2

CELLS FOR TISSUE ENGINEERING

Learning Objectives:

After completing this chapter, students should be able to:

1. Explain why cells are important for tissue engineering.
2. Describe the structure and function of organelles in eukaryotic cells.
3. Describe the structure and function of the mammalian extracellular matrix.
4. Discuss different mechanisms for cell signaling.
5. Describe the structure and function of cellular junctions.
6. Discuss cell-cell and cell-matrix interactions in terms of tissue engineering.
7. Discuss potential cell sources for tissue engineering and tissue fabrication.
8. Explain the principles of cell transplantation, and discuss how this field relates to tissue engineering and the tissue fabrication process.
9. Provide examples of cells that have been used for cell transplantation.
10. Compare the fields of cell transplantation and tissue engineering.
11. Describe the process of 2D monolayer cell culture, and explain how it relates to tissue engineering and the tissue fabrication process.
12. Provide examples of applications of monolayer cell culture.
13. Compare the fields of 2D cell culture and 3D tissue engineering.

Introduction to Tissue Engineering: Applications and Challenges, First Edition. Ravi Birla.
© 2014 The Institute of Electrical and Electronics Engineers, Inc. Published 2014 by John Wiley & Sons, Inc.

14. Describe characteristics of stem cells, and explain the concepts of cell differentiation and cell potency.
15. Discuss the use of human embryonic stem cells, induced pluripotent stem cells, and adult stem cells for fabrication of 3D artificial tissue.

CHAPTER OVERVIEW

We begin this chapter by explaining the importance of cells during the tissue fabrication process. We proceed with a discussion of cell structure and function and then discuss the dynamic extracellular matrix. We then discuss cell signaling and cellular junctions. We next discuss the role of cell biology in tissue engineering and follow this up with discussion on cell sourcing for tissue fabrication. The last three sections of this chapter are focused on mammalian cell culture, cell transplantation, and stem cell engineering. We describe the process of cell transplantation, including steps that are involved in the development of cell transplantation therapies. During our discussion on mammalian cell culture, we describe the process of cell culture and the relative importance of cell culture during tissue fabrication. We also provide a discussion comparing the relative advantages and disadvantages of cell transplantation and tissue engineering. We next move on to describe mammalian cell culture. We describe several steps in the cell culture process, including isolation, culture, and expansion of primary cells. We discuss applications of cell culture and provide a comparison of cell culture versus tissue engineering. We then move on to discuss stem cells and the role of stem cell engineering in tissue engineering. We describe the properties of stem cells, along with several stem cell sources that have been used in tissue engineering, including human embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells.

2.1 CELLS AND TISSUE ENGINEERING

An understanding of cells is central to the field of tissue engineering and regenerative medicine. As we have seen in Chapter 1, cells are the functional components of 3D artificial tissue and are one of the building blocks of the field. Isolation, culture, and expansion of cells are routinely conducted as a part of the tissue fabrication process. In addition, stem cell engineering has evolved to play a critical role in tissue engineering (1–4). Genetic programming of stem cells can drive the differentiation fate of these cells toward a given lineage, and these cells can be used to support fabrication of artificial tissue (5–7). An understanding of cell biology, cell culture, and stem cell engineering is critical for success in tissue engineering and will be the focus of this chapter.

Cells are the fundamental units of life and are one of the building blocks for tissue engineering. There are several aspects of cell biology that are important for the tissue fabrication process, and, as illustrated in Figure 2.1, cell biology, cell culture, cell transplantation, and stem cell engineering are important during the

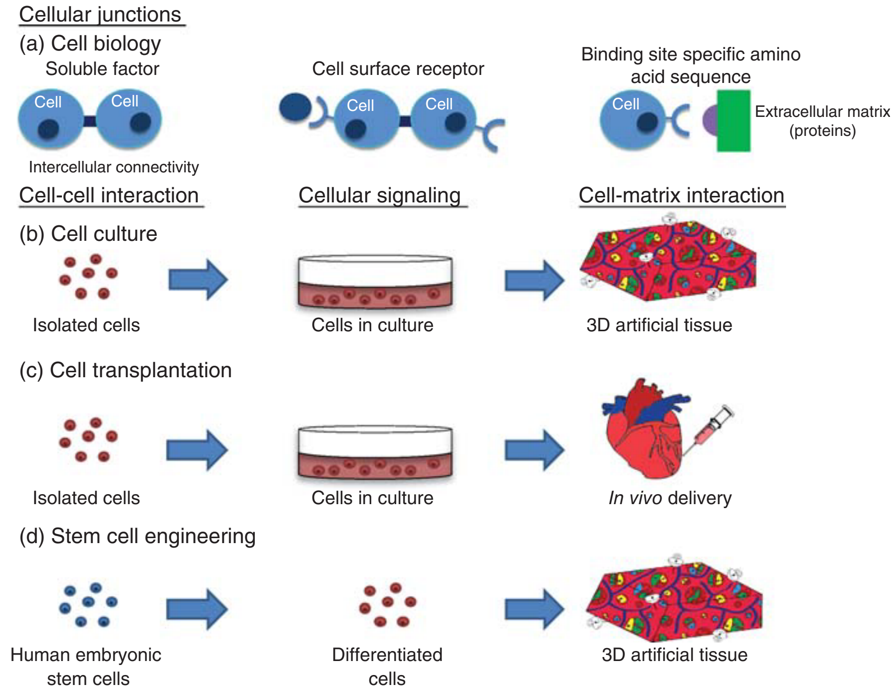

Figure 2.1 Cells and Tissue Engineering—Cells are important for tissue engineering, and four important areas are (a) Cell Biology, (b) Cell Culture, (c) Cell Transplantation, and (d) Stem Cell Engineering.

tissue fabrication process. These four topics are the subject of this chapter and are briefly introduced in this section.

Let us begin this discussion with cell biology as it relates to tissue engineering. Some of the concepts are illustrated in Figure 2.1a and will be discussed throughout this chapter, including cell-cell interactions, cell-matrix interactions, intercellular connectivity, and cellular signaling. Individual cells form the basic unit of mammalian tissue and are a critical function component of 3D artificial tissue. Mammalian tissue is composed of different cell types, each of which has a unique role to support functional performance of tissue. In addition to cells, mammalian tissue is composed of an extracellular matrix (ECM), which provides structural support during tissue function. There are functional interactions between adjacent cells and between cells and mammalian ECM; these are referred to as cell-cell interactions and cell-matrix interactions respectively, both of which are critical determinants of tissue function. Cells do not function in isolation; they are part of a large biological system. As such, cells have a sophisticated mechanism to sense and respond to changes in the local environment through a process known as cell signaling.

In addition to understanding cell behavior as it relates to the tissue fabrication process, another important area related to tissue engineering is cell culture

(8–11) (Figure 2.1b). In order to undertake any tissue engineering experiment, it is important to isolate, maintain, and expand cells in culture. In order to achieve this objective, a technique known as cell culture is used. Cell culture techniques are important for the isolation of primary cells, along with the culture and expansion of these cells prior to utilization for fabrication of 3D artificial tissue. There are several fundamental principles related to mammalian cell culture, which are discussed later in this chapter.

Cell transplantation was introduced in Chapter 1 and involves the delivery of isolated cells *in vivo* in order to improve functional performance of damaged or diseased tissue (12–14) (Figure 2.1c). Prior to *in vivo* transplantation, isolated cells are cultured using cell culture techniques to increase the number of cells, as large cell numbers are required for cell transplantation studies.

The most important question relating to cells during the tissue fabrication process is: *where exactly will the cells come from?* There are many options available for cell sourcing. Stem cell engineering has received a lot of attention in recent literature (Figure 2.1d), with human embryonic stem (hES) cells being one example (15–17). The primary advantage of hES cells is the totipotent potential of these cells, which means that they can be differentiated to form any cell type in the human body. Therefore, starting with a single cell type (hES cells), researchers have the ability to obtain many different cell types, which can then be used to fabricate artificial tissue. During the course of this chapter, we will study many scientific challenges associated with achieving this objective and will also study different sources for stem cells.

2.2 CELL STRUCTURE AND FUNCTION

Cells are the fundamental units of life and building blocks of all mammalian tissue, which is made up of multiple cell types interconnected with extracellular matrix components. Mammalian cells are extremely complex and can undertake hundreds of functions at any given time. Cells consist of many different structures known as organelles, each of which has a specialized function. Cells are categorized as prokaryotes if they do not contain a nucleus; they are categorized as eukaryotes if the cells contain a nucleus. Both prokaryotic and eukaryotic cells have DNA. In the case of prokaryotic cells, DNA is not separated in membrane bound structures from the cytoplasm; rather, it is concentrated within a region of the cell known as the nucleoid. One the other hand, the nucleus of eukaryotic cells is contained in a specialized membrane-bound nucleus which separates it from the cytoplasm.

Nucleus—The nucleus serves as a storage site for genetic material in eukaryotic cells and consists of several components: nuclear envelope (NE), nuclear pore complex (NPC), chromosomes, chromatin, and nucleolus (18–26).

The NE consists of two lipid bilayer membranes which surround the nucleus and separate it from the cytoplasm (27–30). The membrane that is closer to the cytoplasm is known as the outer nuclear membrane (ONM) and the membrane that is on the nuclear side is known as the inner nuclear membrane (INM). The NE

also consists of nuclear lamina and the nuclear pore complex (NPC). As many as 80 proteins that make up the ONM and INM have been identified, many of which participate in specific interactions with cytoplasmic proteins and proteins in the nuclear lamina and are important in maintaining the proper assembly and structure of the NE. Proteins in the nuclear lamina, including the lamin proteins LA, LC, LB1, and LB2, are known to interact with proteins in the INM, including emerin, SUN1/2, LAP2β, and LBR (31–35). There are also several proteins in the ONM that interact with proteins in the cytoplasm. Nesprin 1, 2, 3 and 4 are known to bind to microtubules and actin molecules as well as participating in plectin-mediated binding to intermediate filaments. These proteins serve to anchor and stabilize the nucleus relative to the cytoplasm by specific protein-protein interactions.

NPCs are porous channels that connect the nucleus and the cytoplasm and serve to regulate flow of molecules in both directions. NPCs consist of proteins known as nucleoporins (NUPs), and have a central pore that is connected to the nuclear envelope, cytoplasmic rings with eight associated filaments, and a nuclear ring, which also has eight associated filaments. Small molecules with a diameter of 4–5 nm or less can passively diffuse through the NPCs, to and from the cytoplasm and nucleus, without any selective membrane transport mechanisms in place. Large molecules are transported through the NPCs by selective membrane transport via specialized transport proteins known as importins and exportins (21). The transport of molecules from the cytoplasm through the NPC to the nucleus is known as nuclear import and makes use of transport proteins known as importins (21). Similarly, the transport of molecules from the nucleus through the NPC to the cytoplasm is known as nuclear export and makes use of transport proteins known as exportins. In the case of nuclear import, the molecule of interest binds to the importin on the cytoplasmic side and is transported through NPCs to the nucleus; once inside the nucleus, the importin undergoes a conformational change after binding Ras-related nuclear protein (RAN) guanosine triphosphatase GTPase, (RAN.GTP) which allows release of the molecule of interest inside the nucleus (21). The reverse is true for nuclear export, where the molecule of interest binds to exportin proteins and RAN.GTP complex in the nucleus; this allows transport of the molecule to the cytoplasm, where conformation changes in the exportin proteins cause release of the transported molecule (21).

The genetic material within the nucleus is organized into chromosomes (36–39). Chromosomes are organized into primary, secondary, and tertiary structures using proteins to form these complex structures; this results in the formation of chromatin. The organization of chromosomes to form chromatin requires the use of an equal mass of proteins. Nucleosomes are repeating units of chromatin and consist of 145–147 base pairs of DNA around a histone octamer core. Histones are proteins that are used to compact and package DNA to form nucleosomes. Histones serve in compaction of DNA and serve to stabilize nucleosome structure by protein-protein interactions, which stabilize the histone core. In addition, electrostatic interactions and hydrogen bonds between DNA and the histone protein help stabilize the entire nucleosome structure. Individual nucleosomes are held together by linker DNA and this leads to the formation of the primary chromatin structure, resembling beads on

a string. Compaction and organization of primary chromatin structures leads to the formation of secondary and tertiary chromatin structures.

The nucleolus is the largest compartment within the nucleus of eukaryotic cells; the core function of the nucleolus is synthesis and assembly of ribosomal RNA (rRNA) (40–45). The nucleolus has three major compartments known as the fibrillar center, dense fibrillar region, and granular component, each with a specialized function. The nucleolus also contains genes for pre-rRNA, which are transcribed into ribosomal proteins. The inner fibrillar center of the nucleolus is where transcription of pre-rRNA takes place while rRNA processing occurs in the dense fibrillar region, and early steps of ribosome assembly occur in the granular region of the nucleolus.

Ribosomes—Ribosomes are the sites of protein synthesis in eukaryotic cells and are made in the nucleolus of cell nucleus, as we have seen before (46–52). Ribosomes can either be suspended in the cytosol or bound to the endoplasmic reticulum. Free ribosomes translate proteins that are used in the cytoplasm, where membrane-bound ribosomes translate proteins that are used in the membrane or exported from the cell. The number of ribosomes present in a cell relates to the protein synthesis activity of the cell, and certain cells can have up to 10 million ribosomes. Ribosomes are characterized based on their rate of sedimentation, and are designated as 80S for eukaryotic cells. Eukaryotic ribosomes consist of two subunits, the larger of which is known as the 60S subunit and contains about 45 proteins; the smaller one is known as the 40S subunit and contains about 30 proteins. Consistent with their role in protein synthesis, eukaryotic ribosomes have a binding site for messenger RNA (mRNA) and three binding sites for transfer RNA (tRNA), known as the E site, P site and the A site.

Mitochondria—Mitochondria are the primary organelles for energy generation within eukaryotic cells (53–57). Mitochondria are responsible for the production of ATP, which is the primary currency of energy in cells, from breakdown of carbohydrates and fatty acids via oxidative phosphorylation. The number of mitochondria present in eukaryotic cells is related to the metabolic activity of the specific cell type; cells like cardiac myocytes, which have a high metabolic demand, are known to have a high concentration of mitochondria. The mitochondria of eukaryotic cells consist of two membranes, referred to as the outer membrane and the inner membrane; the space between the outer and inner membranes is known as the intermembrane space. The inner region of mitochondria is known as the matrix, and the inner membrane folds into matrix-forming indentations known as cristae. The inner region of the mitochondria contains enzymes responsible for the citric acid cycle, which is the primary mechanism for production of ATP in eukaryotic cells. The citric acid cycle in the mitochondria interfaces with glycolysis, which takes place in the cytoplasm. Glycolysis is responsible for the breakdown of glucose to pyruvate, which then feeds into the citric acid cycle for ATP production. Through this process, mitochondria are able to meet the metabolic demands of the cells.

