

6 Mechanotransduction

6.1 Motivation

The process of converting physical forces into biochemical signals and integrating these signals into the cellular response is referred to as mechanotransduction [11, 20]. To fully understand the molecular basis for mechanotransduction, we need to know how externally applied forces are transmitted into and throughout the cell. Different techniques have been developed to probe mechanotransduction by mechanically stimulate cells. The goal of this chapter is to discuss the advantages and disadvantages of these techniques to address the following questions.

What do we study in mechanotransduction? How do cells respond to mechanical forces? ◦ How do mechanical forces lead to biochemical and molecular responses? ◦ How can we strengthen bone? ◦ How can we grow cartilage? ◦ How can we strengthen muscle? ◦ How can we improve cardiac contractility? ◦ How can we engineer tissues for artificial organs? ◦ How can we mimic the mechanical loading environment of cells in vitro? ◦ What can we learn from mechanical stimulation of cells with precisely controlled forces?

It is useful to divide the process of mechanotransduction into three stages:

- (i) Mechanoreception: Detection of the stimulus and transmission of the signal from outside the cell to its inside.
- (ii) Intracellular signal transduction: Transduction of the stimulus to location in the cell where a molecular response can be generated.
- (iii) Target activation: Activation of proteins that cause alterations in cell behavior through a variety of different mechanisms.

6.1.1 Mechanoreception

Mechanoreceptors respond to extracellular signals and relay stimuli from the outside to the inside. They are thus located right in the cell membrane. Three different types of mechanoreceptors can be identified in the membrane: (i) integrins, (ii) stretch-activated ion channels, and (iii) cell-surface receptor proteins. We will discuss their individual functions in the sequel.

| disease | dysfunctional cell type |
|----------------------------------|--|
| deafness | hair cells in the inner ear |
| glaucoma, loss of vision | optical neurons |
| muscular dystrophy | myocytes, endothelial cells, fibroblasts |
| cardiomyopathy | cardiomyocytes |
| osteoporosis | bone cells |
| arteriosclerosis | endothelial cells, smooth muscle cells |
| immune system disorders | leukocytes |
| central nervous system disorders | neurons |

Table 6.1: Typical diseases associated with defects in mechanotransduction.

Integrins

Integrins are transmembrane proteins that interact with the extracellular matrix and mediate various intracellular signals. Integrins are heterodimers, they have an extracellular binding domain, a transmembrane domain, and a cytosolic domain. As cell surface receptors, integrins recognize binding domains on collagen, laminin, fibronectin, and other matrix proteins. Their cytosolic associates with a large protein group called focal adhesion complex which attaches to F-actin filaments to connect the cytoskeleton with the fibrous material outside the cell. Since integrins form a direct connection between the extracellular matrix and the intracellular cytoskeleton, a mechanical stimulus applied to integrins can directly alter the architectural structure of the cytoskeleton. Deformations of the cytoskeleton can (i) change the physical properties of the cell, (ii) activate other receptors in the cell, and (iii) regulate biochemical and molecular events. The signals the cell receive through the integrins can be related to cell growth, cell division, cell survival, cellular differentiation, and cell death.

Stretch-activated ion channels

Ion channels are proteins that span the cell membrane connecting the cytosol to the cell exterior. Their permeability is highly controlled allowing Na^+ , K^+ , Ca^{2+} , and Cl^- ions to selectively pass the otherwise impermeable cell membrane. Ion channels are usually closed. Their rapid opening enables the flow of ions down their electrochemical gradient, either into or out of the cell. The opening of the channel is controlled, or gated, by specific stimuli. It is common to classify ion channels by their gating mechanism: voltage-gated, ligand-gated, or mechanically-gated. Mechanically gated ion channels are sensors for a number of systems including the senses of touch, hearing and balance, as well as participating in cardiovascular regulation and osmotic homeostasis. Two mechanisms are thought to be responsible for the activation of mechanically gated ion channels: (i) physical deformation of the cell membrane which causes conformational changes that affect the membrane-embedded proteins (ii) deformation of the actin cy-

toskeleton which is attached to the channel to directly regulate its opening. Depending on the type of channel, stretch might either activate or inactivate its activity.

