Tissue Engineering

Tractional Forces of Cells

Cells are able to exert tractional forces on their substrates, and these forces appear to be vital in wound contraction and cell migration. Cellular traction has also been shown to direct the formation of complex cellular patterns found in tissues. An understanding of the mechanical interaction of cells with their substrates is needed for the rational design of scaffolds for tissue engineering applications.

One consequence of cell traction that is relevant to tissue engineering is the resulting change in porosity, pore size, or pore structure of the scaffold. Changes in material properties will affect cell growth or the subsequent infiltration of other cell types needed, for example, in vascularization of tissue, and they will also affect the diffusion rates for oxygen, nutrients, and waste products. Moreover, stress and strain in the network may modulate cell differentiation and protein and DNA synthesis.

The molecular mechanism of cell traction should not be confused with cell adhesion. Most mammalian cells are anchorage-dependent, meaning that they must attach to a surface in order to survive. Cell traction also requires the contractile activity of the cytoskeleton via actin-myosin interactions and exertion of tension through specific sites connecting the substrate to the cytoskeleton.

These sites most likely correspond to either focal adhesions or focal complexes that consist of transmembrane extracellular matrix receptors known as integrins and actin-associated molecules such as vinculin, talin, and α-actinin, as well as signaling molecules.

An active area of research is the identification of the molecules and signaling pathways involved in the regulation of cell traction. Advances in biotechnology have made it possible to investigate the effects of specific biochemical inhibitors or genetically engineered cell lines on the biomechanical properties of cells. It is possible to directly test various proposed molecular mechanisms of cell traction by examining the biomechanical behavior of cells that have specific molecules inhibited or knocked out.

One of the first observations of cellular traction forces was in an in vitro model of the wound healing process called the fibroblast-populated collagen assay. Wound healing begins with acute inflammation marked by migration of activated fibroblasts into the wound interface. Migration is followed by cell proliferation and by synthesis of a matrix containing collagen, producing the transitory "granulation tissue." Wound contraction then takes place, and the wound healing process proceeds to scar formation.

The in vitro fibroblast-populated collagen assay showed that traction forces are involved in wound contraction. Cells are able to grab hold of the collagen matrix and cause a restructuring of collagen fibrils that, over time, leads to gel compaction. During compaction of a disc-shaped matrix, there is an isotropic (uniform) reduction in diameter if the disc is floating. However, if the matrix is anchored at one end to a substrate, the confinement leads to a reduction in height. The initial geometry and stress conditions of the fibroblast-containing collagen gel are important factors that govern the final state of the tissue.

Morphological and proliferative features of cells also depend on the initial stress conditions. For example, the phenotype that develops after contraction differs dramatically depending on whether the matrices are floating or anchored. In the case of the floating matrix, where tension is distributed isotropically, the morphology of fibroblasts is stellate, with long processes and a cytoskeletal meshwork. In the anchored case, where tension is distributed anisotropically, cells develop prominent stress fibers and fibronexus junctions, and they resemble myofibroblasts.

Furthermore, cell proliferation and collagen biosynthesis are attenuated in floating collagen matrices when compared with cells in anchored matrices. Interestingly, contraction of the floating matrix leads to a tissue resembling dermis, whereas the anchored matrix leads to a tissue resembling granulation tissue. The mechanical properties of the matrix clearly play a role in determining the properties of the final structure.

Studies of cells cultured on elastic substrates illustrate the role of traction in the motility of individual cells, though it is not known whether the traction forces involved in cell locomotion are of the same origin as those involved in the remodeling of tissue matrices. Cell types able to exert traction forces include fibroblasts, liver parenchyma cells, Kupffer cells, pigmented retina cells, neurons, neuroglia, kidney epithelial cells, and keratinocytes. As cells migrate, wrinkles and distortions develop in the elastic substrate.

Incorporation of marker beads into the elastic substrate allows quantitative measurement of the

