CHAPTER

6

Tissue Engineering

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OUTLINE

AT THE CONCLUSION OF THIS CHAPTER, STUDENTS WILL BE ABLE TO:

- Discuss the growing area of cellular therapies.
- Discuss the three general categories of extracorporeal bioreactors.
- Understand the cellular dynamics underlying tissue function.
- Qualitatively describe the importance of stem cells in tissue function.
- Quantitatively describe cellular fate processes.
- Analytically describe mass transfer in three different configurations.
- Explain the parameters that characterize the tissue environment and how to approach mimicking them in vitro.
- Describe the issues fundamental to scale-up.
- Define functional tissue engineering and how the "-omics" sciences are driving this newly created research area.
- Discuss the issues that one encounters when implementing cellular therapies or bioartificial organs to patients.

6.1 WHAT IS TISSUE ENGINEERING?

Tissue engineering is a biomedical engineering discipline integrating biology with engineering to create tissues or cellular products outside the body (*ex vivo*) or to use the gained knowledge to better manage the repair of tissues within the body (*in vivo*). This discipline requires understanding of diverse biological fields, including cell and molecular biology, physiology and systems integration, stem cell proliferation and differentiation, extracellular matrix chemistry and compounds, and endocrinology. It also requires knowledge of many engineering fields, including biochemical and mechanical engineering, polymer sciences, bioreactor design and application, mass transfer analysis of gas and liquid metabolites, and biomaterials. Translation of tissue engineering constructs to clinical applications will involve yet other scientific disciplines so novel engineered tissues will be easily accepted and used by clinicians. The combination of these sciences has spawned the field of *regenerative medicine*, which is closely aligned with tissue engineering but has a focus on strategies that use the body's natural regeneration mechanisms to repair damaged tissues. Two of the main goals of these fields are cell therapies for the repair of damaged tissues, involving injection or engraftment of cells or cellular suspensions, sometimes in combination with scaffolding material, or establishing tissue ex vivo for use as grafts or extracorporeal organs to assist or supplement ailing in vivo organs. Clinical trials with cell therapies or extracorporeally created tissues have begun to be undertaken in the area of skin, cartilage, bone, heart, neural, and liver tissues, and the first tissue-engineered products have become available in the last decade. In addition, tissue engineering strategies are being employed to develop improved in vitro diagnostic and screening techniques, as well as creating improved tissue models to study disease. Both scientific and economic issues will define the success of these and future therapeutic modalities.

6.1.1 The Challenges Facing the Tissue Engineer

Some of the fundamental challenges that face the tissue engineer "in the implementation of cell therapies or creation of grafts and bioartificial organs" are shown in Figure 6.1. In particular, the following issues will impact the field as it progresses toward larger-scale clinical application:

1. Reconstitution of physical (mass transfer) and biological (soluble and insoluble signals) microenvironments for the development and control of tissue function.
2. Overcoming scale-up challenges in order to generate cellular microenvironments on a clinically and commercially meaningful scale.
3. Systems automation to provide appropriate process and quality control on clinically and commercially meaningful scales.
4. Implementation of tissue engineering technologies in clinical settings, including appropriate cell handling and preservation procedures that are required for cell therapies and the transplantation of viable tissues.

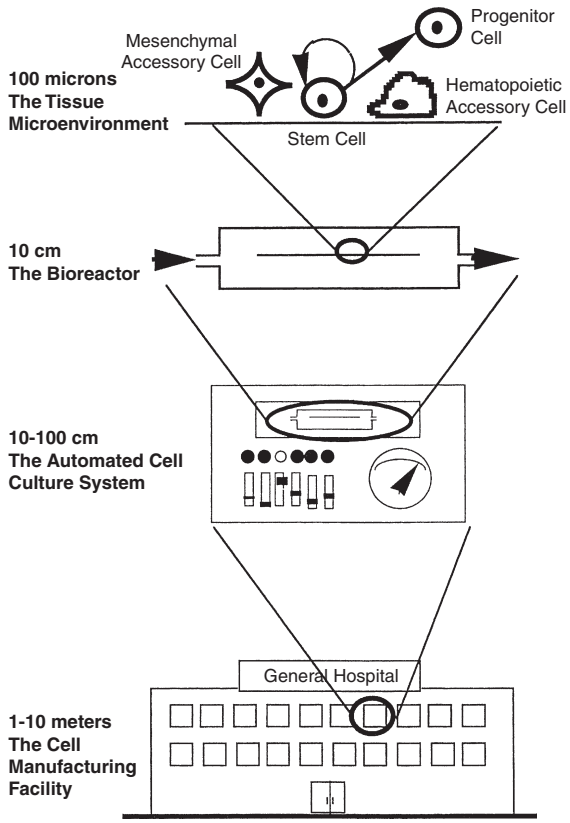


FIGURE 6.1 The four principal size scales in tissue engineering and cellular therapies.

This chapter concentrates on items 1 and 2, although some of the challenges faced with items 3 and 4 are discussed in Section 6.6. For an overall understanding, items 1 and 2 are further illustrated in Figure 6.2 from the viewpoints of "every cell in the body."

In the center of Figure 6.2 is a cell. The figure represents the environment that influences every cell in the body. This environment includes the chemical components of the microenvironment: the extracellular matrix, hormones, cyto/chemokines, nutrients, and gases. Physically, it is characterized by its geometry, the dynamics of respiration, and the removal of metabolic by-products. From these characterized observations, expanded details of each component will be discussed such that biological understandings precede the physical considerations. Finally, both biological and physical environments are combined to help integrate research and clinical activities—for the future development of tissue engineered products.

6.1.2 Cellular Therapies, Grafts, and Extracorporeal Bioartificial Organs

The development of cellular therapies initially arose from advancing knowledge within the cell and molecular biology science domains. Transferring these developments into the clinical arena is a design challenge that requires organized culture control and exploitation

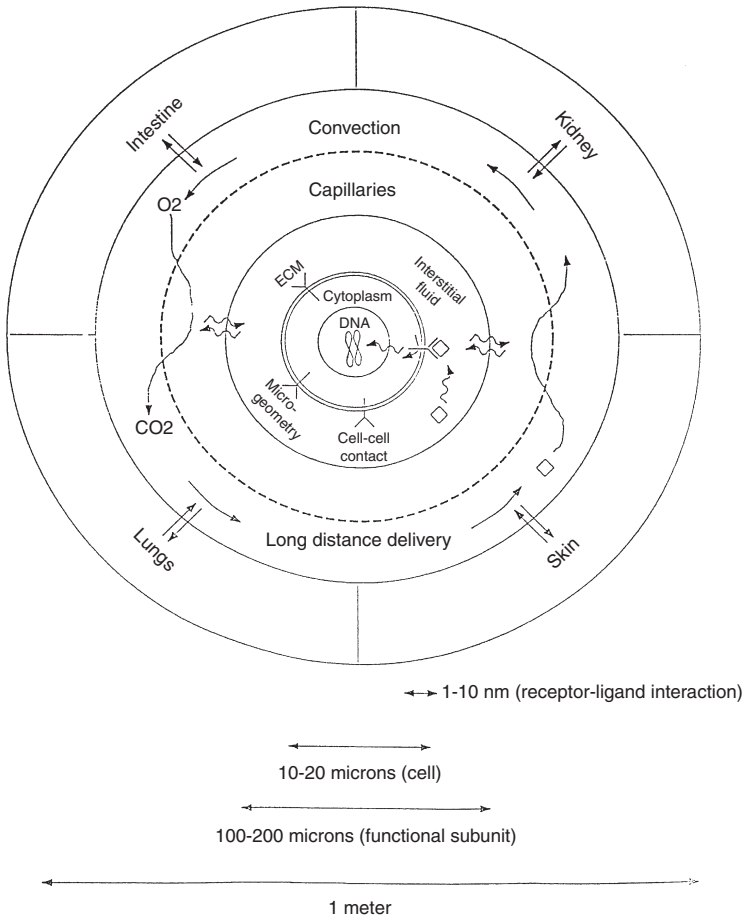


FIGURE 6.2 A cell and its communication with other body parts. *Modified from [11].*

of cell metabolites. For this reason, many scientific fields such as bioengineering, biochemical engineering, and biomaterial sciences are needed for the implementation of cell therapies. A significant challenge in tissue engineering is isolating and growing sufficient numbers of cells for device/therapy designs for clinical and commercial programs. As a historical example, it should be noted that the discovery of penicillin alone was not enough to affect the delivery of health care. Methods for the mass production of clinical grade material had to be developed. The development of such large-scale production of antibiotics arguably represents the most significant contribution of engineering to the delivery of health care. In a similar fashion, the development of industrial-scale methods for isolation, expansion, and cryopreservation of human cells will enable routine uses for cell therapies. In order for tissue engineering to have a tangible impact on modern medicine, the therapies that are developed must be both scientifically and commercially viable.

Tissues are comprised of multiple cell types that interact dynamically with each other. Therefore, tissue-specific functions are often observed only with cocultures of those multiple cell types or with cultures of a particular cell type in combination with the signals from the others. Those signals include insoluble factors in the extracellular matrix, signals from direct cell-cell contact, and soluble signals from autocrine, paracrine, and endocrine interactions. To use these signals as bioengineers, several basic concepts in cell biology need to be understood and quantitatively characterized. These include the key cellular processes of cell differentiation, hyperplastic and hypertrophic growth, migration (motion), protein synthesis, and death (necrosis or apoptosis), all of which combine to define tissue function. Basic information about stem cell and maturational lineage biology and the role of determined stem cells in organ function, genesis, and repair will be presented.

The creation of new engineered tissues requires that many bioengineering challenges be met. For example, bioengineering considerations in cell therapies include injection needle design and procedure protocols. For this application, needles must be optimized to reduce shear stress on cell membranes. Nutrient mass transfer must be analyzed to determine the range of cell aggregate sizes that can be sustained as viable tissues. Engraftment techniques and seed site selection criteria must be established so cells will prosper and assist in system homeostasis. Detrimental events, such as the formation of emboli, need to be prevented. For more complex implantable devices and bioreactors, other challenges will be faced. In these systems, the function, choice, manufacturing, and treatment of biomaterials are important for cell growth and device construction. Fluid mechanics and mass transfer play important roles in normal tissue function and therefore become critical issues in ex vivo cellular device designs. System analysis of metabolism, cell-cell communication, and other cellular processes can be used to define bioartificial organ specifications. A properly designed ex vivo culture system must appropriately balance the rates of biological and physicochemical processes to obtain desired tissue functions. By mathematically modeling this balancing effect, dimensionless parameter groups can be formulated that describe characteristic ratios of time constants. In this way, new dimensionless values will evolve to relate ratios of "physical times" with "biological times."

Finally, the implementation of cell therapies and tissue grafts in the clinic requires the recognition and resolution of several difficult issues. These include tissue harvest, cell processing and isolation, safety testing, cell activation/differentiation, assay and medium development, storage and stability, and quality assurance and quality control issues. These challenges will be described in this chapter but are not analyzed in detail.

6.1.3 Human Cells and Grafts as Therapeutic Agents

Cell therapies use human cells as therapeutic agents to alleviate a pathological condition. It is important to note that some cell therapies are already an established part of medical care. One existing type of cell therapy is blood transfusion, which has been practiced for decades with great therapeutic benefit. This therapy uses red blood cells (RBC) as the transplant product into anemic patients to help to restore adequate oxygen transport. Similarly, platelets have been transfused successfully into patients who have blood clotting problems. Bone marrow transplantation (BMT) has been practiced for almost two decades, with tens of thousands of cancer patients undergoing high-dose chemo- and radiotherapies followed by

BMT. More recently, transplantation of hemopoietic stem cells has occurred with increasing frequency to correct hematological disorders. These are all applications of cell therapies associated with blood cells and blood cell generation (hematopoiesis). (The term *hematopoiesis* comes from the Greek *hemato*, meaning "blood," and *poiesis*, meaning "generation of.") Therefore, a large population of patients already has benefitted from cell therapies, and this benefit can be extended by developing new therapies using other progenitor cell sources.

Transplants can be xenogeneic (donor and recipient are members of different species), allogeneic (donor and recipient are members of the same species but are not genetically identical), or syngeneic (donor and recipient are genetically identical—e.g., clones in the case of animals, or identical twins). Syngeneic transplants include autologous transplants (cells from a patient being isolated and given back to the same person). The issues associated with allogeneic transplants are well known because of the widespread use of organ transplantation and chiefly involve prevention of immune rejection as well as longer-term negative responses to transplanted tissues. However, with the advent of ex vivo cell culture and advances in cell manipulation procedures, autologous transplantation is becoming more common. In addition there are efforts under way in several laboratories to create "universal donor" cell sources and cell lines that would alleviate many of these issues.

The ability to reconstitute tissues ex vivo and produce cells in clinically meaningful numbers has broad implications. Table 6.1 summarizes the supply and demand of organs and tissues versus the number of procedures performed annually in the United States. Although the number of procedures is limited, the overall cost of these procedures was still estimated at a staggering $400 billion per year. The potential socioeconomic impact

TABLE 6.1 Incidence of Organ and Tissue Deficiencies, or the Number of Surgical Procedures Related to These Deficiencies in the United States[a]

Indicator	Procedure or Patients per Year
Skin	
Burns[b]	2,150,000
Pressure sores	150,000
Venous stasis ulcers	500,000
Diabetic ulcers	600,000
Neuromuscular disorders	200,000
Spinal cord and nerves	40,000
Bone	
Joint replacement	558,200
Bone graft	275,000
Internal fixation	480,000
Facial reconstruction	30,000

TABLE 6.1 Incidence of Organ and Tissue Deficiencies, or the Number of Surgical Procedures Related to These Deficiencies in the United States[a]—Cont'd

Indicator	Procedure or Patients per Year
Cartilage	
Patella resurfacing	216,000
Chondromalacia patellae	103,400
Meniscal repair	250,000
Arthritis (knee)	149,900
Arthritis (hip)	219,300
Fingers and small joints	179,000
Osteochondritis dissecans	14,500
Tendon repair	33,000
Ligament repair	90,000
Blood Vessels	
Heart	754,000
Large and small vessels	606,000
Liver	
Metabolic disorders	5,000
Liver cirrhosis	175,000
Liver cancer	25,000
Pancreas (diabetes)	728,000
Intestine	100,000
Kidney	600,000
Bladder	57,200
Ureter	30,000
Urethra	51,900
Hernia	290,000
Breast	261,000
Blood transfusions	18,000,000
Dental	10,000,000

[a]*From Langer and Vacanti (1993).*
[b]*Approximately 150,000 of these individuals are hospitalized and 10,000 die annually.*

of cellular therapies is therefore substantial. Progress in decreasing the costs of these therapies also will encourage investment into new types of treatments. In this way, new medical products will be developed to greatly improve the quality of life and productivity of affected individuals.

The concept of directly engineering tissues was pioneered by Y. C. Fung in 1985. The first symposium on this topic was organized by Richard Skalak and Fred Fox in 1988, and since then the field of tissue engineering has grown rapidly. Thousands of scholarly articles have been written on the topic, and in 1995 a peer-reviewed journal called *Tissue Engineering* was established. Since that time, the original journal has sprouted into three separate journals (*Part A: Primary Papers*, *Part B: Reviews*, and *Part C: Methods*) to more broadly cover the field, and numerous other journals in the topic area have emerged, including the *Journal of Tissue Engineering and Regenerative Medicine*. The field also has received considerable attention in the lay press because of the opportunities it presents to revolutionize medicine for an aging population.

The last two decades have seen remarkable advances in biology that have enabled tangible progress in tissue engineering. Cutting-edge cell therapies that have reached the advanced stages of development include various forms of immunotherapies, chondrocytes for cartilage repair, liver and kidney cells for extracorporeal support devices, β-islet cells for diabetes, skin cells for patients with ulcers or burns, and genetically modified myocytes for treatment of muscular dystrophy. In addition, engineered tissues such as blood vessels, bladders, urethras, and other tissues are rapidly moving toward the clinic. As would be expected based on tissue complexity, the challenges faced with each tissue are different. A few examples are provided in the following sections for illustrative purposes.

Bone Marrow Transplantation

Bone marrow is the body's most prolific organ. It produces on the order of 400 billion myeloid cells daily, all of which originate from a small number of pluripotent stem cells (Figure 6.3). The bone marrow is comprised of 500 to 1,000 billion cells and regenerates itself every two to three days, which represents normal hematopoietic function. Individuals under hematopoietic stress, such as systemic infection or sickle cell anemia, will have blood cell production rates that exceed the basal level. The prolific nature of bone marrow cells makes it especially susceptible to damage from radio- and chemotherapies. Bone marrow damage limits the extent of these therapies, and some regimens are fully myoablative. Without any hematopoietic support, patients who receive myoablative dose regimens will die due to hematopoietic failure.

Bone marrow transplantation (BMT) was developed to overcome this problem. In an autologous setting, the bone marrow is harvested from the patient prior to radio- and chemotherapies. It is cryopreserved during the time period that the patient undergoes treatment. After chemotherapeutic drug application and several half-lives of the drug have passed, the bone marrow is rapidly thawed and returned to the patient. The bone marrow cells are simply introduced into the circulation, and the bone marrow stem cells naturally "home" to the marrow cavity and reconstitute bone marrow function. In other words, the hematopoietic tissue is rebuilt in vivo by these cells. This process takes several weeks to complete, during which time the patient is immunocompromised.

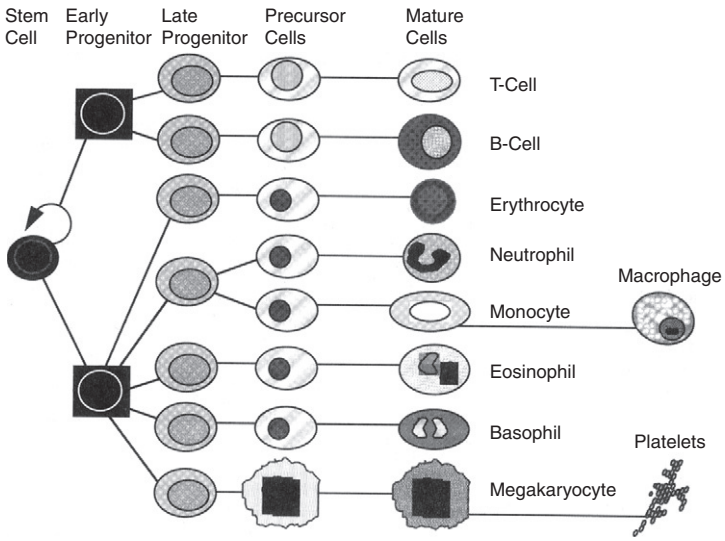


FIGURE 6.3 Hematopoietic cell production. The production fluxes through the lineages can be estimated based on the known steady-state concentration of cells in circulation, the total volume of blood, and the half-lives of the cells. Note that the 400 billion cells produced per day arise from a small number of stem cells. *From [8].*

Current forms of autologous BMT as a cellular therapy simply involve removing the cells from the patient and storing them temporarily outside the patient's body. There are several advantages to growing and increasing the number of harvested cells, and therefore newer therapies and treatments are being developed based on ex vivo culture of hematopoietic cells. In addition, new techniques to harvest bone marrow stem cells have been developed. These methods rely on using cytokines or cytotoxic agents to "mobilize" the stem cells into circulation. The hematopoietic stem and progenitor cells are then collected from the circulation using leukopheresis.

Myoablative regimens are used in allogeneic settings. In the case of leukemia, this not only removes the bone marrow but hopefully also the diseased tissue. The donor's cells migrate to the marrow and repopulate the bone cavity, just as in the autologous setting. The primary difficulty with allogeneic transplants is high mortality (10 to 15 percent), primarily due to Graft-versus-Host Disease, in which immune cells in the transplanted marrow recognize the recipient's tissues as foreign and mount an immunologic attack. Overcoming this rejection problem would significantly advance the use of allogeneic transplantation in BMT as well as other cell-based therapies.

BMT is a well-developed and accepted cellular therapy for a number of indications. These include allogeneic transplants for diseases such as leukemia and autologous transplants for diseases such as lymphoma and breast and testicular cancer. Significant growth has occurred in the use of BMT since the mid-1980s, and tens of thousands of patients are now treated using this family of therapies each year. Improvements continue to be made in tissue harvesting, processing, and transplantation, and these advances help to inform the field of tissue engineering as new cell-based therapies are developed.

Skin and Vascular Grafts

Skin is a highly proliferative and regenerative tissue. It consists of two main layers: the dermis, whose main cellular components are stromal cells or fibroblasts, and the epidermis, whose main cellular components are epidermal cells at various stages of differentiation into keratinocytes (Figure 6.4). Both cell types grow well in culture, and ex vivo cultivation is not the limiting factor with this tissue. Interestingly, transplanted dermal fibroblasts have proven to be surprisingly nonimmunogenic.

Skin transplants and engineered skin technologies have been applied to victims of burns and patients with diabetic ulcers who have severe problems with skin healing. To treat these problems, skin may be cultured ex vivo and applied to the affected areas. Technologically this cell therapy is relatively well developed, and currently there are several "engineered skin" products available, including Apligraf® (Organogenesis Inc.) and Dermagraft® (Advanced BioHealing Inc.). Figure 6.5 shows an expansion bioreactor for creating skin grafts from human foreskins developed by Advanced Tissue Sciences Inc., the now defunct company that originally developed Dermagraft®. The bioreactor is constructed with one mechanical hinge and two ports for constant "bleed-feed" flow (left panel). The graft products—which are similar to autologous skin grafts without hair follicles—are easily removed from the bioreactor, as shown in the right panel. In spite of these successes, important challenges remain in bringing such products to wide clinical use. The relatively high cost of the

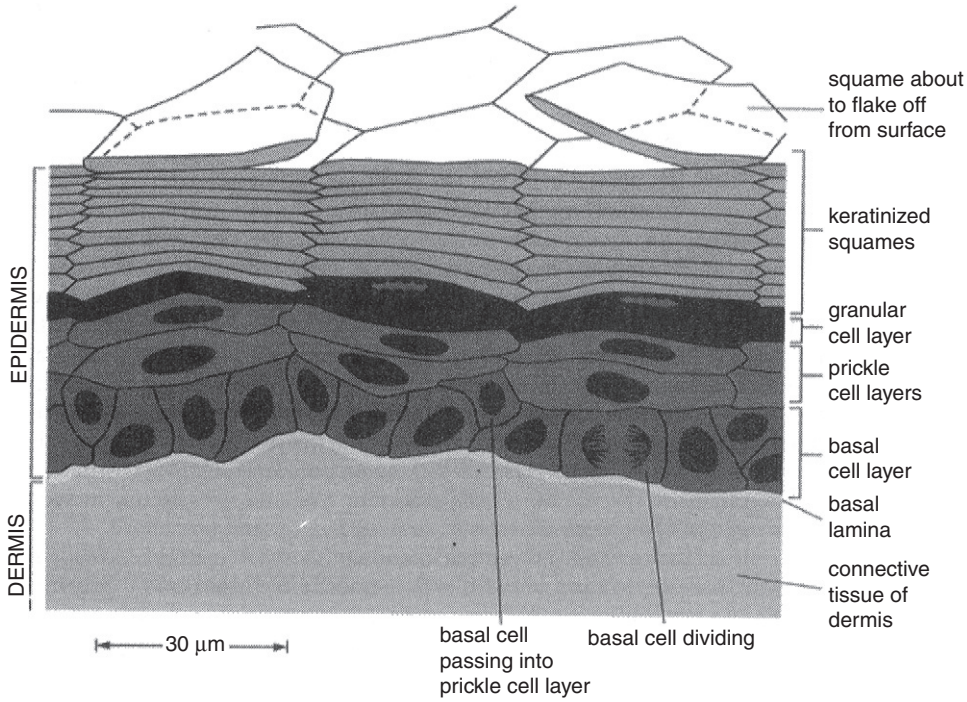


FIGURE 6.4 The cellular arrangement and differentiation in skin. The cross section of skin and the cellular arrangement in the epidermis and the differentiation stages that the cells undergo. *From [1].*

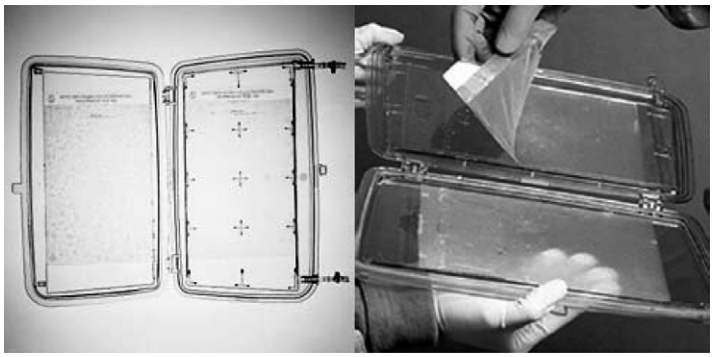


FIGURE 6.5 Advanced Tissue Sciences bioreactor for culture of their skin product, Trancyte, derived from human foreskins.

extensive testing, processing, and quality control that is required for such allogeneic products are a significant barrier to adoption for procedures for which effective therapies already exist. However, these products have introduced tissue engineering technologies to the clinic and have paved the way for future products.

Pancreatic β-Cells

Insulin-dependent diabetes mellitus is characterized by the inability of the pancreas to produce and secrete insulin, a hormone required for normal glucose metabolism. Pancreata derived from cadaveric donors can be used as sources of islets of Langerhans, which contain the insulin-secreting β-islet cells. These cells can be injected into the portal vein leading to the liver, where they lodge and secrete life-sustaining insulin. Unfortunately, at present it is not possible to expand the number of β-cells in culture without losing their essential properties, and thus this procedure is constrained by the severely limited supply of tissue. However, recently there has been exciting progress in the derivation of insulin-secreting β-cells from stem cells. This form of cellular therapy is an allogeneic transplantation procedure, and the duration of the graft can be prolonged by immunosuppressing the recipient.

The immune rejection problem is an important concern in cellular therapies and is treated in a separate section following. However, ways to overcome rejection include the physical separation of the donor's cells from the immune system by a method that allows exchange between the graft and the host across a semipermeable membrane (Figure 6.6). This approach is the basis for many of the encapsulation strategies that have been used to improve the efficacy of islet transplantation. In addition, there have been many efforts to develop "artificial pancreas" devices that allow cells to secrete insulin while being protected from the host's immune system.

Cartilage and Chondrocytes

Cartilage is an unusual tissue in that it is avascular, alymphatic, and aneural. It consists mostly of extracellular matrix in which chondrocytes are dispersed at low densities on the order of 1 million cells/cc (whereas most tissues contain several hundred million cells/cc).

