

Chapter 2

Important Definitions and Concepts

2.1 Cell Biology Related Terms/Concepts

2.1.1 Cell

A cell is defined as a self-contained unit, capable of replicating itself given the proper nutrients and environment. In general, eukaryotic ('truly' nucleated) cells are enclosed by a lipid bilayer and contain the necessary genetic material, needed to direct the continued propagation of the cell (Fig. 2.1a).

Considering the cell density in a natural tissue as $1-3 \times 10^9$ cells/ml, an adult person would have approximately 10^{14} cells. A biological cell contains micro-molecules and macromolecules (macro = large). The most abundant and important macromolecule is protein, as discussed below. Small structures which are aggregates of macromolecules surrounded by membranes are called **organelles**. A number of organelles exist within cells and these organelles perform specialized functions. Further, the cells can be classified as **prokaryotes** (*pro-* = before; *-karyon-* = nucleus, i.e. primitively nucleated cell) and **eukaryotes** (*eu-* = true; *Karyon* = nucleus, i.e. truly nucleated cells). In cell biology, a eukaryote is described as a multicellular species with well-defined membrane-bound organelles and nuclei, while prokaryotes are defined as single-celled organisms with organelles not surrounded by specific membranes and DNA dispersed in cytosol without being enclosed in a well-defined nucleus.

In Fig. 2.1a, various cellular organelles in a eukaryotic cell are shown with more details of a cytoskeleton structure in Fig. 2.1b. As will be emphasized later in this book, cell-material interaction is facilitated by cytoskeleton reorganization. The cytoskeleton consists of three types of polymers: actin filaments, microtubules and a group of polymers, known collectively as intermediate filaments. Together, these polymers control the shape and mechanics of eukaryotic cells. All three are

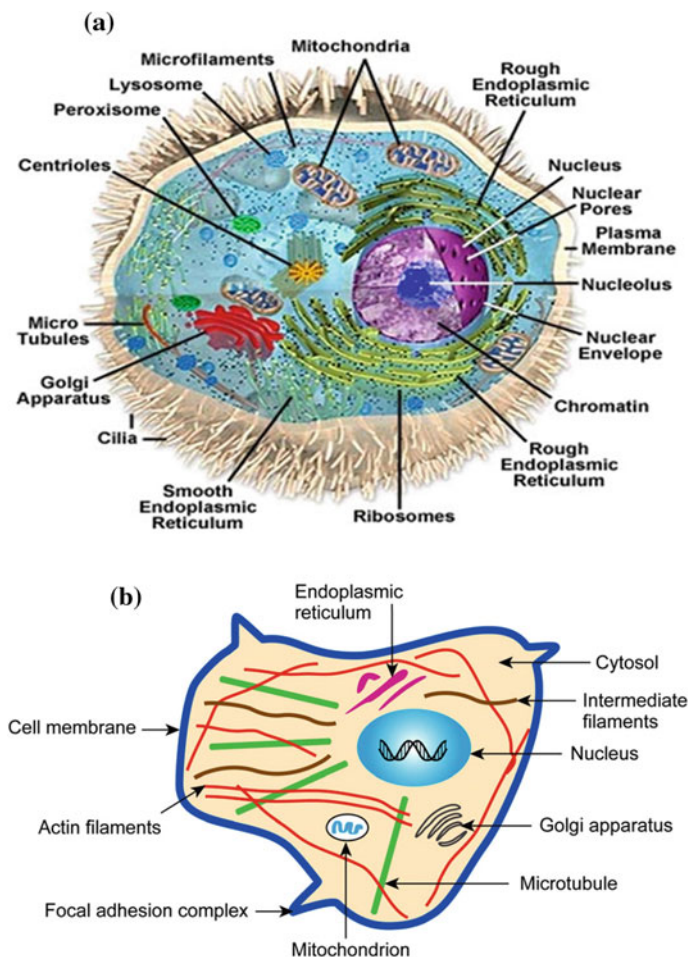


Fig. 2.1 An animal cell with different organelles stained with different fluorescent dyes (a) and cytoskeleton structure (b)

organized into networks that resist deformation but can reorganize in response to externally applied forces and stimuli [1]. All the cells have specific transmembrane proteins, which act like ‘tong’ to establish physico-chemical interaction with adsorbed proteins.

The identification of various cellular organelles under a microscope is based on the use of appropriate fluorescent dyes. For example, Alexa Fluor Phalloidin is used to stain actin filaments in the cytoskeleton. Another fluorescent dye, propidium iodide (PI) can intercalate with the nucleus of the cells, and, thereby, fluorescence the nucleus as a blue stained region under fluorescence microscope. Another dye,

microtracers, can be used to tag the mitochondria of cells. Similarly, a host of other dyes can be useful in other organelles. A fluorescence microscope, based on the principle of exciting fluorescently tagged molecules with a light ray (the excitation wavelength being shorter than the reflected wavelength), is widely used in cell biology research.

Apart from cellular organelles and the abundant presence of proteins, another important point to be noted is that a cell also contains two important nucleic acids, DNA and RNA. In molecular biology, the transcription process inside the nucleus involves the transfer of information from DNA to RNA and, thereafter, transfers of information from RNA to protein. The translation process takes place in the cytoplasm (outside the nucleus). Also, reverse transcription is defined as the transfer of information from RNA to DNA.

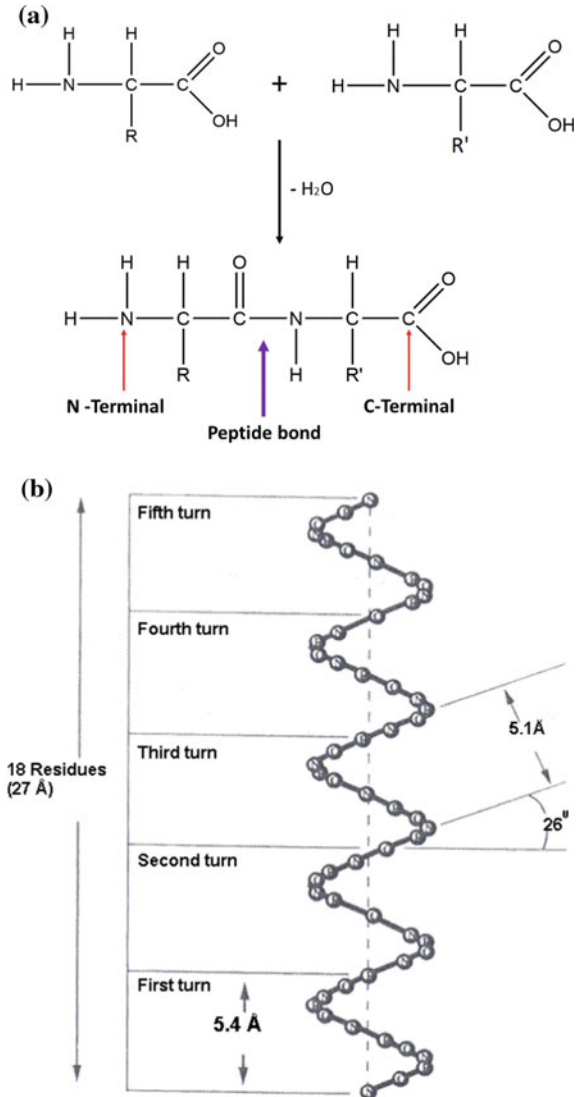
An important characteristic of the cell is that a cell can undergo various cell fate processes, i.e. cell division, cell differentiation, cell migration etc. and these are also discussed in this chapter. Another characteristic is that depending on the cellular microenvironment, a cell has unique adaptability characteristics, which can be **atrophy** (decrease in cell size), **hypertrophy** (increase in cell size), **hyperplasia** (increase in cell number), **metaplasia** (change in cell type) and **dysplasia** (disordered cellular growth or abnormal changes in cellular shape/size, resulting from chronic irritation or infection). Summarising, a eukaryotic cell is, in general, uniquely characterised by a set of properties, (a) transcription-translation, (b) cell adaptation, (c) cell fate and (d) compartmentalised cellular organelles with double-layered membranes.