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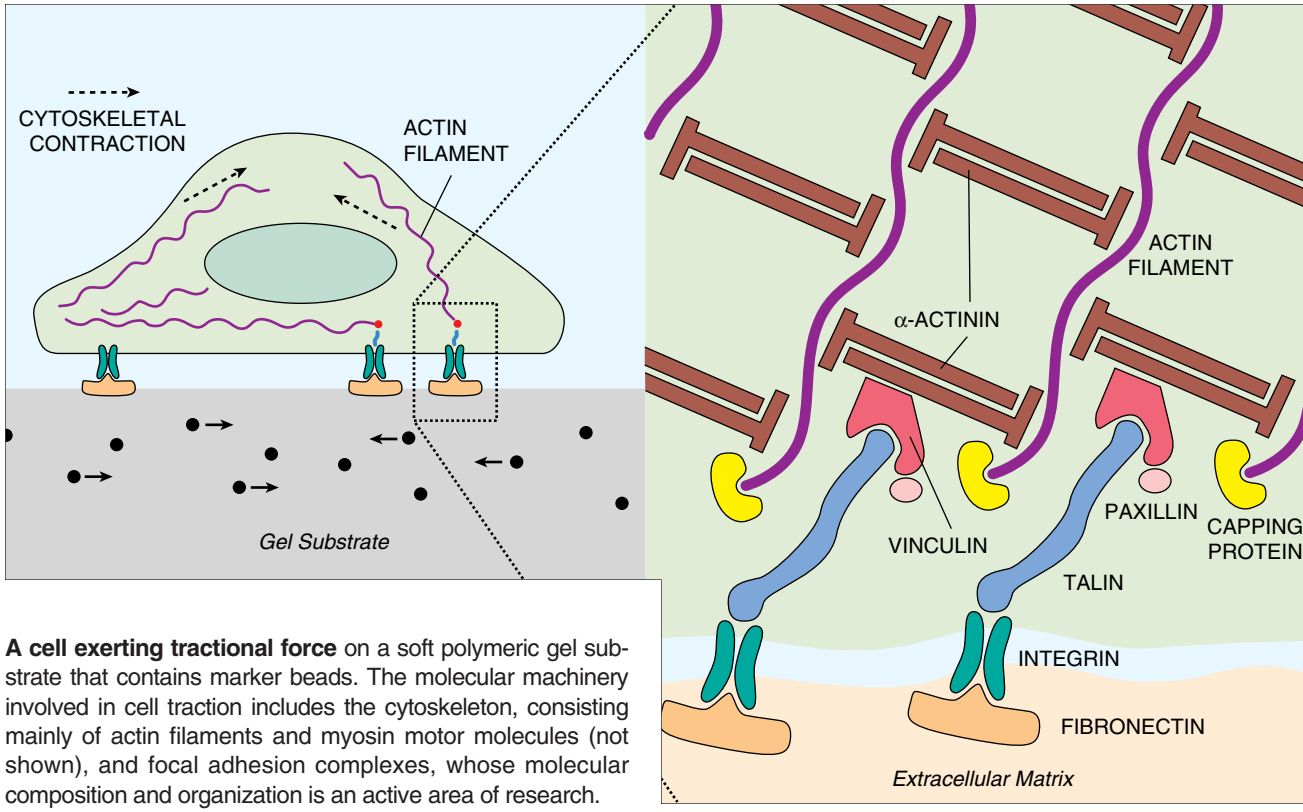

A cell exerting tractional force on a soft polymeric gel substrate that contains marker beads. The molecular machinery involved in cell traction includes the cytoskeleton, consisting mainly of actin filaments and myosin motor molecules (not shown), and focal adhesion complexes, whose molecular composition and organization is an active area of research.

traction force. The motion of the beads can be converted by computational methods into a "traction map," which reveals the magnitude, direction, and spatial location of the traction forces.

Substrates used to measure cell traction must satisfy several requirements. First, they must deform elastically, allowing movement of the embedded beads to be translated into traction forces using standard equations for material elasticity. Second, they should have tunable mechanical properties to allow for investigation of different cell types, because traction forces are dependent on cell type; fibroblasts exert higher traction forces than keratinocytes, for example.

Third, substrates should be biocompatible and allow the attachment of cells, which can be achieved by chemically cross-linking extracellular matrix adhesion molecules such as fibronectin or collagen to the surface of the substrate. Fourth, substrates should be transparent to permit observation by video microscopy.

To date, silicone sheets and polyacrylamide gels have been used as substrates for measuring traction of single cells. It is important to characterize the mechanical properties of the substrate, as this information is needed to generate the traction maps. Standard mechanical testing techniques can be used to determine Young's modulus (of elasticity), compliance, and Poisson's ratio (of the lateral and axial strains).

During migration, a cell exerts forces on its substrate to propel itself. One model of cell migration proposes that adhesions in the tail of the cell are intrinsically weaker than those in the front. Contraction of the cytoskeleton results in a tension pulling on both ends of the cell, but because of weaker adhesion, bonds at the trailing end break first and cause both cytosolic flow and membrane protrusion at the front of the cell. The process is repeated as new adhesions are formed and broken.

Traction maps of fibroblasts on substrates have shown that the traction forces are exerted along the axis of locomotion, supporting the differential adhesion model of cell migration. In contrast, fish epidermal keratinocytes exert forces perpendicular to the axis of motion, indicating a pinching mechanism. In both cases, traction maps show that the forces exerted on the substrate are much greater than needed to simply propel the cell, suggesting that the additional force may have a role in tissue development and wound contraction.

Quantitative relationships between cellular traction and substrate properties are essential for creating models that will predict cellular response based on the properties of the biomaterial surface. These models may eventually lead to the establishment of fundamental engineering principles of cell-substrate interactions that could then be used for the rational design of novel biomaterials that would elicit a desired biological response.

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Gene Therapy

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