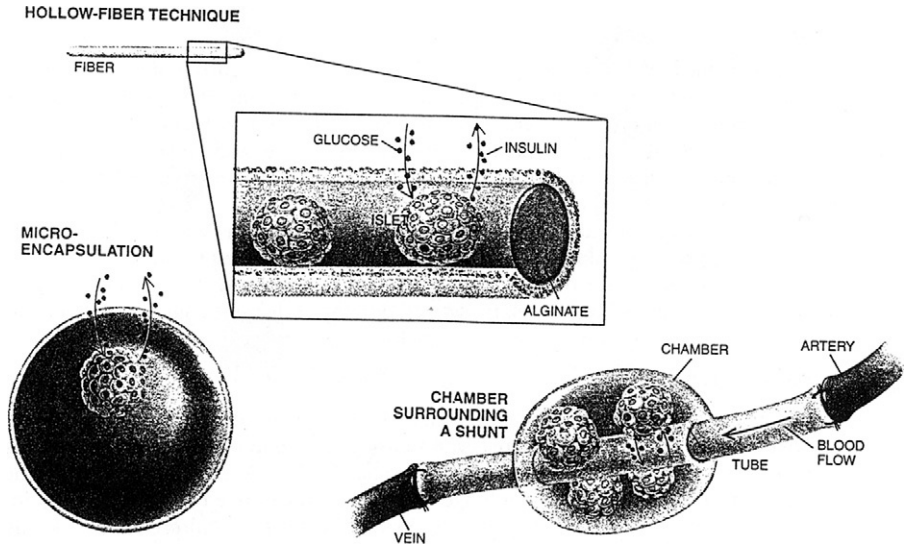


FIGURE 6.6 Encapsulation of islets in semiporous plastic is one promising way to protect them from attack by the immune system. *From Lacey, 2001.*

Chondrocytes can be cultured ex vivo to increase their numbers by up to tenfold. Using this technique, deep cartilage defects in the knee can be treated by autologous cell transplantation. In this case, a biopsy is collected from the patient's knee outside the affected area (Figure 6.7) and the chondrocytes are liberated from the matrix by an enzymatic treatment. The cells are allowed to grow in a two-stage cell ex vivo culture process to increase their number. The cells are then harvested and reintroduced into the affected area within the knee joint. This type of therapy has been shown to aid in healing of cartilage defects and is the basis of the Carticel® cell-therapy product (Genzyme Inc.). Numerous patients are afflicted with knee problems that effectively leave them immobile. It is estimated that over 200,000 patients are candidates for this type of cellular therapy annually in the United States alone.

Hepatic, Neural, and Cardiovascular Cell Therapies

Other forms of cell therapies are being developed to treat complex tissues such as the liver, brain, and heart. It is difficult to obtain and culture the main cell types of these complex tissues, and therefore progenitor and stem cell–based approaches are being actively investigated. Clinical trials for each of these tissues have been initiated using both adult mesenchymal stem cells as well as tissue-specific stem cells. The derivation of liver cells from stem cells is of interest both to treat hepatic failure, as well as to produce improved in vitro screens for drugs. Bone marrow and stem cell transplantation into the heart to treat ischemia and prevent the progression to heart failure has shown promise in the clinic, but there are still many important issues that need to be resolved for such procedures to be used widely. Cell transplantation to the brain also has been attempted clinically with mixed results. Recently, Geron Corporation was given clearance to start the first human clinical trial of

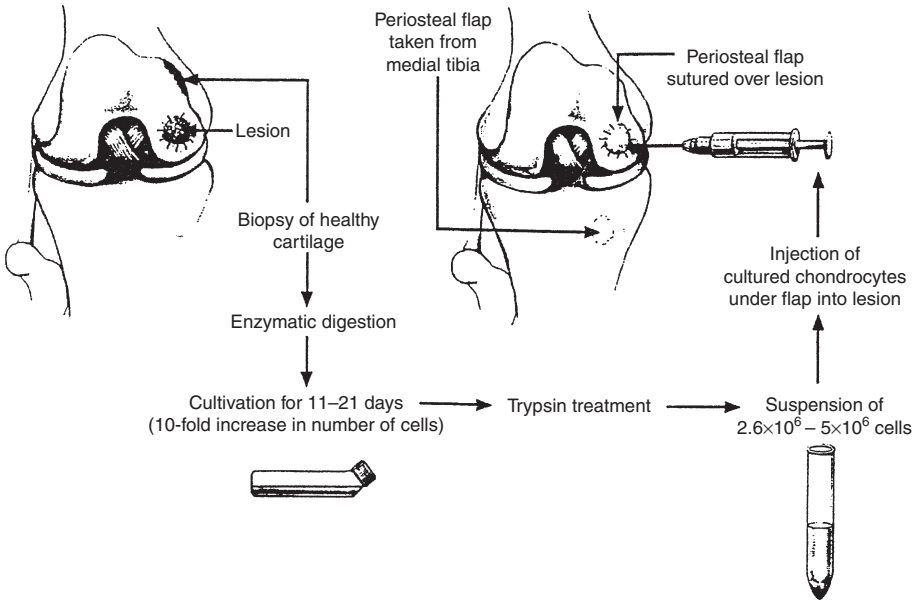


FIGURE 6.7 Chondrocyte transplantation in the right femoral condyle. *From [2].*

embryonic stem cells to treat acute spinal cord injury. These therapies, while exciting and of high potential, are still at a very early stage of development. From the tissue engineering perspective, there are still many unanswered questions regarding the most appropriate cell sources, mass transfer limitations, delivery methods, control of the microenvironment, integration with the host, and provision of an acceptable functional outcome.

6.1.4 Mechanisms Governing Tissues

Normal tissue functions can be used to define the engineering specifications of tissues created in the lab. In addition, our understanding of biology can be used to control cell function to generate tissues. Two important mechanisms that govern tissue dynamics are the relationship between epithelial cells and mesenchymal cells, and stem cell and maturational lineage biology. An understanding of these biological processes can inform the design of engineered tissues. For example, the smallest physiological unit defines mass transfer dimensions, while the number of cells required to replace the desired physiological functions defines the overall dimensions of an engineered product. These numbers help set overall design specifications and goals of the clinical device or engineered tissue.

The Epithelial-Mesenchymal Relationship

A fundamental paradigm defining tissues is the epithelial-mesenchymal relationship, since many tissues are composed of a layer of epithelial cells bound onto a layer of mesenchymal cells (e.g., fibroblasts, smooth muscle cells, etc.), as shown in Figure 6.8. Normal

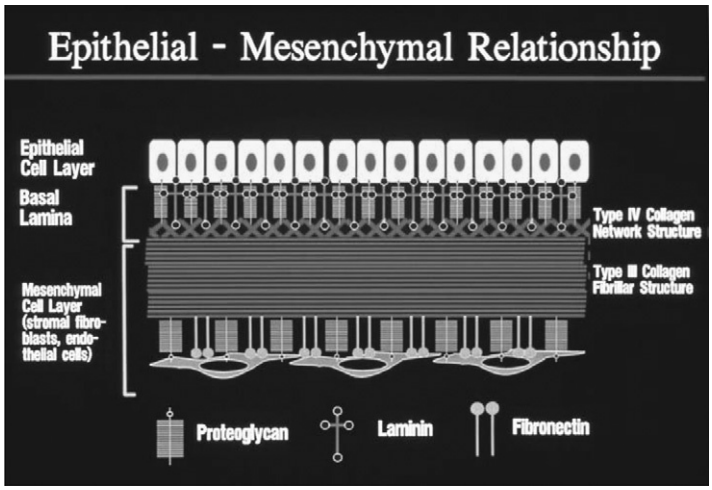


FIGURE 6.8 The epithelial–mesenchymal relationship.

epithelial cells require constant interaction with an appropriate mesenchymal partner or with matrix and soluble signals mimicking that relationship. Communication between and within the two cell layers coordinates local cell activities and is effected by soluble signals (autocrine, paracrine, and endocrine factors) working synergistically with extracellular matrix.

The Soluble Signals

Dissolved growth factors, hormones, and small molecules are often characterized in the following way:

- *Autocrine* factors are factors produced by cells that then act on those same cells.
- *Paracrine* factors are factors produced by cells that then act on neighboring cells in the same tissue.
- *Endocrine* or *systemic* factors are factors produced by cells that are then carried through the blood or lymphatic fluid to target cells in a distant tissue.

There are multiple large families of factors that operate as autocrine and paracrine signals. All of them typically can act as mitogens (i.e., elicit growth responses from cells) or can drive differentiation (i.e., induce expression of specialized tissue-specific functions), depending on the characteristics of the extracellular matrix associated with the cells. Some of the most well studied of these factors include the insulin-like growth factors (IGF), epidermal growth factors (EGF), fibroblast growth factors (FGF), colony stimulating factors (CSF), platelet derived growth factors (PDGF), transforming growth factors (TGF), and cytokines such as interleukins. It should be noted that these molecules are highly pleiotropic and can produce multiple effects on many cell types. The name "growth factor" comes from the fact that most were initially identified by assays in which a growth response was being examined.

The Extracellular Matrix

The extracellular matrix (ECM) is the material that underlies or surrounds cells in tissues. The ECM is diverse and abundant in most tissues and comprises up to half the proteins in the body. Indeed, the collagen family of ECM proteins contains over 25 members and accounts for 2 to 30 percent of the proteins in the body. For many years, the ECM was thought to play an entirely mechanical role binding together cells in specific arrays. Over the last 30 years, we have learned that the ECM is a major regulator of cell and tissue function. In addition to mechanical structure, it provides direct biochemical signals to cells in tissues and also acts as a regulator of many of the soluble growth factors in tissues.

In epithelial cells the ECM between homotypic cells can be referred to as the "lateral" ECM, while the proteins underlying the cells (and separating them from underlying mesenchymal cells) are often referred to as the basal matrix or "basement membrane." Two of the primary components of the lateral extracellular matrix are: cell adhesion molecules, or "CAMs," which are age- and tissue-specific, and proteoglycans, molecules containing a protein core to which polymers of sulfated (negatively charged) sugars called glycosaminoglycans (e.g., heparan sulfates, heparins, chrondroitin sulfates, or dermatan sulfates) are attached. The basal extracellular matrix consists of basal adhesion molecules (e.g., laminins or fibronectins) that bind the cells via matrix receptors (integrins) to one or more types of collagen scaffoldings. The collagens of one cell layer are cross-linked to those of the adjacent cell layer to provide stable coupling between the layers of cells. In addition, proteoglycans are bound to the basal adhesion molecules, to the collagens, and/or to the basal cell surface.

The lateral and basal extracellular matrix components of epithelial tissues provide direct signaling to cells in the form of chronic or persistent signaling. Indirectly, the components also facilitate signaling by stabilizing cells in appropriate configurations of ion channels, receptors, antigens, and so on; by influencing intracellular pathways; and by inducing appropriate cell shapes (flattened or three-dimensional). These interactions enable the cells to respond rapidly to soluble signals that may derive from local or distant sources. The soluble factors act by binding to high-affinity molecules called receptors, which can be on the cell surface or present in the interior of the cell. When the signal binds to its receptor, a signal initiation response is triggered.

Cell Numbers In Vivo and Orders of Magnitude

The cell densities in human tissues are on the order of 1 billion to 3 billion cells/ml. The volume of a 70 kg human is about 70,000 ml. Therefore, the human body consists of about 100 trillion (trillion $= 10^{12}$) cells. The volume of a "typical" organ is around 100 to 500 ml, and therefore a typical organ contains about 100 to 1,500 billion (10^9) cells. Organs are comprised of functional subunits. Their typical linear dimensions are approximately 100 μm. The cell number in a cube that is 100 μm on each side is estimated to be about 500 to 1,000. These cell numbers are summarized in Table 6.2.

Based on these estimations, a typical organ will have a few hundred million functional subunits, each of which is quite small. This number is dictated by the capability of each subunit and the overall physiological need for its particular function. For example, the

TABLE 6.2 Cell Numbers in Tissue Biology and Tissue Engineering: Orders of Magnitude

Cell numbers *in vivo*	
Whole body	10^{14}
Human organ	10^{9}–10^{11}
Functional subunit	10^{2}–10^{3}
Cell production *in vivo*	
Theoretical maximum from a single cell (Hayflick limit)	$2^{30–50} < 10^{15}$
Myeloid blood cells produced over a lifetime	10^{16}
Small intestine epithelial cells produced over a lifetime	5×10^{14}
Cell production *ex vivo*	
Requirements for a typical cellular therapy	10^{7}–10^{9}
Expansion potential[a] of human tissues	
Hematopoietic cells	
Mononuclear cells	10-fold
CD34 enriched	100-fold
Two or three antigen enrichment	10^{6}- to 10^{7}-fold
T cells	10^{3}- to 10^{4}-fold
Chondrocytes	10- to 20-fold
Muscle, dermal fibroblasts	$>10^{6}$-fold

[a]*Expansion potential refers to the number of cells that can be generated from a single cell in culture.*

number of nephrons in the kidney is determined by the maximal clearance need of toxic by-products and the clearance capability that each nephron possesses. These estimations provide insight into tissue structure and function. The fundamental functional subunit of most tissues contains only a few hundred cells, and in most cases this is a mixed-cell population, since most organs have accessory cells that can be as much as 30 percent of the total cell number. Further, as illustrated following, the tissue-type-specific cells may be present at many stages of differentiation.

The nature of tissue microenvironments, along with cellular dynamics, communication, and metabolic processes, must be understood in order to reconstitute tissue function accurately. In addition, generating a therapeutic dose of cells requires a large number of microenvironments. These microenvironments must be relatively similar to have all the functional subunits perform in comparable fashions. Therefore, the design of cell culture devices must provide uniformity in supporting factors, such as nutrient, oxygen, and growth factor/hormone concentrations. These inputs must be reasonably homogeneous down to 100 μm distances. Below this size scale, nonuniformity would be expected and in fact needed for proper functioning of tissue function subunits.

6.1.5 Clinical Considerations: Important Questions

What are clinically meaningful numbers of cells?

Currently, the cells estimated to be needed for clinical practice and experimental cell therapy protocols fall into the range of "a few tens of millions" to "a few billion" (Table 6.2). Since tissue-like cultures require densities above 10 million cells per milliliter, the sizes of the cell culture devices for cell therapies appear to fall into the range of 10 to a few hundred milliliters in volume.

What are the fundamental limitations to the production of normal cells?

The number of divisions a cell can undergo is dependent on its maturational lineage stage, with the key stages being (1) stem cells (diploid, pluripotent), (2) diploid somatic cell subpopulations (unipotent), and (3) polyploid cell subpopulations. Stem cells can self-replicate and undergo unlimited numbers of divisions (stem cells are also a potential source of immortalized cell lines and of tumor cells). Diploid cells are limited in the number of times they can divide and are subject to the so-called Hayflick limit. Normal, somatic, diploid human cells can undergo about 30 to 50 doublings in culture. Therefore, a single diploid cell can theoretically produce 10^{10} to 10^{15} cells in culture. Given the requirements for cell therapies, the Hayflick limit is not a major issue for cell therapies that utilize diploid cells, either stem or somatic phenotypes. However, the expansion potential is minimal or negligible for subpopulations of polyploid cells, which are found in all tissues. Conversely, expansion potential is very high in various quiescent tissues such as the liver and heart. Regenerative stimuli can cause polyploid cells to undergo DNA synthesis with negligible capability to undergo cytokinesis. This results in an increase in their level of ploidy, an increase in cell volume, and a phenomenon called hypertrophy. Thus, the regeneration of tissues is a combination of hyperplasia (the diploid subpopulations) and of hypertrophy (the polyploid subpopulations) activities.

How rapidly do normal cells grow in culture?

Normal cells vary greatly in their growth rates in culture. Hematopoietic progenitors have been estimated to have 11- to 12-hour doubling times, which represent the minimum cycle time known for adult human cells. Dermal foreskin fibroblasts grow with doubling times of 15 hours, a fairly rapid rate that may be partially attributable to the fact that they are isolated from neonatal tissue containing a high proportion of stem/progenitor cells. In contrast, adult chondrocytes grow slowly in culture, with doubling times of about 24 to 48 hours.

How are these cells currently produced?

Expansion of cells can be performed in a variety of culture containers (plates, flasks, roller bottles, bags), and in some cases also in suspension cultures (e.g., certain hemopoietic cells or cell lines). Novel scaffolding material is permitting ex vivo expansion of cell types that previously have proved difficult to culture. The ability to maximize expansion potential for specific cell types requires precise culture conditions. These conditions may comprise specific forms of extracellular matrix, defined mixtures of hormones and growth

factors, nutritional supplements, and basal media containing specific concentrations of calcium, trace elements, and gases. Reviews of these conditions for many cell types are available in recently published textbooks and journal articles.

6.2 BIOLOGICAL CONSIDERATIONS

6.2.1 Stem Cells

It is now thought that essentially all tissues contain stem cell populations that can produce cellular progeny that differentiate into mature tissue phenotypes. The maturational process includes two branches: the "commitment" branch, in which pluripotent stem cells produce daughter cells with restricted genetic potentials appropriate for a single set of cell activities (unipotent), and the "differentiation" branch, in which sets of genes are activated and/or altered in their levels of expression. The following sections discuss how these major tissue subdivisions work together to generate or repair tissues in order to provide tissue functions.

Stem Cells and the "Niche" Hypothesis

Pluripotent stem cells are cells that are capable of producing daughter cells with more than one fate; they can self-replicate, and they have the ability to produce daughter cells identical to the parent. Totipotent stem cells are cells that can generate all the cell types of the organism. Determined stem cells are cells in which the genetic potential is restricted to a subset of possible fates; they can produce some, but not all, of the cell types in the organism.

Determined stem cells of the skin can produce all the cell types in the skin but not those of the heart. Similarly, determined stem cells of the liver can produce all liver cell types but not brain. The lay press often refers to determined stem cells as "adult stem cells," which is a misnomer because determined stem cells are present in fetal and adult tissue. The determined stem cells give rise to unipotent progenitors, also called committed progenitors, with genetic potential restricted to only one fate. These unipotent progenitors rapidly proliferate into large numbers of cells that then differentiate to mature cells. The stem cells and the unipotent progenitors are the normal counterparts to tumor cells and to immortalized cell lines. Determined stem cells identified to date are small in size (typical diameters of 6–10 μm), have a high nucleus to cytoplasmic ratio (blast-like cells), and express certain early genes (e.g., alpha-fetoprotein) and antigens (e.g., CD34, CD117). They have chromatin that binds particular dyes at levels lower than that of the chromatin in mature cells, enabling them to be isolated as "side-pocket" cells using flow cytometric technologies. Stem cells express an enzyme, telomerase, that maintains the telomeres of their chromosomes at constant length, a factor in their ability to divide indefinitely in vivo and ex vivo. Multiple parameters must be used to permit isolation and purification of any determined stem cell type, since there is no one parameter (antigen, size, cell density) sufficient to define any determined stem cells. Furthermore, they appear to grow very slowly in vivo and may commit to growth and differentiation in a stochastic manner. A first-order rate constant for hemopoietic stem cells is about one day and their cycling times have been measured

by means of time-lapse videography. They commit to differentiation in culture. The first and second doubling take about 60 hours, and then the cycling rate speeds up to about 24 hours cycling time. By the fifth and sixth doubling, they are dividing at a maximal rate of 12 to 14 hours doubling time.

What evidence is there that stem cells exist?

Lethally irradiated mice that would otherwise die from complete hematopoietic failure can be rescued with as few as 20 selected stem cells. These animals reconstitute the multiple lineages of hematopoiesis as predicted by the stem cell model. In sublethally irradiated animals, genetically marked mesenchymal stem cells found in bone marrow will give rise to cells in multiple organs over a long time period. These investigations and many others have established conclusively the presence of stem cells, their multilineage potential, and their ability to persist over long periods of time in vivo.

Stem Cell Niches

The field of stem cell niches is rapidly expanding due to its importance in regulating stem cell fate. The goal is to define and understand the local microenvironment of the stem cell compartment. The field is still new but already has yielded generalizations that are proving to be useful guides for defining ex vivo expansion conditions for the cells:

- Stem cells do not have the enzymatic machinery to generate all their lipid derivatives from single lipid sources and so require complex mixtures of lipids for survival and functioning.
- Calcium concentrations are quite critical in defining whether stem cells will expand or undergo differentiation. The mechanisms underlying the phenomenology are poorly understood.
- Specific trace elements, such as copper, can cause more rapid differentiation of some determined stem cell types. It is unknown whether this applies to all stem cells, and the mechanism(s) is not known.
- Specific mixtures of hormones and growth factors are required, with the most common requirements being insulin and transferrin/Fe. Addition of other factors can result in expansion of committed progenitors and/or lineage restriction of the stem cells toward specific fates.
- The matrix chemistry of known stem cell compartments consists of age-specific and cell-type-specific cell adhesion molecules, laminins, embryonic collagens (e.g., type III and IV collagen), hyaluronans, and certain embryonic/fetal proteoglycans. With maturation of the stem cells toward specific cell fates, the matrix chemistry changes in a gradient fashion toward one typical for the mature cells. Although the matrix chemistry of the mature cells is unique for each cell type, a general pattern is the inclusion of adult-specific cell adhesion molecules, various fibrillar collagens (e.g., type I, II collagen), fibronectins, and adult-specific proteoglycans. A major variant is that for skin and neuronal cells, in which mature cells lose expression of collagens, fibronectins, and laminin. Additionally, the matrix chemistry of these cell types is dominated by CAMs and proteoglycans.

- Stem cells are dependent on signals from age- and tissue-specific stroma. The signals from the stroma are only partially defined but include signals such as Leukemia Inhibitory Factor (LIF), various fibroblast growth factors or FGFs, and various interleukins (e.g., IL 8, IL11).
- The most poorly understood of all the signals defining the stem cell niche(s) are those from feedback loops, which are initiated from mature cells, and where these signals inhibit stem cell proliferation. Implicit evidence for feedback loops is that cell expansion ex vivo requires separation between cells capable of cell division (the diploid subpopulations) and the mature nonproliferating cells.

Which tissues have stem cells?

For decades it was assumed that stem cell compartments exist only in the rapidly proliferating tissues such as skin, bone marrow, and intestine. Now, there is increasing evidence that essentially all tissues have stem cell compartments, even the central nervous system. There are now numerous reports of the isolation and characterization of tissue-specific stem cells, and they are actively being investigated as a potential cell source for tissue engineering.

The Roles of Stem Cells

The stem cell compartment of a tissue is the fundamental source of cells for turnover and regenerative processes. Stem cell commitment initiates cell replacement and genesis of the tissue, resulting in tissue repair and maintenance of tissue functions. Stem cell depletion due to disease or toxic influences (e.g., drugs) eventually leads to partial or complete loss of organ function. Mutational events affecting the stem cells can result in tumors for which both altered and normal stem cells are actively present. Thus, tumors are now considered transformed stem cells, an idea originally proposed by Van Potter, Sell, and Pierce, and now confirmed by current stem cell biologists.

6.2.2 The Maturational Lineages of Tissues

All stem cells are pluripotent, giving rise to multiple, distinct lineages of daughter cells that differentiate, stepwise, into all of the mature cells of the tissue. A general model for the production of mature cells arising from tissue-specific stem cells is shown in Figure 6.9. Determined stem cells (pluripotent) replicate slowly in vivo, with rates influenced by various systemic signals. Their immediate descendants are committed progenitors (unipotent) capable of rapid proliferation and shown in some tissues (e.g., skin) to be the acute responders to mild to moderate regenerative stimuli. The unipotent progenitors mature in a stepwise fashion through intermediate stages into fully mature cells. Characteristically, various tissue-specific functions are expressed in cells throughout the maturational lineage and in a lineage-dependent fashion. One can generalize about these gradual phenotypic changes by categorizing the functions as “early,” “intermediate,” and “late” taskings. In some cases, a specific gene is expressed uniquely only at a specific stage. In others, there are isoforms of genes that are expressed in a pattern along the maturational lineage, while in yet others there are changes in the levels of expression of the gene. Finally, the cells progress to senescence, a phenomena that typifys aging cells. The maturation of the cells is dictated in part by mechanisms inherent in the cells (e.g., changes in the chromatin)

	Stem Cell	Early Progenitor	Late Progenitor	Precursor Cell	Mature Cell
Cell Number	Potential 2^{30} to 2^{50} per cell				Need About 10^{16} total over lifetime
Cell Cycling	Very slow (t_d ~ 1/6wks.)	Slow (t_d~60–100 hrs.)	Very rapid (t_d ~ 12 hrs.)	Slow	Zero (can be activated in special cases)
Apoptosis	Inactive	Inactive	Very Active (1:5,000 survives)	Slow	Inactive (can be induced)
Motility	Zero (except during homing)	Zero	Low	Higher	Function of Physiological State
Regulation	Cell-Cell Contact	Cell-Cell Contact	Soluble Growth Factors	Soluble Growth Factors	Soluble Growth Factors

FIGURE 6.9 Model for cell production in prolific tissues. This model was derived from decades-long research in hematology. The columns represent increasingly differentiated cells, while the rows indicate the cellular fate processes (Figure 16.4) and other events that cells undergo at different states of differentiation. (t_d denotes doubling time.)

and in others by matrix and/or soluble signals in their microenvironment. The microenvironment can include signaling affecting growth, differentiation, or apoptosis. For example, some growth factors are survival factors with antiapoptotic effects.

Examples of Stem Cell–Fed Maturational Lineages

The stem cell models best characterized are the hemopoietic stem cells, the intestinal stem cells, and the skin stem cells. In addition, bone marrow–derived mesenchymal stem cells and stem cells of the liver are being increasingly investigated. These systems are described further in the following sections.

Bone Marrow and Blood Cell Formation

Hematopoiesis was the first tissue function for which a stem cell model was established (see Figure 6.3). The reconstitution of the multiple lineages of hematopoietic cells following a stem cell transplant in mice has been demonstrated to occur with a surprisingly small number of cells. Once the cells have reached certain maturational stages, they leave the bone marrow and enter the circulation, where they perform their mature cell functions. The mature cells eventually die and must be replaced. The rate of death of mature cells (by apoptosis or necrosis) sets the need for the cell production rate in the tissue. Ultimately, this death rate determines the number of stem cell commitments that are required. The specific hematopoietic lineage cell production is given in Figure 6.3.