Lysosomes—Lysosomes are membrane-bound structures, consisting of a single membrane which contains digestive enzymes (58–61). Lysosomes can be viewed as membrane-bound sacs of digestive enzymes with a primary catabolic function

in eukaryotic cells. The primary function of eukaryotic lysosomes is digestion and breakdown of biological molecules, including proteins, nucleic acids, carbohydrates, and lipids. Lysosomes contain about 50 different digestive enzymes and are involved in phagocytosis and autophagy (62,63). Phagocytosis is the process by which external agents like bacteria are internalized within the cell and then destroyed by digestive enzymes of the lysosome. The external agent is first engulfed by the cell membrane of a host cell and then internalized to form a membrane-bound vesicle known as a phagosome. The phagosome fuses with a host lysosome to form phagolysosomes. The digestive enzymes of the lysosome destroy this external agent within the phagolysosome. Lysosomes are also responsible for a process known as autophagy, which involves digestion of internal organelles. The organelle to be digested is first enclosed within a membrane that is derived from the endoplasmic reticulum. The newly formed vesicle is known as an autophagosome. As in the case for phagocytosis, the autophagosome fuses with lysosomes, and digestive enzymes in the lysosome degrade this organelle.

Endoplasmic Reticulum (ER) and Golgi Apparatus—The ER is an extensive network of membranes found in the cytoplasm of eukaryotic cells that functions in protein synthesis and distribution (64,65). The membrane network of the ER accounts for more than half of the total membrane structures in eukaryotic cells, and the luminal space of the ER can account for up to ten percent of total cell volume. There are two types of ER, referred to as rough ER and smooth ER, each with distinct functions. The rough ER is covered with ribosomes on the outer surface and is involved in protein synthesis. The smooth ER does not have ribosomes on the surface and is involved in synthesis of lipids and in carbohydrate metabolism. While the ER is involved in protein synthesis, the Golgi apparatus is involved in storage and modification of these proteins and then further distribution of the modified proteins to other organelles in eukaryotic cells. The ER and the Golgi apparatus define the secretory pathway of proteins within the cells. Proteins are synthesized in the rough ER and then transferred to the Golgi apparatus for protein modification, and from the Golgi apparatus, the proteins are transported in vesicles to other organelles within the cell and can also be transported outside of the cell.

Cytoskeleton—The cytoskeleton is a network of fibers that provides structural and mechanical support for eukaryotic cells (66). The composition, distribution, and alignment of the cytoskeleton's fibers varies based on cell type and physiological state of cells, but the cytoskeleton consists of three types of fibers: microtubules, microfilaments, which are intertwined strands of actin, and intermediate filaments. Microtubules have a diameter of about 25 nm, intermediate filaments have a diameter of about 10 nm, and actin filaments have a diameter of about 7 nm. Microtubules are involved in cell motility and movement of chromosomes and movement of cell organelles and function to maintain cell shape. Some examples of microtubules include α-tubulin and β-tubulin. Actin filaments are one of the most abundant cytoskeletal proteins and, together with the contractile protein myosin, are involved in muscle contraction. Intermediate filaments play a structural role by providing mechanical support to eukaryotic cells and play an important role in maintenance of cell shape. Some examples of intermediate filaments include

desmin in muscle cells, nuclear lamins in the nuclear lamina of all cell types, and nestin found in stem cells of the central nervous system.

Plasma Membrane—The plasma membrane provides a barrier around eukaryotic cells and provides a selective barrier to regulate the flow of molecules in and out of the cell. The plasma membrane consists of several phospholipids that are organized asymmetrically into two regions, with the inner leaflet toward the cytosol of the cell and the outer leaflet toward the outside of the cell (67–70). The plasma membrane contains several phospholipids, glycolipids, cholesterol, and protein molecules. Most eukaryotic plasma membranes contain an equal amount of lipid and protein molecules on a weight basis. The four most abundant phospholipids in the plasma membrane are phosphatidylcholine and sphingomyelin in the outer leaflet and phosphatidylethanolamine and phosphatidylserine in the inner leaflet. The lipid molecules provide structural support to the plasma membrane while the protein molecules play a functional role. The proteins in the plasma membrane are categorized as either peripheral proteins or integral membrane proteins. Peripheral proteins are not embedded in the phospholipid bilayer, but are associated with the membrane through protein-protein interactions. Integral membrane proteins, on the other hand, are embedded within the phospholipid layer of the plasma membrane. Integral membrane proteins, which span the lipid bilayer, are known as transmembrane proteins. Many transmembrane proteins serve as transport channels that selectively allow specific molecules to enter and leave the cell. This transport function of these proteins and selective permeability of plasma membranes to specific molecules is a critical function for eukaryotic cells.

2.3 THE DYNAMIC EXTRACELLULAR MATRIX

The ECM provides structural support for cells during normal tissue formation and function (71–74). The ECM in mammalian tissue is a complex mixture of proteins and other molecules and has specific binding sites for cells. During 3D tissue formation, cells form intercellular connections with neighboring cells and attach in a specific way to the ECM. These cell-cell interactions and cell-matrix interactions modulate tissue function, thereby allowing changes in the physiological state of the tissue. Cell-matrix interactions refer to the binding of cell surface receptors known as integrins to specific domains on ECM molecules. Binding of cell surface integrins to specific regions on ECM molecules triggers a cascade of intracellular signaling events, leading to changes in cell behavior and/or tissue organization. There are specialized cell types within any given tissue responsible for ECM production. Some examples of ECM-producing cells are fibroblasts, chondrocytes, and osteoblasts.

Structure and Function of ECM—The ECM can be viewed as the structural component of mammalian tissue and functions to stabilize 3D tissue by providing mechanical support. The ECM also functions to modulate tissue properties

through specific cell-matrix interactions. The ECM consists of two major components, which are protein molecules and glysoaminoglycans (GAGs) (71–74). Some examples of ECM proteins include collagen, elastin, fibronectin, and laminin, while some examples of GAGs include hyaluronan, chondroitin sulfate, and heparin. The specific composition, distribution, and alignment of ECM proteins and GAGs varies from one tissue to another and changes in response to the physiological state of the tissue, including changes in stress environment or tissue remodeling in response to specific pathological conditions. While the ECM has many different functions, two primary functions of the ECM are to provide mechanical support/stability and to regulate cellular properties through specific cell-matrix interactions, which elucidate intracellular signaling events leading to changes in cell behavior/phenotype.

Cell-Matrix Interactions and Integrins—We have seen that cells and the ECM are major components of mammalian tissue. We have also seen that ECM components are large macromolecules like collagen and fibronectin and consist of hundreds of amino acids. Cells and ECM talk to each other in a very specific manner known as cell-matrix interaction. It is very intriguing to think about the way in which cells and the ECM communicate and/or interact. ECM proteins and other components are very large macromolecules with hundreds of amino acids. *How exactly does the cell "see" this large ECM protein and how exactly does the cell interact or communicate with the ECM protein?* Cells have cell surface receptors known as integrins, which recognize specific amino acid sequences on ECM proteins (75–80). Integrins consists of two subunits known as α and β subunits. Eighteen α and eight β subunits that form 24 distinct integrins have been identified, each with a specific ECM binding site. When a cell sees any given ECM protein, the cell scans the protein molecule to identify specific binding sites for which it has integrins; for example, the integrin α5β1 binds to the RGD site of the fibronectin molecule (81,82). Although the fibronectin molecule is large, there is only a sequence of three amino acids that are recognized by cells having the α5β1 integrin (81,82); the binding of the α5β1 integrin to the RGD site on the fibronectin molecule is referred to as a specific cell-matrix interaction.

Why is cell-matrix interaction important? Cell-matrix interaction is the only way for cells to functionally interact with ECM proteins. In the absence of specific cell-matrix interactions, cells will passively interface with ECM proteins without any functional benefit to cells. When cell surface integrins interact with specific ECM protein binding sites, this interaction triggers a series of intracellular signaling events that impact cell survival and proliferation, regulation of transcription and protein synthesis, and cytoskeletal organization and remodeling. Cell-matrix interaction is therefore important for cell function, behavior, and phenotype, and the absence of cell-matrix interaction leads to abnormal cell behavior and phenotype.

2.4 CELL SIGNALING

In the previous section, we studied the ECM and ways in which cells communicate with components of the ECM via cell-matrix interactions. Cells also communicate

with other cells via cell-cell interactions, and these are critical in maintaining cell phenotype and tissue function (83). There are four types of cell signaling, known as endocrine, paracrine, autocrine, and contact-dependent signaling (83). The general principle of cell signaling in all four cases is the same—the signaling molecule is secreted by a specific cell and acts on a second cell (or the same cell in the case of autocrine signaling) by binding to a cell surface receptor specific to the signaling molecule. The binding of the signaling molecule to the cell surface receptor leads to a cascade of signaling events that regulate cell behavior and phenotype.

Endocrine Signaling—In the case of endocrine signaling, hormones are secreted by specialized cells in endocrine glands, and secreted hormones are distributed throughout the body by the circulatory system (84,85). Some examples of molecules that participate in endocrine signaling are thyroid hormone and adrenaline. Thyroid hormone is secreted by thyroid glands and released into the circulatory system; it acts on several cell types to regulate the rate of metabolism. Adrenaline is secreted by the adrenal gland and is responsible for the well-known fight and flight response, which causes an increase in blood pressure and heart rate during time of stress and anxiety.

Paracrine Signaling—In the case of paracrine signaling, the molecule is secreted into the local environment and rather than being transported through the circulatory system, the molecule acts on cells that are in close proximity (86–89). Neurotransmitters like acetylcholine use paracrine signaling; these molecules are released by nerve endings and travel across a synapse to act on the target cell.

Autocrine Signaling—In the case of autocrine signaling, the molecule is released by a cell and binds to cell surface receptors on the same cell from which it is released. Using this mechanism, cells can modulate their own behavior and phenotype in response to any given external cues (90–92). An example of autocrine signaling is in the case of T lymphocytes in response to antigenic stimulation. When T lymphocytes are exposed to specific antigens, they release a growth factor into the local environment; this growth factor then binds to cell surface receptors on T lymphocytes. Binding of the growth factor to cell surface receptors triggers an intracellular signaling cascade; this in turn results in T lymphocyte proliferation (93–95). This process leads to an increase in the number of T lymphocytes and therefore provides a mechanism to increase the intensity of the immune response by host cells.

Direct Cell-to-Cell Signaling—In this case, the signaling molecule is not released into the local environment or into the circulatory system; rather, the signaling molecule remains attached to the secretory cell. An adjacent cell that has a cell surface receptor for this molecule binds to the molecule while remaining attached to the secretory cell. Direct cell-to-cell signaling plays an important role in human embryology and development. During human development, cells start off as unspecialized stem cells and eventually give rise to specialized cells. Initially, there is a large pool of unspecialized stem cells, and only a certain proportion of these cells differentiate toward a specific lineage, while additional cells remain undifferentiated or differentiate to a different lineage. Direct cell-to-cell signaling plays an important role in regulating the differentiation fate of stem cells toward a

specific lineage. In the case of neural differentiation, direct contact of developing nerve cells with neighboring cells inhibits differentiation of the neighboring cells to a neural lineage (96,97).

Intracellular Signaling—In the previous section, we looked at cell-matrix interaction and subsequent intracellular signaling events that modulate cell behavior and phenotype. The same thing occurs with cell-cell interactions—when signaling molecules bind to specific cell surface receptors, this binding triggers a series of intracellular signaling events that can lead to changes in cell function, including regulation of metabolic activity, regulation of gene expression, and changes in cytoskeletal organization and distribution.

2.5 CELLULAR JUNCTIONS

Introduction—In the previous section, we looked at several mechanisms used by cells for signaling, which depend on binding of a specific molecule to a cell surface receptor. We examined autocrine, paracrine, endocrine, and direct cell signaling. In this section, we continue our discussion on cellular signaling and look at cellular junctions, which provide various functions at the cell-cell or cell-matrix interface. There are five categories of cellular junctions: tight junctions, adherens junctions, desmosome junctions, gap junctions, and hemidesmosome junctions. Cellular junctions can be classified based on function: occluding junctions, anchoring junctions, or communication junctions. Tight junctions are an example of occluding junctions, which prevent the movement of molecules between adjacent cells. Anchoring junctions connect one cell to another cell or to components of the ECM and include adherens junctions, desmosome junctions, and hemidesmosome junctions. Communicating junctions allow the flow of molecules between adjacent cells and include gap junctions.

Tight Junctions—Tight junctions are found between adjacent cells, are prevalent in epithelial tissue, and serve to provide adhesion and barrier functions, hold cells together, and provide a semipermeable barrier (98–101). Tight junctions provide cells with a semipermeable size-specific and ion-specific barrier and restrict the diffusion of apical and basolateral membrane components. Tight junctions also function as landmarks by spatially confining signaling molecules and polarity cues and serving as docking site for vesicles. The structural organization of proteins has been identified, and at least three major components come together to at the site of tight junctions: claudins, occludins, and the family of junctional adhesion proteins (JAMs). The claudin family of proteins spans the intracellular space between epithelial cells and consists of at least 24 members, which vary significantly between tissue systems and organs. These variations in the claudin family of proteins between different tissue systems serve to induce selectivity of tight junctions. Occludin also spans the extracellular space, and although a specific function for this protein has not been elucidated, occludins have been implicated in the organization of tight junctions and as a regulatory protein. JAMs are a group of transmembrane structural proteins that anchor the adjacent cells

together at the tight junction. In addition to claudin, occulin, and the JAM family of proteins, tight junctions also contain scaffolding proteins known as the zonula occludens (ZO) group of proteins. The ZO group of proteins directly interacts with claudins, occulins, and JAMs and provides a link with intracellular actin; ZO proteins provide a scaffolding function by stabilizing tight junction proteins and anchoring these proteins to intracellular components.

Adherens Junctions—The primary function of adherens junctions is to connect adjacent cells together (102). In addition to bridging adjacent cells, adherens junctions connect the intracellular actin bundles of adjacent cells, thereby providing increased structural stability and a mechanism to translate changes in the extracellular environment to intracellular remodeling of cytoskeletal proteins. There are two major protein complexes at adherens junctions: cadherin-catenin complexes and nectin-afadin complexes. In the case of cadherin-catenin complexes, the cadherin molecule is a transmembrane protein with five repeating subunits in the extracellular space that are stabilized by calcium ions. The intracellular portion of cadherin interacts with p120-catenin, β-catenin, or plakpglabin, which in turn interacts with intracellular cytoskeletal proteins. The nectin-afadin complex acts similarly to the cadherin-catenin complex, with nectin providing the primary scaffolding function and associating cytoskeletal proteins by the intracellular protein afadin.

Desmosome Junctions—Desmosome junctions anchor adjacent cells together and perform a function similar to tight junctions and adherens junctions (103,104). Desmosome junctions contain components of three protein families: cadherins, armadillo proteins, and desmoplakin. The cadherins span the extracellular space between two adjacent cells and provide physical anchoring of cells. The cadherin proteins are connected to plakins on the intracellular side and the plakin proteins are connected to keratin filaments; this mechanism provides continuity in protein interactions, stabilizes participating cells, and provides a direct link between extracellular and intracellular components. The armadillo family of proteins facilitates tethering of desmoplakin and keratin filaments to desmosomes; this serves to regulate clustering of desmosomal components and mediate signal transduction pathways.