2.1.2 Protein

Proteins are biological polymers built up from amino acid and monomers. Structurally, amino acids are characterized by a central carbon (the alpha carbon) atom bonded to four groups—a hydrogen atom (H), a Carboxyl group ($-\text{COOH}$), an Amino group ($-\text{NH}_2$) and a “variable” group or “R” group. The reaction of two amino acids to form a peptide bond is shown in (Fig. 2.2a). The “R” group varies among amino acids and the differences between the proteins are made in terms of amino acid sequences, which determine the information in the cellular genetic code. According to the accepted convention, the sequence is written always with an N-terminal at the left and a C-terminal at the right. An important example of a protein is collagen, which is the most abundant protein in the human body. A helical structure of a protein molecule is shown in Fig. 2.2b, which also shows the typical nanoscale dimension.

Structurally, a protein molecule is described by primary, secondary, tertiary and quaternary levels and a higher level of structure is characterised by more complexity. Also, a more complex structural characteristic at the higher level is often analysed using computational software.

Fig. 2.2 The peptide bond formation as a result of reaction between two amino acids (a) and typical helical structure of a protein molecule (b)



In a stable or lowest energy configuration, the protein molecules are in the folded state and if they are in a straightened configuration, it will be in the **‘denatured’** state. The protein, in the denatured state cannot express its desired function and, therefore, the **‘folded’** protein conformation is necessary after the proteins are adsorbed on a material substrate to mediate further with the cell surface receptors. Also, protein-protein interaction is mediated by weak H-bonds or Van Der Waals bonds.

2.1.3 Extracellular Matrix (ECM)

The biological space in a tissue outside the cytoplasm bounded by the cell membrane of a given cell is called an extracellular matrix, and this contains different types of collagen, elastic fibers, proteoglycans and hyaluronans, fibronectin, laminin and integrins (see Fig. 2.3). It is important to note that the ECM composition varies between different tissues. In particular, ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs), which are essentially a class of carbohydrate polymers and are linked to ECM proteins to form proteoglycans. The presence of hyaluronan in the ECM essentially enables the tissues with the required ability to resist compression and therefore is found in abundance in the ECM of load-bearing joints.

Among the fibrous proteins contained in the ECM, Collagens, by far is the most abundant protein as it accounts for 90 % of the bone matrix. In particular, collagens are present as fibrillar proteins to provide structural support to the cells. The collagens exist in different structural forms, like Fibrillar (type I, II, III, V, XI), short chain (type VIII, X), basement membrane (Type IV), etc. In contrast to collagens, the presence of elastins, which are synthesized by fibroblasts and smooth muscle cells, provide elasticity to tissues so that the tissues can stretch when needed, and recoil back to the original state in the absence of a mechanical stimulus. As far as

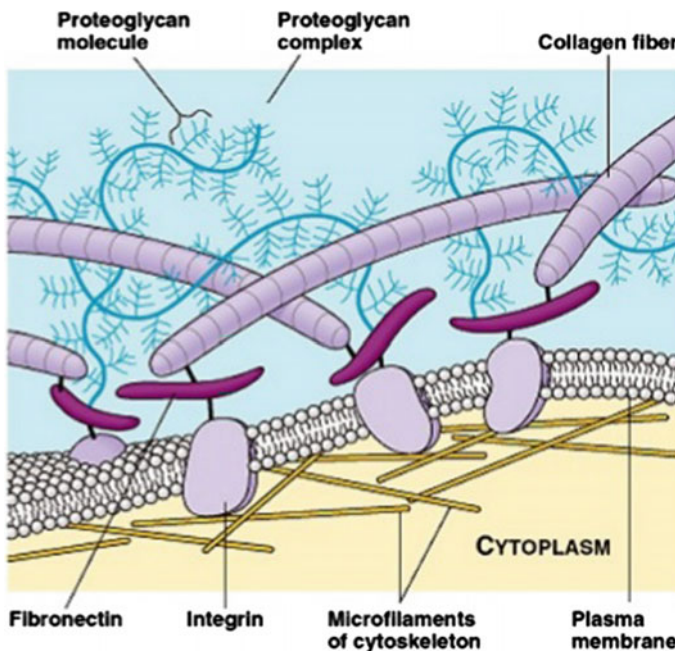


Fig. 2.3 Schematic of the cell membrane structure together with the structure of extracellular matrix (ECM)

other fibrous proteins are concerned, fibronectins are glycoproteins, which bridge cells with collagen fibers, and such a characteristic arrangement allows cells to move through the ECM.

The wide-ranging elastic property of the ECM can be attributed to the distribution of collagen and elastin concentrations. Also, the ECM provides structural and biochemical support to the surrounding biological cells. In particular, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM. The stiffness and elasticity of the ECM have important implications in various cellular processes, including cell migration, gene expression, and differentiation, cell proliferation and cell death (apoptosis).

2.1.4 Tissue

A tissue can be defined as a self-assembly of similar cells of identical origin with the ability to perform a specific function. Tissues can be classified into four basic types, connective, muscle, nervous, and epithelial. Among these four tissue types, the connective tissues are fibrous tissues which provide structural support to an organ and are made up of cells separated by the ECM. Spindle-shaped fibroblasts are contained in connective tissues. Typical examples of connective tissues are blood, bone, tendons, ligaments and adipose tissues. As shown in Fig. 2.4, muscle tissue contains muscle cells with an active contractile nature, and its typical functions are to produce force and to cause motion, (locomotion or movement). Among the different muscle tissues, cardiac muscle is found in the heart and it allows the heart to contract and pump blood throughout an organism. Neural tissue contains the characteristic neurons. In the central nervous system, neural tissues are contained in the brain and spinal cord, while in the peripheral nervous system, neural tissue forms the cranial nerves and the spinal nerves. The epithelial tissues contain closely-spaced epithelial cells, which cover organ surfaces. The presence of semi-permeable, tight junctions in epithelial tissue provides a barrier between the external environment and the underlying organ.