Cell-surface receptors

Cell surface receptors are proteins that bind extracellular signaling molecules to initiate an intracellular response. These receptors are either (i) G protein linked, or (ii) enzyme linked. Typically, they respond to soluble molecules such as proteins, peptides, steroids, or dissolved gases. It is hypothesized that some cell-surface receptors are also involved in sensing mechanical signals. Similar to stretch-activated ion channels, cell surface receptors may be activated by (i) membrane deformation, or (ii) deformation of the actin cytoskeleton. G protein-linked and enzyme-linked receptors initiate a cascade of intracellular signaling pathways that distribute the signal throughout the entire cell.

6.1.2 Intracellular signal transduction

Physical transduction

Within the cell, the cytoskeleton and its associated regulatory molecules are believed to serve as a scaffold for the transduction of mechanical signals into biochemical signals. Integrins can initiate the deformation of the cytoskeleton which can affect the biochemical state of the cell. Since the cytoskeleton is a dynamic, highly interconnected network of cellular filaments, its deformation at one location will immediately influence other locations within the cell. A local perturbation of an integrin can, for example, lead to the movement of organelles, distort the cell nucleus, and influence gene expression. Cytoskeletal deformation also activate mechanoreceptors such as mechanically gated ion channels and G protein-linked receptors. In addition, it has been hypothesized that the some regulatory molecules which are attached to the cytoskeleton sense its mechanical deformation which could, in turn, affect their own kinetic behavior and biochemical activity.

Biochemical transduction

The activation of a receptor initiates a cascade of events mediated by a series of signaling molecules. These will alter target proteins which will then change the behavior or the cell. The intracellular signal is relayed by (i) small intracellular mediator molecules which are referred to as second messengers, and (ii) a network of intracellular signaling proteins. In response to the activation of a receptor, large numbers of second messengers are generated diffusing rapidly through the cytosol and along the cell membrane. Second messengers then bind to the signaling proteins and target proteins and alter their behavior. Signaling proteins propagate the signal further downstream either by generating additional small-molecule mediators or by activating another signaling protein.

6.1.3 Target activation

Mechanical signals can influence cellular function in various different ways depending on the targets of the signaling pathway. The cellular response to a single type of stimulus can be quite complex since the activation of a single type of receptor usually activates multiple parallel signaling pathways. Typical examples of target proteins are those that regulate gene expression and the transcription of mRNA from DNA. The production of proteins and their secretion from a cell can affect the function of neighboring cells, thereby propagating the effect of the mechanical signal from one cell to another.

6.2 Probing Mechanotransduction

In their physiological environment, cells are subjected to various combinations of mechanical stimuli and it is difficult to predict which stimulus is responsible for which change within the cell. In an attempt to better understand the response of the cell to individual mechanical stimuli, experiments are performed under controlled laboratory conditions in which different loading scenarios can be applied in a selective way. Because of the complexity of the cellular biomechanics, a single cell experiment which isolates the cell from its environment does not mimic the *in vivo* conditions properly. That's why it has become common practice to culture cells *in vitro* and stimulate the entire tissue culture. Let's discuss some of the classical devices that are used to probe mechanotransduction in living cells. From a mechanical point of view, existing devices can be classified according to the type of loading; tension, compression, and shear.

6.2.1 Tension - Uniaxial and biaxial

The most common approach to apply tension to cells is to culture them on a stretchable surface and apply tension once the cells have adhered to it. Various modifications of this technique have been developed to apply both uniaxial and biaxial stretch, either statically or dynamically, see figure 6.1. It's important to keep in mind that the dynamic motion of a sheet with cells will in general also move the surrounding liquid which, in turn, applies additional shear or pressure forces to the cell sheet. The sheet on which