Gap Junctions—The first three cellular junctions (tight junctions, adherens junctions, and desmosome junctions) are examples of anchoring junctions, which couple adjacent cells and provide structural and mechanical stability. On the other hand, gap junctions are functional coupling points between adjacent cells and regulate the flow of molecules between coupled cells (105–108). Gap junctions are composed of repeating units of a protein family known as the connexin family of proteins. Gap junctions consist of porous channels formed between adjacent cells and are made of six protein connexin subunits; the actual channel is referred to as a connexon. Gap junctions act as physical channels that connect adjacent cells and allow the flow of ions or other molecules through these cells. This is particularly important in excitable tissues like heart muscle, where the flow of ions through gap junctions is critical for depolarization of the heart.

Hemidemosome Junctions—Hemidemosomes junctions anchor cells to the underlying basement membrane by connecting intermediate filaments within the

cells to specific components of the ECM via integrin mediated binding (109). Hemidemosome junctions contain many different proteins on the intracellular side, including the plectin and plakin family of proteins. The primary function of the hemidesmosome junction is to anchor cells to the underlying ECM.

2.6 MAMMALIAN TISSUE AND ARTIFICIAL TISSUE

During the course of this chapter, we have looked at cell architecture and organelle function, ECM structure and function, and cell-cell and cell-matrix interactions. In this section, we provide a brief description of how many of the concepts introduced in this chapter are relevant in the formation and function of mammalian tissue; we can also apply these concepts to the fabrication of artificial tissue.

Let us begin our discussion with a review of some of the concepts as they relate to mammalian tissue. As we have seen, mammalian tissue is composed of different cell types and ECM components, which provide functional and structural support, respectively. Individual cells connect with other cells using cellular junctions that include tight junctions, adherens junctions, desmosome junctions, and gap junctions. In addition, cells communicate with other cells using a variety of cellular signaling methods including autocrine, paracrine, endocrine, and direct cell-cell signaling. *The functional coupling of cells with other cells is known as cell-cell interaction*. In addition to cells, mammalian tissue consists of the ECM, which serves to provide mechanical support; components of the ECM functionally couple with cells by binding to cell surface receptors known as integrins. *The functional coupling of cells with the ECM is known as cell-matrix interaction*. Cell-cell and cell-matrix interactions trigger a cascade of intracellular signaling events, which modulate cell behavior and phenotypes and, as a result, have a direct impact on 3D tissue architecture and function.

Now that we have seen how many of the concepts presented in this chapter relate to mammalian tissue, the next critical question is—*how do these concepts apply to tissue engineering and fabrication of 3D artificial tissue?* As we have seen in chapter one, tissue engineering is focused on the development of technologies to support the fabrication of artificial tissue. As we have seen during our discussion of the tissue fabrication process, isolated cells are cultured within 3D scaffolds to support the formation of 3D artificial tissue. During the tissue fabrication process, it is critical to support the formation of functional cell-cell and cell-matrix interactions between isolated cells and biomaterials. Successful implementation of strategies to support cell-cell and cell-matrix interactions is critical to the development of 3D artificial tissue.

2.7 CELL SOURCING

Requirements of an Ideal Cell Source—During the tissue fabrication process, isolated cells are coupled with biomaterials to fabricate 3D artificial tissue.

During this process, one critical decision needs to be made regarding the source of cells—*where exactly will the cells come from?* In order to help researchers determine the most suitable cell source for any given tissue engineering application, the following criteria have been established for an ideal cell source (110): 1) cells need to be safe and not trigger tumor creation, 2) improve functional performance of host tissue, 3) functionally integrate with host tissue and host vasculature, 4) cells need to be tolerant of noninvasive delivery methods, which are increasingly common in the operating room, 5) not trigger the host immune response, 6) in the case of stem cells, be sensitive to social and ethical issues, 7) when working with stem cells, the cells should have a clearly demonstrated potential to be differentiated with high efficiency to the cell type of interest, 8) any cell type should tolerate the processing conditioning required to develop off-the-shelf therapies. While there are several choices for cells in tissue engineering, none satisfy all of the stated design requirements. There are four cell sources which have been used extensively in tissue engineering: cell lines, animal derived cells, stem cells (adult-derived mesenchymal stem cells, embryonic stem cells, or induced pluripotent stem cells), and, finally, human derived cells. We will discuss these in detail in subsequent sections and start here by presenting some general considerations for cell sourcing, as shown in Figure 2.2.

Autologous versus Allogeneic—Cells can either be autologous or allogeneic. Autologous cells are cells that have been isolated from a tissue biopsy of the person who will also be recipient of these cells; the donor and recipient for autologous cells is the same. This strategy has clear advantages, as it circumvents issues related

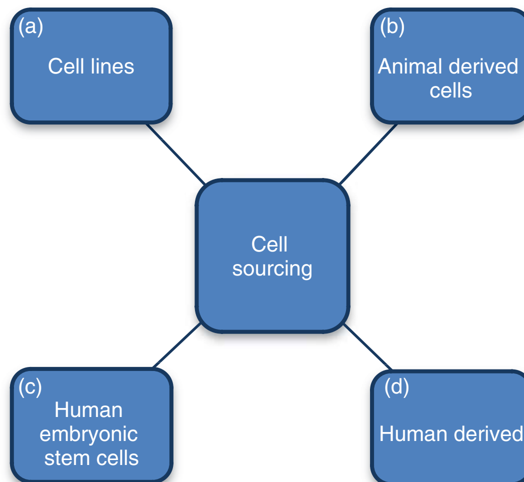

Figure 2.2 Cell Sourcing for Tissue Engineering—There are four sources of cells that are commonly used during the fabrication of 3D artificial tissue: (a) Cell lines, (b) Animal Derived Cells, (c) Human Embryonic Stem Cells, and (d) Human Donor Cells.

to host immune rejection and eliminates any issues related to identifying potential donors. Autologous cells are aggressively being evaluated in stem cell therapies involving the use of bone marrow-derived mesenchymal stem cells (MSCs). MSCs can be purified from a patient's bone marrow aspirate and processed *in vitro* and expanded in culture, followed by differentiation to a given cell lineage. The differentiated cells can later be implanted into the same patients at the site of injury. Allogeneic cells are isolated from a donor and then transplanted into a recipient patient, with the donor and recipient being different people. Blood transfusions are the most popular examples of allogeneic cell transplantation. The main limitations of allogeneic-based cells are the challenges in identifying potential donors and the potential for immune rejection by the host.

Animal-Derived or Human-Derived Cells—Cells can either be animal-derived or isolated from human tissue specimens. Clearly, if the cells are being used for clinical applications, cells isolated from humans would be the only choice. Animal-derived cells are used extensively during early stages of research, particularly in proof-of-concept studies, model development and optimization studies, and safety/efficacy studies. The primary advantage of using animal-derived cells is availability, as tissue specimens can be obtained based on need.

Cell Lines—Cell lines are used extensively for tissue engineering studies and can easily be purchased form commercial vendors. Primary cells are maintained in culture for extended periods of time and at regular intervals, can be sub-passaged to increase the cell yield (discussed in a subsequent section of this chapter). After several sub-passages, the cells in most cases tend to decrease in proliferative capacity; however, in some cases, these cells undergo genetic alterations that allow the cells to proliferative for extended periods of time. When this occurs, the cells are known as a cell line (111) . This property of cell lines, the ability to proliferate over extended periods of time, proves to be advantageous for tissue engineering studies. During the tissue fabrication process, a large number of cells are required to support 3D tissue formation. The proliferative capacity of cell lines allows researchers to obtain these cell numbers during routine cell culture.

Stem Cell Engineering—We will discuss stem cell engineering in detail in subsequent sections, but will use this opportunity to introduce the general concept. Cell sourcing for tissue engineering requires cells to have tissue specific functionality—fabrication of artificial heart muscle requires cardiac myocytes, fabrication of artificial cartilage tissue requires chondrocytes, and so forth. It is well-known that during early stages of human embryogenesis, all cells are the same, and during the course of development, they differentiate to become different cell types (112,113). These early stem cells are known as embryonic stem cells. The interest in stem cells, particularly embryonic cells, arises from the differentiate potential of these cells. For example, if a researcher wants to bioengineer vascularized heart muscle, several different cell types would be required, including cardiac myocytes, fibroblasts, endothelial cells, and smooth muscle cells. Since embryonic cells have the potential to differentiate into any cell type, a single cell source can be used to obtain multiple cell types, which in turn can be used to bioengineer multicellular tissue, like vascularized heart muscle.

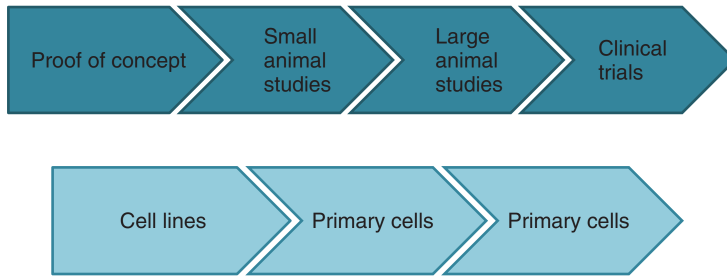

Figure 2.3 Cell Sourcing During the Progression of Tissue Engineering Studies—Different cell sources are suited for different parts of the technology development process for fabrication of 3D artificial tissue.

Cell Sourcing Strategy in Tissue Engineering Research—In tissue engineering, there is a common trajectory used for cell sourcing: progressing from proof-of-concept studies to clinical trials (Figure 2.3).

During the initial stages of project development, cell lines are used to conduct proof of concept studies. Cell lines are considerably easier to work with, reasonably inexpensive, and have reproducible phenotypes during cell culture. Once proof-of-concept studies have been successful, the work is translated to small animal studies, which are designed to validate the results obtained with cell lines. At this stage of the study, primary cells that are isolated from animal tissue are more suitable. The next step in the technology progression is from small animals to large animals; in this case, animal-derived cells would be more suitable. The final stage of the tissue engineering study is clinical trials, which require testing of the artificial tissue in human patients. The cells used in clinical trials have to be autologous, with stem cells being commonly used, particularly bone marrow-derived MSCs.

2.8 THE CELL TRANSPLANTATION PROCESS

Introduction to Cell Transplantation—In the first chapter of this book, we introduced the concept of cell transplantation or cell therapy, both of which refer to the same thing. The concept of cell transplantation is based on delivery of isolated cells to the site of injury; these therapies are based on the hypothesis that transplanted cells will support functional recovery of damaged or diseased tissue (110). From a conceptual standpoint, this is a very intriguing premise and is based on the hypothesis that isolated cells have the capacity to perform tissue-level functions. This is in sharp contrast to the field of tissue engineering, which is based on the premise that lost tissue function can be replaced by transplanted 3D tissue. The primary difference between the field of cell transplantation and tissue engineering is the therapeutic agent, with isolated cells being used in the former and 3D artificial tissue in the latter. Prior to describing the details of the process,

we introduce a few fundamental concepts related to the field and provide some definitions.

- Cell transplantation has been defined as the process by which cells are delivered to the site of injury in order to improve the functional performance of injured tissue (114,115). Whole blood transfusions, packed red cell transfusions, platelet transfusions, and bone marrow transplants are examples of cell therapy.
- Stem cell transplantation is a specialized case of cell transplantation, in which the cells being delivered are stem cells (116). Use of embryonic stem cells, induced pluripotent stem cells, and adult stem cells fall under the classification of stem cell transplantation. Cell transplantation and cell therapy are broad terms and apply when any cell type is used; stem cell engineering is a specialized case of cell transplantation that utilizes stem cells as the therapeutic agent.

Steps in the Process of Cell Transplantation—The process of cell transplantation is shown in Figure 2.4.

While there are several choices for stem cells, the general scheme that we present is based on hES cells (117). In this case, hES cells are isolated from the inner cell mass of a blastocyst at the 64 to 200 cell mass stage. The cells are isolated and

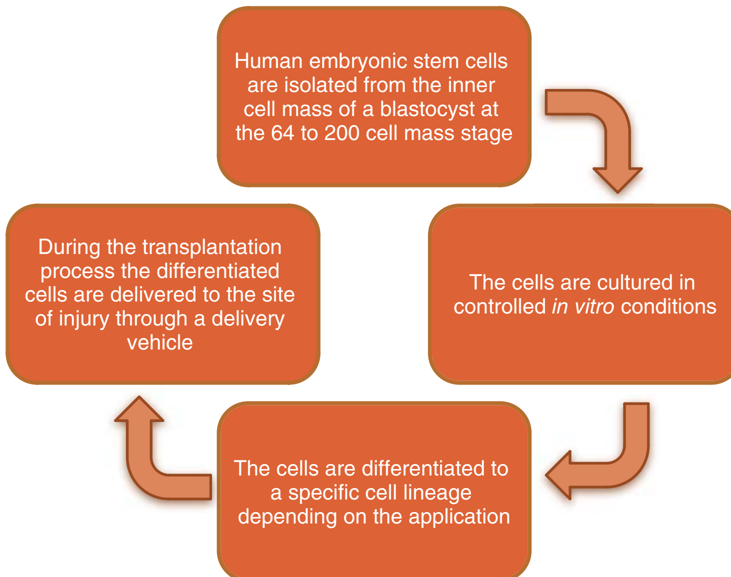

Figure 2.4 The Cell Transplantation Process—Human embryonic stem cells are differentiated to form different cell types that are delivered to the site of injury to support functional recovery.

cultured under controlled *in vitro* conditions. These cells are then differentiated to the specific cell lineage, which varies depending on application; for example, hES cells will need to be differentiated to cardiac myocytes for the treatment of myocardial infarction. The next step in the stem cell transplantation process is delivery of differentiated cells to the site of injury. Differentiated cells are maintained in 2D monolayer culture and will first need to be detached from the culture surface and suspended in a suitable buffer or cell culture media. Once suspended, the cells are placed within the delivery vehicle, with catheter-based delivery systems being commonly used. The differentiated cells are then delivered to the site of injury using these catheter-based delivery systems.

The cell transplantation process may appear to be a simple and straightforward process; however, this is not the case, and cell transplantation therapies are extremely complicated and are faced with many critical scientific and technological challenges.

Critical Challenges in Cell Transplantation—The isolation and culture of hES cells or any other stem cell requires trained technicians with a high degree of expertise. The researcher needs to identify the right culture conditions that will support the differentiation of hES to the required cell lineage. This is often done using a cocktail of chemical compounds and growth factors; the exact composition and concentration requires rigorous optimization, testing, and validation. In addition to using chemical compounds to drive the differentiation of hES to a specific lineage, other strategies have been used. These strategies include genetic engineering to manipulate the gene profile of cells, co-culturing with specific cell types, and electromechanical stimulation.