The Villi in the Small Intestine

The lining of the small intestine is comprised of villi that absorb nutrients, as shown in Figure 6.10. The intestinal epithelial cell layer is highly dynamic. Its cellular content turns over approximately every five days and is a very prolific tissue. In between the villi are tube-shaped epithelial infoldings, known as crypts. All intestinal epithelial cell production

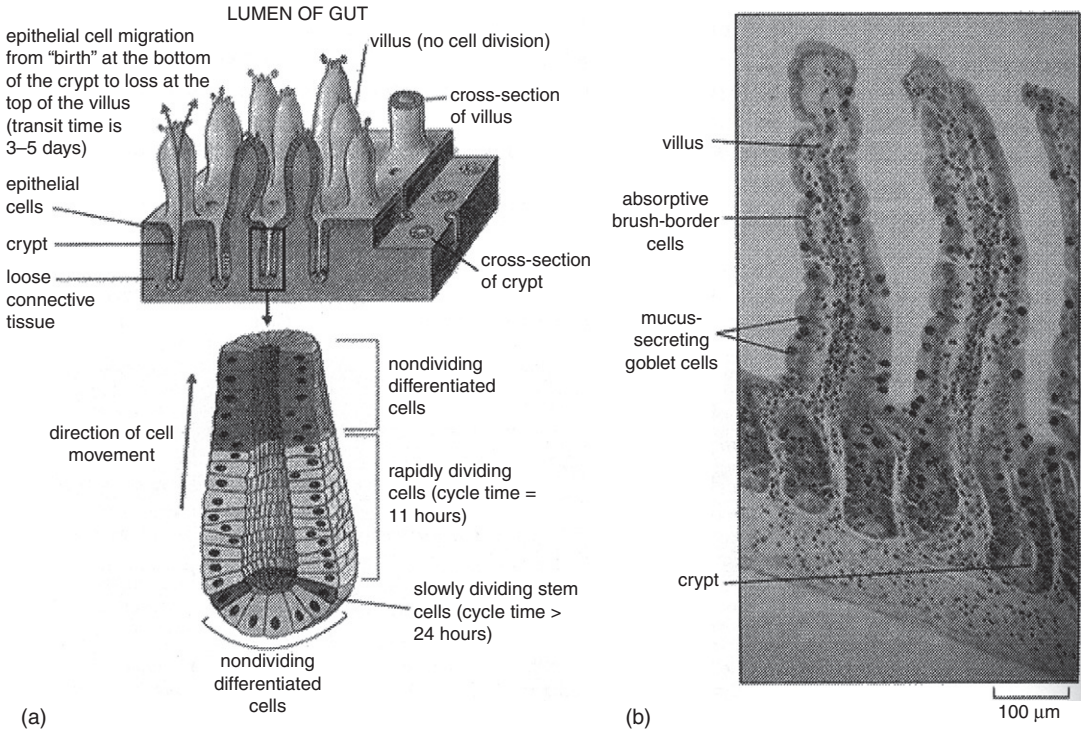


FIGURE 6.10 Villi in the small intestine. (a) The villi and the crypt indicating the mitotic state of the cells in various locations. (b) Rows of villi of epithelial intestinal cells (the diameter of a villi is about 80 mm). *From [1].*

takes place in set locations within the crypt. Once the cells are mature, they migrate to the outer edge of the crypt and then move over a period of about five days from the base of the villus to the top, where they die and slough off. During this passage, the cells carry out their organ-specific function as mature parenchymal cells. They function in the absorption and digestion of nutrients that come from the lumen of the gut. Toward the bottom of the crypt is a ring of "slowly dividing" determined stem cells. The number of stem cells per crypt is about 20. After division, the daughter cell moves up the crypt, where it becomes a rapidly cycling progenitor cell, with a cycling time on the order of 12 hours. The cells that are produced move up the crypt and differentiate. Once they leave the crypt, they are mature and enter the base of the villi.

Skin

Human skin has two principal cell layers, an epidermis and dermis, separated by a form of extracellular matrix called a basal lamina (or basement membrane) (see Figure 6.4). In this extracellular matrix, collagen VII is an important component. The two layers undulate with respect to each other, and the undulations produce deep pockets (distant from the skin surface) and other pockets that are shallower. The pattern of the relationship between layers is unique for each type of skin (e.g., that on the trunk of the body versus that in the palms of

the hand versus that on the face). The dermis is a connective tissue layer under the basal lamina and is comprised primarily of fibroblasts (also called stroma). The epidermis consists of multiple layers of epithelia comprised of differentiating keratinocytes, with the least differentiated cells located at the basal lamina. Thin skin has a squamous columnar organization (each column about 30 μm in diameter). A stem cell compartment has been identified in the deep pockets within the epidermis/dermis undulations and also in bulges near hair follicles. The stem cells produce committed progenitors, also called "transit amplifying cells," which migrate into the shallow pockets and from there into cells that line the entire basal lamina. Only epidermal cells adherent to the basal lamina are cycling, while cells that lose their attachment to the basal lamina move upward and differentiate into succeeding stages of cells. Ultimately, they turn into granular cells, and then into keratinized squames that eventually flake off. Keratins are proteins defining the differentiated cells of the skin and are evident in many forms—from forms that provide skin mechanical protection to those that are present in body hair (or in feathers in birds or scales in other vertebrates). The net proliferative rate of skin depends on the region of the body. In particular the turnover of skin is on the order of a few weeks.

The Liver

The liver is a maturational lineage system, including a stem cell compartment, that is analogous to those in the bone marrow, skin, and gut. The liver's lineage is organized physically within the acinus, the structural and functional unit of the liver. In cross section, the acinus is organized like a wheel around two distinct vascular beds: six sets of portal triads, each with a portal venule, hepatic arteriole, and a bile duct, form the periphery, and the central vein forms the hub. The parenchyma, effectively the "spokes" of the wheel, consist of plates of cells lined on both sides by the fenestrated sinusoidal endothelium. By convention, the liver is demarcated into three zones: zone 1 is periportal; zone 2 is midacinar; and zone 3 is pericentral. Blood flows from the portal venules and hepatic arterioles at the portal triads, through sinusoids that line plates of parenchyma, to the terminal hepatic venules and into the central vein. The stem cell compartment is present around the portal triads, zone 1, and identified in anatomical entities called Canals of Hering. The stem cells of the liver are at least bipotent and produce daughter cells that become either biliary cells (bile duct epithelia) or hepatocytes. Hepatocytes display marked morphologic, biochemical, and functional heterogeneity based on their zonal location. The size of hepatocytes increases from zone 1 to zone 3, and one can observe distinctive zonal variations in morphological features of the cells, such as mitochondria, endoplasmic reticulum, and glycogen granules. Hepatocytes show dramatic differences in DNA content from zone 1 to zone 3, with periportal cells being diploid and midacinar to pericentral cells shifting toward polyploid distinctions. Octaploid cells in the pericentral zone show evidence of apoptosis. Adult rodent livers (rats and mice) are 90 to 95 percent polyploid; adult human livers are 40 to 50 percent polyploid, whereas fetal and neonatal liver cells are entirely diploid. The transition to adult ploidy patterns is observed by age 3 to 4 weeks in rats and mice, and by late teenage years in humans. With age, the liver becomes increasingly polyploid in all mammalian species surveyed. This may help to explain the reduction in regenerative capacity of the liver with age.

6.2.3 Models for Stem Cell Proliferative Behavior

It should be clear by now that the replication functions of stem cells are critical to tissue function and tissue engineering. How do stem cells divide, and what happens when they divide? Three models describe the dynamic behavior of the stem cell population.

The Clonal Succession Concept

Cellular systems are maintained by a reservoir of cells that either grows very slowly or may be in a dormant state. In these cases, the reservoir of cells is available throughout the tissue's lifespan and can be routinely challenged to enter the complex process of cell proliferation and differentiation. Once triggered, such a stem cell would give rise to a large clonal population of mature cells. Any one of these clones would now have a limited life span, since feedback signals dissipate the need to maintain cell production fluxes. After time, such a clone will "burn out," and, if needed, a new stem cell clone would take over the cell production role.

Deterministic Self-Maintenance and Self-Renewal

This model relies on an assumption that stem cells can self-replicate. Following a stem cell division, there is a 50 percent probability that one of the daughter cells maintains the stem cell characteristics, while the other undergoes differentiation. The probability of self-renewal is regulated and may not be exactly 50 percent, depending on the dynamic state of the tissue.

Stochastic Models

This model considers that the progeny of a stem cell division can generate zero, one, or two stem cells as daughter cells (notice that the clonal succession model assumes zero, and the deterministic model assumes one). The assumption is that each of the three outcomes has a particular property.

6.2.4 Stem Cells and Tissue Engineering

Stem Cells Build Tissues

Stem cells are the source of the cells that make up all tissues in the body during development. In addition, certain tissues in adults have reservoirs of resident stem cells that can be mobilized to repair damage when needed. Thus, stem cells build and maintain tissues in vivo and are of great interest because of their potential use to also generate tissue ex vivo. The use of stem cells in tissue engineering has rapidly gained momentum because of their very high proliferative capacity and ability to differentiate into multiple cell types.

Ex Vivo Growth and Manipulation of Stem Cells

The classical definition of a stem cell holds that it has both the ability to divide into a new stem cell without differentiating and to differentiate into a specialized cell type. This makes stem cells attractive as a cell source for tissue engineering because the number of stem cells can be expanded greatly before they are differentiated toward a specific tissue lineage. This scenario offers a theoretically inexhaustible source of cells with which to create new tissues. However, the ex vivo conditions for truly self-replicative expansion have not been

fully determined for any stem cell family, though there have been considerable improvements toward this goal.

Isolating Stem Cells for Scientific and Clinical Purposes

Methods have been developed to isolate stem cells or enrich the stem cell content of a cell population. At present no single marker has been identified for use as a definitive stem cell marker. Therefore, most effective isolation protocols use multiparametric isolation strategies. These strategies may comprise immunoselection for cells with specific antigenic profiles that are used in combination with cell selection methods such as diameter, cell density, and levels of "granularity" (the extent of cytoplasmic particles such as mitochondria). The published protocols include flow cytometry and/or immunoselection with magnetic columns, affinity columns, and counterflow elutriation. Once purified, validation of the identity of stem cells can be achieved either ex vivo or in vivo. Ex vivo assays typically involve clonogenic expansion assays, in which a single cell is expanded in culture under precise conditions, and the ability to give rise to daughter cells of more than one fate identifies the original cell as a stem cell. In vivo assays involve transplanting the putative stem cell into an animal to observe whether multiple tissue types can be regenerated.

Types of Stem Cells

Embryonic stem cells are the most pluripotent cell type, since they can give rise to essentially any tissue in the body. These cells are typically derived from very early stages of embryonic development (usually the blastocyst stage). They have high potential to create large populations of specialized cell types, but their use is controversial because of ethical issues. In addition, their use for therapeutic cell transplantation is in question because they have the immune profile of the original embryo and therefore are necessarily an allogeneic therapy. For these reasons, scientists have very actively searched for alternatives to embryonic stem cells. The technique of somatic nuclear transfer involves replacing the nuclear material of an egg cell with that from a specialized somatic cell. The resulting cell is thereby "reprogrammed" to form a blastocyst with the genetic identity of the original somatic cell. This technique is the basis for cloning in animals and is being investigated for "therapeutic cloning," which involves creating cell banks that are genetically identical to the somatic cell donor for potential therapeutic use. It also is controversial because of the potential to be used for "reproductive cloning," which involves recreation of an entire organism based on the original reprogrammed cell.

A recent exciting discovery is the ability to reprogram cells by transfecting a few key regulatory genes (as opposed to the entire nuclear material). The resulting cells are termed *inducible pluripotent stem (iPS) cells* to reflect the fact that they have been induced to revert to a pluripotent state. In this technique, a specific set of two to six genes is introduced into the nucleus of a somatic cell. The gene set is chosen because of its ability to reprogram the target cell to a near-embryonic state, and currently there are several different gene sets that can achieve this goal. The resulting cell line then can theoretically be propagated and then differentiated into the desired specialized cell type. This technique has energized the stem cell research community, and the applications to tissue engineering are being investigated.

In the last decade there has been a large amount of research devoted to finding, characterizing, and using adult stem cells for tissue engineering and regenerative medicine. It is now recognized that there are a number of cell types in various tissues of the adult that

exhibit multipotent capabilities. The hematopoietic stem cells of the bone marrow have been widely studied and are known to create the cellular components of blood. The bone marrow also contains a rare population of so-called mesenchymal stem cells. These are isolated based on their ability to adhere to culture substrates, which distinguishes them from other cell types in the marrow, including hematopoietic stem cells. The source and fate of these cells is an active topic of discussion, and these cells are sometimes referred to as bone marrow stromal cells. A number of other adult tissue have also yielded multipotent progenitor and stem cells, including blood (endothelial progenitor cells), fat (adipose stem cells), brain (neural stem cells), and heart (cardiac stem cells). Putative stem cells also have been identified in the stroma of the umbilical cord, as well as in amniotic fluid. Not all researchers agree that each of these cell types is a true stem cell, but the potential for pluripotent and multipotent stem cells to impact tissue engineering is great.

6.2.5 Stem Cell Aging

Telomerases, DNA Stability, and Natural Cell Senescence

When linear DNA is replicated, the lagging strand is synthesized discontinuously through the formation of the so-called Okazaki fragments. The last fragment cannot be initiated, and therefore the lagging strand will be shorter than the leading strand. Linear chromosomes have noncoding repeating sequences on their ends that are called telomeres. These telomeres can be rebuilt using an enzyme called telomerase. Telomerase is a ribonucleoprotein DNA polymerase that elongates telomeres. When expressed, telomerase maintains the telomere length in growing cells. The telomere hypothesis implicates short telomere length and telomerase activation as critical players in cellular immortalization. This enzyme is active in microorganisms, in stem cells, and in transformed derivatives of stem cells (i.e., tumor cells). Commitment of stem cells toward their unipotent descendants results in the loss of telomerase activity. Thus, normal somatic cells lack this activity, and the telomeres are shortened by about 50 to 200 bp per replication. This shortening gives rise to the so-called mitotic clock. The length of the telomeres is about 9 to 11 kbp, and when it reaches about 5 to 7 kbp, the chromosomes become unstable and replication ceases (Figure 6.11). This mechanism is believed to underlie the Hayflick limit.

Telomerase activity is found in somatic hematopoietic cells but at a low activity level. There is evidence that telomeres in immature hematopoietic cells do shorten with ontogeny and with increased cell doublings in vitro. The rate of telomere shortening in stem cells is finite, but it may be slower than in other somatic cells. Numerical evaluation of the consequences of stem cell aging strongly suggests that there has to be some form of self-renewal of stem cells in adults (see Exercise 1).

6.2.6 Tissue Dynamics

Tissues are comprised of many different cell types of various developmental origins (Figure 6.12). The dynamic behavior of cells and their interactions determine overall tissue formation, state, and function. The activities of individual cells are often substantial.

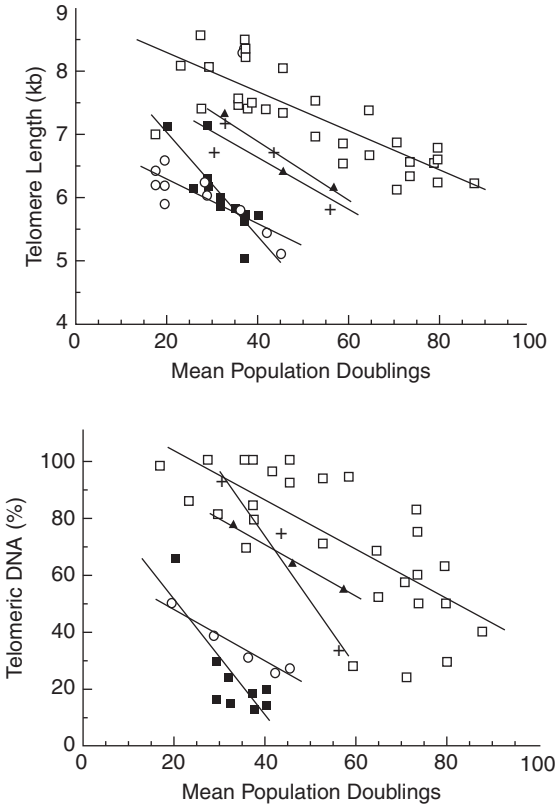


FIGURE 6.11 Primary experimental data showing the shortening of telomere length with increasing cellular doubling in cell culture. *From [5].*

However, the time scales that relate cellular activities with tissue function are relatively long, and therefore their importance tends to be overlooked. As examples, some cellular-level activities that underlie dynamic states of tissue function fall into the following categories:

1. *Hyperplasia:* Cell replication or proliferation resulting in an increase in cell number due to complete cell division (both DNA synthesis and cytokinesis).
2. *Hypertrophy:* DNA synthesis not accompanied by cytokinesis and instead results in an increase in the cell volume.
3. *Cell maturation and differentiation:* Changes in gene expression and the acquisition of particular functions, with the changes occurring in a maturationally lineage-dependent pattern.
4. *Cell apoptosis:* Aging cells undergoing "programmed cell death," a process distinguishable from necrosis.
5. *Cell adhesion:* The physical binding of a cell to its immediate environment, which may be a neighboring cell, an extracellular matrix, or an artificial surface.
6. *Cell motility:* The motion of a cell into a particular niche or location.

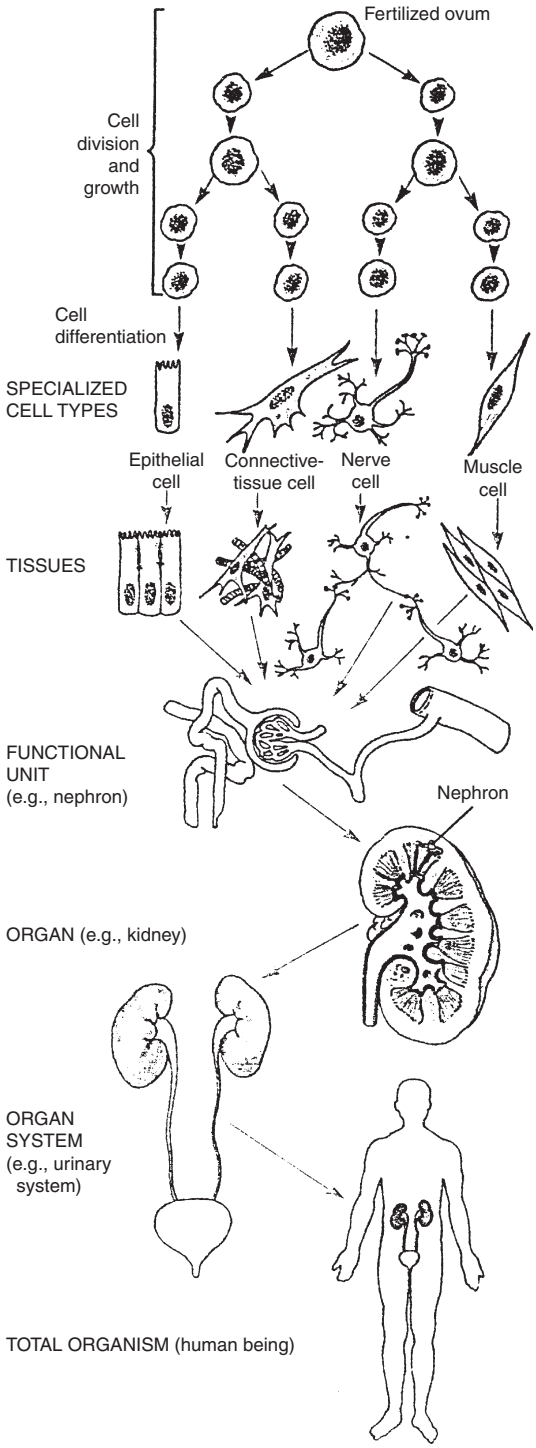


FIGURE 6.12 Levels of cellular organization in tissues and the diverse developmental origins of cells found in tissues. *From [17].*

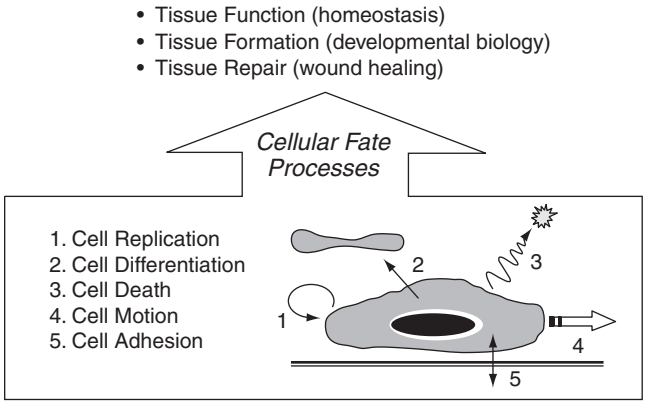


FIGURE 6.13 Tissue dynamics. The three dynamic states of tissues and the underlying cellular fate processes.

These processes are illustrated in Figure 6.13. What is known about each one of these processes will be briefly described in the following sections, with particular emphasis on quantitative and dynamic descriptions. The processes contribute to three dynamic states at a tissue level:

1. *Histogenesis:* The maturational lineages of cells derived from a tissue's stem cell compartment. A tissue's overall functions are the net sum of contributions from all the cells within the maturational lineages of a tissue.
2. *Tissue formation:* The formation of tissue has been characterized by studies comprising the field of developmental biology. Tissues vary in their proportion of stem cells, diploid cells, and polyploid cells, depending on age. The tissue, when isolated from young donors (e.g., infants) will have tissues with maturational lineages skewed toward the young cells in the lineage (stem cells, diploid somatic cells), whereas those from geriatric donors will have tissues skewed toward the later stages of the lineage (polyploid cells). This phenomenon is the explanation for why tissues procured from young donors have greater expansion potential ex vivo and are probably going to have greater potential for most forms of cell therapy programs.
3. *Tissue repair:* Repair of damaged tissues involves production of cells from the stem cell compartment, proliferation of the cells, and their differentiation into the fully mature cells. The repair process can also involve migration of cells, if need be, to a site of damage.

The preceding dynamic processes involve interplay among many different cell types. The cells communicate and coordinate their efforts through the principal cellular processes shown in Figure 6.13. The biology and dynamics of these processes are discussed in detail in Section 6.3.4.

Tissue Histogenesis

All tissues are dynamic. For instance, tissue dynamics can be illustrated by comparing the cell numbers that some organs produce over a lifetime to the total number of cells in the human body. As stated before, the human bone marrow produces about 400 billion myeloid cells daily in a homeostatic state. Over a 70-year lifetime, the cell production from

bone marrow accumulates to a staggering 10^{16} cells. This cell number is several hundred times greater than the total number of cells that are in the body at any given time. Similarly, the intestinal epithelium, the body's second most prolific tissue, produces about 5×10^{14} cells over a lifetime—ten times the total number of cells in the human body.

Tissues have their own characteristic turnover rates (Table 6.3). Bone marrow is the most proliferative tissue in the body, followed by the lining of the small intestine, and then by the epidermis. The turnover rate of these two tissues is on the order of a few days; that for the epidermis is in terms of weeks. The turnover in quiescent tissues is on the order of months to even years. For example, the turnover of the liver of rodents is estimated to be about one year; the turnover rate in the livers of humans is also slow. Even the tissue of the central nervous system is now known to turn over, though at a very slow rate, through the action of stem cells in compartments that line the ventricles of the brain.

Tissue Genesis

The preceding overview relates mainly to steady-state tissues that develop and regenerate through stem cell compartments and maturing lineages of cells. This steady state is achieved gradually during embryonic development and the process of organogenesis, which is quite complicated, as exemplified by hematopoiesis or the formation of blood cells. During vertebrate ontogeny, hematopoiesis sequentially occupies the yolk sac, fetal liver, spleen, and bone marrow. Variations in this pattern exist among vertebrate species. The earliest identification of hematopoietic cells is their assignment to the progeny of the C4 blastomere in the 32-cell embryo. The blastula grows to about 1,000 cells (ten doublings) and assumes a spherical shape. Then, the blastula undergoes gastrulation, not unlike

TABLE 6.3 Cell Renewable Rates in Tissues

Tissue	Species	Turnover Time (days)
Erythropoiesis	Rat	2.5
Myelopoiesis	Rat	1.4
Hematopoiesis	Human	2.5
Small intestinal epithelium	Human	4–6
	Rat	1–2
Epidermis	Human	7–100
Corneal epithelium	Human	7
Lymphatic cells	Rat (thymus)	7
	Rat (spleen)	15
Epithelial cells	Rat (vagina)	3.9
	Human (cervix)	5.7
Spermatogonia	Human	74
Renal interstitial cells	Mouse	165
Hepatic cells	Rat	400–500

pushing a finger into an inflated balloon. The point of invagination is the endoderm, which eventually forms the gut. After gastrulation, the ectoderm is brought into position relative to the endoderm and a third germ layer is formed between the two: the mesoderm. This middle layer is formed via cell-cell interactions and soluble growth factor action. Several determined stem cells originate from the mesoderm, including hematopoietic tissue, mesenchymal tissue, muscle, kidney, and notochord. Blood cells originate from the ventral mesoderm. Some hematopoietic cells migrate into the yolk sac to form blood islands consisting mainly of erythroid cells ("primitive" hematopoiesis). Intraembryonic hematopoiesis originates from the aortic region in the embryo and leads to "definitive" hematopoiesis. It appears that the embryonic origin of hematopoietic cells is from bipotent cells that give rise to both the vasculature (the endothelium) and hematopoietic cells. Hematopoietic stem cells are then found in the liver in the fetus. Around birth, the hematopoietic stem cells migrate from the liver into the bone marrow, where they reside during postnatal life. Interestingly, the umbilical cord blood contains hematopoietic stem cells capable of engrafting pediatric, juvenile, and small adult patients. This developmental process illustrates the asymmetric nature of stem cell division during development and increasing restriction in developmental potential. Furthermore, the migration of stem cells during development is important. Understanding the regulatory and dynamic characteristics of the stem cell fate processes is very important to tissue engineering.

Tissue Repair

When tissue is injured, a healing response is induced. The wound healing process is comprised of a coordinated series of cellular events. These events vary with ontological age. Fetal wound healing proceeds rapidly and leads to the restoration of scarless tissue. In contrast, postnatal healing is slower and often leads to scarring, which generally permits satisfactory tissue restoration, while not always fully restoring normal tissue structure. Some pathological states resemble wound healing. A variety of fibrotic diseases involve similar processes to tissue repair and subsequent scarring. The increasing appreciation of stem cell compartments and their descendant maturational lineages and the changes that occur in them with age are likely to provide an improved understanding of wound healing phenomena.

THE SEQUENCE OF EVENTS IN WOUND HEALING

Immediately following injury, control of bleeding starts with the rapid adhesion of circulating platelets to the site of damage. Within seconds, the platelets are activated, secrete contents from their storage granules, spread, and recruit more platelets to the thrombus that has started to develop. Within minutes of injury, the extent of hemorrhaging is contained through the constriction of surrounding blood vessels.

The next phase of the wound healing process involves the release of agents from the platelets at the injured site that cause vasodilatation and increased permeability of neighboring blood vessels. The clotting cascade is initiated and results in the cleavage of fibrinogen by thrombin to form a fibrin plug. The fibrin plug, along with fibronectin, holds the tissue together and forms a provisional matrix. This matrix plays a role in the early recruitment of inflammatory cells and later in the migration of fibroblasts and other accessory cells.

Inflammatory cells now migrate into the injured site. Neutrophils migrate from circulating blood and arrive early on the scene. As the neutrophils degranulate and die, the abundance

of macrophages at the site increases. All tissues have resident macrophages, and their number at the injury site is enhanced by macrophages migrating from circulation. They act in concert with the neutrophils to phagocytose cellular debris, combat any invading microorganisms, and provide the source of chemoattractants and mitogens. These factors induce the migration of endothelial cells and fibroblasts to the wound site and stimulate their subsequent proliferation. If the infiltration of macrophages into the wound site is prevented, the healing process is severely impaired.