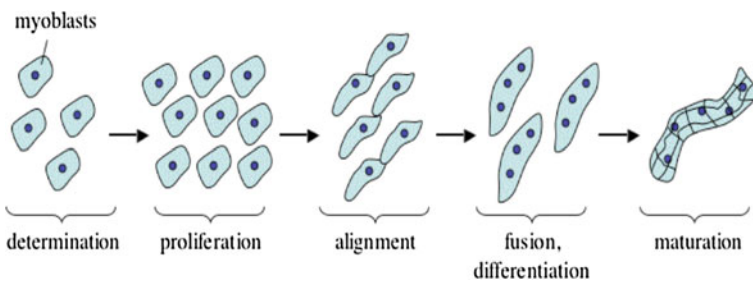


Fig. 2.4 Schematic illustration of the formation of myotube in muscle tissue, starting from the myoblast cells

According to another classification, the tissues can be grouped as soft and hard tissues and such a distinction is primarily based on the elastic stiffness or mechanical strength. As the name suggests, hard tissues are, by far, mechanically superior to soft tissues, which are essentially epithelial or neural tissues. Hard tissue, also known as mineralized tissue, contains hard minerals and soft collagenous matrices. Some examples include bone, tendons, cartilages, tooth enamel and dentin. Due to the presence of minerals (inorganic component) and collagenous proteins (organic component), mineralized tissues have an outstanding combination of stiffness, low weight, strength and toughness. Despite several decades of research to develop synthetic materials for biomedical applications, often via biomimicking approaches, researchers are yet to discover a synthetic material with matching mineralized tissue properties and, therefore, newer manufacturing techniques are constantly under development. Some of these approaches will be described in subsequent chapters.

2.1.5 Stem Cell

Stem cells are unspecialized cells that have the remarkable ability to differentiate into cells of specific function and can also divide to produce more stem cells. They are characterized by the following properties that make them fundamentally different from other cells.

- (a) Self-renewal—The ability of stem cells to divide, usually after long periods of time to produce more stem cells while maintaining their undifferentiated state.
- (b) Potency—The ability to differentiate into specialized cell types. Stem cells can be totipotent (can differentiate into any kind of cell of a total organism), pluripotent (derived from totipotent cells, can differentiate into all cell types), multipotent (can differentiate into a number of closely related cell types), oligopotent (can differentiate into only two cell types) and unipotent (can differentiate into a single cell type).
- (c) Clonality—This implies the state of a cell being derived along a specific lineage or from one source. In cell biology, a clone represents a group of identical cells sharing a common ancestry.

There are two major types of stem cells, (i) Embryonic stem cells (ESC) , which are obtained from the human embryo by the process of *in vitro*-fertilization. ESCs can differentiate into all the cell types. (ii) Adult/somatic stem cells, which are found in the differentiated cells of tissues or organs that can renew themselves and differentiate into major cell types. These stem cells are mainly involved in the repair and maintenance of tissues/organs. Based on the source, the stem cells can be classified into three types, (a) Hematopoietic stem cells (HSCs), that can differentiate into blood cells, (b) Mesenchymal stem cells (MSCs) , that are extracted from the bone marrow and can differentiate into multiple cell types, (c) Adipose stem cells

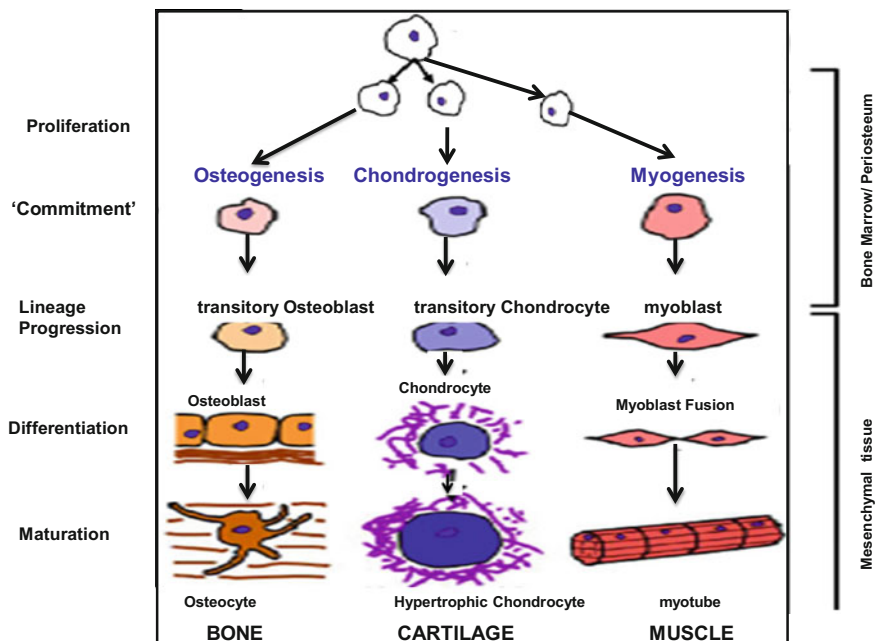


Fig. 2.5 The schematic illustration of differentiation of human mesenchymal stem cells along three different lineages to form bone cells cartilage cells and muscle cells

(ASCs)-Adipose derived stem cells extracted from fat cells. Figure 2.5 illustrates the differentiation of MSCs through three different lineages, i.e. osteogenic (bone cells), chondrogenic (cartilage cells) and myogenic (muscle cells).

2.1.6 Cell Adhesion and Cell-Cell Interaction

Cellular recognition on a biomaterial substrate becomes possible due to the interaction of adhesion receptors (integrins) with adhesion proteins. Such interactions between cell receptors and ligands adsorbed on the substrate lead to the formation of focal adhesion points.

While interacting with the adsorbed protein layer, the cells reorganize the cytoskeleton and nuclei over the surface. The physical and chemical properties of the implanted material decide the type, concentration and conformation of surface adsorbed proteins, which consequently control the adhesion and proliferation of cells. If cells coming in contact with the surface find the surface compatible, they adhere, and these adhered cells, in turn, send signals to other cells to adhere on the biomaterial surface.

The cytoskeleton plays a major role during cellular recognition on a biomaterial surface. It is important to mention that cell adhesion is an important cell fate

process. The interaction of cells with each other as well as with their substrate (extracellular matrix), is a primary feature of the architecture of many tissues. Cells interact through specialized multi-protein adhesive structures, also known as cell surface proteins (CAMs-cell-adhesion molecules). These proteins are commonly present on plasma-membranes, that contact other cells and the cytosol-facing domains of these proteins are usually connected to elements of the cytoskeleton. Despite the differences between these cell-surface proteins, their primary functions remain the same; that is to enable cellular communication and the transduction of mechanical signals.

Once a single cell adheres to a biomaterial substrate, it can communicate with other cells in the cellular microenvironment using various cell-signalling processes.

Through these signalling pathways, an adhered cell also establishes 'cross-talk' with neighbouring cells and such biophysical mechanisms have a significant influence on cell morphological changes as well as cell fate processes.

2.1.7 Cell Signalling

Cell signalling involves the way a group of cells communicates with each other in a co-ordinated manner in the neighbourhood of a biomaterial. In this context, cell signalling can be defined as part of a complex cellular communication network which will determine cell-fate processes in a co-ordinated manner.

In a cell culture medium, cells often receive multiple signals from the immediate microenvironment and then integrate the information they receive into a unified action plan.

Therefore, cell communication results in coordinated cellular activities and occurs in three principal ways such as the, (a) secretion of soluble signals, (b) secretion of insoluble signals and (c) direct cell-cell contact. Communication by extracellular signals usually involves six steps: (1) synthesis and (2) release of the signalling molecule by the signalling cell; (3) transport of the signal to the target cell; (4) detection of the signal by a specific receptor protein; (5) a change in cellular metabolism, function, or development triggered by the receptor-signal complex; and (6) removal of the signal, which often terminates the cellular response.

Some reference will be made to this aspect when cell-material interaction is discussed in one of the subsequent chapters of this book.