Another critical issue is the ability to maximize differentiation efficiency, which refers to the percentage of cells that differentiate to the desired cell lineage. The differentiation efficiency will never be 100%, and only a certain percentage of cells will be differentiated to any given lineage. One of the challenges in the field is the rigorous optimization of protocols to maximize differentiation efficiency. Another important challenge is the development of strategies to purify and separate differentiated cells from undifferentiated cells. At the end of any differentiation strategy, there will be a mixed cell population that contains differentiated and undifferentiated cells. The differentiated cells need to be separated from the undifferentiated cells prior to transplantation. Again, experimental strategies need to be developed for the separation of differentiated and undifferentiated cells, and this is no trivial task.

There is one more critical challenge associated with the differentiation of hES cells to specific lineages. *After differentiating hES cell to any given cell lineage, how effective is the differentiation strategy in producing functional cells?* For example, if the objective is to obtain cardiac myocytes, then we will develop and optimize differentiation strategies that drive the differentiation of hES cells to a cardiac myocyte lineage. Any given differentiation strategy will have finite differentiation efficiency, and a certain percentage of hES cells will form cardiac myocytes. Then the critical question becomes—*how close are the differentiated cardiac myocytes in form and function to mammalian cardiac myocytes?* In the case of cardiac myocytes, this

question can be answered by obtaining functional and biological data and comparing the results to mammalian heart muscle. Functional tests will be designed to measure the contractile and electrical properties and the changes in calcium transients. In order to assess the biological properties of cardiac myocytes, researchers can undertake studies to measure changes in gene and protein expression using rt-PCR and Western blotting, respectively. In addition, information about the localization of specific proteins can be obtained by immunohistochemistry strategies, and 3D volumetric rendering can be accomplished using confocal imaging. Collectively, this data will provide a comprehensive assessment of cardiac myocytes' function. Similar studies can be conducted for any cell type. This data can be compared to published and experimental values for mammalian tissue to judge the effectiveness of hES differentiation. While the results vary significantly based on the tissue system, differentiation strategy, and between research laboratories, the functional performance of differentiated cells will not approach that of mammalian cells, and, in most cases, the functional performance will be significantly lower. Therefore, before these cells can be considered a true therapeutic option for patients, strategies will need to be developed to bridge the gap between the functional performance of differentiated and mammalian cells.

One of the most significant limitations of the cell transplantation strategy is low cell retention upon transplantation. *What percentage of total transplanted cells is actually retained at the site of injury?* This may come as somewhat of a surprise, but cell retention is extremely low and has been reported in the range of 1–2%. This means that for every one hundred cells that are transplanted to the site of injury, only 1–2 cells actually are retained at the site of delivery. This is a major limitation of cell transplantation as a potential therapeutic strategy, as a small population of cells cannot support tissue-level function and support recovery of injured tissue.

2.9 CELLS FOR CELL TRANSPLANTATION

A large number of cells have been evaluated as therapeutic agents for cell transplantation. Human embryonic stem cells, induced pluripotent stem cells, and bone marrow-derived mesenchymal stem cells have been used extensively for cell transplantation. Hematopoietic stem cells have been used as a source of blood cells and can be used to treat patients with blood disorders. MSCs derived from adipose tissue are also an attractive option, as adipose tissue is widely available, can easily be obtained from a tissue biopsy, and does not require any invasive procedures. Umbilical cord tissue and blood have received a lot of attention recently, as specimens can be preserved at birth for future use; cord tissue is a source for MSCs, while cord blood is a source for hematopoietic stem cells. Resident stem cells refer to stem cells that are present in tissue in a dormant state under normal physiological conditions; however, in times of injury, become activated and participate in the tissue repair process. Most mammalian tissues have resident stem cells, and as one common example, satellite cells are known to be resident stem cells, which are present in skeletal muscle tissue.

2.10 MODE OF ACTION OF CELLS DURING CELL TRANSPLANTATION

The purpose of cell transplantation is to elicit functional recovery of host tissue. Let us recall the process of cell transplantation—isolated cells are delivered to the site of injury to support functional recovery of host tissue. *What are some possible mechanisms by which this can occur?* There have been several hypotheses postulated, but not yet conclusively proven. There are four primary mechanisms by which transplanted cells can support functional recovery: trans-differentiation to host cells, secretion of paracrine factors, recruitment of circulating stem cells, and increase in vascularization of host tissue (Figure 2.5).

Trans-differentiation of Transplanted Cells—This hypothesis states that transplanted cells differentiate to form functional cells that support functional recovery of damaged or diseased tissue (118–122). The transplanted cells functionally interact with cells of host tissue, and cell-cell interactions lead to differentiation of transplanted cells. Stated another way, the transplanted cells senses the local environment and identifies the need to differentiate to form functional host cells. Stem cells are plastic and have the potential to become many different cell types. By sensing the local environment and interfacing with cells of the host tissue, the transplanted cells differentiate to the lineage of the host tissue.

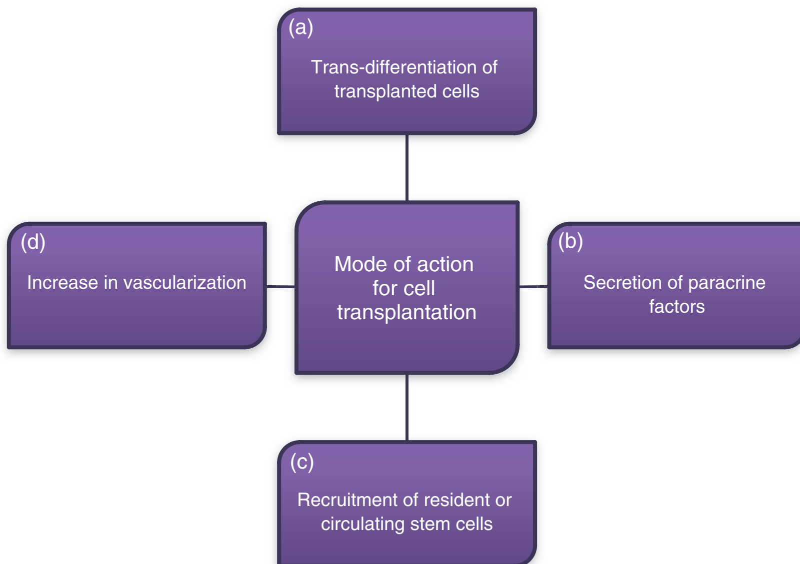

Figure 2.5 Mode of Action for Cell Transplantation—There have been four mechanisms postulated for the mode of action for cell transplantation. (a) Trans-differentiation of Transplantation Cells, (b) Secretion of Paracrine Factors, (c) Recruitment of Resident or Circulating Stem Cells, and (d) Increase in Vascularization.

Secretion of Paracrine Factors—The second potential mechanism is by secretion of paracrine factors—factors which are released into the local environment and act on neighboring cells (123–126). This hypothesis states that transplanted cells either secrete paracrine factors into the local environment or stimulate cells of the host tissue to secrete these factors. These paracrine factors then act by reducing cell apoptosis, increasing cell proliferation, and increasing angiogenesis and neovascularization of injured tissue. These actions lead to an increase in the functional performance of injured tissue.

Recruitment of Circulating Stem Cells—The third mechanism by which transplanted cells can improve functional performance of host tissue is by recruitment of resident or circulating stem cells (127–129). Resident stem cells are present within the tissue, while circulating stem cells refers to those present in the circulation, like hematopoietic stem cells. This hypothesis states that transplanted cells serve to recruit resident or circulating stem cells to the site of injury. Resident stem cells migrate from neighboring regions to the site of injury, while circulating stem cells can be recruited via capillaries. This process results in an increase in the number of stem cells present at the site of injury, which in turn leads to functional recovery of host tissue.

Increase in Vascularization of Host Tissue—The fourth mechanism by which transplanted stem cells can improve function is by promoting angiogenesis and neovascularization (130). Any increase in vascularization of host tissue will have a direct impact on tissue survival and will lead to an increase in cell viability, which in turn will lead to an increase in functional performance. Transplanted stem cells are postulated to support vascularization in one out of two possible ways; either by direct incorporation with host vasculature or by the release of angiogenic factors into the local environment. These two mechanisms will lead to a direct increase in host vascularization, which in turn will support functional recovery of the host tissue.

2.11 CELL TRANSPLANTATION AND TISSUE ENGINEERING

We will conclude our discussion of cell transplantation by presenting a comparison between cell transplantation and tissue engineering.

Which one is better, cell transplantation or tissue engineering? Let us begin by a brief discussion of the two fields. Cell transplantation is focused on delivery of isolated cells to the site of injury, while tissue engineering is focused on the fabrication of artificial tissue or entire organs that can be transplanted to the site of injury to support or replace lost tissue functionality. One of the most attractive features of cell transplantation is the simplicity of the approach, which utilizes isolated cells as a therapeutic option. From a methodological standpoint, isolated cells can easily be expanded and manipulated in culture and then delivered to the site of injury. There are, however, many limitations to the approach, the most significant of which is low cell retention, reported in the range of 1–2%.

Tissue engineering is focused on the design and fabrication of artificial tissue under controlled *in vitro* conditions, followed by transplantation of artificial tissue *in vivo* to support and/or replace lost tissue functionality. Tissue engineering addresses two significant drawbacks of cell transplantation. The first is the basic premise of the field, which is based on the hypothesis that lost tissue function can be recovered by transplantation of isolated cells. The field of tissue engineering takes this premise one step further and suggests that bioengineered tissue and/or organs would be better suited than isolated cells to repair, augment and/or replace lost tissue function. The second major drawback of cell transplantation is low cell retention; tissue engineering addresses the limitation of low cell retention by fabrication of artificial tissue/organs, which does not lead to significant loss of cells at the site of implantation. The reason for this is that the cells are tightly bound to other cells and to biomaterial and have come together to form complex 3D tissue under controlled *in vitro* conditions. The 3D architecture of bioengineered tissue and organs is maintained upon implantation *in vitro*.

The fields of cell transplantation and tissue engineering have made rapid progress during the last decade and both provide novel and innovative therapies to support lost tissue functionality. While the reader of this book is encouraged to formulate his/her opinion about the two fields, it is the view of the author that tissue engineering offers advantages over cell transplantation that make it a better therapeutic option to support lost tissue function.

2.12 THE CELL CULTURE PROCESS

Introduction—Isolated cells can be maintained in monolayer 2D cultures using a technique known as cell culture (131–143). The process of monolayer 2D cell culture has become very common and routine with very well-established protocols and standardized equipment. This can be appreciated by recognizing the abundance of cell culture laboratories in major research universities and medical centers, exceeding one hundred per institution. Cell culture is extremely critical to the development of cell transplantation and tissue engineering technologies. The application of cell culture during cell transplantation studies can easily be appreciated, as isolated cells need to be maintained, manipulated, and expanded in culture prior to implantation; at least fifty percent of cell transplantation studies are based on cell culture experiments. The same is true for tissue engineering studies; cells form the functional components of artificial tissue and, coupled with biomaterials and bioreactors, form one of the building blocks of 3D artificial tissue. The ability to isolate, maintain, manipulate, and expand cells is central to the tissue fabrication process. Development of tissue engineering technologies relies heavily on cell culture; at least fifty percent of tissue engineering research is based on cell culture experiments. Due to the essential role of cell culture techniques in tissue engineering, we will study some of the general principles of cell culture in this section.

The Cell Culture Process—The steps involved in the isolation, culture and expansion of cells are shown in Figure 2.6.

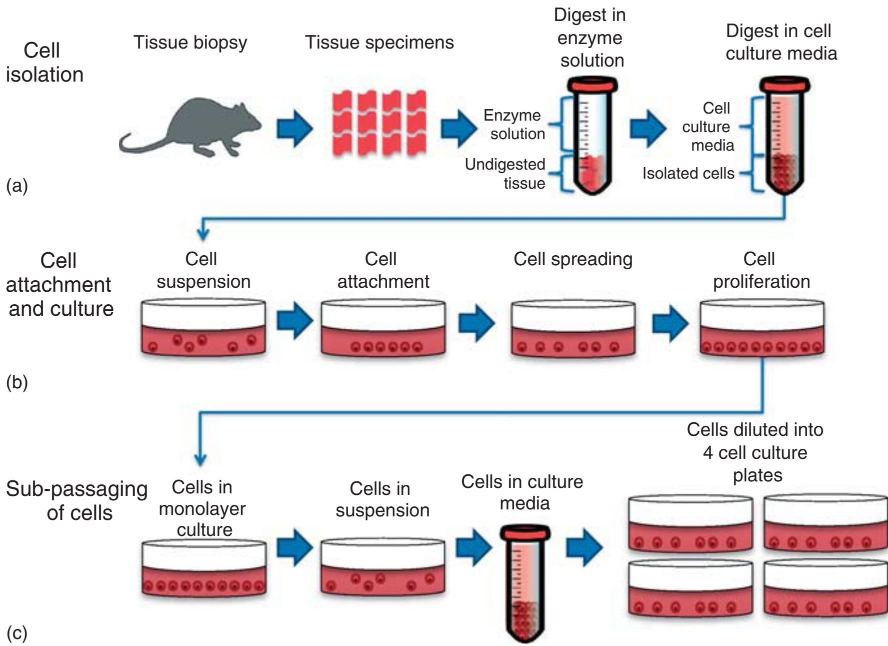

Figure 2.6 The Cell Culture Process—(a) Cell Isolation—A tissue specimen from a biopsy is cut into small pieces and subjected to an enzymatic digestion process to yield isolated cells. (b) Cell Attachment and Culture—The primary cells are cultured on the surface of tissue culture plates where they attach, spread, and proliferate. (c) Cell Expansion—Upon confluency, cells are subjected to treatment with trypsin and then replated to promote cell expansion.

Our discussion here is focused on primary cell culture, cells which are isolated from a tissue biopsy. There are several steps in the cell culture process: cell isolation, cell attachment, cell proliferation, and sub-passaging of cells. The first step in the process is the isolation of primary cells from a tissue biopsy. The tissue specimen is harvested either from animal or human tissue and is maintained in a buffered solution. The tissue specimen is cut into small pieces and suspended in digestion solution, which consists of digestive enzymes, with collagenase and trypsin being commonly used examples. The exact digestion time varies between tissue systems and is a variable that needs to be optimized and validated experimentally; digestion times of 30 to 60 minutes are common. The purpose of the digestive enzymes is to dissociate cells from the ECM, and over time, the cells are completely separated from the tissue. The isolated cells are transferred from the digestive solution to cell culture media. The tissue digestion process separates cells from the ECM, and the isolated cells are then maintained in culture and used for a variety of applications.