The result of these initial processes is the formation of a so-called granulation tissue. It is comprised of a dense population of fibroblasts, macrophages, and developing vasculature that is embedded in a matrix comprised mainly of fibronectin, collagen, and hyaluronic acid. The invading fibroblasts begin to produce collagen, mostly types I and III. The collagen increases the tensile strength of the wound. Myofibroblasts actively contract at this time, shrinking the size of the wound by pulling the wound margins together.

Over time, the matrix then undergoes remodeling, which involves the coordinated synthesis and degradation of connective tissue proteins. Remodeling leads to a change in the composition of the matrix as healing progresses. For instance, collagen type III is abundant early on but gives way to collagen type I with time. The balance of these processes determines the degree of scar formation. Although the wound appears healed at this time, chemical and structural changes continue to occur within the wound site. The final step of the wound healing process is the resolution of the scar, though in most cases this process is incomplete and some form of scar tissue remains after healing. The formation and degradation of matrix components take place over many months. The healing process is essentially complete when the composition of the matrix and the spatial location of the cells have returned to close to the original state. Understanding the wound healing process is important to the tissue engineer, since the placement of disaggregated tissues in ex vivo culture induces responses reminiscent of the wound healing process.

6.2.7 Cell Differentiation

The coordinated activity of the cellular fate processes determines the dynamic state of tissue function (see Figure 6.13). There is growing information available about these processes in genetic, biochemical, and kinetic terms. The dynamics considerations that arise from the interplay of the major cellular fate processes are introduced at the end of the chapter, and the associated bioengineering challenges are described.

Describing Cellular Differentiation from a Biological Perspective

Differentiation is the process by which a cell undergoes phenotypic changes to become a particular specialized cell type. This specialized cell type is characterized by its physiological function and its corresponding role as part of a tissue and/or organ. This process begins with a lineage and differentiation commitment and is followed by a coordinated series of gene expression events.

The term *differentiation* is derived from differential gene expression. Differentiation involves a change in the set of genes that are expressed in the cell, and this change is usually an irreversible change toward a particular functional state. This process involves a carefully orchestrated switching off and on of gene families. The final set of genes expressed is those that pertain to the function of the mature cell.

EXAMPLE PROBLEM 6.1

How can the production of red blood cells be modeled?

Solution

Erythropoiesis replaces decaying mature red blood cells. About 200 billion of these cells need to be produced daily in a human adult. Many of the changes that a cell undergoes during the process of erythropoiesis are well known (Figure 6.14). The cell size, the rates of RNA and

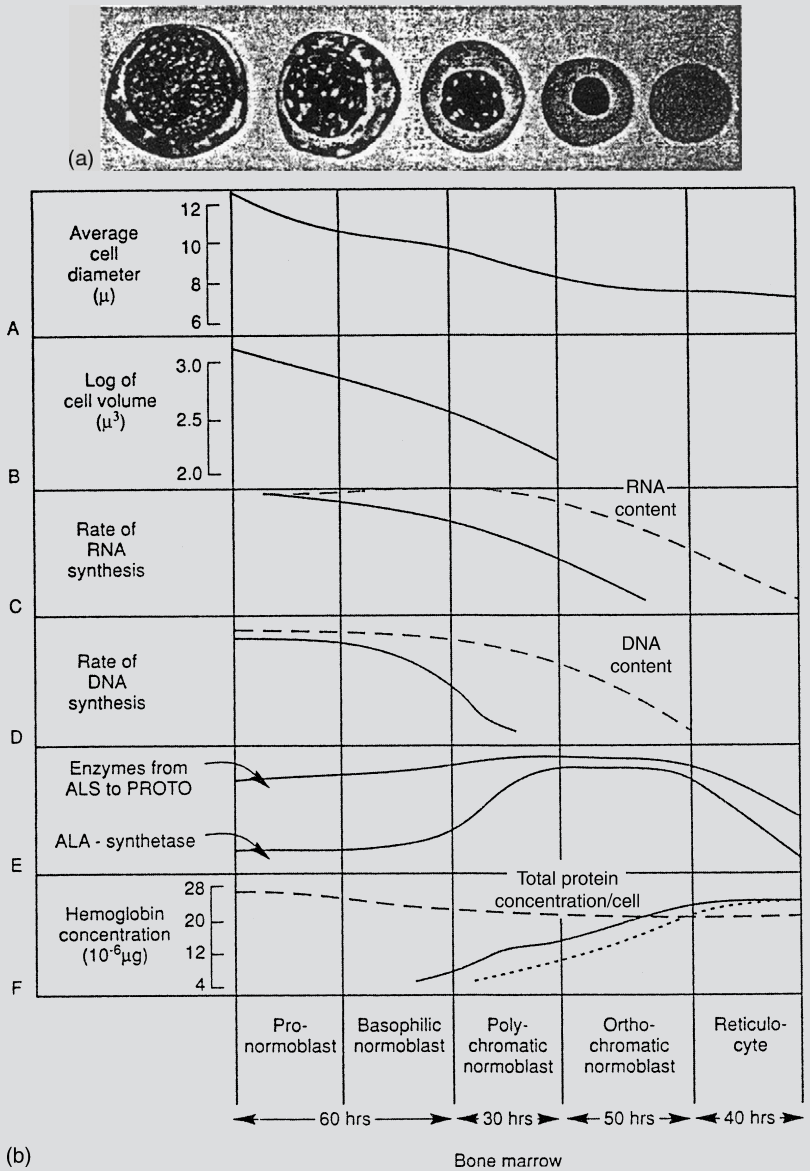


FIGURE 6.14 The erythroid maturation sequence. *From [4].*

Continued

DNA synthesis, and protein content all change in a progressive and coordinated fashion. The differentiation from a pronormoblast (earlier precursor stage) to a fully mature enucleated erythrocyte takes about 180 hours, or about one week. The replication activity is the highest at the preprogenitor and progenitor stages, but once the precursor stage is reached, replication activity ceases sharply. This information can be used to derive and solve equations that describe the process of erythropoeisis.

Experimental Observations of Differentiation

The process of differentiation can be observed directly using fluorescent surface markers and/or light microscopy for morphological observation. Ultrastructural changes are also used to define the stage of differentiation.

A flow cytometer is often used to monitor the process of cellular differentiation. The basis for this approach is the fact that characteristic surface proteins are found on cells at different stages of differentiation. These surface markers can be used as binding sites for fluorescently conjugated monoclonal antibodies. The flow cytometer can be used to trace the expression of several surface markers, and by performing such studies over time, it is possible to track the differentiated state of the cells and cell population.

For example, erythropoiesis can be traced based on expression of the transferrin receptor (CD71) and glycophorin-A. The latter is an erythroid-specific surface protein that is highly negatively charged and serves to prevent red cell aggregation in dense red cell suspensions. The transferring receptor plays a critical role during the stages in which iron is sequestered in hemoglobin. The measurement of this process is shown in Figure 6.15.

DESCRIBING THE KINETICS OF CELL DIFFERENTIATION

The process of differentiation is a slow one, often taking days or weeks to complete. The kinetics of this complex process can be described mathematically using two different approaches:

A. Compartmental models. The traditional approach to describing cell growth and differentiation is to use compartmental models. The differentiation process involves a series of changes in cell phenotype and morphology, typically becoming more pronounced at the latter stages of the process.

$$X_0 \rightarrow X_1 \rightarrow X_2 \rightarrow \ldots\ldots X_i \rightarrow \ldots\ldots X_n \rightarrow \textit{turnover} \tag{6.1}$$

where n can be as high as 16 to 18.

In the use of compartmental models in the past, the transition from one stage to the next was assumed to represent cell division. Thus, these models coupled the dual process of cell differentiation with cell replication. Mathematically, this model is described by a set of ordinary differential equations as

$$\frac{dX_i}{dt} = 2k_{i-1}X_{i-1} - k_iX_i \tag{6.2}$$

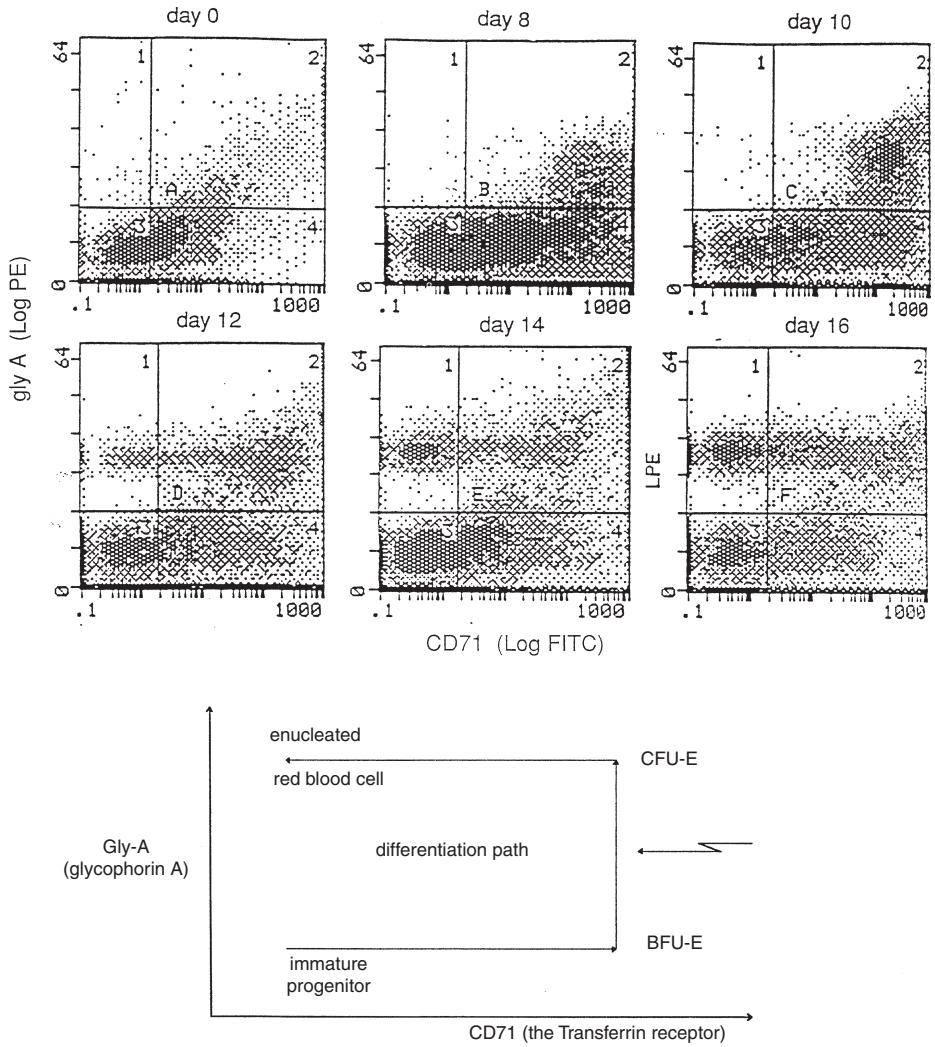


FIGURE 6.15 Two-parameter definition of erythropoietic differentiation. Glycophorin A is found on erythroid cells post the blast-forming unit-erythroid (BFU-E) stage, whereas transferrin (CD71) is expressed at the progenitor stage (BFU-E and colony-forming unit-erythroid (CFU-E)). By measuring the two simultaneously using a flow cytometer, this differentiation process can be traced as a U-shaped path on a bivariate dot plot. *From [16].*

The transition rate from one stage to the next is proportional to the number of cells present at that stage. This transition rate can clearly be a function of growth factor concentration and a number of other variables.

B. Differentiation as a continuous process. An alternative view is to consider the differentiation process to be a continuous process. Once the commitment to differentiation has been

made, the differentiation process is assumed to proceed at a fixed rate. This viewpoint leads to a mathematical description in the form of first-order partial differential equations:

$$\frac{dX}{dt} + \delta \frac{dX}{da} = (\mu(a) - \alpha(a))X \tag{6.3}$$

where δ is the rate of differentiation and a is a parameter that measures the differentiation state of the cell. μ and α are the growth and death rates, respectively, and vary between zero and unity. Both μ and α can be a function of a.

Cell Motion

THE BIOLOGICAL ROLES OF CELL MIGRATION

Cell migration plays an important role in all physiological functions of tissues and also some pathological processes. Cell migration is important during organogenesis and embryonic development. It plays a role in the tissue repair response in both wound healing and angiogenesis. The immune system relies on cell migration, and pathological situations like cancer metastasis are characterized by cell motility. Cell migration represents an integrated molecular process.

Animal cells exhibit dynamic surface extensions when they migrate or change shape. Such extensions, called lamellipodia and filopodia, are capable of dynamic formation and retraction. Local actin polymerization at the plasma membrane is a key process in the generation of these structures. These extensions are also regulated by a complex underlying process involving multiprotein interactions. In neurites, filopodia are believed to play a role in the progression of cell elongation by aiding the assembly of microtubules that are a significant component of these cells. The filopodia in neurites extend from the lamellipodial region and act as radial sensors. Filopodia on neurites have been found to be crucial to growth cone navigation. The filopodia on neurite growth cones have also been found to carry receptors for certain cell adhesion molecules. Mature leukocytes also can extend cytoplasmic extensions. Recently, structures termed uropods have been found on T lymphocytes. These cytoplasmic projections form during lymphocyte-endothelial interaction. There is a redistribution of adhesion molecules, including ICAM-1,-3, CD43, and CD44, to this structure. T cells have been found to use the uropods to contact and communicate directly with other T cells. Uropod development was promoted by physiologic factors such as chemokines. Cytoplasmic extensions, therefore, can perform a spectrum of functions in different cells that are related to migration and communication.

DESCRIBING CELL MOTION KINETICALLY

Whole populations. The motion of whole, nonreplicating cell populations can be described by

$$\frac{dX}{dx} = J \tag{6.4}$$

where J is the flux vector of the system boundary (cells/distance/time in a two-dimensional system), X is the cell number, and x is the flux dimension. The cellular fluxes are then related to cellular concentration and chemokine concentrations using

$$\begin{aligned} \mathrm{J} = &\ \text{random motility} + \text{chemokinesis} + \text{chemotaxis} \\ = &\ \sigma \frac{dX}{dx} - \left(\frac{X}{2}\right)\left(\frac{d\sigma}{da}\right)\left(\frac{da}{dx}\right) + \chi \frac{X da}{dx} \end{aligned} \tag{6.5}$$

where σ is the random motility coefficient, χ is the chemotactic coefficient, and a is the concentration of a chemoattractant. The first term is similar to a diffusion term in mass transfer and is a measure of the dispersion of the cell population. The chemokinesis term is a measure of the changes in cell speed with concentration and is normally negligible.

If $\chi = 0$ and chemokinesis is negligible, then the motion of the cells is represented by a random walk and is formally analogous to the process of molecular diffusion. The biased movement of the cells directly attributable to concentration gradients is given by the chemotaxis term, which is akin to convective mass transfer.

Individual cells. As described, motile cells appear to undergo a random walk process that mathematically can be described in a fashion similar to the diffusion process. Unlike the diffusion process, the motion of each moving entity can be directly determined (Figure 6.16). Thus, the motility characteristics of migrating cells can be measured on an individual cell basis.

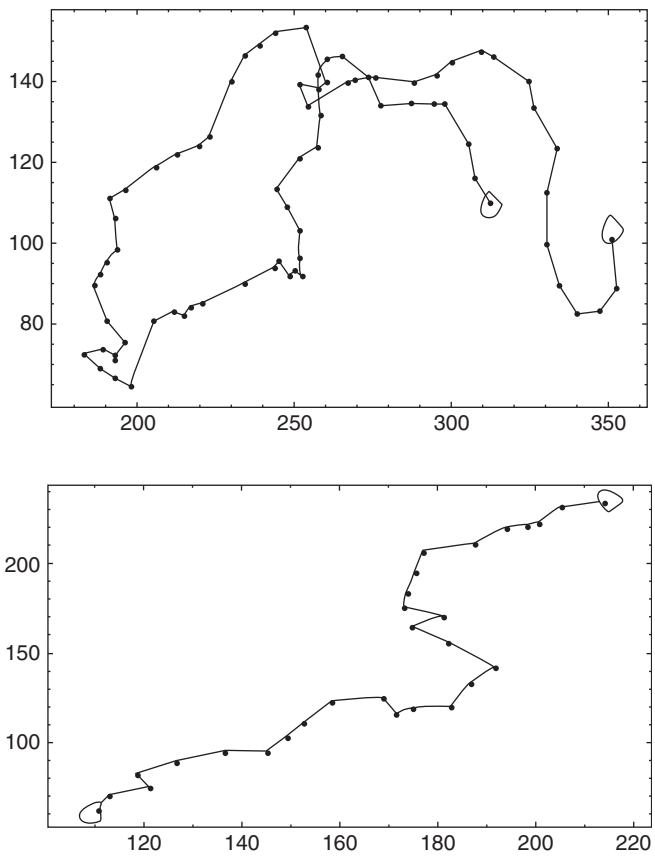


FIGURE 6.16 Experimental paths of individual neutrophil leukocytes undergoing random motility in uniform environments. *From [9].*

TABLE 6.4 Random Motion—Measured Cell Speeds and Persistence Times

Cell Type	Speed	Persistence Time
Rabbit neutrophils	20 μm/min	4 min
Rat alveolar macrophages	2 μm/min	30 min
Mouse fibroblasts	30 μm/h	1 h
Human microvessel endothelial cells	25–30 μm/h	4–5 h

Such a description is given in terms of the cell speed(s), the persistence time (p, the length of time that the cell moves without changing its direction), and the orientation bias (θ) that is due to action of chemoattractants. The random motility coefficient is related to these parameters as

$$\sigma = s^2 p \tag{6.6}$$

Typical values for these parameters are given in Table 6.4.

Cell Replication

THE CELL CYCLE

The process of cell division is becoming increasingly well understood in terms of molecular mechanisms (Figure 6.17). The so-called "cell cycle" of division is driven by a family of regulatory proteins known as cyclin-dependent kinases (cdk proteins). The cyclins exist in phosphorylated and dephosporylated states, basically a binary system. The network goes through a well orchestrated series of switches, during which the cyclins are turned off and on. When in an "on" state, they serve to drive the biochemical processes that are needed during that part of the cell cycle. It should be noted that there are several important decisions associated with moving "in and out of cycle," which is the process of a cell moving from a quiescent state (the so-called G_o) to a cycling (proliferative) state and vice versa. The cell cycle

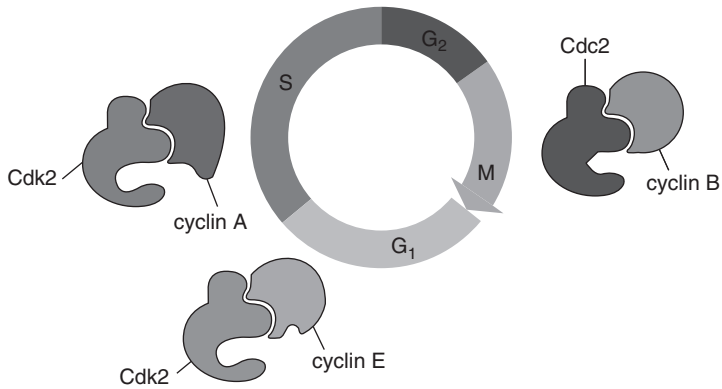


FIGURE 6.17 Schematic representation of the eukarotic cell cycle (G1–S–G2–M) and the presence of cyclin-dependent kinases.

is such that the time duration of the S, G_2, and M phases is relatively fixed. Once a cell determines it needs to and can divide, it initiates DNA synthesis and subsequent cell division. This process has the overall characteristics of a zero-order kinetic process. Once initiated, it proceeds at a certain rate. The minimal cycling time of human cells is about 12 hours. Progenitor cells can cycle at this rate, whereas more differentiated cell types tend to have longer cycle times.

DESCRIBING THE CELL CYCLE DYNAMICS

The dynamics of growth can be described in different ways.

1. *Exponential growth*. If growth is unconstrained, the rate of formation of new cells is simply proportional to the number of cells present

$$\frac{dX}{dt} = \mu X, \Rightarrow X(t) = X_o \exp(\mu t) \tag{6.7}$$

and exponential growth results. The growth rate μ is equal to $\ln(2)/t_d$, where t_d is the doubling time.

2. *Age-time structured descriptions.* If the phases of the cell cycle are to be described, then the status of the cell in the cycle needs to be incorporated in the dynamic description. This leads to first-order partial differential equations in time and cell cycle status:

$$\frac{dX}{dt} + v\frac{dX}{da} = \alpha(a)x \tag{6.8}$$

where v is the rate at which the cell moves through the cell cycle, a is a variable that describes the cell cycle status ($a = 0$ newborn cell, $a = 1$ cell completing mitosis), and α is the death rate of the cell that can be cell cycle dependent. This population balance equation can be solved under the appropriate initial and boundary conditions.

3. *Molecular mechanisms.* The cascade of cyclin-dependent kinases that constitute the molecular mechanism for cell cycling has largely been unraveled. Based on this knowledge, it is possible to describe the cell cycle in terms of the underlying molecular determinants. Such models have many components and typically need to be solved using computational methods.

Interacting Cellular Fate Processes Determine Overall Tissue Dynamics

The differentiation process involves a series of changes in cell phenotype and morphology that typically become more pronounced at the latter stages of the process. The key event is milieu-dependent differentiation (or differential gene expression), which encompasses the organogenic process that yields mature cells of a certain type and function. Similarly, embryonic induction can be described as a series of such events. This process is schematically presented in Eq. (6.1).

This progression of changes is typically coupled to fundamental "driving forces"—for example, cell cycling (mitosis) and cell death (apoptosis). Thus, the basic cellular processes can be accounted for in a population balance on each stage of differentiation:

$$\Delta\text{'s in cell\#} = \text{exit by input} - \text{differentiation} - \text{exit by apoptosis} + \text{entry by cell division}$$

or in mathematical terms:

$$\begin{aligned}\frac{dX}{dt} &= I - \delta X - \alpha X + \mu X \\ &= I - (\delta + \alpha - \mu)X\end{aligned} \tag{6.9}$$

where δ, α, and μ are the rates of differentiation, apoptosis, and replication, respectively. This equation can be rewritten as

$$\frac{dX}{dt} + kX = I, \tag{6.10}$$

where $k = \delta + \alpha - \mu$, $t = 1/k$. The parameter k is the reciprocal of the time constant t that characterizes the dynamics of changes in the number of cells of type X. It is therefore evident that the ratios of μ/δ, α/μ are the key dimensionless groups that determine the overall cell production in tissue.

6.2.8 Cellular Communications

How Do Cells Communicate?

Cells in tissues communicate with one another in three principal ways (Figure 6.18).

1. They secrete soluble signals, known as cyto and chemokines.
2. They touch each other and communicate via direct cell-cell contact.
3. They make proteins that alter the chemical microenvironment (ECM).

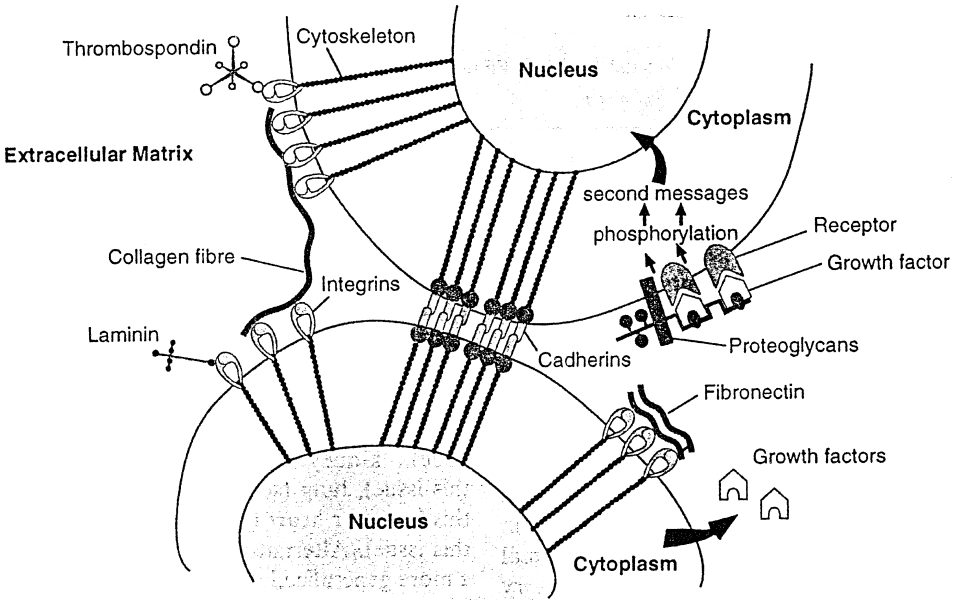


FIGURE 6.18 Cell and extracellular matrix (ECM) protein interactions. *From [13].*

These means of cellular communication differ in terms of their characteristic time and length scales, as well as in their specificity. Consequently, each is most suitable to convey a particular type of a message.

Soluble Growth Factors

Growth factors are small proteins that are on the order of 15,000 to 20,000 Dalton in size (one Dalton is the weight of the hydrogen atom and is a typical unit used to describe the size of molecules). They are relatively chemically stable and have long half-lives unless they are specifically degraded. Initially, growth factors were discovered as active factors that originated in biological fluids. For instance, erythropoetin was first isolated from urine and the colony stimulating factors from conditioned medium from particular cell lines. The protein could be subsequently purified and characterized. With the advent of DNA technology, growth factors can now be cloned directly as ligands for known receptors. This procedure was used to isolate thrombopoietin, as the *c-mpl* ligand and the stem cell factor as the *c-kit* ligand. Growth factors are produced by a signaling cell and secreted to reach a target cell.

EXAMPLE PROBLEM 6.2

What is the maximal secretion rate of protein?

Solution

The maximal secretion rate of a protein from a single gene in a cell can be estimated based on the maximal rates of transcription and translation. Such estimates have been carried out for the production of immunoglobulins (MW = 150,000 D), whose genes are under the control of strong promoters. The estimate shows that the maximal secretion rate is on the order of 2,000 to 8,000 protein molecules per cell per second, which corresponds to about 1 pg per cell per hour. This estimate compares favorably with measured maximal secretion rates. Since growth factors tend to be about one-tenth the size of immunoglobulin, a correspondingly higher secretion rate in terms of molecules per cell per time would be expected, although the total mass would stay the same. The secretion rates of protein from cells are expected to be some fraction of this maximum rate, since the cell is making a large number of proteins at any given time.

