on cell behavior. It has now become well acknowledged that the mechanical microenvironment plays an as important role as the chemical one [1]. Recently, it has been demonstrated by Engler et al. that the mechanical properties of the matrix can even guide the differentiation behavior of human adult stem cells (MSCs) [2]. For potential in vivo applications deeper understanding of the interplay between cells and the extracellular matrix (ECM) a biocompatible mechanically well-defined model is essential. We successfully established a biomimetic ECM model based on hyaluronic acid (HA) that exhibits a widely tunable and well-controllable elasticity and allows for *in vivo* use.

Methods:
Hyaluronic acid was chemically modified using an adapted method published by Shu et al [3] and cross-linked with poly(ethylene glycol)-diacrylate (PEG-DA). The effective Young's modulus *E* of the substrates was determined with an atomic force microscope (AFM) by fitting the force indentation curves with a modified Hertz model [4]. For culturing MSCs collagen I was covalently attached to the surfaces.

Results/Discussion:
Stable HA hydrogels were produced yielding an effective Young's modulus *E* in the range of 0.1 to 150 kPa. The elasticity can be finely tuned by variation of the concentration of HA, and cross-linker. Using covalently attached collagen I as matrix ligand, MSCs were cultured on the HA hydrogels as well as on the conventional PA system.

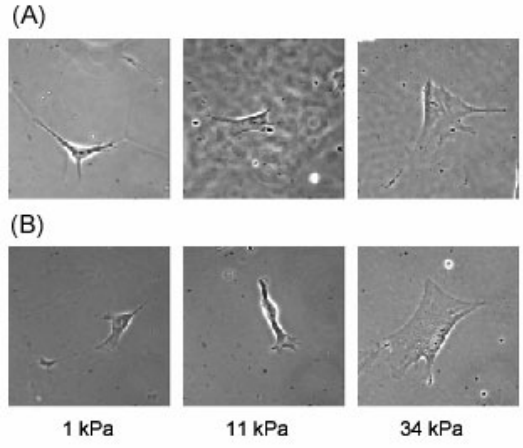

Figure 1 Morphology of MSCs on collagen coated conventional PA hydrogels (A) and on HA substrates (B) at three different elasticities *E* (1kPa, 11kPa, and 34kPa).

Figure 1 shows the cell morphologies in phase contrast microscopy yielding a comparable spreading behavior for both systems, having a branched appearance on soft (1kPa), spindle-like on intermediate (11kPa), and a polygonal shape on stiff (34 kPa) substrates, respectively. Further analysis of non-muscle mysoin IIa (NMM IIa) and actin expression and localization confirms the suitability of the HA matrix model for these biomechanical experiments.
Using this biomimetic ECM model combined AFM and fluorescence microscopy studies will help to better understand the molecular mechanism of mechano-sensing and –transduction.

Conclusions: We successfully established a biocompatible hydrogel based on hyaluronic acid with a tunable and well-controlled elasticity as measured by AFM. MSCs cultured on the novel HA substrates exhibit similar morphology as on conventional PA gels making the HA gels a promising candidate for stem cell guidance in potential *in vivo* applications.

References:
1. D.E. Discher et al. *Science* **2005**;310:1139-1143
2. A.J. Engler AJ. *Cell* **2006**;126:677-689
3. X.Z. Shu *Biomacromolecules* **2002**;3:*1304-1311*
4. E.K. Dimitriadis *Biophysical Journal* **2002**;82:2798-2810