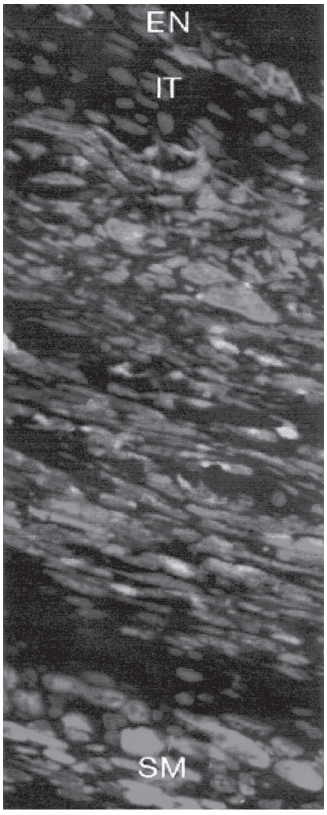
14

CARDIAC REGENERATIVE ENGINEERING



Transformation of bone marrow cells to infarcted heart. Myocardial infarction was induced and EGFP-Lin-c-*kit*^{POS} bone marrow cells were injected into the infarcted heart. Infarcted tissue (IT) can be seen in the subendocardium; spared myocytes (SM) can be seen in the subepicardium. Original magnification, ×250. EN: endocardium. Red: cardiac myosin. Green: EGFP-positive cells. (Reprinted by permission from Macmillan Publishers Ltd.: Orlic D et al: Bone marrow cells regenerate infarcted myocardium, *Nature* 410:701–5, copyright 2001.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

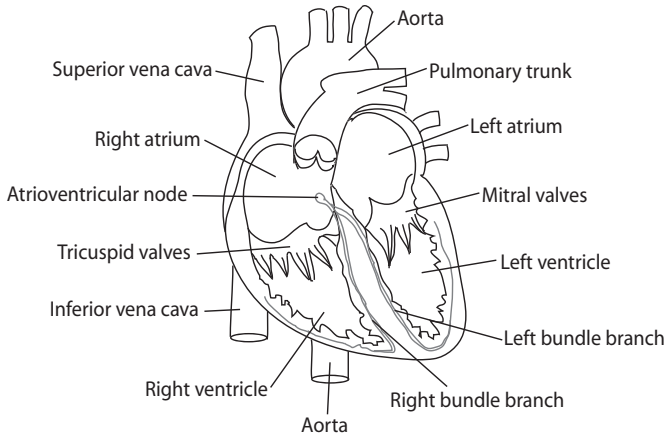


Figure 14.1. Schematic representation of cardiac structure. Based on bibliography 14.1.

ANATOMY AND PHYSIOLOGY OF THE HEART [14.1]

Cardiac Structure

The heart is a muscular organ that provides driving forces for blood circulation. The heart is located within the thoracic cavity, together with the left and right lungs, and is composed of the left atrium and ventricle, the right atrium and ventricle, and the pericardium (Fig. 14.1). The left atrium is a muscular chamber that receives oxygenated blood from the lung via the pulmonary veins and conducts blood to the left ventricle. The left ventricle pumps oxygenated blood to the aorta, the largest artery in the body. Oxygenated blood is delivered to the peripheral tissues and organs via various generations of arteries and capillaries. After oxygen is released and used by peripheral cells, deoxygenated blood is converged into various generations of veins and returned to the right atrium. The right atrium conducts blood to the right ventricle, which pumps deoxygenated blood to the lung via the pulmonary arteries for oxygenation. The pericardium is a double-layered thin sac that encloses the heart. The external layer of the pericardium is composed of tough connective tissue, known as the *fibrous pericardium*, whereas the internal layer consists of epithelial cells, known as the *serous pericardium*.

The structure and geometry differ considerably among the four atrial and ventricular chambers. In a human adult, the left ventricle consists of a chamber about 125 mL in volume and a muscular wall about 1 cm in thickness, which is the highest wall thickness in the heart because the left ventricle pumps blood against the highest blood pressure in the vascular system (80–120 mm Hg under physiological conditions). The right ventricle is about the same size in volume as the left ventricle and its wall is about half of the thickness of the left ventricular wall. The thinner wall of the right ventricle is attributed to the lower work load (pulmonary arterial blood pressure ~20–30 mm Hg) compared to the left ventricle. The left and right atria are smaller chambers compared with the ventricles, and their walls are about half the thickness of the right ventricular wall. The atria encounter lower blood pressure (about 0–5 mm Hg) compared to the ventricles.