The next step in the cell culture process is cell attachment and culture on the surface of monolayer tissue culture plates. Most mammalian cells are anchorage-dependent, which means they need to be anchored to a substrate in order to support

cell viability, growth and proliferation. Anchorage-dependent cells cannot maintain viability and function during suspension culture and will die over time. In order to support cell viability and growth, isolated cells are plated on the surface of tissue culture plates that have been specifically designed to support the attachment and growth of anchorage-dependent mammalian cells. The process of transferring isolated cells from suspension culture to the surface of a tissue culture plate is known as cell plating. The cells are plated at a specific density, which refers to the number of cells per milliliter of culture media or cells per unit area; in order to calculate the plating density, the number of cells will need to be determined using a hemocytometer or an automated cell counter.

Once the cells are plated, they attach to the culture surface. Often, the culture surface is coated with adhesion proteins like collagen, fibronectin, or laminin to promote specific integrin-mediated cell-matrix interactions. The choice of ECM protein and specific concentration depends on the cell type and cell plating density; the protein choice and its concentration needs to be experimentally tested and validated. Cells undergo morphological changes after attachment to the culture surface and start to spread out and occupy a large footprint on the culture surface. This process, referred to as cell spreading, is due to changes in cytoskeletal proteins. Cell spreading is common with mammalian cells and is indicative of a positive cell phenotype in response to cell attachment. If cell spreading occurs, it is considered to be a normal cellular response, while the lack of cell spreading indicates a problem with cell culture process.

So far, we have looked at cell attachment and cell spreading; the next step in the cell culture process is cell proliferation, which refers to the process by which cells divide and increase in number. Cells will only proliferate if they are cultured in the correct environment, including temperature, pH, nutrient composition, and adhesion matrix. Cell proliferation is an important part of the cell culture process and is essential in tissue engineering and cell transplantation studies, as a very large cell number is required for these studies. In the case of cell transplantation technologies, a large number of cells are required for *in vivo* implantation, whereas in the fabrication of 3D artificial tissue, a large number of cells are required to populate synthetic scaffolds.

The next step in the cell culture process is subpassaging. During the course of monolayer culture, attached cells continue to proliferate and increase in number. This process of cell proliferation continues as a function of time. However, there are certain limitations, and the cells cannot continue to proliferate indefinitely. The primary limitation is space—the culture surface has a finite area to support cell attachment; as the cells continue to proliferate, the culture surface gets covered with cells leaving no room for additional cells. The term confluency refers to the percentage of total culture surface that is covered by cells. An increase in cell confluency leads to contact inhibition, which refers to the process by which attached cells no longer proliferate due to constraints imposed by space limitations. As the cells no longer have space to grow and proliferate, they enter a decay or decline phase that leads to a reduction in cell number. This is not a desirable outcome, and prior to contact inhibition, the cells need to be subpassaged. Subpassaging refers

to the process by which attached cells are detached from the culture surface, resuspended in cell culture media and re-plated at a lower cell density. Re-plating cells at a lower density reduces or eliminates confluency-induced contact inhibition and allows cells to proliferate and expand in number. Digestive enzymes like trypsin are used to detach cells from the culture surface, and once cells have detached, they are collected and diluted to the required cell concentration and then replated onto additional culture plates, commonly with a one to four dilution.

2.13 APPLICATIONS OF MONOLAYER 2D CELL CULTURE

Monolayer cell culture techniques have been used extensively in research for many different applications, some of which are summarized in Figure 2.7.

A significant amount of information regarding cell-cell interactions and cell-matrix interactions has been obtained from monolayer 2D cell cultures. Different cell types can be cultured under controlled *in vitro* conditions, and specific interactions between cells or with ECM components can be studied in isolation without the confounding effect of mammalian physiology. Therefore, specific cell-cell and cell-matrix interactions, along with subsequent signaling pathways, can be identified using isolated 2D monolayer cell culture systems. Intracellular signaling pathways,

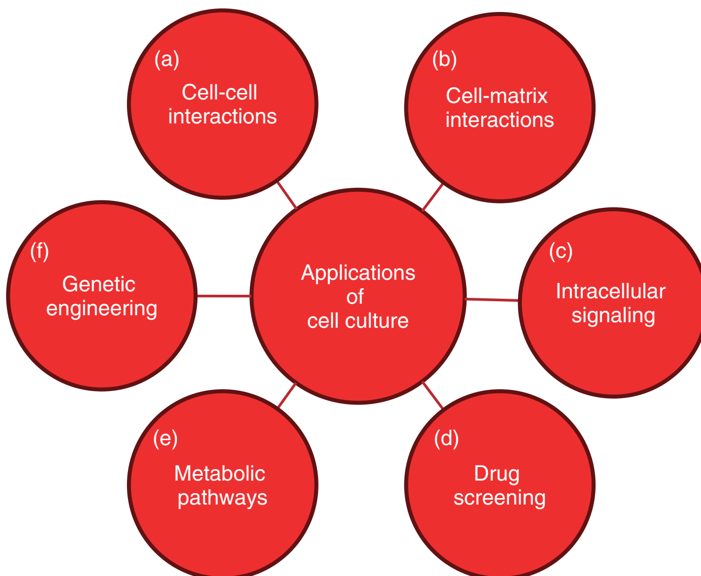

Figure 2.7 Applications of Cell Culture—Cell culture studies have been used for many different applications, some of which include: (a) Cell-Cell Interactions, (b) Cell-Matrix Interactions, (c) Intracellular Signaling, (d) Drug Screening, (e) Metabolic Pathways, and (f) Genetic Engineering.

either in response to cell-cell or cell-matrix interactions, or other modes of stimulation like growth factor conditioning, have been elucidated based on cell culture studies. Another area of research that has benefitted from cell culture studies is drug development. During the early stages of drug development, there are thousands of potential candidates that need to be narrowed down to a manageable number. Cell culture models provide a quick and cost-effective away of screening potential pharmacological compounds. Candidate compounds can be added to cell culture media and exposed to cells during monolayer culture; after a finite expose time, cellular response can be evaluated. Along the same line of thought, cell culture models have proven to be effective in toxicology studies and in the screening of environment toxins, agents that have a negative impact on cell and tissue function. Dose–response studies using cells in monolayer culture can be conducted to determine human exposure limits. Much of the information we know about metabolic pathways have been obtained from isolated cell culture studies. Advances in stem cell engineering, genetic engineering, and strategies to drive the differentiation fate of stem cells toward a cell specific lineage have been a result of cell culture studies.

2.14 CELL CULTURE VERSUS TISSUE ENGINEERING

A Typical Tissue Engineering Experiment—The first step of the process involves the isolation, culture, and expansion of primary cells. In most cases, a large number of cells are required to bioengineer artificial tissue, and several rounds of subpassaging are required. Once a sufficient number of cells have been obtained, cells are detached from the culture surface using digestive enzymes like trypsin, and the suspended cells are collected in a conical flask. These cells are then plated onto the surface of a biomaterial and maintained in culture. The cells attach to the biomaterial, proliferate to increase in cell number, and over time populate the entire scaffold. This process leads to the formation of bioengineered 3D artificial tissue. This example serves to illustrate the interplay between cell culture and tissue engineering and the relationship between the two fields.

Cell Culture versus Tissue Engineering—In the previous example, we looked at the relationship between cell culture and tissue engineering; techniques in cell culture are essential to obtain the large cell numbers required for tissue engineering and tissue fabrication. Cell culture, in a sense, feeds into tissue engineering. However, the two fields can be compared side by side as two separate areas of research. Cell culture techniques have been used extensively in basic research and have provided an understanding of cell behavior and function. In an earlier section, we identified a list of research areas in which cell culture has proven to be a valuable tool, like cell-cell interactions, cell-matrix interactions, intracellular signaling, and others. During cell culture, isolated cells are maintained in 2D culture and do not have the 3D geometry of mammalian tissue; therefore, information obtained from cell culture models is limited.

However, this information can be extended by using tissue engineering models, which provide complex 3D architecture, absent in 2D monolayer culture systems.

Researchers can extract considerably more information using tissue engineering models when studying cell-cell interactions and cell-matrix interactions, as the cells are now cultured in a complex 3D geometry.

Tissue engineering models have a tremendous role to play in basic research and in understanding cell and tissue organization, structure, and function.

2.15 INTRODUCTION TO STEM CELL ENGINEERING

Introduction—Stem cells have proven to be an attractive cell source for tissue engineering and tissue fabrication. Embryonic stem cells, induced pluripotent stem cells, and adult-derived mesenchymal stem cells have received significant attention in recent literature. Advances in stem cell engineering are based on manipulation of the genetic material of stem cells, and in order to understand this process, an understanding of the central dogma of molecular biology is important. We begin our discussion on stem cell engineering with a brief overview of the central dogma of molecular biology.

Central Dogma of Molecular Biology—The central dogma of molecular biology, states that DNA is transcribed to RNA, which is then translated to proteins (144–146). All cells of any given species have the same genetic material, which is stored in the nucleus of cells. The genetic material is stored in the form of DNA molecules, which have a double helical structure with repeating units of four nucleotides: adenine, thymine, guanine, and cytosine. Human DNA contains all the genetic information required to synthesize all proteins that are required for normal function. As such, DNA molecules do not leave the nucleus and are kept within the nucleus for safekeeping. The genetic information necessary for protein synthesis is transferred from the nucleus by messenger RNA (mRNA) molecules. The process by which information stored within segments of DNA molecules is converted to mRNA is known as transcription. Messenger RNA is single-stranded, compared to double-stranded DNA and is also composed of the same four nucleotides found in DNA. The process by which proteins are synthesized using the information within the mRNA molecules is known as translation. Ribosomes are the site of protein synthesis, and mRNA leaves the nucleus and binds to ribosomes, which leads to the formation of a specific peptide by the process known as translation. The central dogma states that DNA is converted to RNA and then to protein.

What exactly does the central dogma of molecular biology tell us regarding stem cell engineering? All mammalian cells have the necessary genetic information to produce all proteins required for normal function; the production of specific proteins distinguishes the function of one cell type from another cell type. For example, cardiac myocytes require the expression of contractile proteins in order to support function of heart muscle. Protein expression in turn is governed by the expression of mRNA, which is regulated by the process known as transcription. Therefore, transcriptional regulation is at the heart of cell specialization and is also a defining concept in the field of stem cell engineering.

Stem Cell Differentiation During Embryogenesis—During human development, fertilization of an ovulated oocyte by spermatozoa leads to the formation of a zygote. The zygote is a single cell, and early on during human development, it undergoes a series of rapid cell divisions to give rise to the 2-cell stage, 4-cell stage, 8-cell stage, and so forth (147–149). This process continues until the formation of a blastocyst, which contains an inner cell mass of embryonic stem cells. This blastocyst develops from an early stage of development known as an early blastocyst to a later stage of development known as a late blastocyst. The late blastocyst is implanted within the walls of the uterus and gives rise to an embryo. All cells within the embryo at this stage are the same and are known as human embryonic stem cells (hES). During the course of human development, hES cells give rise to all cells within the human body. This means that hES cells have the necessary genetic information to form all cells in the human body. *What causes a hES to become a cardiac myocyte versus a hepatocyte?* Different cell types have different functions that are performed by expression of cell specific proteins—cardiac myocytes exhibit contractile functions due to the presence of myosin heavy chain and other contractile proteins. The expression of different proteins is regulated by transcription of DNA to RNA; therefore, the regulation of gene expression is responsible for the expression of specific proteins (Figure 2.8). This means that hES can become any cell type in the human body by regulation of gene expression. This is the basic premise of stem cell engineering, which is also referred to as genetic engineering. The excitement in the field of stem cell engineering has been derived from the fact that a single cell type can, in theory, give rise to all cell types in the human body.

Human Embryonic Stem Cell and Tissue Engineering—In the previous section, we looked at the differentiation of human embryonic stem cells to any type in the

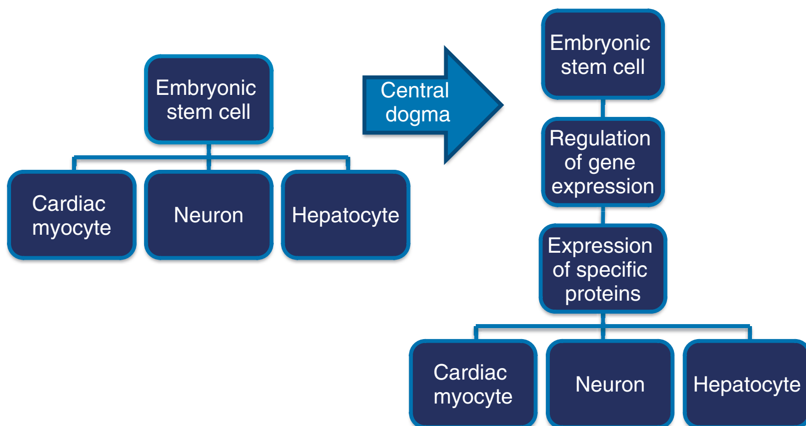

Figure 2.8 Differentiation of Human Embryonic Stem Cells—hES cells have the potential to become any cell type that is present in the human body. This is achieved by regulation of the genetic code, which in turn affects the expression of specific proteins, which in turn dictates cell function.

human body, at least in theory, by gene regulation. *What exactly does this mean for tissue engineering and why is this important?* In order to bioengineer 3D vascularized heart muscle, multiple cell types are required. Some of these required cell types which include cardiac myocytes for contractile function, cardiac fibroblasts for ECM production, and vascular cells, including endothelial cells and smooth muscle cells. Let us begin by using a scheme that relies on primary cells isolated from tissue biopsies. This scheme requires heart muscle specimens to isolate cardiac myocytes and cardiac fibroblasts. Similarly, vascular segments are required to isolate endothelial cells and smooth muscle cells. The isolated cells are then expanded in culture and combined with suitable scaffolds to fabricate 3D vascularized heart muscle.

Now let us look at the same scenario using hES cells. Once we have established isolation and culture conditions for hES, we can drive the differentiation of these cells toward all cell types required to fabricate vascularized heart muscle. This means that a single cell type can give rise to cardiac myocytes, cardiac fibroblasts, endothelial cells, and smooth muscle cells. These cells can then be combined with scaffolding material to fabricate 3D vascularized heart muscle. While controlling the differentiation fate of hES to multiple lineages is challenging and much more difficult than stated here, this example serves to illustrate the potential of hES cells during the tissue fabrication process.

Characteristics of Stem Cells—Stem cells have three important characteristics that distinguish them from other cell types: self-renewal, unspecialized function, and differentiation potential (150–153).

1. Self-renewal—This refers to the property of stem cells by which they are able to proliferate in order to maintain a constant supply of stem cells within any given tissue. We can illustrate this by looking at the case of satellite cells within skeletal muscle. Satellite cells are dormant under normal physiological conditions and become activated in response to muscle injury. The satellite cells proliferate and expand in number and then fuse to form myotubes. However, during this process, satellite cells are, in effect, used up. This means that the total number of satellite cells is reduced, limiting the ability to respond to the next muscle injury. Stem cells overcome this problem by the process of self-renewal. This means that every time a stem cell divides, one of the daughter cells is an identical copy of the parent and is maintained as a stem cell. The second copy may also be an identical copy, and if this occurs, the process is known as symmetrical division. Alternatively, the second daughter cell can differentiate to a specialized cell lineage, and in this case, the cell division process is known as asymmetrical division.
2. Unspecialized Function—Stem cells are unspecialized cells and do not perform any physiological function. Most cells in the human body are specialized and are programmed to undertake a specific function. For example, cardiac myocytes are specialized cells, and their primary function is to generate contractile force, which leads to the pumping action of the heart. In order to perform this specialized function, cardiac myocytes express specific contractile

proteins like actin, myosin heavy chain, tropomyosin, and troponin. However, stem cells do not perform any such specialized functions, and during growth and development, they are maintained in an unspecialized form. When the need arises, either due to disease or injury, stem cells have the potential to be differentiated to form specialized cells.