Growth factors bind to their receptors, which are found in cellular membranes, with high affinities. Their binding constants are as low as 10 to 100 pM. The binding of a growth factor to a receptor is described as

$$G + R \Leftrightarrow G{:}R, \quad \text{and} \quad \frac{[G][R]}{[G{:}R]} = K_a \tag{6.11}$$

where [G], [R], and [G:R] are the concentrations of the growth factor receptor and the bound complex, respectively, and K_a is the binding constant. Since the total number of receptors (R_{tot}) in the system is constant, we have the mass conservation quantity:

$$R_{tot} = [R] + [G{:}R] \tag{6.12}$$

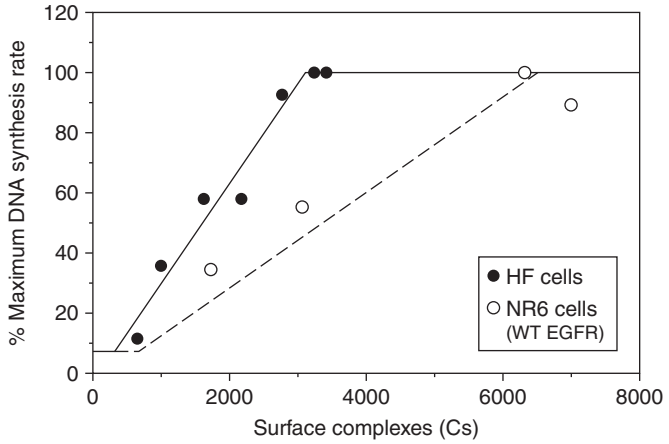


FIGURE 6.19 Relationship between steady-state surface complexes and mitogenic response to EGF for NR6 cells (○) and human fibroblasts (●). *Data from [7].*

and therefore Eq. (6.12) can be written as

$$R_{tot} = [G{:}R]\frac{K_a}{[G]} + [G{:}R] = [G{:}R]\left(1 + \frac{K_a}{[G]}\right) \tag{6.13}$$

or

$$([\mathrm{G{:}R}]/\mathrm{R_{tot}}) = [\mathrm{G}]/([\mathrm{G}] + \mathrm{K_a}) \tag{6.14}$$

Receptor occupancy rates ($[\mathrm{G{:}R}]/\mathrm{R_{tot}}$) need to be on the order of 0.25 to 0.5 to reach a significant stimulation (Figure 6.19), and therefore growth factor concentrations as low as 10 pM are sufficient to generate cellular response.

In many cases, the receptor:ligand complex is internalized, and a typical time constant for internalization is 15 to 30 minutes. The absolute values for growth factor uptake rates have been measured. For instance, interleukin-3 (IL-3) and the stem cell factor (SCF) are consumed by immature hematopoietic cells at rates of about 10 and 100 ng per million cells per day. Further, it has been shown that 10,000 to 70,000 growth factor molecules need to be consumed to stimulate cell division in complex cell cultures.

EXAMPLE PROBLEM 6.3

How far can soluble signals propagate and how long does it take?

Solution

The maximum signaling distance can be estimated from a simple diffusion model that describes secretion from a sphere. Under steady-state conditions, it can be shown that the concentration of a secreted molecule as a function of the distance from the cell is described as

$$\frac{c}{K_a} = \alpha\frac{R}{r} \quad \textit{where} \quad \alpha = \frac{(R^2/D)}{(K_a R/F)} \tag{6.15}$$

where R is the radius of the cell, F is the secretion rate, and D is the diffusion coefficient of the growth factor. The distance that a signal reaches when $c = K_a$ is estimated by

$$r_{critical}/R = \alpha \tag{6.16}$$

Thus, the maximal secretion rate is given by the ratio of two time constants α, the time constant for diffusion away from the cell (R^2/D), and the secretion time constant (K_aR/F). Since it takes an infinite amount of time to reach a steady state, a more reasonable estimate of the signal propagation distance is the time at which the signal reaches one-half of its ultimate value. This leads to a time constant estimate for the signaling process of about 20 minutes and a maximal distance of about 200 μm. This distance is shortened proportionally to the secretion rate (F) and inversely proportionally to the affinity (K_a), both of which are limited.

The binding of a growth factor to its receptor triggers a complex signal transduction process (Figure 6.20). Typically the receptor complex changes in such a way that its intracellular components take on catalytic activities. These activities are in many cases due to

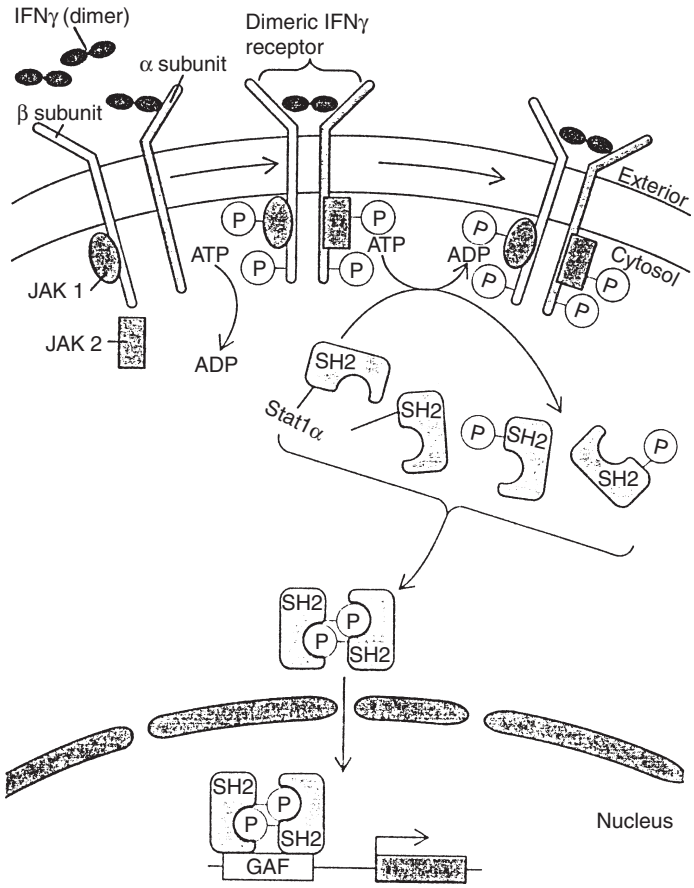


FIGURE 6.20 A schematic representation of the interferon gamma signal transduction pathway. *From [10].*

the so-called JAKs (Janus kinases). These kinases then operate on the STATs (signal transducers and activators of transcription) that transmit the signal into the nucleus. The kinetics of this process are complex, and detailed analyses are becoming available for the epidermal growth factor and other signal transduction processes.

Direct Cell-Cell Contact: Insoluble Factors 1

Cells are equipped with proteins on their surface called cell adhesion molecules (CAMs). These include the cadherins (adhesion belts, desmosome) and connexins (gap junctions). These molecules are involved in direct cell-to-cell contact. Some CAMs are known as the cell junction molecules, since junctions are formed between the adjacent cells, allowing for direct cytoplasmic communication. Such junctions are typically on the order of 1.5 nm in diameter and allow molecules below about 1,000 D to pass between cells.

A growing body of literature is showing how fluid mechanical shear forces influence cell and tissue function. Tissue function has a significant mechanical dimension. At the cellular level, the mechanical role of the cytoskeleton is becoming better understood. Signals may be delivered to the nucleus by cellular stretching in a way that is similar to the method in which growth factor binding to a receptor delivers signals. The cell-surface integrin receptors thus can perform as "mechanical transducers" of important signals. Further, cells have specific locations within a tissue, and long-distance information must be transmitted between cells via weak mechanical interactions. The mechanical characteristics of the cellular microenvironment are thus of importance, as are the mechanical properties of the cells themselves. The study of mechanically induced changes in cell function and mechanotransduction of cell signals is a rapidly growing area with great relevance to tissue engineering.

Cell-Matrix Interactions: Insoluble Factors 2

The extracellular matrix (ECM) is composed of a complex weave of fibers, struts, and gels that fills the spaces between cells in a tissue and interconnects the cells and their cytoskeletal elements. The ECM is multifunctional and provides tissue with mechanical support and cells with a substrate on which to migrate, as well as a place to locate signals for communications. The ECM thus has structural as well as instructional functions. It is dynamic and is constantly being modified. For instance, ECM components are degraded by metalloproteases and can be regenerated via cellular production. Many tissues remodel their matrix to some degree. For example, in cardiac muscle about 3 percent of the matrix is turned over daily.

The ECM is comprised of a large number of components that have varying structural and regulatory functions. On the cell surface, there are a number of adhesion and ECM receptor molecules that facilitate cell-ECM communications. These signals contain instructions for migration, replication, differentiation, and apoptosis. The nature of these signals is governed by the composition of the ECM, which in turn can be altered by the cells found in the tissue. Thus, many cellular fate processes can be directed by the ECM, and it provides a means for cells to communicate. The signals in the ECM are more stable and can be made more specific and stronger than those delivered by diffusible growth factors. The components of the ECM and their functions are summarized in Table 6.5.

TABLE 6.5 Components of the Extracellular Matrix[a]

Component	Function	Location
Collagens	Tissue architecture, tensile strength	Ubiquitously distributed
	Cell–matrix interactions	
	Matrix–matrix interactions	
Elastin	Tissue architecture and elasticity	Tissues requiring elasticity (e.g., lung, blood vessels, heart, skin)
Proteoglycans	Cell–matrix interactions	Ubiquitously distributed
	Matrix–matrix interactions	
	Cell proliferation	
	Binding and storage of growth factors	
Hyaluronan	Cell–matrix interactions	Ubiquitously distributed
	Matrix–matrix interactions	
	Cell proliferation	
	Cell migration	
Laminin	Basement membrane component	Basement membranes
	Cell migration	
Epiligrin	Basement membrane component (epithelium)	Basement membranes
Entactin (nidogen)	Basement membrane component	Basement membranes
Fibronectin	Tissue architecture	Ubiquitously distributed
	Cell–matrix interactions	
	Matrix–matrix interactions	
	Cell proliferation	
	Cell migration	
	Opsonin	
Vitronectin	Cell–matrix interactions	Blood
	Matrix–matrix interactions	Sites of wound formation
	Hemostasis	
Fibrinogen	Cell proliferation	Blood
	Cell migration	Sites of wound formation
	Hemostasis	
Fibrillin	Microfibrillar component of elastic fibers	Tissues requiring elasticity (e.g., lung, blood vessels, heart, skin)

Continued

TABLE 6.5 Components of the Extracellular Matrix[a]—Cont'd

Component	Function	Location
Tenascin	Modulates cell–matrix interaction	Transiently expressed associated with remodeling matrix
	Antiadhesive	
	Antiproliferative	
SPARC[b] (osteonectin)	Modulates cell–matrix interaction	Transiently expressed associated with remodeling matrix
	Antiadhesive	
	Antiproliferative	
Thrombospodin	Modulates cell–matrix interaction	Platelet α granules
Adhesion molecules	Cell surface proteins mediating cell adhesion to matrix or adjacent cells	Ubiquitously distributed
	Mediators of transmembrane signals	
von Willebrand factor	Mediates platelet adhesion	Plasma protein
	Carrier for procoagulant factor VIII	Subendothelium

[a]*Mutsaers et al., 1997.*
[b]*SPARC, secreted protein acidic and rich in cysteine.*

EXAMPLE PROBLEM 6.4

How many receptor sites are needed for various cellular functions?

Solution

The RGD (arginine-glycine-aspartic acid) tripeptide binding sequence has been immobilized on a cell growth surface at varying densities. Cell attachment, spreading, and growth were examined as a function of the surface density of RGD binding sites for fibroblasts. The results showed that an average receptor spacing of 440 nm was sufficient for cell attachment and spreading, and 160 nm for focal point adhesion formation. This type of experiment and analysis can provide insight into how cells adhere and migrate on different substrates.

A main thrust of tissue engineering efforts has been the design and fabrication of materials that mimic the ECM. These matrices can be used as scaffolds to support tissue growth. A variety of natural and synthetic materials have been used for this purpose. In some cases these materials are designed to be bioresorbable to allow the cells to replace the supplied ECM as they establish themselves and reconstruct tissue function. It is challenging to design such matrices because many of the functions of the ECM are still incompletely understood and involve two-way communication between cells and the matrix. The full spectrum of ECM functionality is perhaps something that can only be provided by the cells themselves (Table 6.6).

TABLE 6.6 Adhesion Molecules with the Potential to Regulate Cell-ECM Interactions

Adhesion Molecule	Ligand
Integrins	
$\alpha_1\beta_1$	Collagen (I, IV, VI), laminin
$\alpha_2\beta_1$	Collagen (I–IV, VI), laminin
$\alpha_3\beta_1$	Collagen (I), laminin, fibronectin, entactin, epiligrin
$\alpha_4\beta_1$	$\text{Fibronectin}_{\text{ALT}}$, VCAM-1, thrombospondin
$\alpha_5\beta_1$	Fibronectin, thrombospondin
$\alpha_6\beta_1$	Laminin
$\alpha_V\beta_1$	Fibronectin
$\alpha_L\beta_2$	ICAM-1, ICAM-2, ICAM-3
$\alpha_M\beta_2$	ICAM-1, iC3b, fibrinogen, factor X, denatured protein
$\alpha_x\beta_2$	Fibrinogen, iC3b, denatured protein
$\alpha_V\beta_3$	Vitronectin, fibrinogen, fibronectin, thrombospondin
$\alpha_V\beta_5$	Vitronectin
$\alpha_V\beta_6$	Fibronectin
$\alpha_0\beta_4$	Laminin
$\alpha_4\beta_7$	$\text{Fibronectin}_{\text{ALT}}$, VCAM-1, MAdCAM-1
$\alpha_{1\text{th}}\beta_3$	Fibrinogen, fibronectin, vitronectin, vWF
LRI^h	Fibrinogen, fibronectin, vitronectin, vWF, collagen (IV), entactin

6.3 PHYSICAL CONSIDERATIONS

6.3.1 Organization of Tissues into Functional Subunits

The body has 11 major organ systems (Table 6.7), with the muscular and skeletal systems often considered together as the musculoskeletal system. These organ systems carry out major physiological functions, such as respiration, digestion, circulation, and mechanical motion. Each one of these organ systems in turn is comprised of a set of tissues and organs. The major organs that participate in digestion are shown in Figure 6.21. Each organ system and organ has homeostatic functions that can be defined based on their physiological requirements. These can be thought of as providing the specifications that define an organ's function and provide the parameters needed to design the tissue. An example of such a "spec sheet" is given in Table 6.8.

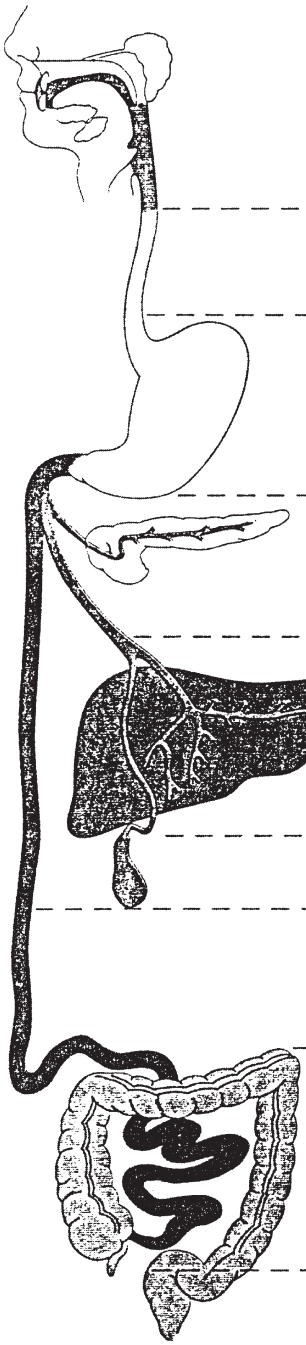
There are several important conclusions that can be arrived at using a simple order of magnitude analysis of the information found in such spec sheets. Insightful and judicious order of magnitude analysis is an important mode of analysis of tissue function, and it

TABLE 6.7 Major Organ Systems of the Body

Circulatory	Heart, blood vessels, blood (some classifications also include lymphatic vessels and lymph in this system)	Transport of blood throughout the body's tissues
Respiratory	Nose, pharynx, larynx, trachea, bronchi, lungs	Exchange of carbon dioxide and oxygen; regulation of hydrogen–ion concentration
Digestive	Mouth, pharynx, esophagus, stomach, intestines, salivary glands, pancreas, liver, gallbladder	Digestion and absorption of organic nutrients, salts, and water
Urinary	Kidneys, ureters, bladder, urethra	Regulation of plasma composition through controlled excretion of salts, water, and organic wastes
Musculoskeletal	Cartilage, bone, ligaments, tendons, joints, skeletal muscle	Support, protection, and movement of the body; production of blood cells
Immune	Spleen, thymus, and other lymphoid tissues	Defense against foreign invaders; return of extracellular fluid to blood; formation of white blood cells
Nervous	Brain, spinal cord, peripheral nerves and ganglia, special sense organs	Regulation and coordination of many activities in the body; detection of changes in the internal and external environments; states of consciousness; learning; cognition
Endocrine	All glands secreting hormones: Pancreas, testes, ovaries, hypothalamus, kidneys, pituitary, thyroid, parathyroid, adrenal, intestinal, thymus, heart, pineal	Regulation and coordination of many activities in the body
Reproductive	Male: Testes, penis, and associated ducts and glands	Production of sperm; transfer of sperm to female
	Female: Ovaries, uterine tubes, uterus, vagina, mammary glands	Production of eggs; provision of a nutritive environment for the developing embryo and fetus; nutrition of the infant
Integumentary	Skin	Protection against injury and dehydration; defense against foreign invaders; regulation of temperature

can be combined with experimental studies to characterize and set organ function parameters. Even with our current advanced state of biological knowledge, detailed theoretical analysis and calculations cannot substitute for focused and well-justified experimental work.

Organs function at a basal rate but have the ability to respond to stress. For example, the total circulation rate and organ distribution under strenuous exercise differs significantly from that at rest. Similarly, under hematopoietic stress, such as infection or in patients with sickle cell anemia, the basal blood cell production rate can significantly exceed the basal rate given in Figure 6.3.



ORGAN	EXOCRINE SECRETIONS	FUNCTIONS
Mouth and pharynx		Chewing (mechanical digestion); initiation of swallowing reflex
Salivary glands	Salt and water	Moisten food
	Mucus	Lubrication
	Amylase	Polysaccharide-digesting enzyme
Esophagus		Move food to stomach by peristaltic waves
	Mucus	Lubrication
Stomach		Store, mix, dissolve and begin digestion of food; regulate emptying of dissolved food into small intestine
	HCl	Solubilization of food particles: kill microbes
	Pepsin	Protein-digesting enzyme
	Mucus	Lubricate and protect epithelial surface
Pancreas		Secretion of enzymes and bicarbonate: also has nondigestive endocrine functions
	Enzymes	Digest carbohydrates, fats, proteins, and nucieic acids
	Bicarbonate	Neutralize HCl entering small intestine from stomach
Liver		Secretion of bile; many other nondigestive functions
	Bile salts	Solubilize water-insoluble fats
	Bicarbonate	Neutralize HCl entering small intestine from stomach
	Organic waste products and trace metals	Elimination in feces
Gallbladder		Store and concentrate bile between meals
Small intestine		Digestion and absorption of most substances: mixing and propulsion of contents
	Enzymes	Food digestion
	Salt and water	Maintain fluidity of luminal contents
	Mucus	Lubrication
Large intestine (colon)		Storage and concentration of undigested matter: mixing and propulsion of contents
	Mucus	Lubrication
Rectum		Defecation

FIGURE 6.21 Functions and organization of the gastrointestinal organs. *From [17].*

TABLE 6.8 Standard American Male[a]

Age	30 years
Height	5 ft 8 in or 1.86 m
Weight	150 lb or 68 kg
External surface	19.5 ft^2 or 1.8 m^2
Normal body temperature	37.0°C
Normal mean skin temperature	34°C
Heat capacity	0.86 cal/(g) (°C)
Capacities	
Body fat	10.2 kg or 15%
Subcutaneous fat layer	5 mm
Body fluids	ca.51 liters or 75%
Blood volume	5.0 liters (includes formed elements, primarily red cells, as well as plasma)
	Hematrocrit = 0.43
Lungs	
Total lung capacity	6.0 liters
Vital capacity	4.2 liters
Tidal volume	500 ml
Dead space	150 ml
Mass transfer area	90 m^2
Mass and energy balances at rest	
Energy conversion rate	72 kcal/h or 1730 kcal/day [40 kcal/(m^2)(h)]
O_2 consumption	250 ml/min (respiratory quotient = 0.8)
CO_2 production	200 ml/min
Heart rate	65/min
Cardiac output	5.01/min (rest)
	3.0 + 8 M in general (M = O_2 consumption in liters per minute)
Systemic blood pressure	120/80 mmHg

[a]*From Lightfoot (1974).*

Organs are comprised of functional subunits. Clear examples of such subunits include the alveoli in the lung and the nephron in the kidney (Figure 6.22). These functional units are comprised of a mixture of different kinds of cells that together constitute tissue function. Separating the functional subunits into their individual cell components often leads to the loss of tissue-specific function, but specific cell properties can be studied using such purified preparations.

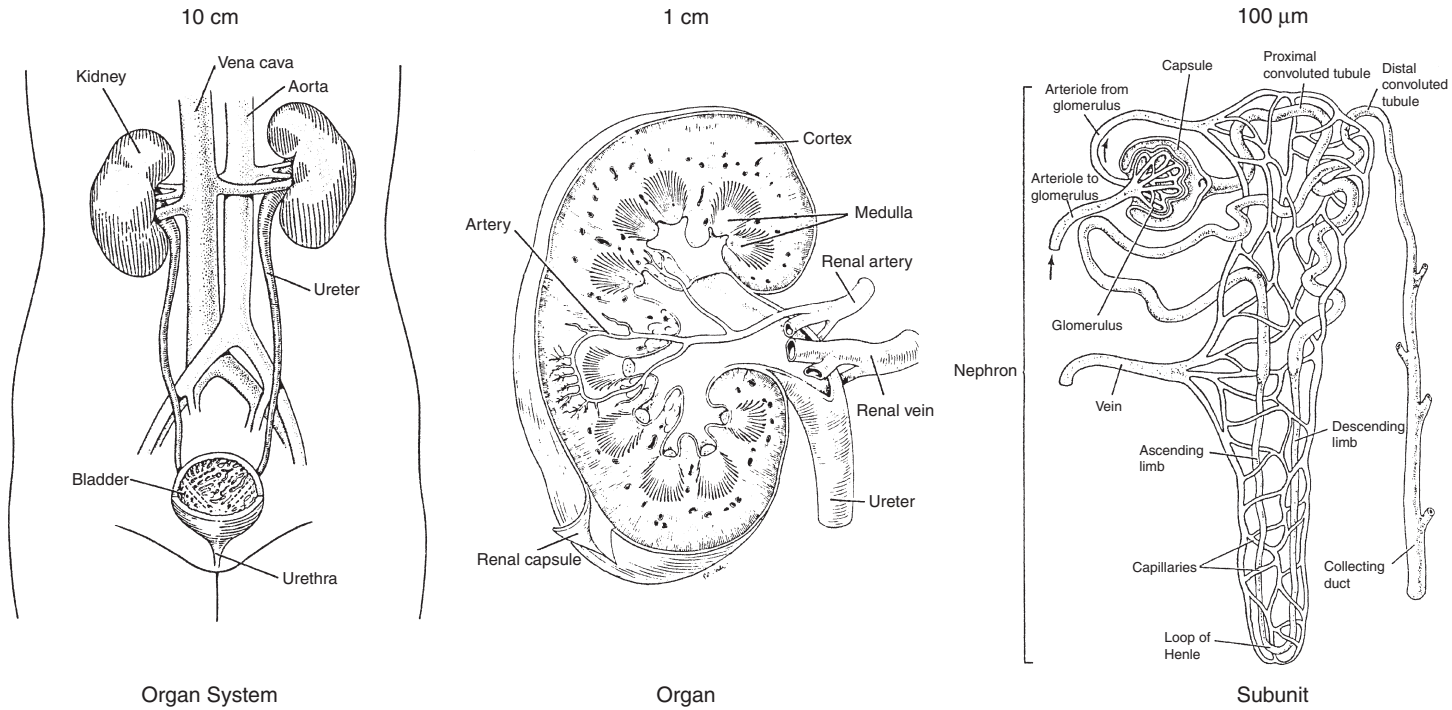


FIGURE 6.22 An organ system, an organ, and a functional subunit.

This observation leads to the conclusion that tissue function is a property of cell-cell interactions. The size of the functional subunits is on the order of 100 μm, whereas the size scale of a cell is 10 μm. Each organ is then comprised of tens to hundreds of millions of functional subunits. The sizing of organs represents an evolutionary challenge that is also faced by tissue engineering in scaling up the function of reconstituted tissues ex vivo.

The tissue and organ microenvironment is thus very complex. To achieve proper reconstitution of organ function, these dynamic, chemical, and geometric variables must be accurately replicated. This is a challenging task, and the following sections are largely devoted to developing quantitative methods to describe the microenvironment. These methods can then be used to develop an understanding of key problems, formulation of solution strategy, and analysis for its experimental implementation.

The microcirculation connects all the microenvironments in every tissue to the larger "whole body" environment. With few exceptions, essentially all metabolically active cells in the body are located within a few hundred μm from a capillary. The capillaries provide a perfusion environment that connects every cell (the cell at the center of Figure 6.22) to a source of oxygen and sink for carbon dioxide (the lungs), a source of nutrients (the small intestine), the clearance of waste products (the kidney), and so forth. The engineering of these functions ex vivo is a main focus of bioreactor design. Such culture devices have to appropriately simulate or substitute for respiratory, gastrointestinal, and renal functions. Further, these cell culture devices have to allow for the formation of microenvironments and thus must have perfusion characteristics that allow for uniformity down to the 100-micron-length scale. These are stringent design requirements.

6.3.2 Estimating Tissue Function from "Spec Sheets"

Most analysis in tissue engineering is performed with approximate calculations and estimations based on physiological and cell biological data—a tissue spec sheet, so to speak (see Table 6.8). These calculations are useful to interpret organ physiology, and they provide a starting point for an experimental program. Some examples follow.

The respiratory functions of blood: Remarkably insightful calculations leading to interpretation of the physiological respiratory function of blood have been carried out. The basic functionalities and biological design challenges can be directly derived from tissue spec sheets. Blood needs to deliver about 10 mM of O_2 per minute to the body. The gross circulation rate is about 5 liters per minute. Therefore, blood has to deliver to tissues about 2 mM oxygen per liter during each pass through the circulation. The pO_2 of blood leaving the lungs is about 90 to 100 mmHg, while pO_2 in venous blood at rest is about 35 to 40 mmHg. During strenuous exercise, the venous pO_2 drops to about 27 mmHg. These facts state the basic requirements that circulating blood must meet to deliver adequate oxygen to tissues.