The cardiac chambers and large blood vessels, including the aorta and pulmonary artery, are separated by four sets of fibrous valves: the mitral, tricuspid, aortic, and

pulmonary valves. The mitral valves divide the left atrium and the left ventricle. They are open during the diastole, allowing blood entering the left ventricle. They are closed during the systole, preventing blood from regurgitation into the left atrium. The tricuspid valves separate the right atrium from the right ventricle, and have function similar to that of the mitral valves. Both mitral and tricuspid valves are referred to as *atrioventricular valves*. The aortic valves separate the left ventricle from the ascending aorta. These valves are open during systole, allowing blood to enter the aorta. They are closed during the diastole, preventing blood from flowing back to the left ventricle. The pulmonary valves separate the right ventricle from the pulmonary arterial trunk, and have function similar to that of the aortic valves. The aortic and pulmonary valves are referred to as *arterial valves*. The driving forces for the opening and closure of the cardiac valves are blood pressure gradients across the valves.

The heart is composed of a blood circulatory system, known as the *coronary circulation*. This system consists of coronary arteries, capillaries, and veins. Two major coronary arteries, the left and right coronary arteries, stem from the ascending aorta and supply blood to the left and right heart, respectively. The left coronary artery is divided into two major branches: the anterior descending artery and the left circumflex artery. The anterior descending artery supplies blood to the anterior wall of the left heart. The left circumflex artery supplies blood to the lateral and posterior wall of the left heart. The right coronary artery is divided into two major branches: the posterior descending artery and the right circumflex artery, which supply blood to the posterior and lateral wall of the right heart, respectively.

The heart consists of a rich capillary network to accommodate its high oxygen consumption activities. Capillaries are distributed within the cardiac muscular bundles. Deoxygenated blood is drained to the coronary venous system. Blood from the left heart is drained to a coronary vein known as the *great cardiac vein*, whereas blood from the right heart is drained to the *small cardiac vein*. These veins converge to the largest coronary vein, known as the coronary sinus, which empties blood into the right atrium.

Cardiac Cells

The heart is composed of a number of cell types, including cardiomyocytes, conducting cells, fibroblasts, epithelial cells, and vascular cells. Cardiomyocytes are elongated, multinucleated, striated, and involuntary contractile muscle cells. These cells consist of actin–myosin filament-containing sarcomeres, the fundamental units of cardiac contraction. Actin and myosin filaments are contractile structures, and their interactions induce sliding motions between the two types of filament, resulting in the contraction of the cardiomyocytes. The ordered arrangement of actin and myosin filaments gives the cardiomyocytes a striated pattern. Other subcellular structures of the cardiomyocytes are similar to those found in other cell types. Cardiomyocytes are organized into muscular bundles, which are further arranged with optimal alignments for the generation of contractile forces.

Cardiac conducting cells are specially differentiated cardiac muscle cells and are found in three structures: the sinoatrial (SA) node, the atrioventricular (AV) node, and the conducting bundles. The sinoatrial node is located at the right atrium near the root of the superior vena cava. The atrioventricular node is located at the right atrium near the intercept of the atrial and ventricular septa. The conducting bundle, known as the

atrioventricular bundle, relays action potentials from the atrium to the ventricle. This bundle is divided into the left and right bundle branches. The left bundle branch is further divided into various generations of branches, which innervate the left ventricle. Similarly, the branches of the right bundle branch innervate the right ventricle. The terminal branches of the bundle branches are called *Purkinje fibers*, which conduct action potentials to the ventricular muscle cells. The conducting cells in the sinoatrial node, atrioventricular node, and conducting branches undergo automatic depolarization and initiate periodic action potentials, which induce cyclic cardiac contraction and relaxation. The conducting cells in these structures emit cyclic action potentials at different frequencies with the highest frequency found in the sinoatrial node (70–80 per minute). Thus, the action potentials from the sinoatrial node override those from other conducting structures and control the frequency of cardiac muscle contraction and heartbeat.

The heart also consists of fibroblasts. These cells are found in the connective tissue, which resides in extramuscular space, and are capable of producing and secreting extracellular matrix components, including collagen, elastin, and proteoglycans. Other cell types found in the heart include epithelial cells and vascular cells. Epithelial cells are the cells that line the internal and external surfaces of the atrial and ventricular wall and the surface of the valves. Vascular cells include endothelial cells and smooth muscle cells, which are found in the coronary blood vessels. Endothelial cells line the internal surface of blood vessels, including arteries, capillaries, and veins. Smooth muscle cells are found in the media of the arteries and veins. The structure and function of vascular cells are discussed further on page 660.

