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Microengineering Approaches for Regenerative Medicine

Yubing Sun

University of Massachusetts Amherst

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Microengineering Approaches for Regenerative Medicine

Yubing Sun, PhD¹

¹Department of Mechanical and Industrial Engineering, University of Massachusetts Amherst

Stem cells, especially human pluripotent stem cells (hPSCs), hold significant promise for modeling developmental and disease processes, drug and toxicology screening, and cell-based regenerative medicine. Most hPSC studies have so far focused on identifying extrinsic soluble factors, intracellular signaling pathways, and transcriptional regulatory networks involved in regulating hPSC behaviors. We focus on the development and applications of some novel synthetic micromechanical systems to understand the mechano-sensitive and -responsive properties of hPSCs and their functional regulation of self-renewal, directed differentiation, and survival of hPSCs. First, we have demonstrated that rigid PDMS micropost arrays (PMAs) support the maintenance of pluripotency of hPSCs. Blocking cytoskeleton contractility by blebbistatin and inhibiting E-cadherin functions by DECMA-1 antibody both impair mechanoresponsive self-renewal of hPSCs on rigid substrates. We have further achieved efficient neuroepithelial induction, caudalization, and motor neuron differentiation from hPSCs combining soft PMAs ($E_{eff} < 5\text{kPa}$) with dual Smad inhibition. The purity and yield of functional motor neurons derived from hPSCs within 23 days of culture using soft PMAs were improved four- and twelve-fold, respectively, compared to coverslips or rigid PMAs. Our mechanistic work has helped reveal for the first time that biomechanical cues, including intracellular contractile forces and cell shape, converge and reinforce signal integration of TGF- β , Wnt, Hippo/YAP, Rho GTPase, and the actomyosin cytoskeleton to regulate the neural plate specification. We also developed a novel acoustic tweezing cytometry (ATC) utilizing ultrasound pulses to actuate functionalized lipid-encapsulated microbubbles (MBs) targeted to cell surface integrin receptors to exert subcellular mechanical forces in the pN - nN range. ATC can robustly induce cell traction force changes through acoustic radiation forces and bubble cavitation induced shear stresses. Importantly, ATC stimulations increased the survival rate and cloning efficiency of hESCs by 3-fold, suggesting its potential application in large-scale expansion of hPSCs.

Contact:

Yubing Sun, Ph.D.

Assistant Professor, Department of Mechanical and Industrial Engineering
University of Massachusetts, Amherst

Office: 317 Engineering Laboratory

T: (413)545-4543 **Web:** <http://blogs.umass.edu/ybsun/>