The solubility of oxygen in aqueous media is low and can be represented by

$$[O_2] = \alpha_{O2} pO_2 \tag{6.17}$$

where the Henry's law coefficient is about 0.0013 mM per mmHg. The oxygen that can be delivered with a partial pressure change of 95 – 40 = 55 mmHg is thus about 0.07 mM, far below the required 2 mM (by a factor of about 30-fold). Therefore, the solubility or oxygen content of blood must be substantially increased, and the concentration dependency

of the partial pressure must be such that the 2 mM are given up when the partial pressure changes from 95 to 40 mmHg. Furthermore, during strenuous exercise, the oxygen demand doubles and 4 mM must be liberated for a partial pressure change from 95 to 27 mmHg.

The evolutionary solution is to put an oxygen-binding protein into circulating blood to increase the oxygen content of blood. To stay within the vascular bed, such a protein would have to be 50 to 100 kD in size. With a single binding site, the required protein concentration for 10 mM oxygen is 500 to 1,000 g/l, which is too concentrated from an osmolarity standpoint, and the viscosity of such a solution may be 10-fold that of circulating blood, which is clearly impractical. Further, circulating proteases would lead to a short plasma half-life of such a protein.

Having four sites per oxygen-carrying molecule would reduce the protein concentration to 2.3 mM, and confining it to a red cell would solve both the viscosity and proteolysis problems. These indeed are the chief characteristics of hemoglobin. A more elaborate kinetic study of the binding characteristics of hemoglobin shows that positive cooperativity will give the desired oxygen transfer capabilities both at rest and under strenuous exercise (Figure 6.23).

Perfusion rates in human bone marrow cultures: The question of how often the growth medium that supports cells should be replenished is important in designing cell culture conditions. Normally, the in vivo situation provides a good starting point for experimental optimization. A dynamic similarity analysis can give insight into this question.

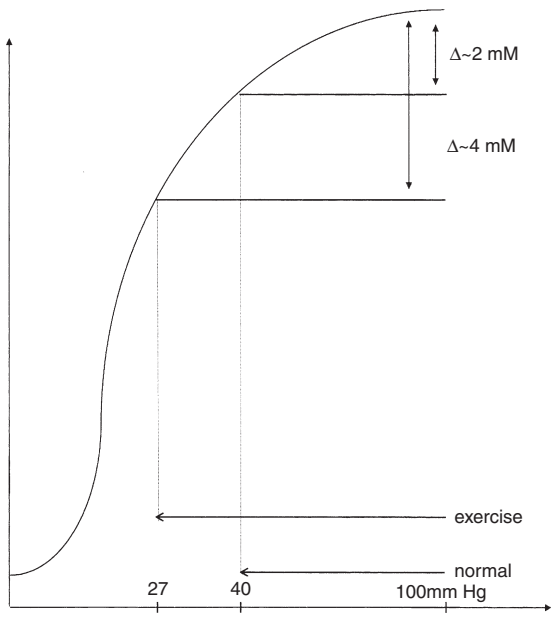


FIGURE 6.23 A schematic of hemoglobin–oxygen binding curves and oxygen delivery. The change in oxygen concentration during passage through tissues (i.e., oxygen delivery) is shown as a function of the concentration of oxygen in blood. *Modified from [3].*

The blood perfusion though bone marrow is about 0.08 ml per cc per minute. Cellularity in marrow is about 500 million cells per cc. Therefore, the cell-specific perfusion rate is about 2.3 ml/10 million cells/day. Cultures of mononuclear cell populations from murine bone marrow were developed in the mid- to late 1970s. These mouse cultures had long-term viability, but attempts to use the same culture protocols for human bone marrow cultures in the early 1980s were largely unsuccessful. The culture protocol called for medium exchange about once per week.

To perform a dynamic similarity analysis of perfusion rates, or medium exchange rates, between in vivo and in vitro conditions, the per cell medium exchange rate in culture is calibrated to that calculated for the preceding in vivo situation. Cell cultures are typically started with cell densities on the order of a million cells per ml. Therefore, 10 million cells would be placed in 10 mls of culture medium, which contains about 20 percent serum (vol/vol). A full daily medium exchange would hence correspond to replacing the serum at 2 ml/10 million cells/day, which is similar to the number calculated previously.

Experiments using this perfusion rate and the cell densities were performed in the late 1980s and led to the development of prolific cell cultures of human bone marrow. These cultures were subsequently scaled up to produce a clinically meaningful number of cells and are currently undergoing clinical trials. Thus, a simple similarity analysis of the in vivo and in vitro tissue dynamics led to the development of culture protocols that are of clinical significance. Such conclusions can be derived from tissue spec sheets.

These examples serve to illustrate the type of approximate calculations that assist the tissue engineering in performing an analysis of a tissue or organ system. Accurate measurements or estimations and well-organized facts (the spec sheets) provide the basic data. Characteristic time constants, length constants, fluxes, rates, concentrations, and so on can then be estimated. The relative magnitudes of such characteristics serve as a basis for order of magnitude judgments, which in turn inform the design of a tissue as well as the process for fabricating that tissue.

6.3.3 Mass Transfer in 3-D Configurations

An understanding of mass transfer in biological systems is essential in tissue engineering in order to design and deliver cell therapy products or extracorporeal organs. Although biological and physiological spec sheets give a global target for mass transfer, the details of the capillary bed of the smallest physiological units of the tissue are required. Mass transfer depends on the diffusion and convection of nutrients and waste to and from tissue, and the consumption of nutrients and production of waste by the tissue. Table 6.9 shows typical convection of blood in the vasculature, and Table 6.12 and Figure 6.29 give the consumption and diffusion rates of nutrients in the human body. Convection is driven by pressure differences and dominates in the vasculature, while diffusion is driven by concentration gradients and dominates in the tissues. Diffusion can be described by Fick's Law, and one can modify this equation to describe mass transfer in three basic configurations: rectangles, cylinders, and slabs.

The key to a successful bioreactor design for extracorporeal support or expansion of stem cells is maintaining adequate mass transfer while at the same time providing a local

TABLE 6.9 Peak Convection Rates of Blood in the Vasculature

Compartment	cm/sec
Aorta	140 ± 40
Common carotid	100 ± 20
Vertebral[a]	36 ± 9
Superficial femoral	90 ± 13
Liver sinusoid[b]	10^{-2}–10^{-3}

Data from DeWitt and Wechsler (1988).
[a]*Data from Jager et al. (1985).*
[b]*Data from McCuskey (1984).*

environment conducive to the differentiated state. The dominant mechanism of transport of low molecular weight nutrients (e.g., oxygen and glucose) within tissue or cell aggregates is diffusion. The length scale for diffusive transport (i.e., the distance over which oxygen penetrates into the tissue from nutrient stream before it is completely consumed by the cells) depends on the volumetric concentration of metabolite in the nutrient stream, C_0; the rate at which the cells consume the nutrient, Q_i; the diffusion coefficient for the metabolite in the tissue, D_t; and the system geometry. The metabolic consumption rate Q_i is generally a function of the nutrient concentration in the cell mass, C. The most common rate expression is the Michaelis-Menton type, which reduces to zero-order kinetics for high concentrations of nutrient (i.e., $C \gg K_m$) and to first-order kinetics for low nutrient concentrations (i.e., $C \ll K_m$). It is reasonable to consider the zero-order limit because the condition $C \gg K_m$ is met for many important nutrients under normal physiological conditions.

An estimate of the length scale for nutrient diffusion in a 3-D cell mass can be obtained by mathematical modeling. The general equation governing the balance between steady-state diffusion and metabolic consumption is

$$D_i \nabla^2 C = Q_i \tag{6.18}$$

where C is the concentration of the nutrient within the cell mass. Three simple geometries amenable to analytical solutions are shown in Figure 6.24: a slab, cylinder, or sphere of cells bathed in medium containing the nutrient at concentration C_0. Expanding the gradient operator for each of these geometries, Eq. (6.18) can be written as

$$\text{slab: } D_t \frac{d^2C}{dx^2} = Q_i \tag{6.19a}$$

$$\text{cylinder: } D_t \frac{1}{r}\frac{d}{dr}\left(\frac{rdC}{dr}\right) = Q_i \tag{6.19b}$$

$$\text{sphere: } D_t \frac{1}{r}\frac{d}{dr}\left(\frac{r^2dC}{dr}\right) = Q_i \tag{6.19c}$$

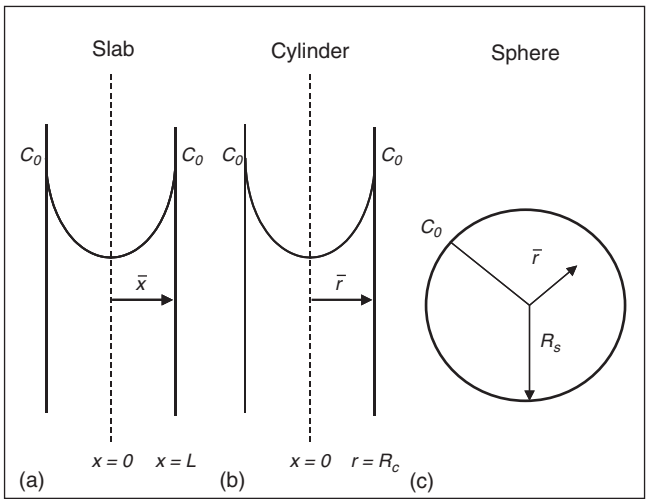


FIGURE 6.24 The three possible configurations considered by the mass transfer equations. *Reproduced from [12].*

The manner in which the distance variables are defined in each case is shown in Figure 6.24. A standard technique in the analysis of differential equations describing transport phenomena is to scale the variables so they are dimensionless and range in value from 0 to 1. This allows the relative magnitude of the various terms of the equations to be evaluated easily and allows the solutions to be plotted in a set of graphs as a function of variables that are universally applicable. For all geometries, C is scaled by its maximum value, C_o—that is, $\bar{C} = C/C_o$. Distance is scaled with the diffusion path length, so for a slab, $\bar{x} = x/L$; for a cylinder, $\bar{r} = r/R_c$; and for a sphere, $\bar{r} = r/R_s$. With these definitions, the boundary conditions for all three geometries are no flux of nutrient at the center $(d\bar{c}/d\bar{x},\, d\bar{c}/d\bar{r} = 0\ at\ \bar{x}, \bar{r}, = 0)$ and that $\bar{C} = 1$ at the surface $(\bar{x}, \bar{r}, = 1)$.

The use of scaling allows the solutions for all three geometries to collapse to a common form:

$$\bar{C} = 1 - \frac{\phi^2}{2(1 - \bar{x}^2)}$$

All of the system parameters are lumped together in the dimensionless parameter ϕ^2, which is often called the Thiele modulus. The Thiele modulus represents the relative rates of reaction and diffusion and is defined slightly differently for each geometry:

$$\phi^2_{slab} = \frac{Q_i/L^2}{C_o D_t}, \qquad \phi^2_{cylinder} = \frac{Q_i/Rc^2}{C_o D_t}, \qquad \phi^2_{sphere} = \frac{Q_i/(L^2 = (R/3)^2)}{C_o D_t}$$

6.3.4 The Tissue Microenvironment: Cell Therapy and Bioreactor Design Principles

A main task in tissue engineering is to recreate tissue function by constructing a tissue. Achieving this goal is challenging, and it is based on some fundamental axioms:

- The developmental program and the wound healing response require the systematic and regulated unfolding of the information on the DNA through coordinated execution of genetic subroutines and programs. Participating cells require detailed information about the activities of their neighbors. Proper cellular communications are of key concern and in many cases the concept of the "stem cell niche" is important.
- Upon completion of organogenesis or wound healing, the function of fully formed organs is strongly dependent on the coordinated function of multiple cell types. Tissues function based on multicellular aggregates, called functional subunits of tissues.
- The microenvironment has a major effect on the function of an individual cell. The characteristic length scale of the microenvironment is 100 μm.
- The microenvironment is characterized by (1) neighboring cells: cell-cell contact, soluble growth factors, and so on; (2) the chemical environment: the extracellular matrix, the dynamics of the nutritional environment; and (3) the local geometry.

Cellular Function In Vivo: The Tissue Microenvironment and Communication

An important requirement for successful tissue function is a physiologically acceptable environment in which the cells will express the desired tissue function. One common way to approach this goal is to attempt to recreate or mimic the physiological in vivo environment. This task involves mimicking a variety of features of the microenvironment including cell-cell and cell-matrix communications, the biochemical and mechanical milieu, as well as communication between tissues.

Examples of tissue microenvironments are shown in Figure 6.25, and some are discussed in Section 6.2.2. The communication of every cell with its immediate environment and other tissues can be examined using a topological representation of the organization of the body. The DNA in the center of the diagram contains the information that the tissue engineer wishes to express and manage. This cell is in a microenvironment that has important spatiotemporal characteristics.

Signals to the nucleus are delivered at the cell membrane and are transmitted through the cytoplasm by a variety of signal transduction mechanisms. Some signals are delivered by soluble growth factors after binding to the appropriate receptors. These growth factors may originate from the circulating blood or from neighboring cells. Nutrients, metabolic waste products, and respiratory gases traffic in and out of the microenvironment in a highly dynamic manner. The microenvironment is also characterized by its cellular composition, the ECM, and local geometry, and these components also provide the cell with important signals. The size scale of the microenvironment is on the order of 100 μm. Within this domain, cells function in a coordinated manner. If this arrangement is disrupted, appropriate cell function will not be obtained.

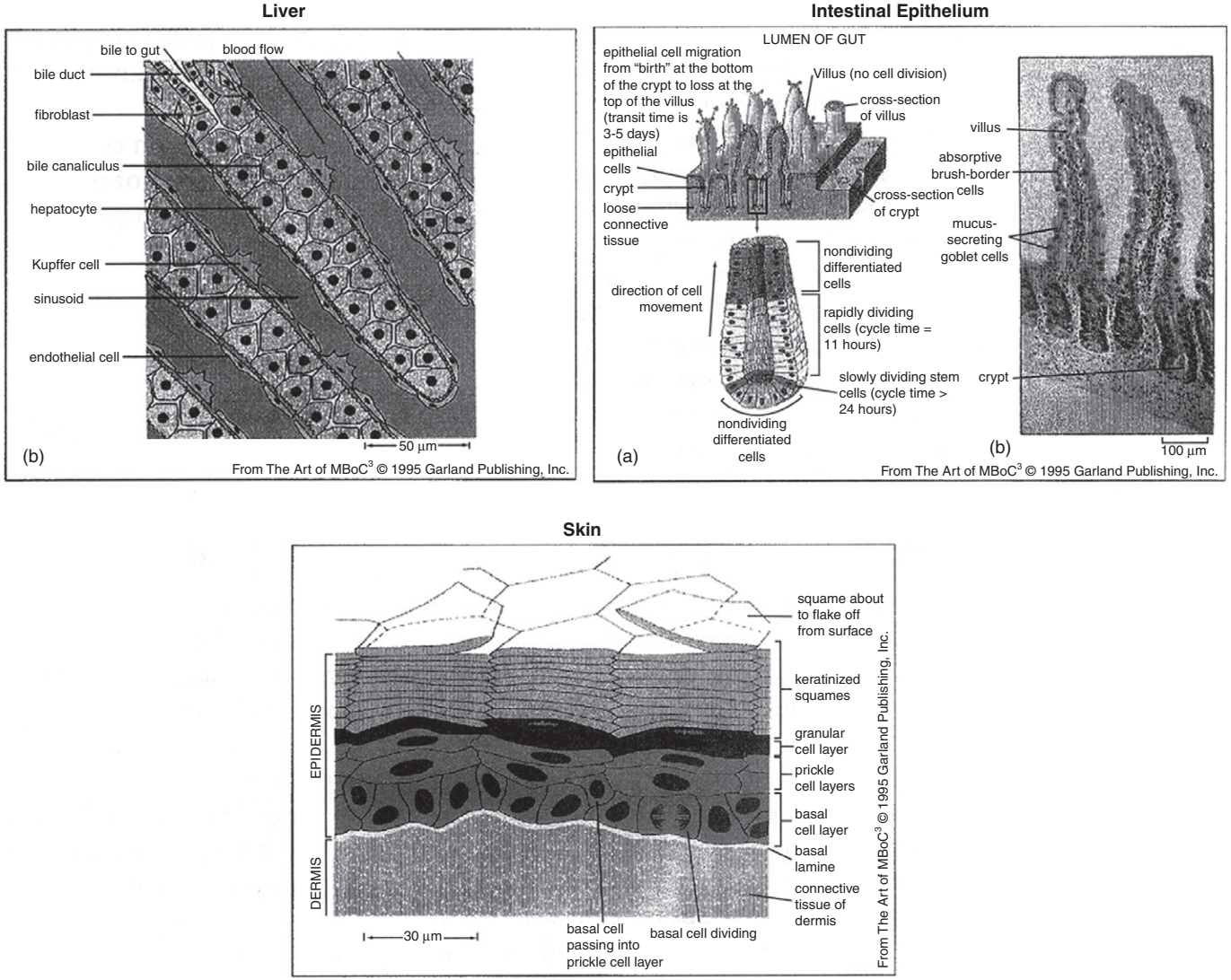


FIGURE 6.25 In vivo tissue microenvironments. *From Alberts et al., 1995.*

EXAMPLE PROBLEM 6.5

The hepatic stellate cell is a mesenchymal cell that is located in the Space of Disse, the area between the sheets of hepatocytes and the hepatic sinusoidal endothelium (Figure 6.26) in the liver. In the normal state, hepatic stellate cells are found in close proximity to a basement membrane-like matrix. This matrix consists of collagen type IV, laminin, and heparan sulphate proteoglycans. If the liver is injured, hepatic stellate cells are activated, and they begin to make matrix proteins, mostly collagen type I and III. As a result of hepatic stellate cell activation, the phenotype of both the hepatocytes (lose their brush border) and endothelium (lose their fenestration) changes. If this condition persists, liver fibrosis results. Thus, a lack of coordination in cellular function in the tissue microenvironment can lead to loss of tissue function. This example can be used to examine how liver dysfunction can be prevented or corrected, including using a tissue engineering approach.

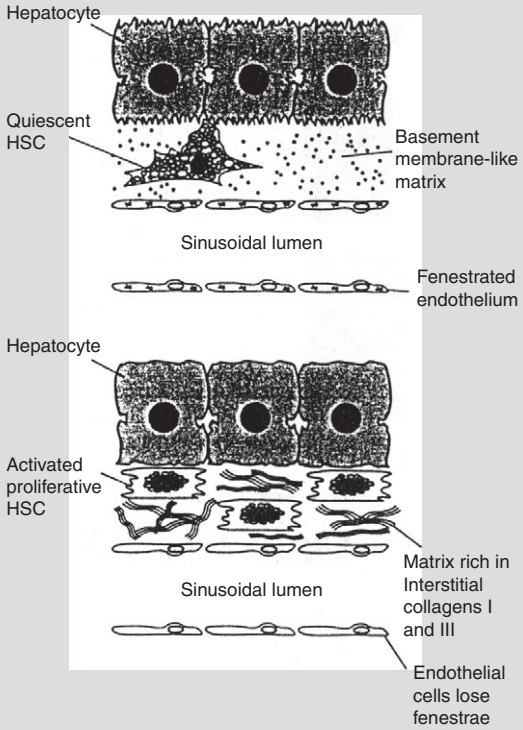


FIGURE 6.26 Schematic of hepatic stellate cell activation and the process of liver fibrosis. *From Iredale, 1997.*

Tissues are perfused by the microcirculation and can be viewed as essentially homogeneous down to a length scale of about 100 μm. The microcirculation then interfaces with the long-distance convective transport system that connects all the tissues in the body and, thus, all cells, to a nutritional supply, exchange of respiratory gases, removal of toxic products, and so on.

From a tissue engineering standpoint, a key challenge is to design the environment at both the micro and macro scales. This problem is characterized by two important considerations:

- The microenvironment (its chemical, geometric, cell architectural, and diffusional characteristics)
- Interactions with other tissues (source of nutrients, exchange of respiratory gases, removal of waste products, and delivery of soluble protein such as growth factors)

Cellularity

The packing density of cells is on the order of a billion cells per cubic centimeter (cc). Tissues are typically operating at one-third to one-half of packing density, such that typical cell densities in tissues are on the order of 100 to 500 million cells per cc. Since the characteristic length scale is about 100 μm, the order of magnitude of the number of cells found in a tissue microenvironment can be estimated: a 100 μm cube at 500 million cells per cc contains about 500 cells. Simple multicellular organisms, such as *C. elegans*, a much-studied small worm, that have about 1,000 cells, provide an interesting comparison.

The cellularity of the tissue microenvironment varies among tissues. An example of a tissue with low cellularity is cartilage. The function of chondrocytes in cartilage is to maintain the extracellular matrix. Cartilage is essentially avascular, alymphatic, and aneural. Thus, many of the cell types found in other tissues are not present, which limits the range of functions that the tissue can perform. The cellularity of cartilage is about a million cells per cc, or about one cell per cubic 100 μm. Most other tissues, in particular those that are highly metabolically active and/or remodel at a high rate, are much more cellular, with hundreds of cells per 100 μm cube.

The tissue microenvironment is characterized by having a number of cell types (Table 6.10). In addition to the parenchymal cells (the tissue type cells—e.g., hepatocytes in liver), a variety of accessory cells are found in all tissues, as described briefly following.

TABLE 6.10 Cells That Contribute to the Tissue Microenvironment

Stromal cells: derivates of a common precursor cell
Mesenchyme
Fibroblasts
Myofibroblasts
Osteogenic/chondrogenic cells
Adipocytes
Stromal-associated cells: histogenically distinct from stromal cells, permanent residents of a tissue
Endothelial cells
Macrophages

TABLE 6.10 Cells That Contribute to the Tissue Microenvironment—Cont'd

Transient cells: cells that migrate into a tissue for host defense either prior to or following an inflammatory stimulus
B lymphocytes/plasma cells
Cytotoxic T cells and natural killer (NK) cells
Granulocytes
Parenchymal cells: cells that occupy most of the tissue volume, express functions that are definitive for the tissue, and interact with all other cell types to facilitate the expression of differentiated function

- Mesenchymal cells (such as fibroblasts and smooth muscle cells) are present in all tissues. These cells are of connective tissue type.
- Monocytes are present in all tissues and can take on a variety of different morphologies (see Figure 6.27). Monocytes can differentiate into macrophages that, once activated, produce a variety of cyto- and chemokines that influence the behavior of neighboring cells.

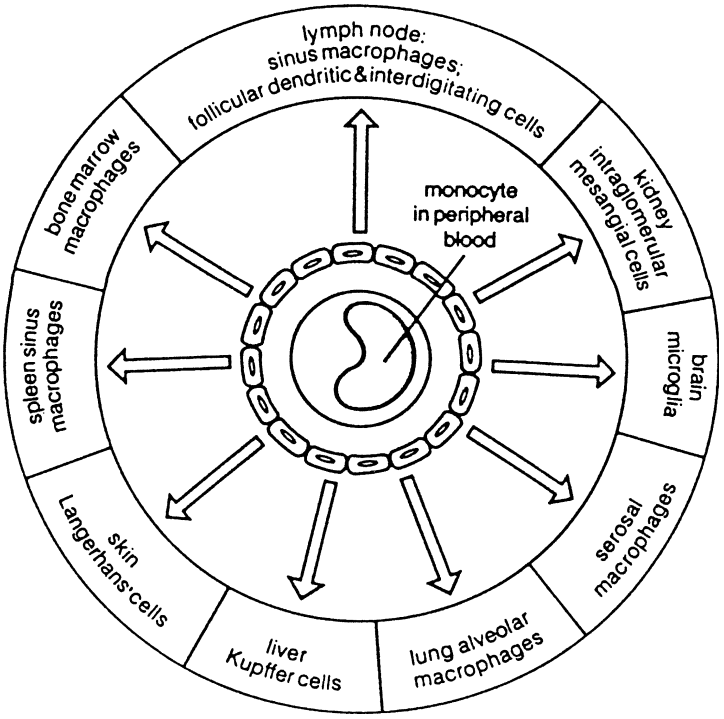


FIGURE 6.27 Distribution of macrophages and their presence in different tissues. *From [6].*

TABLE 6.11 Cells Contributing to the Hepatic Microenvironment

Cell Type	Size (μm)	Relative Percentage of Total Cells
Stroma		
Kupffer cells	12–16	8
Vascular endothelia	11–12	9
Biliary endothelia	10–12	5
Fat-storing cells	14–18	3
Fibroblasts	11–14	7
Pit cells	11–15	1–2 (variable)
Parenchymal cells		
Mononuclear (type I)	17–22	35
Binuclear (type II)	20–27	27
Acidophilic (type III)	25–32	5

- Endothelial cells are associated with the vasculature found in almost all tissues. These cells play a major role in the trafficking of cells in and out of tissue and may play a major role in determining tissue metabolism.
- Lymphocytes and neutrophils have a transient presence in tissues, typically as a part of a host defense response or other cleanup functions.

These accessory cells are typically about 30 percent of the cellularity of tissue, while the parenchymal cells make up the balance. An example is provided in Table 6.11 that lists the cellularity of the liver.

EXAMPLE PROBLEM 6.6

What happens if these accessory cells are removed?

Solution

The role of accessory cells (in some cases called stromal cells) in bone marrow cultures has been systematically studied. Since the immature cells can be isolated based on known antigens, the relative abundance of the key parenchymal cells and the accessory cells can be varied. Such an experiment amounts to a titration of the accessory cell activity. The results from such an experiment are shown in Figure 6.28. All cell production indices (total cells, progenitor cells, and preprogenitor cells) decline sharply as the accessory cells are removed. Supplying preformed irradiated stroma restores the production of total and progenitor cells but not the preprogenitors. This result is consistent with the expectation that specific interactions between accessory cells and parenchymal cells are important for immature cells.