3. Differentiation Potential—Under controlled physiological conditions, stem cells have the potential to become specialized cells. This is the most talked-about property of stem cells and the most extensively studied as well. The ability of stem cells to become one or more specialized cells has broad-reaching applications in tissue engineering. Therefore, any cells that possess these three characteristics—self-renewal, unspecialized function, and differentiation potential—are referred to as stem cells.

Differentiation of Stem Cells—Early in the process of embryogenesis, embryonic stem cells are present and have the potential to give rise to any cell type. Embryonic stem cells give rise to cells of different lineages via a process known as cell differentiation, which refers to an increase in the degree of specialization of stem cells. As previously described, stem cells are unspecialized and do not perform any specialized function; however, stem cells gradually differentiate to form specialized cells. The process of cell differentiation transforms an unspecialized stem cell to a more specialized cell type. Cell differentiation is a result of regulation of gene expression, which in turn alters expression of specific proteins; these proteins undertake specialized functions in any given cell type. Therefore, the process of cell differentiation can be viewed as an increase in the degree of specialization of the cell brought about by expression of specific proteins that are specific to the cell and tissue.

Cell Potency—There are several terms that are used frequently in stem cell literature: cell potency, totipotent stem cells, multipotent stem cells, and unipotent stem cells (154–156) (Figure 2.9).

Cell potency refers to the differentiation potential of stem cells. Certain stem cells, like human embryonic stem cells, have the potential to differentiate to form all cell types, at least in theory, and are known as totipotent stem cells. In order to be truly considered totipotent stem cells, the differentiation potential of hES to all cell types will need to be experimentally validated. While this has not been done, hES are still considered to be totipotent, as it is known that these cells do give rise to all cell types in the human body during embryogenesis. Certain stem cells give rise to many different cell types, but not all, and are referred to as multipotent stem cells; hematopoietic stem cells are a classic example of multipotent stem cells, as they have the potential to become blood cells, but they cannot be differentiated into other cell types. Unipotent stem cells refer to stem cells that can only be differentiated into a single cell type. Most adult tissue has a population of resident stem cells, which are dormant under normal physiological conditions, but can be activated when the tissue is diseased, damaged, or injured. When resident stem cells are activated, they differentiate to form host tissue cells that aide the process of repair and recovery of lost tissue functionality. These resident stem cells are known as unipotent, as they

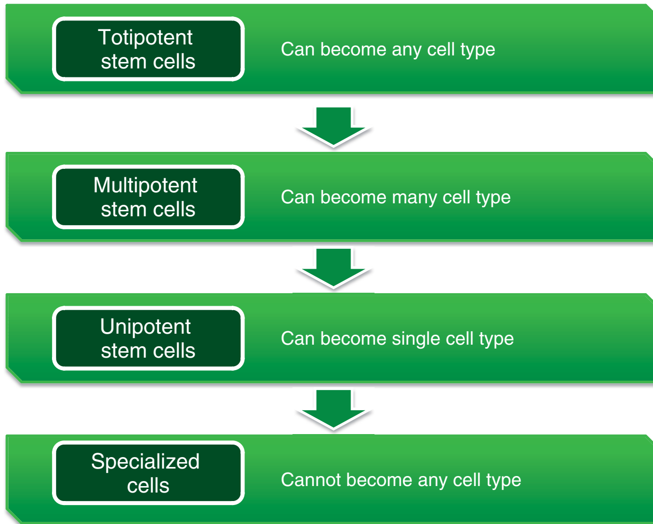

Figure 2.9 Differentiation Potential of Stem Cells—Totipotent stem cells have the potential to be differentiated to any cell type. Multipotent stem cells have the potential to be differentiated to form many cell types. Unipotent stem cells have the potential to be differentiated to form a single cell type. Specialized cells cannot be differentiated to form any cell types.

can only differentiate to a single cell type and cannot be differentiated to form any other cell type.

There have been three sources of stem cells that have been used extensively for tissue engineering studies: human embryonic stem cells (hES), which have been introduced before, induced pluripotent stem cells (iPS), and adult stem cells. We will discuss these three stem cell choices in the next three sections and also present a general scheme in Figure 2.10 outlining the potency of these cell types.

Human embryonic stem cells are totipotent and have the potential to become any cell type. Induced pluripotent stem cells are pluripotent, which means they have the capacity to become almost all types, but cannot be differentiated to form all cell types. Adult stem cells are either multipotent or unipotent, as they have the potential to differentiate only to a few cell types and often can only differentiate to form cells of the tissue in which they reside.

2.16 HUMAN EMBRYONIC STEM CELLS

Human embryonic stem cells hES are found very early during development and give rise to all cells in the body. Human embryonic stem cells are isolated from the inner mass of the human blastocyst and are cultured on tissue culture plates that have been coated with a feeder layer of mouse embryonic fibroblast cells (112,113).

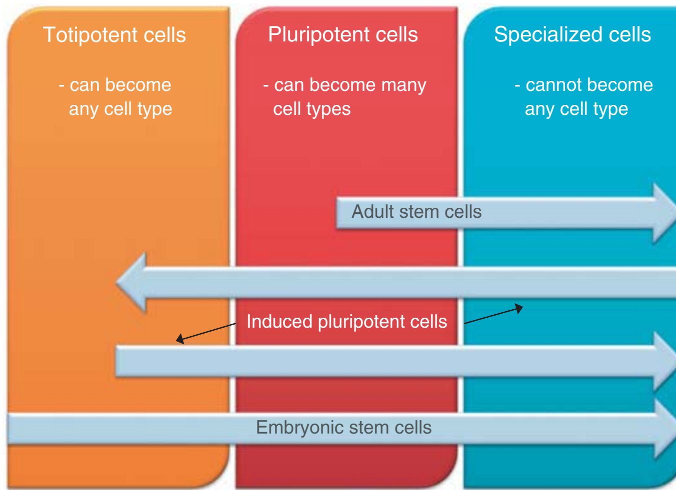

Figure 2.10 Stem Cell Sources for Tissue Engineering—There are three sources of stem cells that have been used extensively for tissue engineering studies: human embryonic stem cells, induced pluripotent stem cells, and adult stem cells.

After several days in culture, the inner cell mass divides and forms clusters of cells. Human embryonic stem cells are separated from the periphery of these clusters and mechanically or chemically dissociated, and then replated onto additional culture plates coated with feeder layers of fibroblast cells. As the cells continue to proliferate in culture, colonies of homogenous cells form and are mechanically/chemically selected and replated on a culture surface coated with feeder fibroblast cells. As this process continues, it leads to the formation of a continuous cell line. Human embryonic stem cells are desirable due to the ability of the cells to differentiate into all cell types, which can be used to support tissue fabrication technologies. However, the use of hES for tissue engineering or any other application has been surrounded by controversy, as the isolation of the cells requires destruction of human embryos.

2.17 INDUCED PLURIPOTENT STEM CELLS

Induced pluripotent stem cells have received a lot of attention in the recent literature and these cells have been extensively used for tissue engineering studies. The most attractive feature of iPS cells is their ability to differentiate into many different cell types without the need to destroy human embryos. Induced pluripotent stem cells are generated from differentiated adult cells, with skin fibroblasts being one common example. The specialized cells are reprogrammed to an embryonic state, and the reprogrammed cells, known as iPS cells, can be differentiated to form may cell types (157–160). The formation of induced pluripotent stem cells was first described in 2006, when it was demonstrated that retroviral overexpression of

four transcription factors (octamer 3/4(Oct 4), SRY box-containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4) and c-Myc) was sufficient to transform murine fibroblasts to an embryonic state (161). The main advantage of iPS cells is that terminally differentiated cells can be transformed to an embryonic state that can then be differentiated to multiple cell types. The use of iPS cells provides the same or comparable advantages as hES in terms of differentiation potential while eliminating the controversial issues associated with hES stem cells. In addition, iPS cells can be generated from terminally differentiated cells like fibroblasts, which can be isolated from a patient-derived biopsy, therefore providing a pathway for the development of autologous treatment strategies. The primary limitation of iPS cells has been the use of retroviral transduction of terminally differentiated cells, which can lead to random insertions and mutations in host genome. However, technology has been developed to address this limitation, and several methods have been developed to reprogram differentiated cells to form iPS cells using fewer transcription factors, viral integration followed by transgene removal, and chemical compounds.

2.18 ADULT STEM CELLS

The concept of adult stem cells or resident stem cells has been introduced earlier in this chapter; these cells refer to stem cells that are present within most tissues in a dormant state and become activated upon disease or injury. Adult stem cells are multipotent, as they can only be differentiated to a few cell types, and at times, they are unipotent and can only be differentiated to a single cell type (162). Adult stem cells isolated from the bone marrow, known as bone marrow-derived mesenchymal stem cells, have been studied extensively for tissue engineering applications (163,164). Bone marrow contains mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). Bone marrow-derived mesenchymal stem cells give rise to a variety of cell types, some of which include bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells, such as those in tendons. Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages, and platelets.

SUMMARY

Current State of the Art—Cells are one of the building blocks of tissue engineering. In this chapter, we have provided an overview of cell biology and looked at the function of many cell organelles. We also looked at cell culture and examined how cell culture techniques are used to maintain and expand isolated cells. We also provided a comparison between monolayer cell culture and tissue engineering, and we looked at the relative advantages and disadvantages of each technique. In this chapter, we also studied cell transplantation and the use of isolated cells, including stem cells, to provide functional support for the repair of damaged and diseased

tissue. In the concluding part of this chapter, we studied stem cell engineering and looked at several sources for stem cells to support the tissue fabrication process.

Thoughts for the Future—Significant research has been dedicated toward understanding the drivers of stem cells toward specific lineages. Many different strategies are currently under investigation to drive the differentiation fate of hES cells, iPS cells, and adult stem cells toward cell specific lineages. However, differentiation efficiency is generally low and differentiated cells do not fully replicate all functions of the cells that are necessary for therapeutic purposes. The ability to regulate the differentiation of stem cells toward a specific cell lineage, with high differentiation efficiencies to form highly functional cells, is a high priority research area.

PRACTICE QUESTIONS

1. Why are cells important for tissue engineering? How are cells used during the tissue fabrication process?
2. There are four aspects of cells that are important in tissue engineering: cell biology, cell culture, cell transplantation, and stem cell engineering. Explain how each of these four aspects relates to the fabrication of 3D artificial tissue.
3. Describe the process of nuclear import and nuclear export.
4. What is the role of the mitochondria in cell function?
5. Explain the process of phagocytosis and autophagy.
6. During the course of this chapter, we provided a description of several organelles and their role in maintaining the function of eukaryotic cells. How does this relate to tissue engineering, and why is it important during the tissue fabrication process?
7. Explain the following terms as they relate to cellular signaling: endocrine signaling, paracrine signaling, autocrine signaling, and direct cell-cell signaling.
8. Why is cellular signaling important for the fabrication of 3D artificial tissue?
9. Explain the following terms as they relate to cellular junctions: tight junction, adherens junction, desmosome junction, gap junction, and hemidesmosome junction.
10. What is the difference between structural and functional cellular junctions? Give examples of each.
11. Why are cellular junctions important during the tissue fabrication process?
12. Describe the steps involved in cell transplantation.
13. What does stem cell transplantation refer to?
14. What are some of the critical challenges in the field of cell transplantation?

15. Describe three mechanisms by which transplanted cells can improve the functional performance of damaged and/or diseased tissue.

16. Explain how cell therapy can be used as a treatment modality for acute myocardial infarction.

17. Compare stem cell therapy and tissue engineering as therapeutic modalities. Discuss the relative advantages and disadvantages of stem cell therapy and tissue engineering. Which one is better and why?

18. Describe the process of maintaining and expanding cells during monolayer 2D culture on the surface of a tissue culture plate. Explain the following steps in the process: cell isolation, cell attachment, cell proliferation, and subpassaging of cells.

19. What does cell confluency refer to? How is it relevant to the cell culture process?

20. Cell culture and tissue engineering are related. Discuss the relationship between cell culture and tissue engineering. What are the relative advantages and disadvantages of each of these two processes?

21. What do the terms cell-cell and cell-matrix interactions mean? Why are cell-cell and cell-matrix interactions important for tissue engineering?

22. We discussed the following cell sources for tissue engineering: cell lines, animal derived cells, human embryonic stem cells, and adult stem cells. Pick any tissue fabrication application of your interest—which cell source will you use and why?

23. What are the relative advantages and disadvantages of human embryonic stem cells and induced pluripotent stem cells to support tissue fabrication?

24. We discussed cell culture, stem cell therapy, and tissue engineering. In order to support the fabrication of artificial heart muscle, discuss how each of these three strategies comes into play.

25. In your opinion, what is the most significant scientific challenge associated with cells as they relate to the tissue fabrication process? What can be done to overcome this scientific challenge?

REFERENCES

1. Yoshida M, Oh H. Stem cell engineering for cardiac tissue regeneration. Cardiology 2010;115(3):191–3.
2. Ashton RS, Keung AJ, Peltier J, Schaffer DV. Progress and prospects for stem cell engineering. Annu. Rev. Chem. Biomol. Eng. 2011;2:479–502.
3. Lim JM, Lee M, Lee EJ, Gong SP, Lee ST. Stem cell engineering: limitation, alternatives, and insight. Ann. N.Y. Acad. Sci. 2011 Jul;1229:89–98.