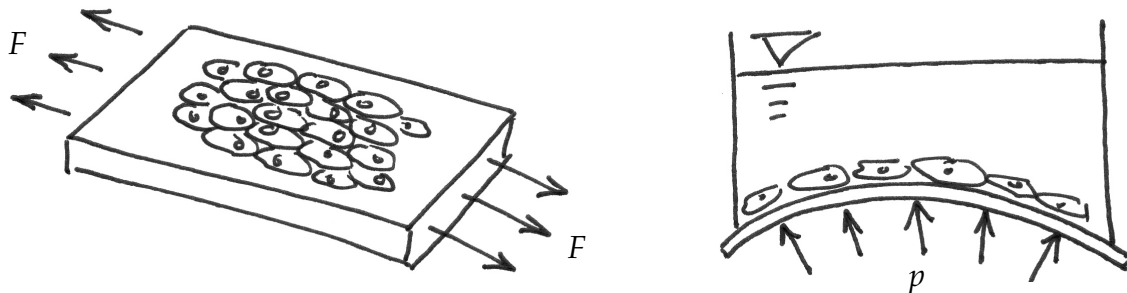


Figure 6.1: Uniaxial and biaxial tension devices stretching cells cultured on a thin sheet.

the cells are cultured must, of course, be biocompatible, ideally also transparent, but can otherwise be fully tuned. For example, it can be coated with well-defined matrix molecules such as collagen or fibronectin to explore the impact of specific cell-matrix interactions on mechanotransduction. From a mechanical point of view, the most challenging aspect is to apply a well-controlled, homogeneous strain profile meaning the cells at different locations of the sheet experience a different strain and might eventually respond differently. Sometimes, this is seen as an advantage because different strain profiles can be tested in only one sample. In general, the interpretation of the results is rather difficult without knowing the exact strain profile. In the following, we will discuss different loading techniques which are relatively successful in generating homogeneous strain fields.

Uniaxial tension

Maybe the most straightforward approach to stimulate cells mechanically is to culture them on a flexible thin sheet, grip the sheet at opposite edges, and stretch it uniaxially. The advantage of this method is that it's really relatively simple. For sufficiently long sheets, the stretch field can be assumed as homogeneous, at least far enough away from the fixed ends. A potential disadvantage is that the membrane is also compressed in the lateral direction due to Poisson's effect.

Biaxial tension

A common way to apply biaxial stretch is not to pull a rectangular cell sheet at all for edges but rather to fix a circular membrane at its edges and load it from underneath. Different devices have been used to either push the membrane up or pull it down with vacuum pressure. Ideally, this configuration stretches the cell sheet in a pure membrane state, blowing it up like a balloon. In that case, all cells would experience the same strain in all directions. In practice, however, a pure membrane state, a state without shear or bending, is difficult to achieve. It requires a very good lubrication at the fixation ensuring that the cell membrane can slide along the frictionless support as it is loaded.

6.2.2 Compression - Hydrostatic and uniaxial

There are two types of compressive loading devices, hydrostatic pressure devices and uniaxial compression platens. The choice of device primarily depends on the cell type to be tested.

Hydrostatic compression

A common approach to compress cells in culture is to increase the gas pressure in the culture system. Ideally, all cells in the liquid medium would then experience a ho-

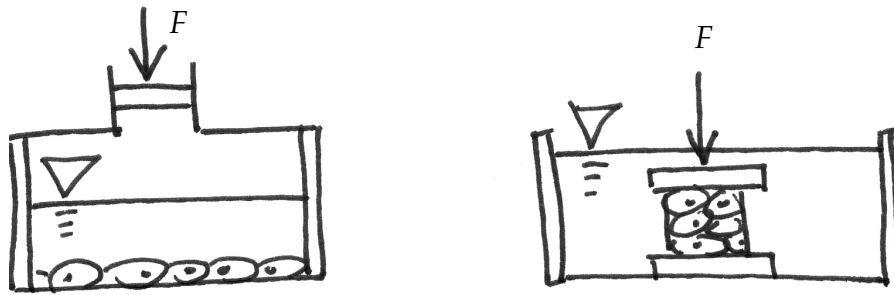


Figure 6.2: Hydrostatic and uniaxial compression devices compressing plain cells and cells in a matrix.

homogeneous increase in pressure. In reality, cells are extremely sensitive to changes in the gas composition; for example changing the O_2 or CO_2 concentration in the culture chamber can have a severe impact on the cell culture. It is therefore difficult to tell whether observed changes can be attributed to the mechanical stimulus, the increased pressure, or result from the chemical stimulus, the different gas composition. In addition, it's important to realize that an increase the hydrostatic pressure will primarily affect the cytoplasm rather than the cells mechanoreceptors. It is thus very unlikely that hydrostatic pressure alone can be sensed by the cell and its real impact on cellular mechanotransduction is presently unclear.