2.1.8 Growth Factors

Cell-signalling processes are facilitated by millions of soluble signalling molecules, which are essentially proteins, e.g. growth factors, defined as a wide variety of signalling molecules that control cell growth and differentiation. They are small proteins that are in the order of 15–20 kDa in size. The growth factors stimulating cell proliferation/differentiation are known as cytokines, while those assisting in cell migration are known as chemokines. The most widely used growth factors in biomaterials research include the transforming growth factor- β (TGF- β), insulin-like growth factor (IGF), fibroblast growth factor (FGF) etc. In cell biology, the growth factors are added to the culture medium to study cell differentiation or other cell fate processes.

2.1.9 Cell Differentiation

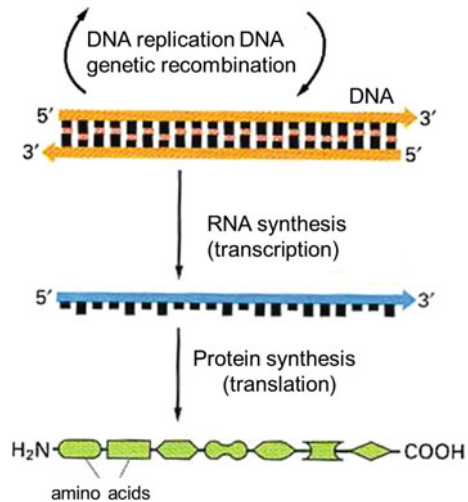
In cell biology, differentiation means ‘differential gene expression’. Therefore, cell differentiation can be formally defined as a cell fate process derived from differential gene expression in which a cell undergoes phenotypic changes to get specialized into another cell type, which performs the destined physiological function.

The process of cell differentiation begins with irreversible changes in a set of specific marker genes that are regulated significantly in a matured cell type, compared to its undifferentiated state.

The process involves the switching off and on of gene families in a co-ordinated manner. During cell differentiation, those genes are expressed, which are more specific to that of the mature cell type and are expressed w.r.t. the housekeeping genes. Gene expression needs to be quantified using molecular biology techniques, like the polymerase chain reaction (PCR). Simplistically, the PCR magnifies the messenger RNA (m-RNA) content extracted from a cultured cell to a level that gene expression changes can be quantified. Apart from m-RNA, two other types of RNA, i.e. ribosomal RNA and transfer RNA (t-RNA) are also present in cells. The process of the change of DNA to RNA (transcription) and RNA to protein (translation) is shown in Fig. 2.6. The reverse transcription of RNA to DNA is also possible.

In biology and biomedical engineering, stem cells have been widely researched to understand various aspects of cell differentiation as stem cells have the unique ability to differentiate to various cell lineages, i.e. to bone cells, neural cells, muscle cells etc. (see also Fig. 2.5).

Fig. 2.6 Schematic illustration of the process of transcription (DNA to RNA) and translation (RNA to protein)



2.1.10 Cell Migration

Cell migration or cell motility can be defined as a cell fate process that is characterised by the movement of a cell on a biomaterial substrate. It is similar to a baby crawling on the floor ('biomaterial substrate'). This process requires a cell to spread different 'podia' in a specific direction, and the stretching of filopodia or lamellipodia, which are analogous to the 'legs' or hands of a baby crawling at a slow speed, is a signature of cell migration. In reality, the speed of cell migration varies from a few tens to a few hundred microns per hour, which is much faster than a newborn baby crawling on the floor. For example, the speed of mouse fibroblasts or endothelial cells is around 30 $\mu\text{m}/\text{h}$, while that of rabbit neutrophils are at the higher side of 1200 $\mu\text{m}/\text{h}$ or 1.2 mm/h. Another parameter that needs to be defined in the context of cell migration is persistence time, which is the timescale over which a cell does not change its direction. The typical persistence time is minutes to hours, e.g. it is 1 h for mouse fibroblasts, while it is 4 min for rabbit neutrophils. The faster the persistence time, the more zigzag or random is the motion of a cell.

Biomechanically, cell migration involves the depolymerisation of actin filaments at the rear part, and repolymerisation at the protrusion part as the cell advances (see Fig. 2.7).

This means the more the actin filaments concentrate at the front, the more the cell will make focal adhesion complexes at the front end. This synchronised depolymerisation will make the focal adhesion points of the cell destabilise enabling a cell to lift-off from the substrate. Therefore, cytoskeletal reorganisation is

