Using light to establish and measure stiffness gradients in three-dimensional engineered tissues

250 word abstract

Cells are known to interact with their local extracellular matrix (ECM) and respond to a variety of biochemical and mechanical cues received from the ECM. The study of these interactions within three-dimensional (3D) naturally derived tissues is complicated by a lack of appropriate technologies. For instance, durotaxis has been studied on two-dimensional substrates and within 3D synthetic hydrogels (such as PEG and hyaluronic acid) in which stiffness gradients can be readily established. However, synthetic hydrogels lack extracellular fibers, limiting their utility as a model of native tissue. Establishing stiffness gradients within 3D fibrous systems such as those comprising collagen or fibrin is a challenge, particularly if the *in situ* stiffness cannot be monitored. Here we present our light-based method for non-invasive patterning of molecular crosslinking combined with multi-axes optical tweezers active microrheology to map ECM stiffness and stiffness anisotropy. Unlike in our previous work, here we crosslink large regions of ECM near to, but not including cells. These regions contract during crosslinking and strain the ECM in which the cell resides. Consequently, we induce strain hardening exclusive of chemical crosslinking. In a series of cell studies we demonstrate that in fact 3D gradients in stiffness are established with observed anisotropies in agreement with predicted strain fields. We further show that migratory cells respond to these gradients as can be assessed by F-actin distribution and morphological properties. Importantly, our method allows us to generate prescribed stiffness gradienties, which we have shown are a good model of the natural peri-cellular ECM.

100 word abstract

Studies of cell-extracellular matrix (ECM) interactions within fibrous systems such as collagen or fibrin are challenging, particularly if peri-cellular stiffness cannot be monitored. Here we present our light-based method for non-invasive patterning of molecular crosslinking combined with multi-axes optical tweezers active microrheology to map ECM stiffness landscapes. This method allows us to generate prescribed stiffness gradients and associated anisotropies, which model stiffness of the natural peri-cellular ECM. Patterned crosslinking induces strain hardening and measured stiffness gradients are in agreement with predicted strain fields. Migratory cells respond to these gradients as assessed by change in F-actin distribution and morphological properties.