Uniaxial compression

A more physiological compressive loading can be achieved by culturing cells in a three-dimensional extracellular matrix and placing the specimen in a uniaxial compression device in which the cell-matrix specimen is loaded by a compression plate. Although this technique seems conceptually simple, the resulting stress state can be quite complex. This has various reasons: the heterogeneous composition of the matrix, the lateral expansion of the specimen due to Poisson's effect, the viscosity of the matrix, and the fluid flow which makes the specimen behave like a sponge. All these effects, however, mimic the true in vivo environment of cartilage and this technique has therefore become very prominent to explore the response of chondrocytes to compressive forces.

6.2.3 Shear - Circumferential and uniaxial

It is obvious that endothelial cells lining blood vessels are subject to shear stresses generated by fluid flow. Surprisingly, also osteocytes in bone have been found to be stimulated by fluid flow caused by locomotion. Two classes of devices have been applied to study the response of cells in well-controlled flow fields, viscometers and flow chambers.

Circumferential flow - Viscometers

A rotational flow field can be generated by two different devices, cone-and-plate viscometers and parallel-plate viscometers. In both cases, cells are cultured on a flat plate.

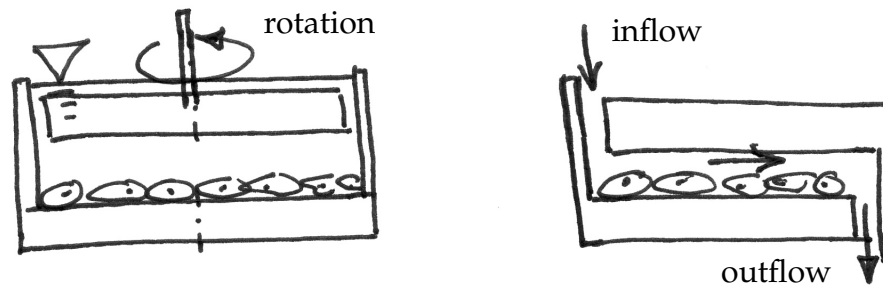


Figure 6.3: Circumferential and uniaxial flow devices applying shear stress to the cell culture.

They are stimulated through a circumferential fluid flow generating by a rotating upper disk. This disk can either be conical or flat. Both devices generate an inhomogeneous fluid profile caused by a zero flow velocity at center that increases radially to reach the maximum flow velocity at the edge of the disk. Again, the interpretation of different shear stresses acting on the cell culture can be difficult. Sometimes, however, it might be advantageous to explore the impact of different shear profiles within one single sample.

Uniaxial flow - Flow chambers

Flow chambers generate a uniaxial flow on top of a cultured substrate by using pressure gradients to drive the fluid. Fluid flow profiles can, in general, be either parallel or radial, but the parallel fluid flow chamber is by far the most common. Parallel flow chambers assume Poiseuille flow, i.e., they are designed such that the flow profile on the culture is laminar and the resulting shear stress is constant for a given flow rate. Parallel flow chambers are very attractive; they are relatively easy to handle and can be put under a microscope to observe the response of cells as the flow profile is changed or reversed. A potential disadvantage of flow chambers is that they apply fully developed laminar flow profiles. It is questionable to what extent laminar flow can fully mimic the in vivo observed stimuli.

6.3 Electrophysiology

The cell membrane can be understood as a boundary separating the internal working cell from its external environment. Maybe the most important feature of this membrane is that it is selectively permeable, regulating the permitted and restricted transport of materials into and out of the cell, see figure 6.4. In chapter 5, we have seen that the cell membrane is a 6-7nm thick double layer of phospholipid molecules. Among other aggregates that move around freely within this double layer, the cell membrane contains water-filled pores with diameters of about 0.8nm, as well as protein-lined pores called channels which allow the controlled passage of specific molecules. The intracellular and extracellular environment consist of a dilute aqueous solution of dissolved salts, primarily NaCl and KCl which dissociate into Na^+ , K^+ , and Cl^- ions.