

# HYDROGEL SUBSTRATE SYSTEM WITH EASY, ROBUST PROTEIN FUNCTIONALIZATION FOR CELL CULTURE

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## Technology Description

Researchers in Prof. Amit Pathak's laboratory have developed a hydrogel system that utilizes an aldehyde-based compound to easily and reliably pre-functionalize polyacrylamide for conjugation of proteins, such as collagen and fibronectin, for cell culture applications.

Currently, polyacrylamide substrates must be treated with a crosslinking agent (e.g., Sulfo-SANPH) in order to attach the proteins necessary for cell culture. This post-hoc functionalization process is messy and has stringent requirements that result in unreliable protein coating. This technology eliminates this crosslinking step with a novel compound that can pre-functionalize the hydrogel when it is added to any standard polyacrylamide precursor. The resulting substrate has a robust protein coating. In addition, fibrous proteins of varying lengths (such as collagen) can be conjugated to the pre-functionalized hydrogel, which is not possible with standard crosslinkers. This feature enables users to mimic native 3D extracellular matrix (ECM) conditions in a 2D cell culture system. End user applications of this technology include cellular mechanobiology research, stem cell differentiation, study of cell migration and proliferation, cancer cell dissemination, tissue engineering, and T-cell expansion.

## Stage of Research

The inventors fabricated pre-functionalized hydrogels of varying stiffness and ECM protein structures. They demonstrated the utility of these substrates by culturing several cell types in sparse (single) cell culture and also in dense colonies. They have studied the effects of collagen fiber architecture on cell migration, cell spreading, and expression of various proteins implicated in stiffness-sensitive cell response.

## Applications

- **Cell culture substrate** – functionalized polyacrylamide hydrogels with tunable stiffness has end user applications such as:
  - Study of cell migration, spreading, and proliferation
  - Stem cell differentiation
  - Cellular mechanobiology research
  - Tissue engineering and regenerative medicine
  - Stiffness- and geometry-induced polarization of macrophages
  - T-cell expansion

## Key Advantages

- **Easy, robust functionalization:**

- aldehyde-based compound can be added to any standard polyacrylamide precursor solution to yield a completely functionalized hydrogel
- polyacrylamide is pre-functionalized, with no messy, post-hoc crosslinking step for protein coating
- dramatically enhances reliability - promotes homogeneous protein coating without triggering non-specific protein binding

- **Mimics extracellular matrix (ECM) in 2D:**

- collagen and other fibrous proteins with varying lengths can form
- tunable collagen length is independent of hydrogel substrate stiffness

### **Publication under review**

### **Patents**

- Provisional U.S. Patent Application Filed

### **Website**

- [Pathak Cellular Mechanobiology Lab](#)