4. Ogle BM, Palecek SP. Editorial: stem cell engineering—discovery, diagnostics and therapies. Biotechnol. J. 2013 Apr;8(4):390–1.
5. Zhang Y, Rockett PI. A Generic multi-dimensional feature extraction method using multiobjective genetic programming. Evol. Comput. 2009;17(1):89–115.
6. Yang ZR, Lertmemongkolchai G, Tan G, Felgner PL, Titball R. A genetic programming approach for Burkholderia pseudomallei diagnostic pattern discovery. Bioinformatics. 2009 Sep 1;25(17):2256–62. PMCID:PMC2734322.
7. Mattick JS. Deconstructing the dogma: a new view of the evolution and genetic programming of complex organisms. Ann. N.Y. Acad. Sci. 2009 Oct;1178:29–46.
8. DePalma A. Enhancement of Cell Culture Techniques. Genetic Engineering & Biotechnology News 2009 Oct 15;29(18):46–8.
9. Helmrich A, Barnes D. Animal cell culture equipment and techniques. Methods in Cell Biology, Vol 57 1998;57:3–17.
10. Mozdziak PE, Petitte JN, Carson SD. An introductory undergraduate course covering animal cell culture techniques. Biochemistry and Molecular Biology Education 2004 Sep;32(5):319–22.
11. Murakami H. Basal Techniques for Serum-Free Animal-Cell Culture. Seikagaku 1988 Jun;60(6):450–3.
12. Dalle JH. Hematopoietic stem cell transplantation in SCD. C.R. Biol. 2013 Mar;336(3):148–51.
13. Haddad IY. Stem cell transplantation and lung dysfunction. Curr. Opin. Pediatr. 2013 Jun;25(3):350–6.
14. Lai PF, Panama BK, Masse S, Li G, Zhang Y, Kusha M, Farid TA, Asta J, Backx PH, Yau TM, et al. Mesenchymal Stem Cell Transplantation Mitigates Electrophysiological Remodeling in a Rat Model of Myocardial Infarction. J. Cardiovasc. Electrophysiol. 2013 Apr 8.
15. Maroof AM, Keros S, Tyson JA, Ying SW, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell 2013 May 2;12(5):559–72.
16. Xie W, Schultz MD, Lister R, Hou Z, Rajagopal N, Ray P, Whitaker JW, Tian S, Hawkins RD, Leung D, et al. Epigenomic analysis of multilineage differentiation of human embryonic stem cells. Cell 2013 May 23;153(5):1134–48.
17. Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, et al. Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer. Cell 2013 May 15.
18. Bilokapic S, Schwartz TU. 3D ultrastructure of the nuclear pore complex. Curr. Opin. Cell Biol. 2012 Feb;24(1):86–91. PMCID:PMC3398480.
19. Grossman E, Medalia O, Zwerger M. Functional architecture of the nuclear pore complex. Annu. Rev. Biophys. 2012;41:557–84.
20. Maimon T, Elad N, Dahan I, Medalia O. The human nuclear pore complex as revealed by cryo-electron tomography. Structure. 2012 Jun 6;20(6):998–1006.
21. Raices M, D'Angelo MA. Nuclear pore complex composition: a new regulator of tissue-specific and developmental functions. Nat. Rev. Mol. Cell Biol. 2012 Nov;13(11):687–99.

22. Clever M, Mimura Y, Funakoshi T, Imamoto N. Regulation and coordination of nuclear envelope and nuclear pore complex assembly. Nucleus. 2013 Mar;4(2):105–14. PMCID:PMC3621742.
23. Sampathkumar P, Kim SJ, Upla P, Rice WJ, Phillips J, Timney BL, Pieper U, Bonanno JB, Fernandez-Martinez J, Hakhverdyan Z, et al. Structure, dynamics, evolution, and function of a major scaffold component in the nuclear pore complex. Structure 2013 Apr 2;21(4):560–71.
24. Imamoto N. Cargo recognition explains nuclear transport regulation induced by nuclear pore complex reorganization. J. Mol. Biol. 2013 Jun 12;425(11):1849–51.
25. Rothballer A, Kutay U. Poring over pores: nuclear pore complex insertion into the nuclear envelope. Trends Biochem. Sci. 2013 Jun;38(6):292–301.
26. Yang W. Distinct, but not completely separate spatial transport routes in the nuclear pore complex. Nucleus. 2013 May 1;4(3).
27. Strasser C, Grote P, Schauble K, Ganz M, Ferrando-May E. Regulation of nuclear envelope permeability in cell death and survival. Nucleus. 2012 Nov;3(6):540–51. PMCID:PMC3515537.
28. Rothballer A, Kutay U. SnapShot: the nuclear envelope II. Cell 2012 Aug 31;150(5):1084.
29. Korfali N, Wilkie GS, Swanson SK, Srsen V, de Las HJ, Batrakou DG, Malik P, Zuleger N, Kerr AR, Florens L, et al. The nuclear envelope proteome differs notably between tissues. Nucleus. 2012 Nov;3(6):552–64. PMCID:PMC3515538.
30. Barcena C, Osorio FG, Freije JM. Detection of nuclear envelope alterations in senescence. Methods Mol. Biol. 2013;965:243–51.
31. Cai S, Zhai Z. Relation between nuclear envelope and nuclear lamina in nuclear assembly in vitro. Sci. China C Life Sci. 1997 Dec;40(6):576–82.
32. Goldberg MW, Fiserova J, Huttenlauch I, Stick R. A new model for nuclear lamina organization. Biochem. Soc. Trans. 2008 Dec;36(Pt 6):1339–43.
33. Righolt CH, Raz V, Vermolen BJ, Dirks RW, Tanke HJ, Young IT. Molecular image analysis: quantitative description and classification of the nuclear lamina in human mesenchymal stem cells. Int. J. Mol. Imaging 2011;2011:723283. PMCID:PMC3065845.
34. Shevelyov YY, Nurminsky DI. The nuclear lamina as a gene-silencing hub. Curr. Issues Mol. Biol. 2012;14(1):27–38.
35. Bank EM, Gruenbaum Y. The nuclear lamina and heterochromatin: a complex relationship. Biochem. Soc. Trans. 2011 Dec;39(6):1705–9.
36. Barbi M, Mozziconacci J, Wong H, Victor JM. DNA topology in chromosomes: a quantitative survey and its physiological implications. J. Math. Biol. 2012 Nov 20.
37. Baumann K. Chromosomes: getting the architecture right. Nat. Rev. Mol. Cell Biol. 2013 Jan;14(1):2–3.
38. Bickmore WA, van SB. Genome architecture: domain organization of interphase chromosomes. Cell 2013 Mar 14;152(6):1270–84.
39. Donev R. Preface: organization of chromosomes. Adv. Protein Chem. Struct. Biol. 2013;90:vii–viii.
40. Cmarko D, Smigova J, Minichova L, Popov A. Nucleolus: the ribosome factory. Histol. Histopathol. 2008 Oct;23(10):1291–8.

41. Boulon S, Westman BJ, Hutten S, Boisvert FM, Lamond AI. The nucleolus under stress. Mol. Cell 2010 Oct 22;40(2):216–27. PMCID:PMC2987465.
42. Pederson T. The nucleolus. Cold Spring Harb. Perspect. Biol. 2011 Mar;3(3). PMCID:PMC3039934.
43. Hernandez-Verdun D. Assembly and disassembly of the nucleolus during the cell cycle. Nucleus. 2011 May;2(3):189–94. PMCID:PMC3149879.
44. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The nucleolus: structure/function relationship in RNA metabolism. Wiley. Interdiscip. Rev. RNA. 2010 Nov;1(3):415–31.
45. Ruggero D. Revisiting the nucleolus: from marker to dynamic integrator of cancer signaling. Sci. Signal. 2012;5(241):e38.
46. Panse VG, Johnson AW. Maturation of eukaryotic ribosomes: acquisition of functionality. Trends Biochem. Sci. 2010 May;35(5):260–6. PMCID:PMC2866757.
47. Gilbert WV. Functional specialization of ribosomes? Trends Biochem. Sci. 2011 Mar;36(3):127–32. PMCID:PMC3056915.
48. Pfeffer S, Brandt F, Hrabe T, Lang S, Eibauer M, Zimmermann R, Forster F. Structure and 3D arrangement of endoplasmic reticulum membrane-associated ribosomes. Structure. 2012 Sep 5;20(9):1508–18.
49. Schutz S, Panse VG. Getting ready to commit: ribosomes rehearse translation. Nat. Struct. Mol. Biol. 2012 Sep;19(9):861–2.
50. Nawy T. Ribosomes, start your engines. Nat. Methods 2012 Aug;9(8):780.
51. Liljas A, Moore P. Ribosomes—structure and function. Curr. Opin. Struct. Biol. 2012 Dec;22(6):730–2.
52. Barna M. Ribosomes take control. Proc. Natl. Acad. Sci. U.S.A 2013 Jan 2; 110(1):9–10. PMCID:PMC3538271.
53. Garcia ML, Fernandez A, Solas MT. Mitochondria, motor neurons and aging. J. Neurol. Sci. 2013 Apr 26.
54. Vannuvel K, Renard P, Raes M, Arnould T. Functional and morphological impact of ER stress on mitochondria. J. Cell Physiol 2013 Sep;228(9):1802–18.
55. Hwang AB, Jeong DE, Lee SJ. Mitochondria and organismal longevity. Curr. Genomics 2012 Nov;13(7):519–32. PMCID:PMC3468885.
56. Nezich CL, Youle RJ. Make or break for mitochondria. Elife. 2013;2:e00804. PMCID:PMC3654434.
57. Desler C, Rasmussen LJ. Mitochondria In Biology and Medicine—2012. Mitochondrion. 2013 May 29.
58. Jerome WG. Lysosomes, cholesterol and atherosclerosis. Clin. Lipidol. 2010 Dec 1; 5(6):853–65. PMCID:PMC3105626.
59. Kurz T, Eaton JW, Brunk UT. The role of lysosomes in iron metabolism and recycling. Int. J. Biochem. Cell Biol. 2011 Dec;43(12):1686–97.
60. Pryor PR. Analyzing lysosomes in live cells. Methods Enzymol. 2012;505:145–57.
61. Hamer I, Van BG, Arnould T, Jadot M. Lipids and lysosomes. Curr. Drug Metab 2012 Dec;13(10):1371–87.
62. Oczypok EA, Oury TD, Chu CT. It's a cell-eat-cell world: autophagy and phagocytosis. Am. J. Pathol. 2013 Mar;182(3):612–22. PMCID:PMC3589073.

63. Garg M, Chandawarkar RY. Phagocytosis: history's lessons. Conn. Med. 2013 Jan;77(1):23–6.
64. Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. Cold Spring Harb. Perspect. Biol. 2013;5(5).
65. Spang A. Retrograde traffic from the Golgi to the endoplasmic reticulum. Cold Spring Harb. Perspect. Biol. 2013;5(6).
66. Moseley JB. An expanded view of the eukaryotic cytoskeleton. Mol. Biol. Cell 2013 Jun;24(11):1615–8. PMCID:PMC3667716.
67. Brini M, Cali T, Ottolini D, Carafoli E. The plasma membrane calcium pump in health and disease. FEBS J. 2013 Feb 18.
68. Ueda Y, Makino A, Murase-Tamada K, Sakai S, Inaba T, Hullin-Matsuda F, Kobayashi T. Sphingomyelin regulates the transbilayer movement of diacylglycerol in the plasma membrane of Madin-Darby canine kidney cells. FASEB J. 2013 May 16.
69. Babcock JJ, Li M. Inside job: ligand-receptor pharmacology beneath the plasma membrane. Sin: Acta Pharmacol; 2013 May 20.
70. Rilla K, Pasonen-Seppanen S, Deen AJ, Koistinen VV, Wojciechowski S, Oikari S, Karna R, Bart G, Torronen K, Tammi RH, et al. Hyaluronan production enhances shedding of plasma membrane-derived microvesicles. Exp. Cell Res. 2013 May 31.
71. Volpato FZ, Fuhrmann T, Migliaresi C, Hutmacher DW, Dalton PD. Using extracellular matrix for regenerative medicine in the spinal cord. Biomaterials 2013 Jul;34(21):4945–55.
72. Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective. Curr. Opin. Biotechnol. 2013 Apr 20.
73. Heinegard D. Extracellular matrix: pathobiology and signaling. Biol. Chem. 2013 Jun 1; 394(6):805–6.
74. Clause KC, Barker TH. Extracellular matrix signaling in morphogenesis and repair. Curr. Opin. Biotechnol. 2013 May 28.
75. Bennett JS. Integrin structure and function in hemostasis and thrombosis. Ann. N.Y. Acad. Sci. 1991;614:214–28.
76. Humphries MJ. Integrin structure. Biochem. Soc. Trans. 2000;28(4):311–39.
77. Shimaoka M, Takagi J, Springer TA. Conformational regulation of integrin structure and function. Annu. Rev. Biophys. Biomol. Struct. 2002;31:485–516.
78. Arnaout MA, Mahalingam B, Xiong JP. Integrin structure, allostery, and bidirectional signaling. Annu. Rev. Cell Dev. Biol 2005;21:381–410.
79. Arcangeli A, Becchetti A. Integrin structure and functional relation with ion channels. Adv. Exp. Med. Biol 2010;674:1–7.
80. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb. Perspect. Biol. 2011 Mar;3(3). PMCID:PMC3039929.
81. Ruoslahti E, Hayman EG, Engvall E, Cothran WC, Butler WT. Alignment of biologically active domains in the fibronectin molecule. J. Biol. Chem. 1981 Jul 25;256(14):7277–81.
82. Venyaminov SY, Metsis ML, Chernousov MA, Koteliansky VE. Distribution of secondary structure along the fibronectin molecule. Eur. J. Biochem. 1983 Oct 3;135(3):485–9.
83. Hunyady L. Cellular signaling in health and disease. Mol. Cell Endocrinol. 2012 Apr 28;353(1–2):1–2.

84. Walker C, Ahmed SA, Brown T, Ho SM, Hodges L, Lucier G, Russo J, Weigel N, Weise T, Vandenbergh J. Species, interindividual, and tissue specificity in endocrine signaling. Environ. Health Perspect. 1999 Aug;107 Suppl 4:619–24. PMCID:PMC1567505.

85. Gough NR. Focus issue: endocrine signaling from clinic to cell. Sci. Signal. 2010;3(143):eg9.

86. Dietze GJ, Taegtmeyer H. Introduction: autocrine and paracrine signaling between contracting myocardium and coronary endothelium during myocardial ischemia: effects of insulin resistance. Am. J. Cardiol. 1997 Aug 4;80(3A):1A-2A.

87. Wang N, De BM, Decrock E, Bol M, Gadicherla A, Vinken M, Rogiers V, Bukauskas FF, Bultynck G, Leybaert L. Paracrine signaling through plasma membrane hemichannels. Biochim. Biophys. Acta 2013 Jan;1828(1):35–50. PMCID:PMC3666170.

88. Mann DA, Oakley F. Serotonin paracrine signaling in tissue fibrosis. Biochim. Biophys. Acta 2013 Jul;1832(7):905–10.

89. Mureli S, Gans CP, Bare DJ, Geenen DL, Kumar NM, Banach K. Mesenchymal stem cells improve cardiac conduction by upregulation of connexin 43 through paracrine signaling. Am. J. Physiol Heart Circ. Physiol. 2013 Feb 15;304(4):H600-H609. PMCID:PMC3566487.

90. Wilson KJ, Mill C, Lambert S, Buchman J, Wilson TR, Hernandez-Gordillo V, Gallo RM, Ades LM, Settleman J, Riese DJ. EGFR ligands exhibit functional differences in models of paracrine and autocrine signaling. Growth Factors 2012 Apr;30(2):107–16.

91. Yi EH, Lee CS, Lee JK, Lee YJ, Shin MK, Cho CH, Kang KW, Lee JW, Han W, Noh DY, et al. STAT3-RANTES autocrine signaling is essential for tamoxifen resistance in human breast cancer cells. Mol. Cancer Res. 2013 Jan;11(1):31–42.