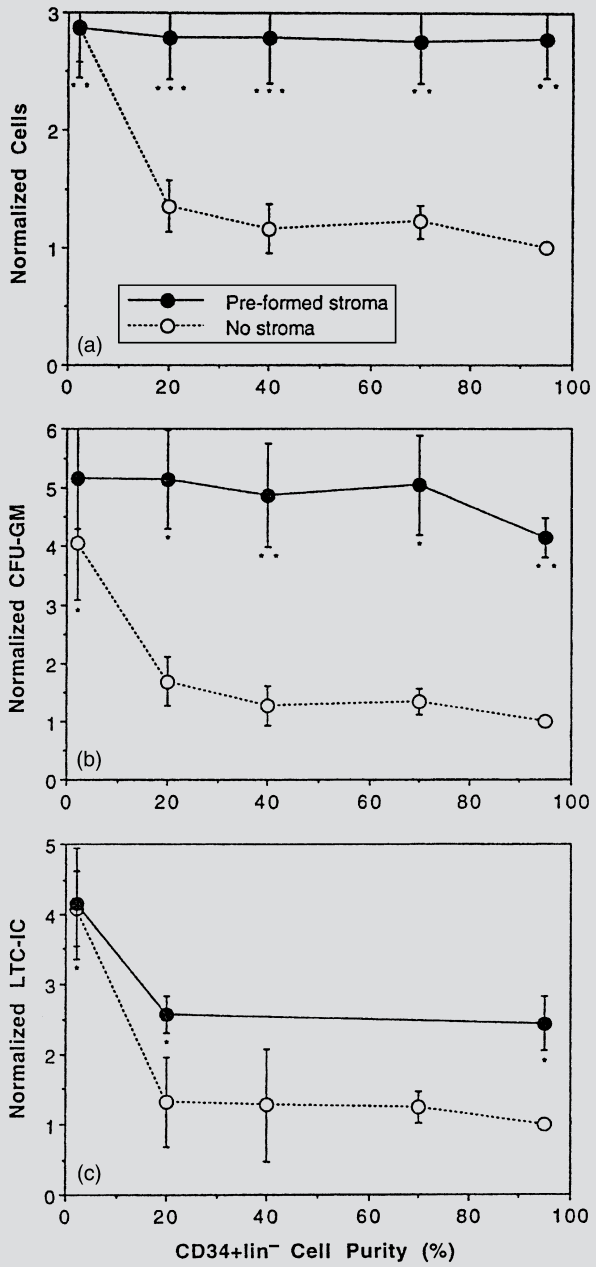


FIGURE 6.28 Effects of CD34+ lin$^-$ cell (a population of primitive hematopoietic cells) purity on culture output. With increasing purity, the performance on a per-cell basis drops due to loss of accessory cell function. CFU-GM ¼ colony-forming units granulocyte/macrophage; LTC-IC ¼ long-term cultureinitiating cells. *From Koller and Palsson, 1993.*

Dynamics

Temporal aspects of the cellular microenvironment also are critical to obtaining desired cell phenotype and function. The microenvironment is highly dynamic and displays a multitude of time constants. Some of the key dynamic processes are described following.

OXYGENATION

Generally, mammalian cells do not consume oxygen rapidly compared to microorganisms, but their uptake rate is large compared to the amount of oxygen available at any given time in blood or in culture media (Table 6.12). At 37°C, air-saturated aqueous media contain only about 0.21 mM oxygen per liter. Mammalian cells consume oxygen at a rate in the range of 0.05–0.5 μmol/10^6cells/hr. With tissue cellularities of 500 million cells per cc, these oxygen uptake rates call for volumetric oxygen delivery rates of 25 to 250 μmol oxygen/cc/hour. This rate needs to be balanced with the volumetric perfusion rates of tissues and the oxygen concentration in blood.

Metabolically active tissues and cell cultures, even at relatively low cell densities, quickly deplete the available oxygen. For instance, at cell densities of 10^6cells/ml, oxygen is depleted in most tissues in about 0.4 to 4 hours. Oxygen thus must be continually supplied. To date, a number of primary cell types (e.g., hepatocytes, keratinocytes, chondrocytes, and hematopoietic cells) have been grown ex vivo for the purpose of cell therapy. The effects of

TABLE 6.12 Measured Oxygen-Demand Rates of Human Cells in Culture

Human	μmol $O_2/10^6$ cells/h
HeLa	0.1–0.0047
HLM (liver)	0.37
LIR (liver)	0.30
AM-57 (amnion)	0.045–0.13
Skin fibroblast	0.064
Detroit 6 (bone marrow)	0.43
Conjunctiva	0.28
Leukemia MCN	0.22
Lymphoblastoid (namalioa)	0.053
Lung	0.24
Intestine	0.40
Diploid embryo WI-38	0.15
MAF-E	0.38
FS-4	0.05

oxygen on hepatocytes have been systematically investigated. The reported specific oxygen uptake rate (OUR) for hepatocytes is around 1.0 μmole/10^6cells/hr, which is relatively high for mammalian cells. Conversely, a much lower oxygen consumption rate of about 0.02 μmole/10^6cells/hr has been reported for rat bone marrow cells.

In addition to the supply requirements, the concentration of oxygen close to the cells must be within a specific range. Oxygenation affects a variety of physiological processes, ranging from cell attachment and spreading to growth and differentiation. An insufficient concentration retards growth, while an excess concentration may be inhibitory or even toxic. For instance, several studies have shown that the formation of hematopoietic cell colonies in colony assays is significantly enhanced by using oxygen concentrations that are 5 percent of saturation relative to air, and an optimal oxygen concentration for bioreactor bone marrow cell culture has been shown to exist. The oxygen uptake rate of cells is thus an important parameter in the design of cell culture and tissue engineering studies.

METABOLISM AND CELL SIGNALING

Typically, there is not a transport limitation for major nutrients, although cells can respond to their local concentrations. The reason is that their concentrations can be much higher than that of oxygen, especially for the nutrients consumed at high rates. Typical uptake rates of glucose are on the order of 0.2 μmoles/million cells/hr, while the consumption rates of amino acids are in the range of 0.1–0.5 μmoles per billion cells per hour. The transport and uptake rates of growth factors face more serious transport limitations. The expected diffusional response times are given in Figure 6.29.

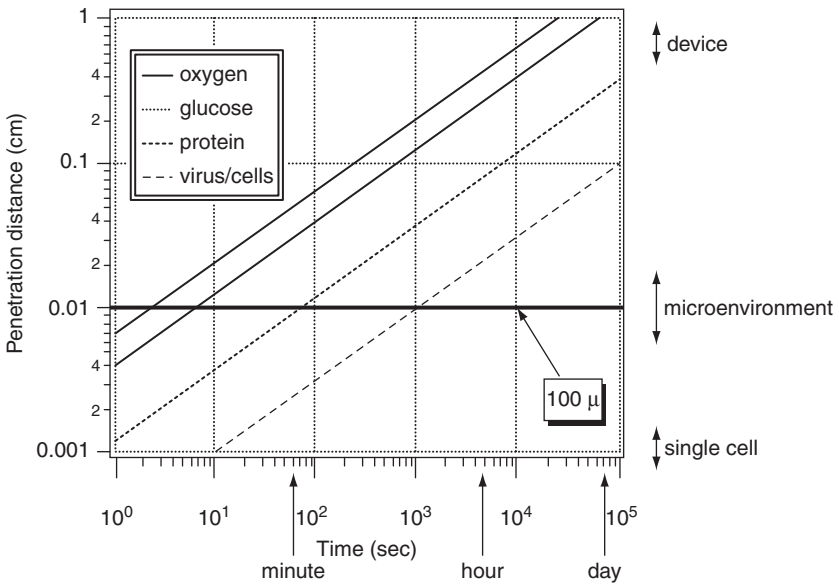


FIGURE 6.29 The diffusional penetration lengths as a function of time for several classes of biomolecules.

PERFUSION

The circulatory system provides blood flow to organs that is then distributed into the microenvironments. Overall, the perfusion rates in a human are about 5 liters/min/70 Kg, or about 0.07 ml/cc/min. With 500 million cells per cc, this is equivalent to 0.14 μl/million cells/min. These numbers represent a whole body average. There are differences in the perfusion rates of different organs that typically correlate with their metabolic activity.

CELL MOTION

As described earlier, cells are motile and move at different rates. Neutrophils can move several cell diameters in a minute, while adherent cell types, such as keratinocytes, fibroblasts, and endothelial cells, move on the order of a cell diameter per hour. These motilities represent rapid processes compared to cell replication and differentiation. Neutrophils have to be able to respond rapidly to invading microorganisms, while the adherent cell types mentioned move in response to dynamic tissue needs.

Size and Geometry

GEOMETRY

The geometric shapes of microenvironments vary (see Figure 6.25), and so do their dimensionalities. Many microenvironments are effectively curved 2-D surfaces. The cellular arrangement in bone marrow has been shown to have a fractal geometry with effective dimensionality of 2.7, while the brain is a 3-D structure.

WHAT DETERMINES THE SIZE OF THE MICROENVIRONMENT?

The answer to this question is not clear. At the lower limit is the size of a single cell, about 10 μm. A cell aggregate must be some multiple of this distance. The factors determining the upper limit are not clear. However, estimates of effective growth factor signal propagation distances and experimental evidence of oxygen penetration distances suggest that the dynamics of cell communication and cell metabolic rates are important in determining the size scale of the microenvironment. These distances are determined by the process of diffusion. In both cases, the estimated length scale is about 100 to 200 μm. The stability issues associated with coordinating cellular functions grow with an increased number of cells and may represent a limitation in practice.

6.3.5 Biomaterials

Biomaterials for tissue engineering present several challenges. There are basically three length scales of interest. The shortest is at the biochemical level, where concerns include the specific chemical features of the ECM and interactions with cellular receptors. Intact ECM components can be used to coat support material to ensure appropriate interactions among the cells and their immediate environment. More sophisticated treatments involve the synthesis of specific binding sequences from ECM protein and presenting them in various ways to the cells. Particular cellular arrangements can be obtained by micropatterning such materials. A combination of material manufacturing, biochemistry, and genetics is required to address these issues.

The next size scale of interest is the 100 micron size scale, the size of a typical organ microenvironment. Many organs have highly specific local geometries that may have to be engineered in an ex vivo system. Hence, a particular microgeometry with particular mechanical properties may have to be produced. Clearly, challenging material manufacturing issues arise. Further, the support matrix may have to be biodegradable after transplantation and the degradation products nontoxic. Lactic and glycolic acid-based polymers are promising materials in this regard. If little restructuring of implants occurs following grafting, then the geometry of the support matrix over larger size scales may be important.

The largest size scale is that of the bioreactor itself. Bioreactors in tissue engineering are likely to be small with dimensions on the order of about 10 cm. The materials issues that arise here are primarily those of biocompatibility. Although manufacturing technology exists for tissue culture plastic, it is likely that additional issues will arise. The tissue culture plastic that is commercially available is designed to promote adhesion, binding, and spreading of continuous cell lines. Although such features may be desirable for continuous cell lines, they may not be so for various primary cells.

6.4 SCALING UP

6.4.1 Fundamental Concept

As just discussed, tissue dynamics are comprised of intricate interplay between the cellular fate processes of cell replication, differentiation, and apoptosis. They are properly balanced under in vivo conditions. The dynamics of the in vivo conditions are a balance of these biological dynamics and the constraining physicochemical processes. The basic concept of design in tissue reconstruction is to engineer a proper balance between the biological and physicochemical rates so normal tissue function can occur.

6.4.2 Key Design Challenges

Within this framework, many of the engineering issues associated with successful reconstitution of tissues can be examined. This section provides an engineering perspective of tissue engineering and helps to define the productive and critical role that engineering needs to play in the ex vivo reconstruction of human tissues.

Important design challenges in tissue engineering include the following:

- Oxygenation—that is, providing adequate flux of oxygen at physiological concentrations
- Provision and removal of cyto- and chemokines
- Physiological perfusion rates and uniformity in distribution
- Biomaterials, including functional, structural, toxicity, and manufacturing characteristics

Other issues associated with the clinical implementation of cellular therapies include the design of disposable devices, optimization of medium composition, initial cell distribution, meeting FDA requirements, and operation in a clinical setting. These cannot be discussed in detail here, but some of these issues are addressed generally in the following section.

6.4.3 Time Scales of Mass Transfer

The importance of mass transfer in tissue and cellular function is often overlooked. The limitations imposed by molecular diffusion become clear if the average displacement distance with time is plotted for diffusion coefficients that are typical for biological entities of interest in tissue function (Figure 6.29). The diffusional penetration lengths over physiological time scales are surprisingly short and constrain the in vivo design and architecture of organs. The same constraints are faced in the construction of an ex vivo device, and high mass transfer rates into cell beds at physiological cell densities may be difficult to achieve.

The biochemical characteristics of the microenvironment are critical to obtaining proper tissue function. Much information exists about the biochemical requirements for the growth of continuous cell lines. For continuous cell lines, these issues revolve around the provision of nutrients and the removal of waste products. In cultures of primary cells, the nutrients may have other roles and directly influence the physiological performance of the culture. For instance, recently it has been shown that proline and oxygen levels play an important role in hepatocyte cultures.

In most cases, oxygen delivery is likely to be an important consideration. Too much oxygen will be inhibitory or toxic, while too little may alter metabolism. Some tissues, like the liver, kidney, and brain, have high oxygen requirements, while others require less. Controlling oxygen at the desired concentration levels, at the desired uniformity, and at the fluxes needed at high cell densities is likely to prove to be a challenge. Further, the oxygen and nutritional requirements may vary among cell types in a reconstituted tissue that is comprised of multiple cell types. These requirements further complicate nutrient delivery. Thus, defining, designing, and controlling the biochemical characteristics of the microenvironment may prove difficult, especially given the constraints imposed by diffusion and any requirements for a particular microgeometry.

EXAMPLE PROBLEM 6.7

How are specific oxygen uptake rates measured?

Solution

Most data on specific oxygen uptake rates are obtained with cells in suspension using standard respirometers. However, to obtain accurate and representative data for primary cells, they need to be adherent. This challenge has led to the design of a novel in situ respirometer, shown in Figure 6.30. This respirometer has been used to measure the oxygen uptake rates of hepatocytes in culture (OUR of 1.0 μmoles/million cells/hr). A similar device has been used to measure the OUR in bone marrow cultures, giving results of 0.03 to 0.04 μmoles/million cells/hr which are similar to the in vivo uptake rates.

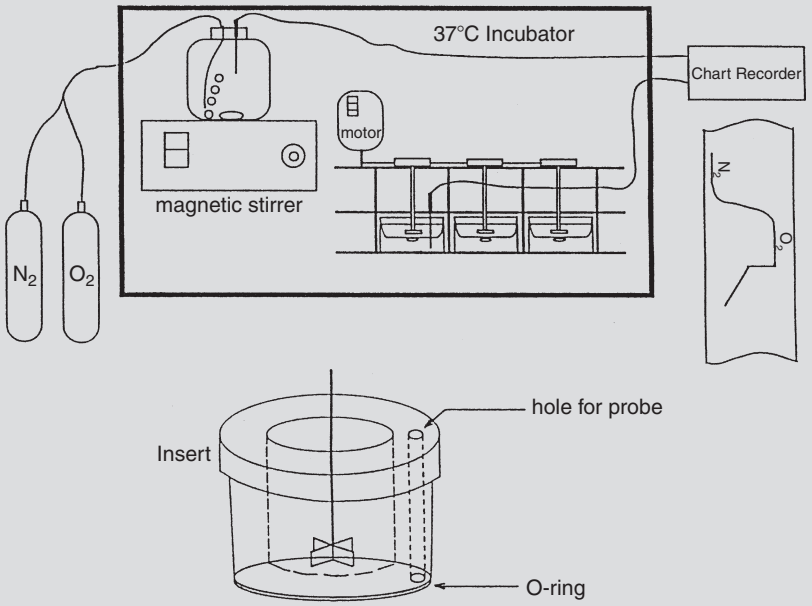


FIGURE 6.30 Schematic diagram of the apparatus for measuring oxygen uptake rate. *From [14].*

EXAMPLE PROBLEM 6.8

Oxygen can be delivered in situ over an oxygenation membrane, as illustrated in Figure 6.31. At slow flow rates compared to the lateral diffusion rate of oxygen, the oxygen in the incoming

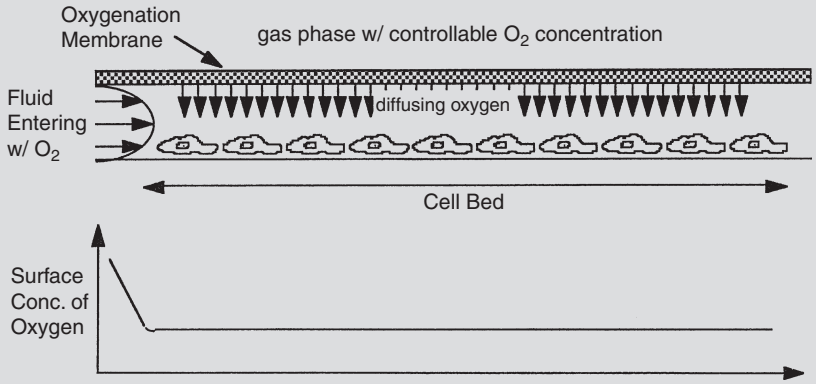


FIGURE 6.31 Oxygen delivery across a membrane that is placed on top of a fluid that is flowing across a cell bed. If the fluid transit time is much slower than the diffusional time for oxygen, then oxygen is delivered primarily via diffusion. This leads to a small entrance effect in which the oxygen in the incoming stream is consumed, while the oxygen concentration over the rest of the cell bed is relatively constant.

Continued

stream is quickly depleted, and the bulk of the cell bed is oxygenated via diffusion from the gas phase. This leads to oxygen delivery that can be controlled independently of all other operating variables and that is uniform. The gas phase composition can be used to control the oxygen delivery. This is an example of how technology can be used to overcome some of the limitations imposed by mass transfer considerations.

6.4.4 Fluid Flow and Uniformity

A device that carries tens of millions of microenvironments (i.e., a reconstituted tissue or organ) must provide uniform delivery and removal of gases, nutrients, and growth factors. Achieving such uniformity is difficult. This difficulty arises partly from the fact that fluid has a no-slip condition as it flows past a solid surface. Thus, there will always be slower-flowing regions close to any side walls in a bioreactor. These slow-flowing regions under conditions of axial Graetz numbers of unity lead to mass transfer boundary layers that extend beyond the hydrodynamic boundary layer.

This problem is illustrated further in Figure 6.32. Fluid is flowing down a thin slit, representing an on-end view of the chamber shown in Figure 6.33. Thin slits with high aspect

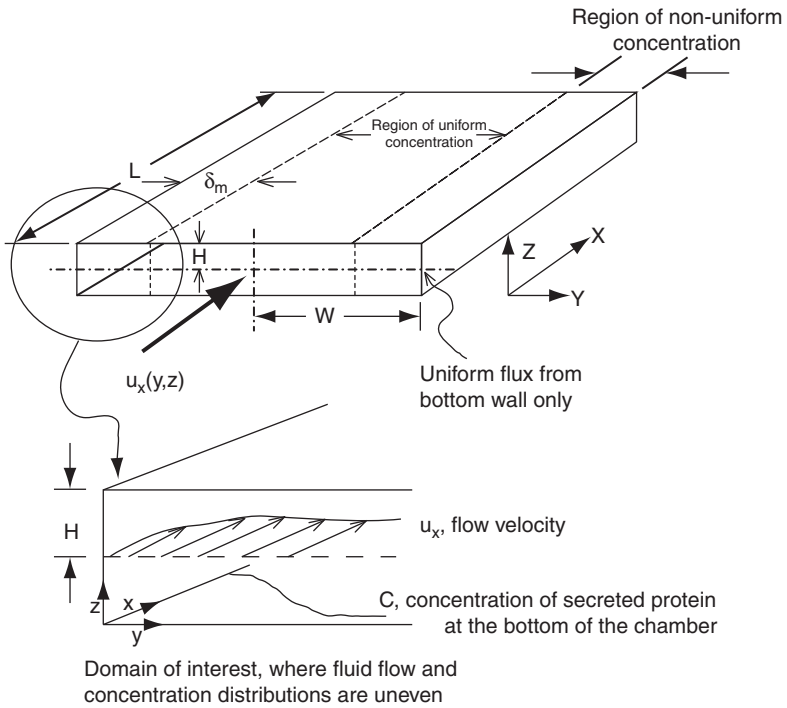


FIGURE 6.32 Coordinate system for a rectangular chamber with production of biological factors secreted by cells lodged on the bottom wall. The fluid is slowed down close to the side wall, creating a different concentration than that found in the middle of the slit. This leads to a very different microenvironment for cell growth and development of tissue function at the wall than elsewhere in the chamber. *From [15].*

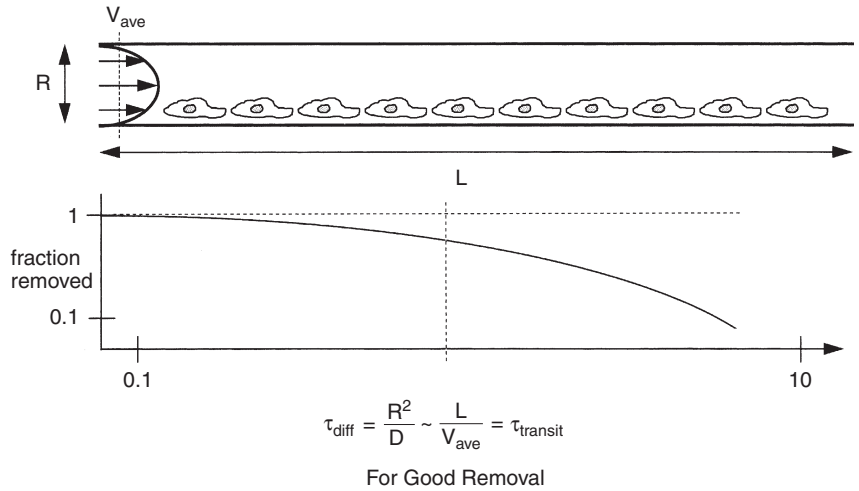


FIGURE 6.33 A schematic of the concentration of growth factors in a liquid that is flowing across a bed of cells that consume it. (Bottom) The fractional removal of the growth factor as a function of the relative time constants of lateral diffusion and transit across the cell bed. The ratio of the two is the Graetz number (Gz). If the diffusional response time is slower than that of transit (Gz >1), then there is insufficient time for the diffusing growth factor molecule to make good contact with the cell bed. Most of the growth factor leaves the system in the exit stream. Conversely, if the diffusion time is much shorter than that of transit, there will be ample time for the growth factor to make it to the cell bed. If it is rapidly consumed there, then negligible amounts will leave the system.

ratios will satisfy the Hele-Shaw flow approximations, in which the flow is essentially the same over the entire width of the slit except close to the edges. The width of the slow-flowing regions is on the order of the depth of the slit (R). Thus, if the aspect ratio is 10, fluid will flow the same way over about 90 percent of the width of the slit. In the remaining 10 percent, the slow flow close to the wall can create microenvironments with a different property than in the rest of the cell bed. Such nonuniformity can lead to differences in cell growth rates and to migration of cells toward the wall. Such nonuniformity in growth close to walls has been reported. This problem can be overcome by using radial flow configurations. The example shown is for one type of a bioreactor for cell culturing. Other configurations will have similar difficulties in achieving acceptable uniformity in conditions, and careful mass and momentum transfer analyses need to be performed to guide detailed design.

6.5 IMPLEMENTATION OF TISSUE ENGINEERED PRODUCTS

6.5.1 Delivering Cellular Therapies in a Clinical Setting

In the last section, the design challenges that the tissue engineer is faced with when it comes to scaling up microenvironments to produce cell numbers that are of clinical significance were surveyed. These problems are only a part of the challenges that must be met in implementing cellular therapies. In this section, some of the other problems that need to be solved will be discussed.

Donor-to-Donor Variability

The genetic variability in the human population is substantial. Therefore, outcome from cell isolations and tissue engineering approaches can vary significantly between donors. Even if the cell growth process, the production of materials, and the formulation of medium are essentially identical, a large variation in outcomes among donors can still result. This variability is due to intrinsic biological factors. Some regularization in performance can be achieved by using a full complement of accessory cells. Although interdonor variability is considerable, the behavior of the same tissue source is internally consistent. An example is shown in Figure 6.34, where the relative uptake rates of growth factors are shown for a number of donor samples. The uptake rates of growth factors can be highly correlated within many donor samples. The quantitative nature of the correlation changes from donor to donor, making it difficult to develop a correlation that would represent a large donor population.

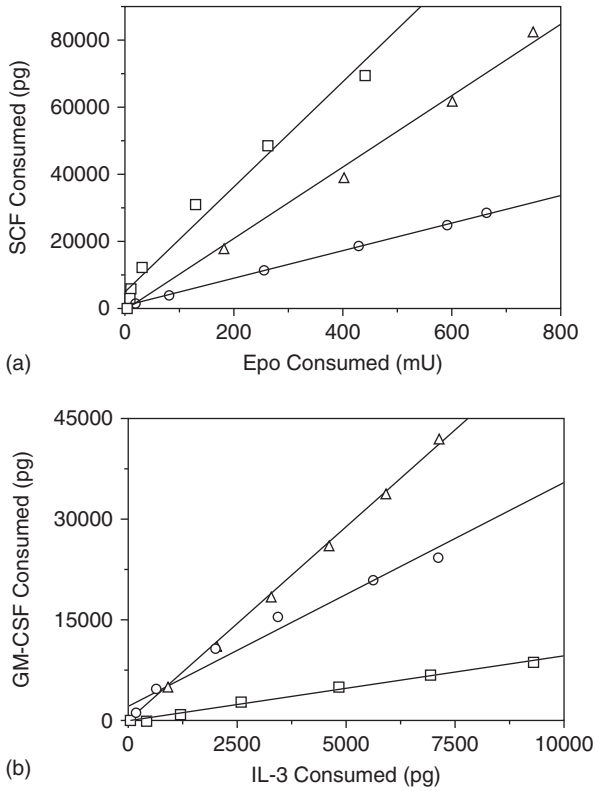


FIGURE 6.34 The uptake of growth factors—that is, stem cell factor (SCF) and erythropoietin (Epo)—is highly correlated in cultures of human bone marrow. The correlation is strong for a single tissue sample. The slopes of the curves vary significantly among donors. *From [8].*

Strongly Interacting Variables

Primary cell cultures are sensitive to many of the variables that define the microenvironment. Many of these variables interact strongly. Thus, any change in a single cell culture variable will change the optimal value for all the others. Statistical experimental design procedures that optimize the search over many experimental variables can be employed to lessen this effect. An example of such a two-dimensional search is shown in Figure 6.35, where the optimal progenitor production performance of human cell cultures is shown as a function of the inoculum density and the medium flow rate. The top panel shows the optimal number of progenitors produced per unit cell growth area. Optimizing this objective would lead to the smallest cell culture device possible for a specified total number of cells that is needed. The bottom panel shows the optimal expansion of progenitor cells—that is, the output number relative to the input. This objective would be used in situations where the starting material is limited and the maximum number of additional progenitors is desired. Note that the two objectives are found under different conditions. Thus, it is critical to clearly delineate the objective of the optimization condition from the outset.

Immune Rejection

Allogeneic transplants face immune rejection by the host. A variety of situations are encountered in such transplantations. Dermal fibroblasts, used for skin ulcers, seem to be effectively nonimmunogenic, though the reason is not clear. This fact makes it possible to make a large number of grafts from a single source and transplant into many patients. In contrast, pancreatic beta cells and islets face almost certain rejection (see Figure 6.6). In allogeneic bone marrow transplantation, the graft may reject the immunocompromised host. This so-called "Graft-vs-Host disease" is the main cause for the mortality resulting from allogeneic bone marrow transplants.

The cellular and molecular basis for the immune response is becoming better understood. The rejection problem is a dynamic process that relies on the interaction between subsets of $CD4^+$ cells ($CD4^+$ is a surface antigen on certain T-cells) Th1 and Th2 that differ in their cytokine secretion characteristics and effector functions. The components of the underlying regulatory network are becoming known. Quantitative and systemic analysis of this system is likely to lead to rational strategies for manipulating immune responses for prophylaxis and therapy.