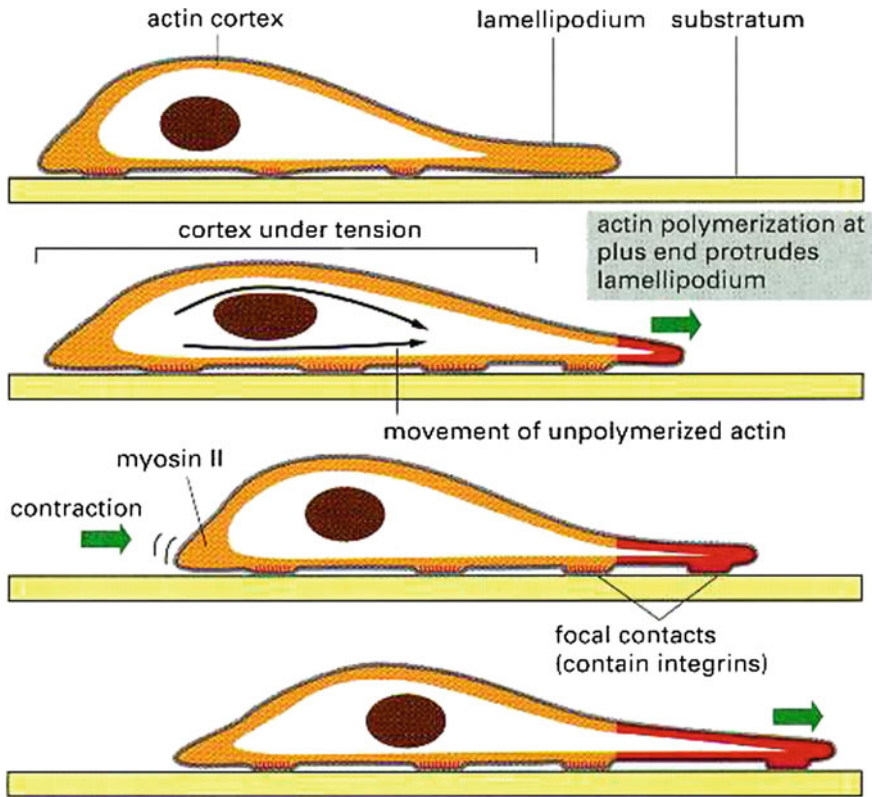


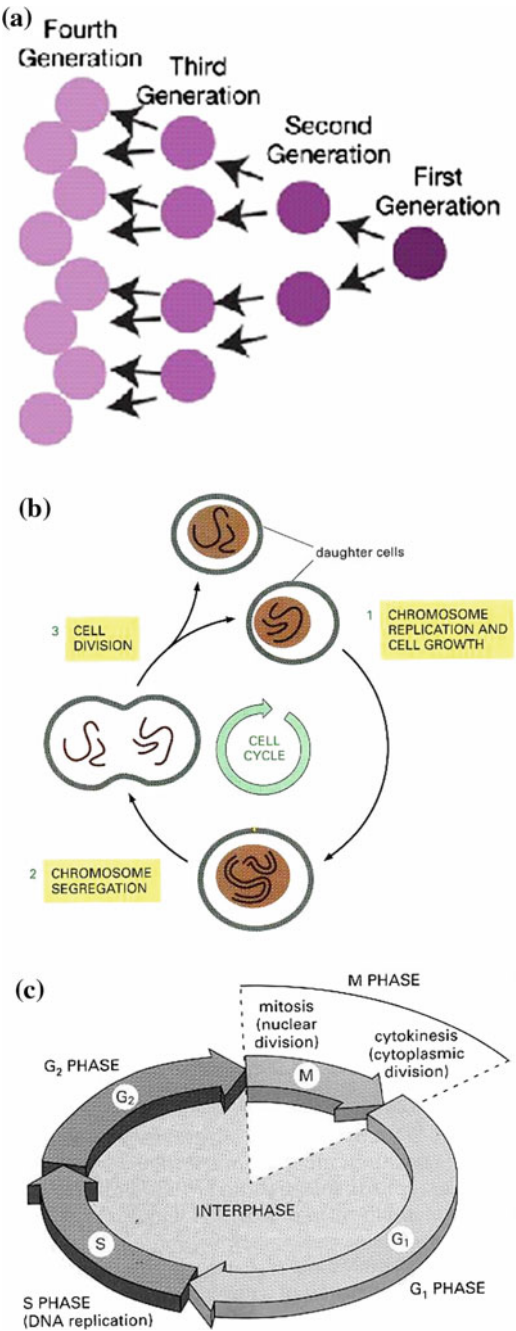
Fig. 2.7 Schematic illustration of cell migration on a biomaterial substrate

the key underlying mechanism for cell migration. This cell-fate process plays an important role in the physiological as well as the pathological process of tissues during organogenesis and embryonic development.

2.1.11 Cell Division

The process of eukaryotic cell division is perhaps the most distinguished feature in biological systems and leads to several generations of cells (see Fig. 2.8a). A eukaryotic cell which grows and divides is known to undergo a repeating series of events or four phases, called the cell cycle (see Fig. 2.8b). During the first phase (G_1), the cell grows and prepares for DNA replication, which occurs in the subsequent S phase. The cell can stay in the G_1 phase for a variable length of time, while the duration of the S phase is about 8 h. In the G_2 phase, a further growth of the cell takes place. A cell stays in this phase for about 2–3 h before entering the M

Fig. 2.8 **a** Schematic of cell division, and **b** biochemical processes involved and **c** different checkpoints that a cell has to cross before entering mitosis and cytokinesis phase



phase, where finally mitosis occurs. The duration of $S + G_2 + M$ is constant (about 12 h), while the time that a cell spends in the G_1 phase is highly variable. The cell growth process depends on the cell doubling time, which can be a few hours for human cells. For example, the cell doubling time for adult chondrocytes is 24–48 h, and that for dermal fibroblasts is 15 h.

One of the important concepts in cell division is the existence of checkpoints, a transition point between the phases of the cell cycle (see Fig. 2.8c).

Once a cell progresses from the G_1 to S or from the G_2 to the M phase, a cell has to satisfy a few criteria, like if protein synthesis leads to an increase in cell size or if the cell microenvironment contains favourable signals etc. If the required checkpoints are verified, then the cell is able to go to the next phase in an irreversible manner. Another concept is that if a cell is stuck in a given phase as all the required checkpoints are not satisfied, then the cell would activate its own suicidal mechanisms, so that it undergoes apoptosis or programmed cell death.

2.1.12 *Cell Apoptosis and Necrosis*

The loss of cell survival can be attributed to necrosis (physical tissue damage due to a sudden mechanical shock or an external electromagnetic field) or due to apoptosis (programmed cell death).

In cell biology, apoptosis (derived from a Greek word meaning ‘falling off leaves’) is a carefully regulated process of cell death that occurs as a normal part of development. In contrast, necrosis is defined as unprogrammed or accidental cell death, characterized by membrane disruption, cell swelling and rupture leading to inflammation.

In normal live cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment (see Fig. 2.9). Experimentally, a fluorescent dye, like Alexafluor 488-Annexin V can be tagged to PS molecules and this principle enables the detection of the fraction of apoptotic cells in a given cell population.

Apoptosis occurs as a part of the co-ordinated function of tissue morphogenesis. Cells undergoing apoptosis first shrink, condense and then fragment into **apoptotic bodies**. This form of cell death is highly regulated, and failure often results in tumor formation. As mentioned before, cellular apoptosis often takes place, when a cell is stuck at one of the checkpoints during cell division. Due to an unfavourable cellular

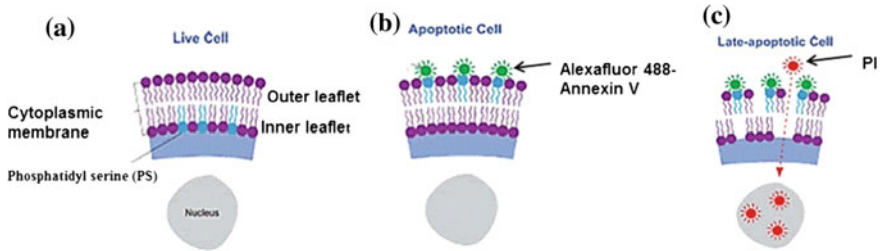


Fig. 2.9 Schematic showing the ‘flip-flop’ mechanism involved in apoptosis with PS molecule being translocated from inner leaflet to outer leaflet of cell membrane and getting it tagged with Alexafluor 488-Annexin V, a biomaterial to detect cell apoptosis (a, b). Another fluorescent dye, propidium iodide (PI) also used to intercalate with DNA of nucleus (c)

microenvironment, a cell is not able to move to the next stage in a cell cycle, and then the cell activates its own suicidal mechanism. Morphologically, cell apoptosis is characterised by (a) shrinkage in cell size, (b) membrane blebbing and (c) nuclear condensation and fragmentation. Overall, apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.

2.1.13 *Bacteria*

Bacteria are unicellular prokaryotic microorganisms or simple associations of similar cells. Typically, they are a few micrometers (0.5–2 μm) in length. Bacteria have a wide range of shapes, ranging from spherical to rods and spirals (see Fig. 2.10).

A bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold proteins, ribosomes and other essential components of the cytoplasm within the cell. As they are prokaryotes, bacteria do not have membrane-bound organelles in their cytoplasm and thus contain few intracellular structures. They, consequently, lack a well-defined nucleus, mitochondria, chloroplasts as well as other organelles such as the golgi apparatus and endoplasmic reticulum. Before the discovery of the DNA sequencing technique, bacteria were mainly classified on the basis of their shape, metabolism biochemistry and response to Gram staining (gram-positive and gram-negative).

