Video Article

# Preparation of compliant matrices for modulating and quantifying cellular contraction

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## Abstract

The regulation of cellular adhesion to the extracellular matrix (ECM) is essential for cell migration and ECM remodeling. Focal adhesions are macromolecular assemblies that couple the contractile F-actin cytoskeleton to the ECM. This connection allows for the transmission of intracellular mechanical forces across the cell membrane to the underlying substrate. Recent work has shown the mechanical properties of the ECM regulate focal adhesion and F-actin morphology as well as numerous physiological processes, including cell differentiation, division, proliferation and migration. Thus, the use of cell culture substrates has become an increasingly prevalent method to precisely control and modulate ECM mechanical properties. To quantify traction forces at focal adhesions in an adherent cell, compliant substrates are used in conjunction with high-resolution imaging and computational techniques in a method termed traction force microscopy (TFM). This technique relies on measurements of the local magnitude and direction of substrate deformations induced by cellular contraction. In combination with high-resolution fluorescence microscopy of fluorescently tagged proteins, it is possible to correlate cytoskeletal organization and remodeling with traction forces. Here we present a detailed experimental protocol for the preparation of two-dimensional, compliant matrices for the purpose of creating a cell culture substrate with a well-characterized, tunable mechanical stiffness, which is suitable for measuring cellular contraction. These protocols include the fabrication of polyacrylamide hydrogels, coating of ECM proteins on such gels, plating cells on gels, and high-resolution confocal microscopy using a perfusion chamber. Additionally, we provide a representative sample of data demonstrating location and magnitude of cellular forces using cited TFM protocols.

## Disclosures

No conflicts of interest declared.

 