Tissue Procurement

The source of the starting material for a growth process is of critical importance. For example, the source of dermal fibroblasts used for skin replacements is often human foreskin obtained from circumcisions. This source is prolific and can be used to generate a large number of grafts. Since the source is always the same, the biological variability in the growth process is greatly diminished. The costs associated with tissue procurement as reflected in the final product are minimal. Conversely, an adult autologous source may be expensive to obtain and will display highly variable performance.

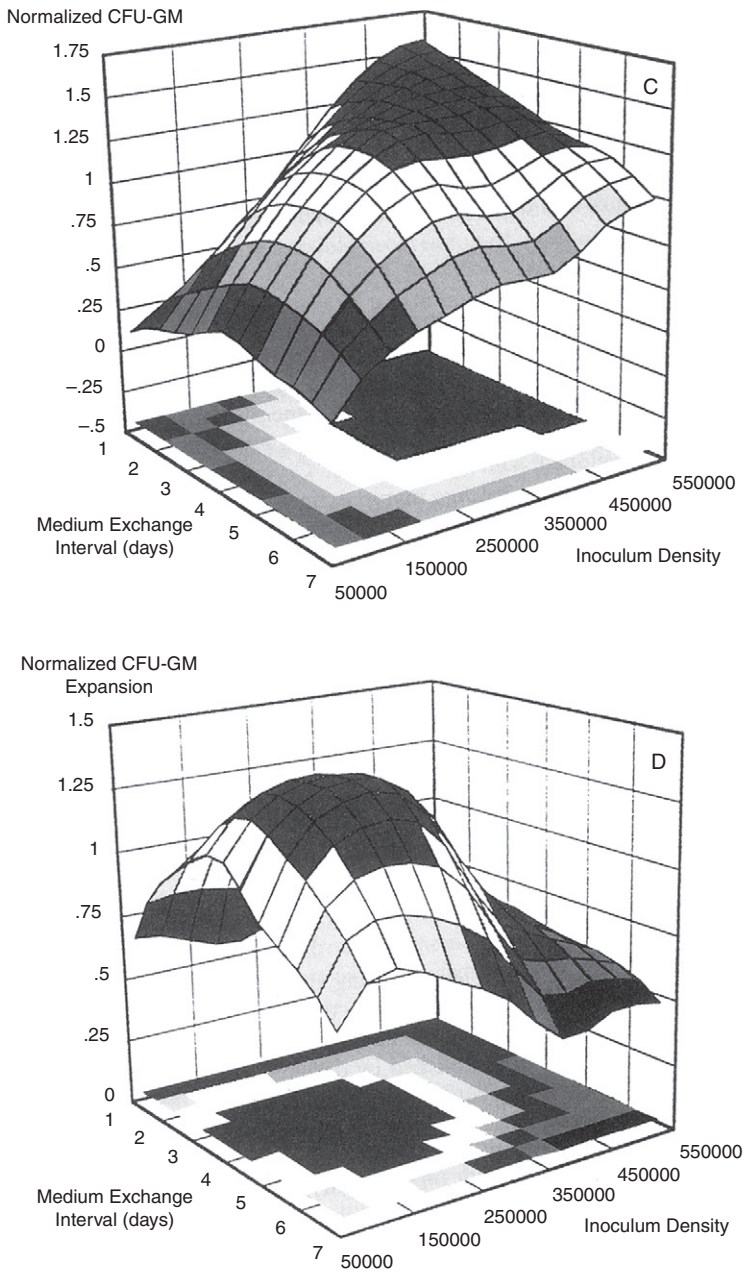


FIGURE 6.35 The performance of a bone marrow culture system over a wide range of inoculum densities and medium exchange intervals. Note that the variables interact. The performance (on the *z*-axis) in the top panel is the total number of progenitor cells (CFU-GM) per unit area, whereas in the bottom panel the expansion ratio is depicted (number out divided by number in). Note that the two measures are optimized under vastly different conditions. *From [8].*

Ultimately, the most desirable starting material for cellular therapies is a tissue-specific stem cell whose rate of self-renewal and commitment to differentiation can be controlled. Further, if such a source could be made nonimmunogenic, most of the problems associated with tissue procurement would be solved. The variability in the tissue manufacturing process would be reduced and would make any quality assurance (QA) and quality control (QC) procedures easier.

Cryopreservation

The scientific basis for cryopreservation involves several disciplines, including basic biophysics, chemistry, and engineering. Current cryopreservation procedures are clinically accepted for a number of tissues, including bone marrow, blood cells, cornea, germ cells, and vascular tissue. Recent experience has shown that, in general, the same procedures cannot be applied to human cells that have been grown ex vivo. New procedures need to be developed and implemented. Any cryopreservation used in existing cellular therapies that rely on ex vivo manipulation call for freezing the primary tissue prior to the desired manipulation.

6.6 FUTURE DIRECTIONS: FUNCTIONAL TISSUE ENGINEERING AND THE "-OMICS" SCIENCES

6.6.1 Cellular Aspects

Some cell populations that are to be transplanted may contain subpopulations of unwanted cells. The primary example of this is the contamination of autologously harvested hematopoietic cell populations with the patient's tumor cells. Ideally, any such contamination should be removed prior to transplantation. Similarly, many biopsies are contaminated with accessory cells that may grow faster than the desired parenchymal cells. Fibroblasts are a difficult contaminant to eliminate in many biopsies, and they often show superior growth characteristics in culture. For this reason, it is difficult to develop primary cell lines from many tumor types.

6.6.2 Functional Tissue Engineering

Tissue engineering is distinguished from cell biology by the focus on the emergent function that arises from the organization of large numbers of cells into higher-order structures, variously called *tissues* or *organs*, depending on the level of anatomical complexity and structural integration. The reengineering of complex human anatomical structures such as limbs or organ systems is by definition a systems-engineering problem. Though it can be argued that all tissue functions arise from fundamental cellular mechanisms, the system-level organization of tissues and organs confers function that is not possible to achieve with individual cells or masses of unorganized cells in a scaffold. A pile of bricks does not provide the functionality of a house, nor does a crate full of car parts function like an automobile. Analogously, engineered tissues must be viewed at the systems level, and the success or failure of the engineering effort ultimately rests upon a quantitative assessment of the organ-level function of the engineered tissue or organ. The use of molecular biological

techniques to verify the presence of one or more critical subcellular constituents is simply not an adequate demonstration of end organ function. Thus, it is important to develop the necessary facilities to actually quantify the organ-level function of tissue engineered constructs. Of course, different tissues and organ systems have different functions. When designing a tissue or organ, it is therefore essential to develop a design specification for the engineered tissue, with well-defined, quantitative functional assessments, also called "figures of merit (FoM)," as well as a defined method by which to assess these values.

Presumably, the tissues or organs will be cultured for some time prior to their use to permit growth and development. Many tissues have measurable function that changes during development, so it is most desirable to identify one or more quantities that may be measured nondestructively during the course of the development of the tissues in culture. A specific example is instructive: the contractility of mammalian skeletal muscle changes throughout the early stages of development into adulthood. Muscle phenotype is defined largely by the myosin heavy chain content of individual muscle fibers, but these can be quantitatively inferred by nondestructive measurements of the isometric and dynamic contractility of the muscle tissue. The same is true for tendon. The tangent modulus, tensile strength, and fracture toughness increase during development, whereas the size of the "toe region" of the stress strain curve tends to decrease, presumably due to the increasingly well-ordered collagen structure during pre- and early postnatal development. It is not possible to nondestructively test the tensile strength and fracture toughness of a cultured tendon specimen, but the tangent modulus and the characteristics of the toe region can be readily measured with minimal disruption to the tendon tissue in culture. With musculoskeletal tissues it often happens to be the case that the electromechanical signals that are required for nondestructive quantitative assessment of the tissues in culture are essentially the same as those that would be applied chronically to the tissues in culture to guide and promote development. For example, electrically elicited contractions of skeletal and cardiac muscle are currently in use in an attempt to promote development, and the application of mechanical strain has been used since the 1980s on many musculoskeletal (muscle, bone, tendon, cartilage, ligament) and cardiovascular tissues to promote development in culture.

An important future challenge is to develop bioreactor systems that permit the application of the stimulus signals, while simultaneously allowing the functional properties of the tissues to be nondestructively measured and recorded. If the functional properties of the developing tissue are measured in real-time in a bioreactor system, it then becomes possible to assess the current developmental status of each tissue specimen and to use this information to modify the stimulus parameters accordingly. This permits stimulus feedback control of the tissue during development and represents a significant increase in the level of sophistication and effectiveness of functional tissue engineering technology. This constitutes an important aspect of current research in the areas of both musculoskeletal and cardiovascular functional tissue engineering.

6.6.3 Bioartificial Liver Specifics

The development of bioartificial liver (BAL) devices arose from the fact that "backup" systems to replace deficient liver functions are nonexistent, in contrast to other tissue in which duplications exist (e.g., as dual lung lobes, two kidneys, fibular crutches). The liver

provides multiple functions to assist with body homeostasis and needs replacement systems when confronted with organ failures such as fulminant hepatic failure (FHF).

Reports of liver treatment date back to the 1950s when low-protein diets were recommended to improve mental impairment and hepatic encephalopathy, and in the 1960s novel concepts of liver assist devices began to emerge. Some of these precedent artificial assist systems (Artif-S) currently remain on the research bench or have entered into preliminary FDA trials due to their intrinsic capabilities of treating patients suffering from FHF or other liver-specific malfunctions. A few of these systems include charcoal filters for ammonia detoxification, mechanical dialysis permitting toxin transfers, and plasmapheresis for removal of diseased circulating substances. Investigations have shown that many Artif-S are successful in their focused purpose, but they are not complete solutions to replace organ function. Although Artif-S are continually being improved, the multitude of tasks performed by the healthy in vivo liver continues to be insufficiently replicated through mechanical mimics of liver cell function.

One successful hurdle in the treatment of patients with FHF is the process of tissue transplantation. This technique exchanges a nonfunctioning liver with a healthy organ capable of performing all metabolic reactions. In this way, successful replacement surgeries alleviate the burden of using mechanical devices in concert with cellular activity. The drawback is that patients must remain on potent immunosuppressants to lessen tissue rejection responses, which decreases quality of life. Even though transplantation options are successful, the limited supply of donor liver organs along with tissue matching requirements illustrate the demand is approximately 300 percent greater than the supply Ultimately, an alternative to bridge or dissolve the *waiting* gap for patients expiring while on liver donor lists must be resolved. This is a clear opportunity for the field of tissue engineering to improve the standard of care and quality of life of a patient population.

6.7 CONCLUSIONS

This chapter outlined some of the key challenges and potential solutions in the field of tissue engineering. To successfully understand tissue function, it must be possible to quantitatively describe the underlying cellular fate processes. Such understanding will allow the tissue engineering to design and control these processes. One aspect of this approach is designing the physicochemical rate processes so they match the requirements of the cellular processes that underlie tissue function. Tissue engineering is an effort that is still in an embryonic stage, but the use of order-of-magnitude and dimensional analysis is proving to be valuable in designing and reconstituting tissue function.

6.8 EXERCISES

1. Given the following data, assess whether human hematopoietic stem cells can truly self-renew in vivo:

- About 400 billion mature hematopoietic cells are produced daily.

Continued

- Best estimate of the Hayflick limit is 44 to 50.
- About 1:5,000 progenitors do *not* apoptose.
- About 50 to 1,000 mature cells are made per progenitor (6 to 10 doublings).
- The entire differentiation pathway may be 17 to 20 doublings (soft fact).
- In vitro, about 10 to 30 million cells maximum can be made from a highly purified single hematopoietic stem/preprogenitor cell.

Present order of magnitude calculations in constructing your decision. Also perform parameter sensitivity analysis of each of the parameters that govern the hematopoietic process. How important are the parameters that govern telomerase activity in determining the total number of mature progeny produced over a person's lifetime?

2. At 1 pM concentration, how many molecules are found in a volume of liquid that is equal to the volume of one cell (use a radius of 5 microns)?
3. Use the continuum approach (Eq. (4.3)) to show that in a steady state the number of cells produced during a differentiation process that involves replication but no apoptosis ($\alpha = 0$) is

$$\frac{X_{out}}{X_{in}} = e^{\mu/\delta}$$

and is thus primarily a function of the ratio δ/μ. $a = 0$ is the completely undifferentiated state, and $a = 1$ is the completely differentiated state. What is X_{out} if μ and δ are the same orders of magnitude and if δ is 10 times slower? Which scenario is a more reasonable possibility in a physiological situation?

(Note that if the rates are comparable, only two mature progeny will be produced. On the other hand, if the differentiation rate is 10 times slower than the replication rate (probably close to many physiologic situations—i.e., 20 hr doubling time, and 200 hr = 8 days differentiation time), then about 1 million cells will be produced. Thus, the overall dynamic state tissue is strongly dependent on the relative rates of the cellular fate processes.)

4. Kinetics of differentiation/continuous model.
   - **i.** Derive the first-order PDE that describes the population balance.
   - **ii.** Make time dimensionless relative to the rate of differentiation.
   - **iii.** Describe the two resulting dimensionless groups (call the dimensionless group for apoptosis A, and the one for the cell cycle B).
   - **iv.** Solve the equation in steady state for $A = 0$.
   - **v.** Solve the equation(s) where A is nonzero for a portion of the differentiation process—that is, between a_1 and a_2.
   - **vi.** Solve the transient equation for $A = 0$.
5. Consider two cells on a flat surface. One cell secretes a chemokine to which the other responds. Show that the steady-state concentration profile of chemokine emanating from the first cell is

$$C(r) = R^2F/D \bullet 1/r$$

where R is the radius of the cell, F is the secretion rate (molecules/area time), and D is the diffusion coefficient of the chemokine. The distance from the cell surface is r. Use the cell flux equation to calculate the time it would take for the responding cell to migrate to the signaling cell if there is no random motion ($\mu = 0$) given the following values:

$\chi(C) = 20\ cm^2/sec\text{-}M$
$D = 10^{-6}\ cm^2/sec$
$R = 5\ \mu m$
Production rate = 5,000 molecules/cell/sec

Hint: Show that the constitutive equation for J reduces to $v = \chi\ dC/dr$, where v is the velocity of the cell.

6. The flux (F) of a molecule present at concentration C through a circular hole of diameter d on a surface that is adjacent to a fluid that it is diffusing in is given by

$$F = 4DdC$$

The total that can be transferred is the per pore capacity times the number of pores formed.

Calculate the flux allowed though each pore if the diffusion coefficient is $10^{-6}\ cm^2/sec$, the concentration is 1 mM, and the pore diameter is 1.5 nm. Discuss your results, and try to estimate how many pores are needed to reach meaningful cell-to-cell communications. With the per-pore flux just estimated, derive the time constant for transfer of a metabolite from a particular cell to a neighboring cell. Assume that these are two epithelial cells whose geometry can be approximated as a box and that the two adjacent boxes are connected with transfer occurring through *n* pores.

7. If the cellularity in cartilage is about 1 million cells per cc, estimate the average distance between the cells. Discuss the characteristics of this microenvironment.

8. Use a one-dimensional analysis of the diffusion of oxygen into a layer of adherent cells to show that the maximum oxygen delivery per unit area (N_{ox}^{max}) in Example Problem 6.8 is given by

$$N_{ox}^{max} = DC^*/R$$

where C^* is the saturation concentration of oxygen and R is the thickness of the liquid layer.

9. Consider a neuron growth cone that is being influenced by a chemoattractant produced by a target cell. The geometry of the model system is shown in the attached figure. The target cell secretes a chemoattractant at a rate, P_r, which diffuses into a three-dimensional volume, with a diffusivity D. The governing equation for mass transport for a spherical source is

$$\frac{\partial C}{\partial t} = D\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial C}{\partial r}\right)$$

a. What are the boundary conditions for the system?

b. Considering a steady state, derive the concentration profile as a function of *r*. It has been found that the growth cone senses a target cell when the concentration difference across the growth cone is higher than 2 percent. It is believed that growth cones develop filopodia that extend radially out of the cone as a means to enhance their chemosensing ability. Let β_1 be the angle that a filopodia makes with the center radius line, R, and β_2 the angle made by a filopodia extending in the diametrically opposite direction. Filopodia can extend radially from the growth cone surface, except where the cone connects to the axon as defined by α.

c. What are the appropriate limits of β_1 and β_2?

d. What are r_1 and r_2 as a function of β_1?

Continued

e. What is the percentage change in concentration, $\Delta C = \frac{|C_1 - C_2|}{C_0}$, across the effective growth cone radius—for example, $R_{gc} + l$—as a function of r_1 and r_2 and consequently β_1?

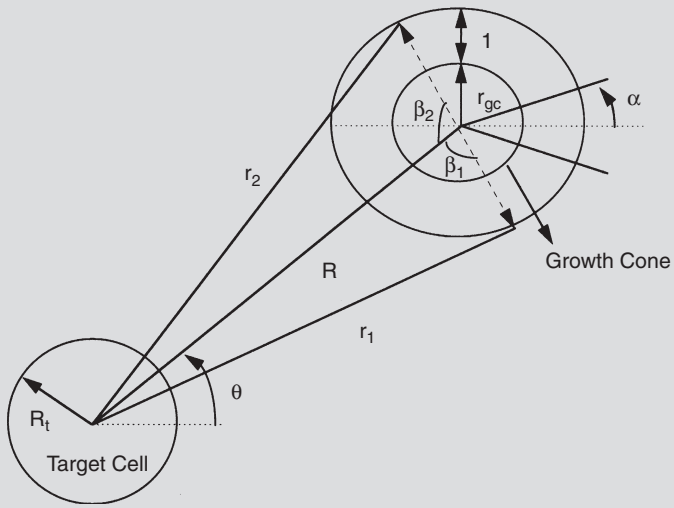
f. What is $\beta_1{}^{max}$, for which ΔC is maximum? Plot the gradient change for the entire range of β_1 for filopodial lengths of 1, 5, and 8 μm compared to no filopodia.

g. The limit for chemosensing ability is a concentration difference of 2 percent. For a target cell 500 μm away, what is the effect of filopodial length on enhancing chemosensing ability? Calculate for $\beta_1{}^{max}$.

$$C_0 = \frac{P_r R_t^2}{DR_o}, P_r = \frac{S_r}{4\pi R_t^2}$$

$$D = 10^{-6}\text{cm}^2/\text{s} \text{ and } S_r = 5{,}000 \text{ molecules/cell/s. } R_0 = R - R_{gc}$$

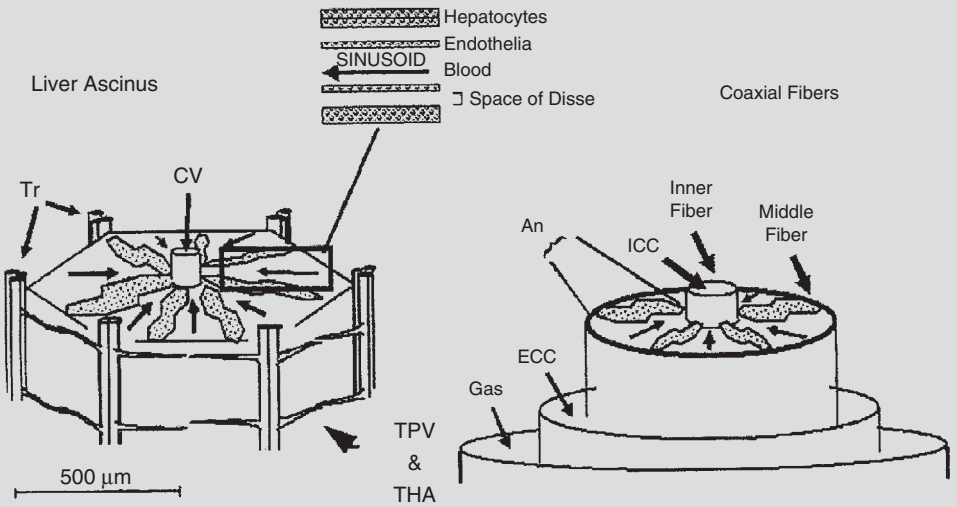
$$R_t = 20\ \mu\text{m},\ R_{gc} = 2.5\ \mu\text{m},\ \alpha = 30^\circ.$$



10. A coaxial bioreactor has been developed to mimic the liver acinus (see the following figure) .

a. Knowing the dimensions of the acinus (shown in figure above), what should be the distance between the two fibers?

b. Knowing what you've learned about biological aspects, what types of cells and soluble and insoluble factors would you choose to induce an environment of cell growth? What types of cells and soluble and insoluble factors would you choose to induce an environment of cell differentiation?



c. From the figure of the liver acinus shown above and in Table 6.9, the flow rate is 10^{-3} cm/s in the liver acinus. Given Darcy's law:

$$Q_r = AL_p[\Delta P - \Delta\pi]$$

where Q_r is the radial flow rate, A is the surface area, L_p is the hydraulic permeability (L_{p1} and L_{p2} are the inner and middle fiber hydraulic permeability, respectively), P is pressure, and π is osmotic pressure. If the bioreactor was filled with water and no cells, and flow is directed outward, flowing from the inner capillary compartment (ICC) outward, through the cell compartment to the extracapillary compartment (ECC), what would be the pressure in the three compartments if Q_r is 10^{-2} cm/s, L_{p1} is 10^{-4} mL/min/mmHg/mm^2, and L_{p2} is 10^{-6} mL/min/mmHg/mm^2? What are the pressures if each compartment flow went in the opposite direction from ECC into the ICC?

d. Assuming a slab configuration in the cell compartment, write a MATLAB program to give the oxygen profile if the diffusion coefficient in the cell matrix is 10^{-6}cm/s^2.

11. Use a compartmental model to calculate the number of mature cells produced from a single cell in a particular compartment. Use a doubling time of 24 hours ($\ln(2)/\mu$) and a mature cell half-life of 8 hours ($\ln(2)/k_d$). Assume that self-renewal can only take place in the first compartment with a probability of 0.5. Use a total of 10 compartments and calculate the number of cells as a function of time with the initial conditions

$$X_i(0) = 0, \text{ except } X_j(0) = 1$$

and vary j between 1 and 8. Plot all curves on the same plot. Discuss the implications of your results for transplantation.

12. Kinetics of differentiation/feedback control in a compartmental model.

Continued

i. Consider a six-stage differentiation process (N = 6), in which the last population, X_6, produces a cytokine, G, at a per-cell rate of q_G. This cytokine has a half-life of $t_{0.5}$ (= 2 hrs) and influences the growth rate of the stem cells—that is, $u_1 = fn([G])$, where [G] is the concentration of the growth factor G. Extend the base set of differential equations to describe the dynamics of [G].

ii. Incorporate into the equations

$$u_1([G]) = u/(1 + K[G])$$

where K is the binding constant for the growth factor. What does the function f([G]) describe physiologically?

iii. Make the equations dimensionless using the growth rate as the scaling factor for time, and K for the cell concentration.

iv. Describe the meaning of the dimensionless groups and estimate their numerical values.

v. Obtain the numerical values for a simulation starting from a single stem cell. Examine the effect of varying the numerical values of the parameters.

vi. Obtain the numerical values for a simulation starting from the steady-state solution and perturb the value of X_3 by 20 percent. Discuss your results.

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Suggested Readings

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Glossary

Allogeneic Transplantation of cells or tissues from a donor into a recipient of the same species but a different strain.
Apoptosis A cellular process of aging and that leads to cell death. This process is initiated by the cell itself.
Autologous transplant Cells or tissue removed from a donor and then given back to the donor.
BMT Bone marrow transplantation.
Cellular therapies The use of grafted or transfused primary human cells into a patient to affect a pathological condition.
Chondrocytes Cells found in cartilage.
Colony-forming assay Assay carried out in semisolid medium under growth factor stimulation. Progenitor cells divide, and progeny are held in place so that a microscopically identifiable colony results after two weeks.
Cytokinesis The process occurring after DNA synthesis and resulting in completion of cell division from one cell into two.
Differentiation The irreversible progression of a cell or cell population to a more mature state. Distinct sets of genes are expressed and at varying levels in the cells during the differentiation process.
Engraftment The attainment of a safe number of circulating mature blood cells after a BMT.
Extracellular matrix (ECM) An insoluble complex of proteins and carbohydrates found between cells. These proteins serve to physically connect cell populations and to persistently signal cells to behave in specific ways.
Flow cytometry Technique for cell analysis using fluorescently conjugated monoclonal antibodies which identify certain cell types. More sophisticated instruments are capable of sorting cells into different populations as they are analyzed.
Functional subunits The irreducible unit in organs that gives tissue function—that is, alveoli in lung and nephron in kidney.

Graft-vs-Host disease The immunologic response of transplanted cells against the tissue of their new host. This response is often a severe consequence of allogenic BMT and can lead to death (acute GVHD) or long-term disability (chronic GVHD).

Hematopoiesis The regulated production of mature blood cells through a scheme of multilineage proliferation and differentiation.

Hyperplasia Growth process involving complete cell division, both DNA synthesis and cytokinesis

Hypertrophy Growth process involving DNA synthesis but absence of cytokinesis and resulting in cells of higher ploidy which, secondarily, causes cells to become larger. The late stages of many, if not most, tissue lineages have cells that undergo hypertrophy in response to regenerative stimuli.

Lineage Refers to cells at all stages of differentiation leading to a particular mature cell type.

Long-term culture-initiating cell Cell that is measured by a 7- to 12-week in vitro assay. LTC-IC are thought to be very primitive, and the population contains stem cells. However, the population is heterogeneous so not every LTC-IC is a stem cell.

Mesenchymal cells Cells of connective type tissue, such as fibroblasts, osteoblasts (bone), chondrocytes (cartilage), adipocytes (fat), and so on.

Microenvironment Refers to the environment surrounding a given cell in vivo.

Mitosis The cellular process that leads to cell division.

Mononuclear cell Refers to the cell population obtained after density centrifugation of whole bone marrow. This population excludes cells without a nucleus (erythrocytes) and polymorphonuclear cells (granulocytes).

Myoablatron The death of all myeloid (red, white, and platelet) cells, as occurs in a patient undergoing high dose chemotherapy.

Parenchymal cells The essential and distinctive cells of a particular organ (i.e., hepatocytes in the liver or myocytes in muscle).

Progenitor cells Unipotent cells that derive from stem cells and will differentiate into mature cells.

Self-renewal Generation of a daughter cell with identical characteristics to the parent cells. Most often used to refer to stem cell division, which results in the formation of new stem cells.

Stem cells Pluripotent cells that are capable of self-replication (and, therefore, unlimited proliferative potential). Malignant tumor cells are aberrant forms of stem cells.

Stromal cells Mesenchymal cells that partner with epithelial cells. They are age- and tissue-specific. Their roles in regulating the expansion and/or differentiation of epithelia have long been known. For example, heterogeneous mixture of support or accessory cells of the BM, also referred to as the adherent layer, is requisite for BM cultures.

Syngeneic Transplantation of cells or tissues from a donor into a genetically identical recipient.

Xenogeneic Transplantation of cells or tissues from a donor of one species into a recipient of a different species.