The membrane structure of bacteria is characteristically different from that of a eukaryotic cell. The cell membrane in prokaryotes is usually made up of peptidoglycan, a network comprised of sugars and amino acids. Also, cell membranes have a polysaccharide capsule, which enables these unicellular microorganisms to adhere to their surface or to other individuals in a colony. The thickness of this wall varies depending on the type of prokaryotic cell. Gram-positive bacteria have simpler but

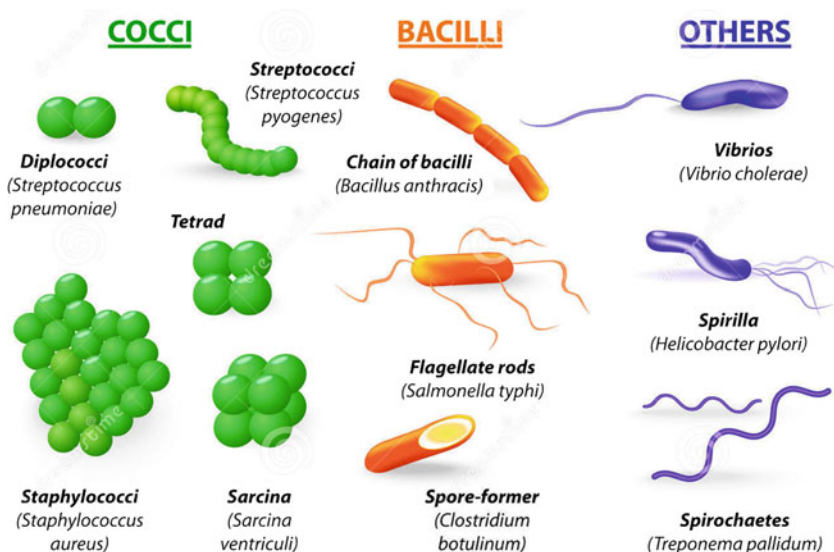


Fig. 2.10 Morphology of different bacteria

thick walls with a relatively large amount of peptidoglycan. Gram-negative bacteria have a thinner layer of peptidoglycan, which is located in a layer between the plasma membrane and an outer membrane.

The differences between prokaryotic and eukaryotic cells can be summarised now. A prokaryotic cell is a simple, single-celled organism, which lacks a complex level of organization, a nucleus and any other membrane-bound organelle (Fig. 2.4). In sharp contrast, eukaryotic cells contain well-defined intracellular membrane-bound organelles and a nucleus containing genetic material (DNA) (Fig. 2.1). Eukaryotes are much larger (25–30 μm) than prokaryotes (2–3 μm). Further, the doubling time of prokaryotes is around 20–60 min, while that of eukaryotes is around 12 h or longer. In a prokaryotic cell, the genetic material is a single circular DNA strand, while in a eukaryotic cell, a membrane-bound nucleus is present. Eukaryotic cells (10–100 μm in diameter) are typically 1–2 orders of length in size while prokaryotic cells are shorter (0.1–5.0 μm in diameter).

2.2 Biomaterials Science Related Terms/Concepts

2.2.1 Biomaterial

Any material, natural or synthetic, constituting a whole or part of a living structure or a biomedical device, which performs, enhances, or replaces a natural function without evoking any undesired toxic reactions to the surrounding tissues/bones (i.e.

non-toxic, non-immunogenic, non-thrombogenic, non-carcinogenic, non-irritant material) can be called as biomaterial. According to Williams,

A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine [2].

An alternative definition can be, “*Biomaterials* are those materials—be it natural or synthetic, alive or lifeless, and usually made of multiple components—that interact with biological systems”. Biomaterials are often used in medical applications to augment or replace a natural function [3]. Biomaterial is a term used to indicate materials that constitute parts of medical implants, extracorporeal devices, and disposables that have been utilized in medicine, surgery, dentistry, and veterinary medicine as well as in every aspect of patient health care.

The implications of all the above definitions can be summarised as: *Biomaterials* are materials of natural or manmade origin for interfacing with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body. When these materials display universally “good” or harmonious behaviour in contact with tissues and body functions and evoke a minimal biological response, they are considered to have good biocompatibility [4].

2.2.2 Biocompatibility

Ratner et al. in 1996 defined the term “*biocompatibility*” of a medical device in terms of the success of that device in fulfilling its intended function. According to a later definition, ‘Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy. All the definitions above are integrated to provide a more thoughtful definition proposed by William, “*Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy*” [5]. It is worthwhile to mention that targeted application specific *in vitro* and *in vivo* experiments are to be conducted for assessing the biocompatibility of any newly synthesized material. The common denominator in all the definitions that have been proposed for “*biomaterials*” is the undisputed recognition that biomaterials are distinct from other classes of materials because of the special *biocompatibility* criterion that these materials must satisfy. For example, a biomaterial can exhibit good biocompatibility with reference to bone replacement applications, but

the same material may not be biocompatible in cardiovascular applications. This tissue-specific biocompatibility is primarily because each cell type likes to adhere, proliferate/differentiate on a material with a specific substrate composition, elastic stiffness, surface energy and surface wettability characteristics.

2.2.3 Cytocompatibility

This is generally related to the behaviour of biomaterials in the context of cell culture *in vitro*. This term reflects the ongoing development of insights into how biomaterials interact with *in vitro* cultured cells, and, eventually, how these interactions determine the cellular fate processes (differentiation, proliferation, migration) of the cells. Cytocompatibility can also be defined as the “ability of biomaterials to be in contact with proliferating cells without producing an adverse effect *in vitro*”. It qualitatively describes how living cells are compatible with a synthetic (non-living) material substrate and is typically measured by various *in vitro* assays.

2.2.4 Cytotoxicity

Cytotoxicity is the quality of being toxic to cells. It is the degree to which an agent/compound/material has a specific toxic action (particularly with reference to lysis) on specific cell types. Cells exposed to these toxic materials can respond in a number of ways resulting into a variety of cellular fate processes. A number of assays involving colorimetric, fluorescence or luminescence detection techniques are widely utilised to measure cytotoxicity *in vitro*, or for toxicological studies. For example, the LDH leakage assays, are the most common assays, which are employed for the detection of cytotoxicity, or cell viability, following exposure to toxic substances, respectively.

2.2.5 Haemocompatibility

Haemocompatibility can be defined as an *in vitro* assessment of the compatibility of a synthetic material with blood and blood cells. Although blood-level compatibility is regularly evaluated for blood-contacting devices, like cardiovascular stents, pacemakers, cardiac patches, etc., it is also considered important in bone tissue engineering as every implant is expected to interact with blood upon implantation. An ideal haemocompatible material should not cause platelet adhesion and should be non-thrombogenic. It should not disturb the natural haemolytic balance between coagulation and fibrinolysis. It should be pro-healing and should not be pro-inflammatory.

2.2.6 *Host Response*

In a nutshell, **host response** is the reaction of a living system to the presence of a synthetic material *in vivo*. The formation of structural and biological bonds with the host tissue is a key aspect of the host response. It can be envisioned that upon biomaterial implantation/injury to the tissue concerned, a cascade of inflammatory and wound-healing responses are elicited. The host response or foreign body response is the reaction of a living system to the presence of a foreign material *in vivo* [6]. The implantation of any biomaterial elicits a local or systemic inflammatory response. This response is termed the host response. It occurs irrespective of the method of introduction of the biomaterial in the body (e.g. surgery or injection), as all biomaterials cause a disruption of the local host tissue environment.

2.3 Biocompatibility Assessment Related Terms

2.3.1 *in vitro*

In vitro is a Latin word which means, “test tube, culture dish or glass”. It is an artificial environment outside a living organism such as a test tube. *In vitro* assays, also called test-tube experiments, are a type of scientific experiment performed with cells or biological molecules in a physiologically simulated environment, but outside the normal biological context. It is used as a preliminary or first step towards biocompatibility assessment, but the results should not be extrapolated directly to predict biocompatibility clinically.

