

SEED: MICROMECHANICS OF CELL ADHESION

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Cell Power: Quantification of the Forces Driving Self-assembly of 3D Micro-tissues. In a non-adhesive environment, cells will self-assemble into micro-tissues, a process with direct relevance to tissue engineering. While this has been recognized for some time, as yet there is no basis for quantitative characterization of this complex process. Here, we describe a recently developed assay, designed to quantify aspects of the process, and discuss its application to compare behaviors between cell types.

Cells were seeded into non-adhesive micro-molded wells, each well with a circular trough at its base formed by the cylindrical sidewalls and by a central peg in the form of a right circular cone. Cells settled into the trough and coalesced into a toroid, which was then driven up the conical peg by the forces of self-assembly. The mass of the toroid and its rate of upward movement were used to calculate the cell power expended in the process against the force of gravity. The power of the toroid was found to be 0.31 ± 0.01 pJ/hr and 4.3 ± 1.7 pJ/hr for hepatocyte cells and fibroblasts, respectively. Blocking rho kinase by means of Y-27632, resulted in a 50% and greater reduction in power expended by each type of toroid, indicating that cytoskeletal mediated contraction plays a significant role in the self-assembly of both types. While the driving force for self-assembly has often been viewed as the binding of surface proteins, these data show that cellular contraction is important for cell to cell adhesion. The power measurement quantifies its contribution and will be useful for dissection of the concerted action of the contributing mechanisms that drive self-assembly.

Characterizing the resistance generated by a molecular bond as it is forcibly separated. The goal of measurements of the resisting force generated by a molecular bond as it is being forcibly separated under controlled conditions is to determine functional characteristics of the bond. We have established the dependence of force history during unbinding on those parameters chosen to characterize the bond itself and the controllable loading parameters. This was pursued for the practical range of behavior in which unbinding occurs diffusively rather than ballistically, building on the classic work of Kramers. For a bond represented by a one-dimensional energy landscape, modified by a second time-dependent energy profile representing applied loading, we carried out a mathematical analysis to show reveal the dependence of the resistance of the bond on bond well shape, general time dependence of the imposed loading, and stiffness of the loading apparatus. The quality of the result was verified through comparison with full numerical solutions of the underlying Smoluchowski equation. The result provides the first model for bond separation under nonsteady force, and it establishes the central importance of associating average bond force over an ensemble, rather than average force on unbroken bonds, in a statistical mechanical description.