92. Leibiger B, Moede T, Muhandiramlage TP, Kaiser D, Vaca SP, Leibiger IB, Berggren PO. Glucagon regulates its own synthesis by autocrine signaling. Proc. Natl. Acad. Sci. U.S.A 2012 Dec 18;109(51):20925–30. PMCID:PMC3529083.

93. Datta S, Sarvetnick N. Lymphocyte proliferation in immune-mediated diseases. Trends Immunol 2009 Sep;30(9):430–8.

94. Zagon IS, Donahue RN, Bonneau RH, McLaughlin PJ. B lymphocyte proliferation is suppressed by the opioid growth factor-opioid growth factor receptor axis: Implication for the treatment of autoimmune diseases. Immunobiology 2011 Jan;216(1–2):173–83.

95. Luckerath K, Kirkin V, Melzer IM, Thalheimer FB, Siele D, Milani W, Adler T, Aguilar-Pimentel A, Horsch M, Michel G, et al. Immune modulation by Fas ligand reverse signaling: lymphocyte proliferation is attenuated by the intracellular Fas ligand domain. Blood 2011 Jan 13;117(2):519–29.

96. Abraham R, Verfaillie CM. Neural differentiation and support of neuroregeneration of non-neural adult stem cells. Prog. Brain Res. 2012;201:17–34.

97. Vilchez D, Boyer L, Lutz M, Merkwirth C, Morantte I, Tse C, Spencer B, Page L, Masliah E, Travis BW, et al. FOXO4 is necessary for neural differentiation of human embryonic stem cells. Aging Cell 2013 Mar 7;

98. Shen L. Tight junctions on the move: molecular mechanisms for epithelial barrier regulation. Ann. N.Y. Acad. Sci. 2012 Jul;1258:9–18.

99. Cording J, Berg J, Kading N, Bellmann C, Tscheik C, Westphal JK, Milatz S, Gunzel D, Wolburg H, Piontek J, et al. In tight junctions, claudins regulate the interactions between occludin, tricellulin and marvelD3, which, inversely, modulate claudin oligomerization. J. Cell Sci. 2013 Jan 15;126(Pt 2):554–64.
100. Sapra B, Jindal M, Tiwary AK. Tight junctions in skin: new perspectives. Ther. Deliv. 2012 Nov;3(11):1297–327.
101. Campbell M, Humphries P. The blood-retina barrier: tight junctions and barrier modulation. Adv. Exp. Med. Biol. 2012;763:70–84.
102. Franke WW. Discovering the molecular components of intercellular junctions–a historical view. Cold Spring Harb. Perspect. Biol. 2009 Sep;1(3):a003061. PMCID:PMC2773636.
103. Penn EJ, Hobson C, Rees DA, Magee AI. Structure and assembly of desmosome junctions: biosynthesis, processing, and transport of the major protein and glycoprotein components in cultured epithelial cells. J. Cell Biol. 1987 Jul;105(1):57–68. PMCID:PMC2114930.
104. Wheeler GN, Parker AE, Thomas CL, Ataliotis P, Poynter D, Arnemann J, Rutman AJ, Pidsley SC, Watt FM, Rees DA, et al. Desmosomal glycoprotein DGI, a component of intercellular desmosome junctions, is related to the cadherin family of cell adhesion molecules. Proc. Natl. Acad. Sci. U.S.A 1991 Jun 1;88(11):4796–800. PMCID:PMC51753.
105. Kleber AG. Gap junctions and conduction of cardiac excitation. Heart Rhythm. 2011 Dec;8(12):1981–4.
106. Saffitz JE, Kleber AG. Gap junctions, slow conduction, and ventricular tachycardia after myocardial infarction. J. Am. Coll. Cardiol. 2012 Sep 18; 60(12):1111–3.
107. Li MW, Mruk DD, Cheng CY. Gap junctions and blood-tissue barriers. Adv. Exp. Med. Biol. 2012;763:260–80.
108. Nielsen MS, Nygaard AL, Sorgen PL, Verma V, Delmar M, Holstein-Rathlou NH. Gap junctions. Compr. Physiol 2012 Jul 1;2(3):1981–2035.
109. Manz BN, Groves JT. Spatial organization and signal transduction at intercellular junctions. Nat. Rev. Mol. Cell Biol. 2010 May;11(5):342–52.
110. Niethammer D, Bader P, Handgretinger R, Klingebiel T. Stem Cell Transplantation. Klin. Padiatr. 2013 May;225(S 01):94–6.
111. Maqsood MI, Matin MM, Bahrami AR, Ghasroldasht MM. Immortality of cell lines: Challenges and advantages of establishment. Cell Biol.Int. 2013 May 30.
112. Amit M. Sources and derivation of human embryonic stem cells. Methods Mol. Biol. 2013;997:3–11.
113. Kaur J, Tilkins ML. Methods for culturing human embryonic stem cells on feeders. Methods Mol. Biol. 2013;997:93–113.
114. Cao Q, Whittemore SR. Cell transplantation: stem cells and precursor cells. Handb. Clin. Neurol. 2012;109:551–61.
115. Segers VF, Lee RT. Stem-cell therapy for cardiac disease. Nature 2008 Feb 21;451(7181):937–42.
116. Teng M, Geng Z, Huang L, Zhao X. Stem cell transplantation in cardiovascular disease: an update. J. Int. Med. Res. 2012;40(3):833–8.

117. Leor J, Gerecht S, Cohen S, Miller L, Holbova R, Ziskind A, Shachar M, Feinberg MS, Guetta E, Itskovitz-Eldor J. Human embryonic stem cell transplantation to repair the infarcted myocardium. Heart 2007 Oct;93(10):1278–84. PMCID:PMC2000918.
118. Terai S, Sakaida I, Yamamoto N, Omori K, Watanabe T, Ohata S, Katada T, Miyamoto K, Shinoda K, Nishina H, et al. An in vivo model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes. J. Biochem. 2003 Oct;134(4):551–8.
119. Choi KS, Shin JS, Lee JJ, Kim YS, Kim SB, Kim CW. In vitro trans-differentiation of rat mesenchymal cells into insulin-producing cells by rat pancreatic extract. Biochem. Biophys. Res. Commun. 2005 May 20;330(4):1299–305.
120. Hombach-Klonisch S, Panigrahi S, Rashedi I, Seifert A, Alberti E, Pocar P, Kurpisz M, Schulze-Osthoff K, Mackiewicz A, Los M. Adult stem cells and their trans-differentiation potential—perspectives and therapeutic applications. J. Mol. Med. (Berl) 2008 Dec;86(12):1301–14. PMCID:PMC2954191.
121. Laco F, Grant MH, Flint DJ, Black RA. Cellular trans-differentiation and morphogenesis toward the lymphatic lineage in regenerative medicine. Stem Cells Dev. 2011 Feb;20(2):181–95.
122. Joo KM, Jin J, Kang BG, Lee SJ, Kim KH, Yang H, Lee YA, Cho YJ, Im YS, Lee DS, et al. Trans-differentiation of neural stem cells: a therapeutic mechanism against the radiation induced brain damage. PLoS One 2012;7(2):e25936. PMCID:PMC3277599.
123. Li H, Zuo S, He Z, Yang Y, Pasha Z, Wang Y, Xu M. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. Am. J. Physiol Heart Circ. Physiol 2010 Dec;299(6):H1772-H1781. PMCID:PMC3006287.
124. Burdon TJ, Paul A, Noiseux N, Prakash S, Shum-Tim D. Bone marrow stem cell derived paracrine factors for regenerative medicine: current perspectives and therapeutic potential. Bone Marrow Res. 2011;2011:207326. PMCID:PMC3195349.
125. Xu S, Zhu J, Yu L, Fu G. Endothelial progenitor cells: current development of their paracrine factors in cardiovascular therapy. J. Cardiovasc. Pharmacol. 2012 Apr;59(4):387–96.
126. Bell GI, Meschino MT, Hughes-Large JM, Broughton HC, Xenocostas A, Hess DA. Combinatorial human progenitor cell transplantation optimizes islet regeneration through secretion of paracrine factors. Stem Cells Dev. 2012 Jul 20;21(11):1863–76.
127. de FP, Gonzalez M, Meloni G, De Propris MS, Bellucci R, Cordone I, Gozzer M, Leone G, Mandelli F. Monitoring of CD34+ cells during leukapheresis allows a single, successful collection of hemopoietic progenitors in patients with low numbers of circulating stem cells. Bone Marrow Transplant. 1999 Jun;23(12):1229–36.
128. Hillebrands JL, Klatter FA, Rozing J. Origin of vascular smooth muscle cells and the role of circulating stem cells in transplant arteriosclerosis. Arterioscler. Thromb. Vasc. Biol. 2003 Mar 1;23(3):380–7.
129. Hennessy B, Korbling M, Estrov Z. Circulating stem cells and tissue repair. Panminerva Med. 2004 Mar;46(1):1–11.
130. Seebach C, Henrich D, Wilhelm K, Barker JH, Marzi I. Endothelial progenitor cells improve directly and indirectly early vascularization of mesenchymal stem cell-driven bone regeneration in a critical bone defect in rats. Cell Transplant. 2012;21(8):1667–77.
131. Walker BA, Brown BB, Krohmer JS, Bonte FJ. Adaptation of disposable plastics to quantitative mammalian cell culture. Tex. Rep. Biol. Med 1962;20:686–92.

132. Perlman D. Value of mammalian cell culture as a biochemical tool. Science 1968 Apr 5; 160(3823):42–6.
133. Gospodarowicz D, Moran JS. Growth factors in mammalian cell culture. Annu. Rev. Biochem. 1976;45:531–58.
134. Oxender DL, Lee M, Cecchini G. Regulation of transport in mammalian cell culture. Prog. Clin. Biol. Res. 1976;9:41–7.
135. Tolbert WR, Schoenfeld RA, Lewis C, Feder J. Large-scale mammalian cell culture: Design and use of an economical batch suspension system. Biotechnol. Bioeng. 1982 Jul;24(7):1671–9.
136. Harakas NK, Lewis C, Bartram RD, Wildi BS, Feder J. Mammalian cell culture: technology and physiology. Adv. Exp. Med. Biol 1984;172:119–38.
137. Linhardt RJ. Mammalian cell culture. Patents and literature. Appl. Biochem. Biotechnol. 1986 Oct;13(2):167–74.
138. Nilsson K. Mammalian cell culture. Methods Enzymol. 1987;135:387–93.
139. Finter NB, Garland AJ, Telling RC. Large-scale mammalian cell culture: a perspective. Bioprocess. Technol. 1990;10:1–14.
140. McKeehan WL, Barnes D, Reid L, Stanbridge E, Murakami H, Sato GH. Frontiers in mammalian cell culture. In Vitro Cell Dev. Biol. 1990 Jan;26(1):9–23.
141. Hu WS, Piret JM. Mammalian cell culture processes. Curr. Opin. Biotechnol. 1992 Apr;3(2):110–4.
142. Reiter M, Bluml G. Large-scale mammalian cell culture. Curr. Opin. Biotechnol. 1994 Feb;5(2):175–9.
143. Hu WS, Aunins JG. Large-scale mammalian cell culture. Curr. Opin. Biotechnol. 1997 Apr;8(2):148–53.
144. Crick F. Central dogma of molecular biology. Nature 1970 Aug 8;227(5258):561–3.
145. Cooper S. The central dogma of cell biology. Cell Biol. Int. Rep. 1981 Jun;5(6):539–49.
146. Thieffry D, Sarkar S. Forty years under the central dogma. Trends Biochem. Sci. 1998 Aug;23(8):312–6.
147. Dvash T, Ben-Yosef D, Eiges R. Human embryonic stem cells as a powerful tool for studying human embryogenesis. Pediatr. Res. 2006 Aug;60(2):111–7.
148. Vaillancourt C, Lafond J. Human embryogenesis: overview. Methods Mol. Biol. 2009;550:3–7.
149. Yi H, Xue L, Guo MX, Ma J, Zeng Y, Wang W, Cai JY, Hu HM, Shu HB, Shi YB, et al. Gene expression atlas for human embryogenesis. FASEB J. 2010 Sep;24(9):3341–50. PMCID:PMC2923361.
150. Kim ND, Oberley TD, Yasukawa-Barnes J, Clifton KH. Stem cell characteristics of transplanted rat mammary clonogens. Exp. Cell Res. 2000 Oct 10;260(1):146–59.
151. Sottile V, Halleux C, Bassilana F, Keller H, Seuwen K. Stem cell characteristics of human trabecular bone-derived cells. Bone 2002 May;30(5):699–704.
152. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005 Nov;23(10):1549–59.
153. Pfeiffer MJ, Schalken JA. Stem cell characteristics in prostate cancer cell lines. Eur. Urol. 2010 Feb;57(2):246–54.

154. Jiao J, Milwid JM, Yarmush ML, Parekkadan B. A mesenchymal stem cell potency assay. Methods Mol. Biol. 2011;677:221–31.
155. Polejaeva I, Mitalipov S. Stem cell potency and the ability to contribute to chimeric organisms. Reproduction 2013 Mar;145(3):R81–R88.
156. Romli F, Alitheen NB, Hamid M, Ismail R, Abd Rahman NM. Current techniques in reprogramming cell potency. J. Cell Biochem. 2013 Jun;114(6):1230–7.
157. Ferreira LM, Mostajo-Radji MA. How induced pluripotent stem cells are redefining personalized medicine. Gene. 2013 May 10;520(1):1–6.
158. Scott CW, Peters MF, Dragan YP. Human induced pluripotent stem cells and their use in drug discovery for toxicity testing. Toxicol. Lett. 2013 May 10;219(1):49–58.
159. Garate Z, Davis BR, Quintana-Bustamante O, Segovia JC. New frontier in regenerative medicine: site-specific gene correction in patient-specific induced pluripotent stem cells. Hum. Gene Ther. 2013 May 15.
160. Takahashi K, Yamanaka S. Induced pluripotent stem cells in medicine and biology. Development 2013 Jun;140(12):2457–61.
161. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006 Aug 25;126(4):663–76.
162. Gonzalez MA, Bernad A. Characteristics of adult stem cells. Adv. Exp. Med. Biol. 2012;741:103–20.
163. Kang Y, Kim S, Khademhosseini A, Yang Y. Creation of bony microenvironment with CaP and cell-derived ECM to enhance human bone-marrow MSC behavior and delivery of BMP-2. Biomaterials 2011 Sep;32(26):6119–30. PMCID:PMC3130069.
164. Pelekanos RA, Li J, Gongora M, Chandrakanthan V, Scown J, Suhaimi N, Brooke G, Christensen ME, Doan T, Rice AM, et al. Comprehensive transcriptome and immunophenotype analysis of renal and cardiac MSC-like populations supports strong congruence with bone marrow MSC despite maintenance of distinct identities. Stem Cell Res. 2012 Jan;8(1):58–73.