Cardiac Performance and Cycle

The heart conducts cyclic contractile activities and pumps blood into the arterial system. The cyclic activities include two mechanical events: contraction and relaxation (Fig. 14.2). Contraction occurs in a phase called *systole*, which lasts about 0.3–0.4 s in healthy humans, and relaxation occurs in another phase called *diastole*, which lasts about 0.4–0.5 s. Systole is defined as the period from the beginning to the end of ventricular contraction, and the diastole is from the beginning to the end of ventricular relaxation. Systole and diastole constitute a cardiac cycle. A cardiac cycle is defined from the beginning of ventricular contraction to the ending of ventricular relaxation or the beginning of next ventricular contraction. Since action potential waves recorded by electrocardiography are consistent with cardiac mechanical events, these waves are usually used to identify cardiac cycles. The QRS wave complex, which reflects the spreading of action potentials within the ventricles and initiates ventricular contraction, is used to indicate the beginning of the systole and the ending of the diastole, although the beginning of the QRS wave complex is slightly earlier than the beginning of ventricular contraction.

A sequence of mechanical events occurs during the systole. When the ventricular muscle cells are excited by action potentials from the sinoatrial node, the ventricular muscle cells start contraction almost at the same time (owning to the fast-conducting activity of the Purkinje fibers). The contraction of the left ventricle rapidly increases ventricular blood pressure from about 5 to 120 mm Hg. The increased ventricular blood pressure rapidly closes the mitral valves due to the pressure gradient across the valves. There is a short period when the mitral valves are closed, but the aortic valves are not yet open. This period is known as the period of isometric contraction, since blood is not flowing from or to the ventricle. A continuous increase in the ventricular blood pressure results

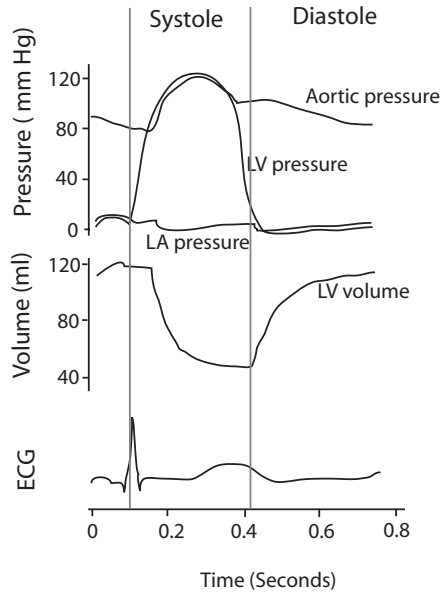


Figure 14.2. Electrical and mechanical events during a cardiac cycle. Based on bibliography 14.1.

in a pressure gradient across the aortic valves, leading to the opening of these valves and blood ejection from the left ventricle to the aorta. A similar sequence of mechanical events occurs in the right ventricle, except that blood pressure in the right ventricle is lower than that in the left ventricle. At the same time, the left and right atria are in the relaxing mode. The two atria collect blood from the pulmonary veins and vena cava, respectively. Approaching the end of ventricular contraction, the ventricles reduce contractile strength in association with a significant decrease in the ventricular blood pressure and volume. At the point when the ventricular blood pressure reaches a level just below the aortic blood pressure (for the left ventricle) and the pulmonary arterial blood pressure (for the right ventricle), the aortic and pulmonary valves are closed owning to the reversed pressure gradients across these valves. The closure of the aortic and pulmonary arterial valves indicates the ending of the systole and the beginning of the diastole.

During early diastole following the closure of the arterial valves, there is a period when the ventricular blood pressure is higher than that in the atria and the atrioventricular valves (mitral and tricuspid valves) are still closed. This period is called the *period of isometric relaxation*, during which blood is not flowing from or to the ventricles. Continuous ventricular relaxation results in a rapid decrease in the ventricular blood pressure. Soon, when the ventricular blood pressure drops below the atrial blood pressure, the atrioventricular valves are open, owing to the reversed pressure gradients across these valves, and blood flows from the left and right atria to the left and right ventricles, respectively. Two factors facilitate ventricular blood filling: continuous expansion of the ventricles and atrial contraction, which occurs in the late stage of diastole. The ventricles are completely filled before the next QRS-exciting wave complex arrives at the ventricle and initiates another cardiac cycle.

Regulation of Cardiac Performance

Cardiac activities undergo constant changes in response to various demands of bloodflow and oxygen consumption in the peripheral tissues and organs. A typical example is the increase in cardiac contraction and heartbeat in response to increased physical activities such as running and other forms of exercise. The change in cardiac activity is regulated by two mechanisms: intrinsic and extrinsic. The *intrinsic* mechanism is controlled by the natural properties of the cardiomyocytes, such as the length of the sarcomere or the fraction of the actin filaments that overlap with the myosin filaments. The contractile strength is proportional to the sarcomere length within a limited range. The muscle generates maximal forces at an optimal sarcomere length. With a further stretch or lengthening of the sarcomere, the contractile strength reduces. Such properties are described by *Starling's law*, which is represented by a sarcomere length–tensile force curve. Under physiological conditions, the heart works in the ascending range of the sarcomere length–tensile force curve; that is, an increase in sarcomere length results in enhanced cardiac contraction. In a beating heart, the sarcomere length of the ventricular muscles is controlled by the diastolic blood volume of the ventricle. An increase in the diastolic blood volume results in an increase in the sarcomere length within a certain range.