2.3.2 *in vivo*

This means experiments conducted inside living organisms to simulate a physiologically and functionally similar micro-environment around a biomaterial in relation to its targeted application. These experiments (pre-clinical) are, by far, more relevant to assess the biocompatibility of a biomaterial. Depending on the applications, specific animal models are used. For example, rabbit models are used for conducting a biocompatibility assessment of bone replacement materials, while sheep models are used for cardiovascular implants. It is important to mention that an institutional ethical committee’s approval must be required before conducting any *in vivo* experiments.

2.3.3 Assay

An **assay** is an investigative (analytic) procedure in laboratory medicine, pharmacology, environmental biology and molecular biology for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity (the analyte). The analyte can be a drug or biochemical substance or a cell in an organism or organic sample or a biomaterial. The assay usually aims to measure an intensive property of the analyte and expresses it in the relevant measurement unit (e.g. molarity, density, functional activity in enzyme international units, degree of some effect in comparison to a standard, etc.).

2.3.4 Bioassay

This is a type of scientific experiment, which involves the use of live animals or plants (*in vivo*) or tissues or cells (*in vitro*) to determine the biological compatibility of a drug or any non-living substance, like biomaterials or scaffolds. Bioassays are typically conducted to measure the quantitative/qualitative effects of a substance on a living organism, and are essential in the development of new drugs or biomaterials.

Bioassays are different from any standard material characterization technique, as the assays use specific biochemical reagents, which are added to a cell culture medium to assess their influence on cells growing on a synthetic/non-living material substrate. In every bioassay, some controls or references are used for standardization and the expressions are normalized w.r.t control sample. In many toxicity studies, both positive and negative controls are used. For example, H_2O_2 treatment is considered a positive control for dead cells, while a non-toxic substance is used to provide a basis for a lack of toxicity. In many cell or bacterial culture experiments, which are widely used for *in vitro* cytocompatibility assessment, only one control, i.e. a tissue culture polystyrene (TCPS) plate or glass slide is used, wherein it is expected that cells or bacteria would happily attach and grow. At each time point in the culture, the assay expression can be normalized w.r.t. the control unit to compare the results among different time points for statistical significance. In addition, one can use widely accepted biocompatible materials, like HA as another reference sample for comparing the results of any biological assay on other HA-based materials for comparison. The use of a control or reference is equally important in the case of *in vivo* studies. For example, in the case of an osseointegration study, a known biocompatible implant material of identical size/shape can be implanted into an experimental animal. For bone regeneration applications, researchers use HA or bioglass as a control implant.

2.3.5 Histology

Histology is the science of the microscopic anatomy of the tissues of plants and animals. It is commonly performed by examining cells and tissues which have been sectioned, stained and mounted on a microscope slide under a light microscope or electron microscope. Histological studies may be conducted using tissue culture, where live human or animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool to qualitatively and quantitatively assess the tissue compatibility of a biomaterial or a scaffold.

A related term i.e. histopathology, the microscopic study of diseased tissue, is an important tool in anatomical pathology, since an accurate diagnosis of cancer and other diseases usually requires a histopathological examination of the samples. In research on biomaterials, it is instructive to use ‘histology’ as an appropriate expression and not histopathology.

2.3.6 Staining

This is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), and organelles within individual cells. To some extent, staining is analogous to chemical etchants used in conventional metallography studies, wherein a chemical reagent is used to etch a polished material surface in order to image microstructural features. Biological staining is also used to distinguish cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis.

2.4 Bone Tissue Engineering Related Terms/Concepts

2.4.1 Scaffold

Typically, the term scaffold implies porous constructs with interconnected pores of typically 10–100 μm , that facilitate growth in tissue and reduce limitations due to the diffusion of nutrients and oxygen on account of its high porosity. While the morphology and porosity are of primary importance in scaffolds, mechanical properties are of secondary importance. Cells are often ‘seeded’ or cultivated into a

three dimensional synthetic porous structure to facilitate tissue formation *in vitro*. Scaffolds usually serve at least one or most of the following purposes, (a) allow cell attachment and migration, (b) deliver and retain cells and biochemical factors, (c) enable diffusion of vital cell nutrients and expressed products and (d) exert certain mechanical and biological influences to modify cell behaviour [7].

2.4.2 Implant

An implant is a general term used to describe any material that may be placed in direct contact with living tissues. The Food and Drug Administration (FDA, USA) defines medical implants as devices or tissues that are placed inside or on the surface of the body. Many implants are prosthetic, intended to replace missing body parts. Other implants deliver medication, monitor body functions, or provide support to organs and tissues. It is important to strike a distinction between an implant and a scaffold, as both these terms are interchangeably used more often in the biomaterials community.

While scaffolds, too, may be considered implants, all implants are not scaffolds. An implant essentially conveys a foreign body that is not essentially porous, and whose main function lies in providing mechanical support to the osseous structure, while exhibiting good osseointegration properties. For instance, the total hip prosthesis is an implant and not a scaffold. Hence, load-bearing properties, like strength, elastic modulus, fracture toughness and fatigue resistance of implants together with acceptable biocompatibility are of prime consideration from a materials perspective.

Conceptually, a porous scaffold and a nonporous implant would have different levels of interaction with the host tissues. For example, a porous scaffold will promote tissue in-growth and tissue regeneration, while an implant is expected to augment lost tissue function.

2.4.3 Tissue Engineering

Tissue engineering can be conceptualized as the means of orchestrating cells, engineering materials and suitable biological factors to enable relevant biological functions. In a nutshell, *“Tissue engineering is the creation of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals”* [6].

Conceptually, there are three general components to tissue engineering systems: (1) isolated cells from the tissue matrix, (2) biomaterial scaffolds that function as carriers to promote cell activity and tissue production, and (3) bioactive factors that regulate and induce cellular behavior in a controlled manner.

2.4.4 Vascularization

Vascularization is a medical term used to describe the presence of vessels in a tissue. In animals, this generally denotes the presence of blood vessels. Vascularization is one of the key challenges in tissue engineering. Below a critical pore size, mass and oxygen transport in scaffolds is severely limited by diffusion, leading to the formation of a necrotic core in scaffolds that consists of dead cells. The pore architecture of a scaffold therefore determines and facilitates vascularisation *in vivo*.

2.4.5 Angiogenesis

Angiogenesis or neoangiogenesis is a term used to describe the formation of new blood vessels. Neoangiogenesis is common in tumours or to a lesser extent in the tissues surrounding implants. While efforts have been directed at reducing angiogenesis in tumours, more angiogenesis around an implant is a positive feature, suggesting better acceptance of the implant. Here again, the pore architecture significantly influences the angiogenic property of a scaffold, when implanted *in vivo*. The terms vascularization and angiogenesis are used interchangeably in the field of tissue engineering.

2.4.6 Osteointegration/Osseointegration

Osseointegration is defined as a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant. This property is critical for implant stability, and is considered a prerequisite for implant loading and the long-term clinical success of end-osseous dental implants [8].

Osseointegration is also defined as a time-dependent healing process, whereby a clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading. Osseointegration may be divided into three stages, (a) incorporation of woven bone, (b) adaptation of bone mass to load (lamellar and parallel-fibered bone deposition) and (c) adaptation of bone structure to load (bone remodeling) [8]. Osteointegration may be qualitatively assessed by a clinical mobility test or from radiographs and is qualitatively determined by the use of a micro-computed tomography (micro-CT) analysis of explanted bone (e.g. bone volume/total volume ratio).

