Exploring the link between human embryonic stem cell organization and fate using tension-calibrated extracellular matrix functionalized polyacrylamide gels.

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Public Summary:
Human embryonic stem cells (hESc) and their induced counterparts can give rise to many functional cells of the body and thus hold enormous potential for treatment of diseases or traumas characterized by the loss of cell functionality. The “how to” of preparation of these derivatives in sufficient numbers is one major challenge in translating this potential into real world therapies. The first step in this process is to convert hESc into cells belonging to one of three descendents referred to as endoderm, mesoderm, or ectoderm. Each of these lineages is restricted in its potential to become one or other useful cell type (ex heart tissue from mesoderm). Thus the numbers of cells that undergo early lineage commitment will in part determine the overall efficiency of the process. During fetal development in the uterus this occurs during a stage referred to as gastrulation. We have found that one determinant of this efficiency is how soft or hard is the surface on which hESc are growing; soft surfaces closer to that found in the body as opposed to the standard hard surfaces used to grow cells outside the body result in cells that look more like the cells in the body and express genes that suggest they are more prone to undergoing gastrulation. In this chapter we describe methods to prepare surfaces with a range of “softnesses” and how to plate and grow hESc on these surfaces as a means for other researchers to study the how and why of this important variable in the early commitment of hESc.

Scientific Abstract:
Human embryonic stem cell (hESc) lines are likely the in vitro equivalent of the pluripotent epiblast. hESc express high levels of the extracellular matrix (ECM) laminin integrin receptor alpha6beta1 and consequently can adhere robustly and be propagated in an undifferentiated state on tissue culture plastic coated with the laminin rich basement membrane preparation, Matrigel, even in the absence of supporting fibroblasts. Such cultures represent a critical step in the development of more defined feeder free cultures of hESc; a goal deemed necessary for regenerative medical applications and have been used as the starting point in some differentiation protocols. However, on standard non-deformable tissue culture plastic hESc either fail or inadequately develop the structural/morphological organization of the epiblast in vivo. By contrast, growth of hESc on appropriately defined mechanically deformable polyacrylamide substrates permits recapitulation of many of these in vivo features. These likely herald differences in the precise nature of the integration of signal transduction pathways from soluble morphogens and represent an unexplored variable in hESc (fate) state space. In this chapter we describe how to establish viable hESc colonies on these functionalized polyacrylamide gels. We suggest this strategy as a prospective in vitro model of the genetics, biochemistry, and cell biology of pre- and early-gastrulation stage human embryos and the permissive and instructive roles that cellular and substrate mechanics might play in early embryonic cell fate decisions. Such knowledge should inform regenerative medical applications aimed at enabling or improving the differentiation of specific cell types from embryonic or induced embryonic stem cells.