2.4.7 Osteoinduction

This is the process that induces *osteogenesis*, which is qualitatively described as bone cell functionality in the surrounding region of an implant, similar to its activity in the host bone structure. It is a phenomenon regularly seen in any type of bone healing process. Osteoinduction indicates the recruitment of immature cells and their simultaneous stimulation into preosteoblasts. Bone healing processes, as in fractures, are primarily dependent on osteoinduction [9].

2.4.8 Osteoconduction

This is a phenomenon regularly encountered in bone implants and this signifies bone growth on a biomaterial surface. Implant materials of low biocompatibility such as copper, silver and bone cement show little or no osteoconduction [9]. The assessment of osteoconduction, osteoinduction and osteointegration, therefore, require an extensive qualitative and quantitative understanding of *in vivo* biocompatibility. Therefore, a combination of X-ray radiography, histology, including other microscopic analyse (SEM, TEM), micro-CT analysis is required to develop such understanding.

2.5 Antimicrobial Property Related Terms/Concepts

2.5.1 Bacteriostatic

A bacteriostatic agent is a chemical or biological agent that inhibits bacterial multiplication, while not necessarily killing them. A characteristic feature of the bacteriostatic effect is the absence of turbidity or visible growth of the broth culture. The logarithmic phase of the bacterial growth curve is absent under the effect of a bacteriostatic agent and such dosage is referred to as the minimum inhibitory concentration (MIC).

2.5.2 Bactericidal

A bactericidal agent kills bacteria or destroys the bacterial cell structure, often leading to their non-culturability on nutrient agar. It is characterized by the inability of bacteria to form colonies on solid agar media. A bactericidal agent is essentially

bacteriostatic, while a bacteriostatic agent need not result in a bactericidal effect. Often, at a lower dosage of antimicrobials, a bacteriostatic effect is observed, while a higher dosage can elicit a bactericidal response.

2.5.3 Colony Forming Unit (CFU)

In traditional microbiology, the viability of a bacterium or fungus is evaluated in terms of the ability of the bacterium to form a colony on nutrient enriched solid agar media. The fundamental assumption for characterizing bacterial viability is that a single viable bacterium can multiply and form a single colony on agar media. The number of viable bacteria in a culture is enumerated by serial dilutions of the culture, followed by plating of the cultures using the spread plate method.

Number of colony forming units = $\text{Number of colonies counted} / (\text{Volume of dilution plated} \times \text{dilution factor})$

2.5.4 Biofilm

A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of, primarily, polysaccharide material [10]. The extracellular polysaccharide (EPS) envelope permits the exchange of gases and nutrients, while preventing the entry of drug molecules into the biofilm. Further, the lower metabolic activity of cells within the biofilm due to a limited nutrient supply, in comparison with their planktonic counterparts, confers resistance against antibiotics. A large number of variables influence bacterial adhesion and biofilm formation on material surfaces and these include,

- (a) Environment—temperature, pH, nutrient supply, presence of antibiotics, etc.
- (b) Bacterial parameters—cell hydrophilicity/hydrophobicity and cellular appendages such as pili/fimbriae
- (c) Material parameters—surface roughness/topography, wettability and charge
- (d) Serum proteins—albumin inhibits bacterial adhesion, while fibrinogen encourages bacterial attachment
- (e) Competitive adhesion—Fibronectin (Fn) has integrin receptor binding ligands which are also recognized by Fn-binding sites on *Staphylococcus aureus* cell surfaces.

2.5.5 Biomaterial Associated Infection (BAI)

Biomaterial-associated infection occurs on both permanent implants and temporary devices due to the bacterial colonization of the material surface as a result of pre-/peri-/post-operative surgical procedures. The infection can arise by the entry of microorganisms into the wound site (pre-operative contamination), attachment of bacteria onto the implants during surgery (peri-operative contamination) or during hospitalization before wound closure (post-operative contamination). Implant failure due to BAI necessitates implant removal and revision surgery. Often, secondary infections arise following revision surgery due to the incomplete removal of bacteria from the primary implant infected site. In order to prevent secondary infection, antimicrobial/drug releasing implants are used in revision surgery [11].

2.5.6 Antibiotic

Antibiotics are natural, semi-synthetic or synthetically-derived molecules that prevent the growth of micro-organisms or even lead to the killing of bacterial cells. Based on their origin, the different classes of antibiotics include natural (Pencillin, the first-discovered antibiotic derived from the fungus *Penicillium notatum* and other *Penicillium* sps.), semi-synthetic (Penicillin derivatives, aminoglycosides, tetracyclines, etc.) and synthetic (Trimethoprim and fluoroquinolones) [12].

References

1. Fletcher, D.A., Mullins, R.D.: Cell mechanics and the cytoskeleton. *Nature* **463**, 485–492 (2010)
2. Williams, D.F.: On the nature of biomaterials. *Biomaterials* **30**(30), 5897–5909 (2009)
3. <http://www.nature.com/subjects/biomaterials>
4. Black, J.: Biological performance of materials: fundamentals of biocompatibility, 3rd edn. CRC Press (1999)
5. Williams, D.F.: On the mechanisms of biocompatibility. *Biomaterials* **29**(20), 2941–2953 (2008)
6. Morais, J.M., Papadimitrakopoulos, F., Burgess, D.J.: Biomaterials/tissue interactions: possible solutions to overcome foreign body response. *AAPS J.* **12**(2), 188–196 (2010)
7. Mitra, J., Tripathi, G., Sharma, A., Basu, B.: Scaffolds for bone tissue engineering: role of surface patterning on osteoblast response. *RSC Adv.* **3**(28), 11073–11094 (2013)
8. Parithimarkalaignan, S., Padmanabhan, T.V.: Osseointegration: an update. *J. Indian Prosthodontic Soc.* **13**(1), 2–6 (2013)
9. Albrektsson, T., Johansson, C.: Osteoinduction, osteoconduction and osseointegration. *Eur. Spine J.* **10**(2), S96–S101 (2001)

10. Donlan, R.M.: Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* **8**(9), 881–890 (2002)
11. Busscher, H.J., van der Mei, H.C., Subbiahdoss, G., Jutte, P.C., van den Dungen, J.J., Zaat, S. A., Schultz, M.J., Grainger, D.W.: *Sci. Transl. Med.* **4**(153r), v110 (2012)
12. Radecka, I., Martin, C., Hill, D.: *Novel Antimicrobial Agents and Strategies*. Wiley-VCH Verlag GmbH & Co. KGaA, Editon edn., pp. 1–16

Further Readings

All the figures are adapted from similar figures, which appear in the following books:

1. Alberts, Johnson, Lewis, Raff, Rberts and Walter; *Molecular Biology of the Cel*, Fourth Edition, Garland Science, Taylore & Francis Group, New York, 2002
2. Ratner, Hoffman, Schoen and Lemons, *Biomaterials Science: An Introduction to Materials in Medicine*, Elsevier Academic Press, 2004

Biomaterials for Musculoskeletal Regeneration

Concepts

Basu, B.

2017, XXXIII, 420 p. 172 illus., 109 illus. in color.,

Hardcover

ISBN: 978-981-10-3058-1