The *extrinsic* mechanism is controlled by neural and hormonal factors. The heart is innervated with parasympathetic and sympathetic nerves. Activation of the parasympathetic nervous system suppresses the cardiac activity or contractility, whereas activation of the sympathetic nervous system exerts an opposite effect. The two nerve systems coordinately control the performance of the heart. In addition, the performance of the heart is regulated by hormones, including epinephrine and norepinephrine. Epinephrine and norepinephrine are produced by the adrenal medulla and released into the blood. The secretion of these hormones are controlled by the sympathetic nervous system and elevated in response to increased physical activities and emotional changes. The function of these hormones, especially epinephrine, is to stimulate cardiac contraction. These hormones can bind to and activate the β-adrenergic receptors in the membrane of the cardiomyocytes, inducing the activation of G-protein signaling pathways (see page 217). As a result, calcium is released into the cytoplasm. As calcium is required for the initiation of actin–myosin interactions, an increase in calcium concentration enhances actin–myosin interactions and the muscular contractile strength.

CARDIAC DISORDERS

Heart Failure and Cardiomyopathy

Pathogenesis, Pathology, and Clinical Features of Heart Failure [14.2]. Heart failure is a pathophysiological state in which the heart is unable to pump sufficient blood to the arterial system, resulting in the dysfunction of tissues and organs due to the lack of blood supply. Heart failure is an end event of cardiac and pulmonary disorders, which are among the most popular diseases. Each year about 960,000 patients are admitted to hospital because of heart failure in the United States. About 20% patients are associated with life-threatening heart failure. Heart failure can be induced by a variety of disorders, including cardiac infarction, congenital heart disease, cardiomyopathy, myocarditis, anemia, hemorrhage, cardiac trauma, cardiac valve rupture, cardiac valvular diseases, arrhythmia, systemic hypertension, and excessive physical exercise. Although these disorders involve

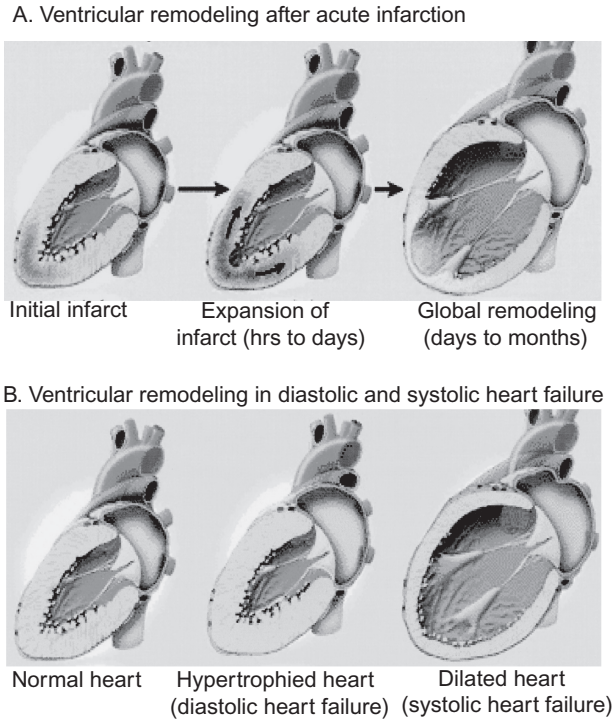


Figure 14.3. Ventricular remodeling after infarction (panel A) and in diastolic and systolic heart failure (panel B). At the time of an acute myocardial infarction, there is no clinically significant change in overall ventricular geometry (panel A). Within hours to days, the area of myocardium affected by the infarction begins to expand and become thinner. Within days to months, global remodeling can occur, resulting in overall ventricular dilatation, decreased systolic function, mitral valve dysfunction, and the formation of an aneurysm. The classic ventricular remodeling that occurs with hypertensive heart disease (middle of panel B) results in a normal-sized left ventricular cavity with thickened ventricular walls (concentric left ventricular hypertrophy) and preserved systolic function. There may be some thickening of the mitral valve apparatus. In contrast, the classic remodeling that occurs with dilated cardiomyopathy (right side of panel B) results in a globular shape of the heart, a thinning of the left ventricular walls, an overall decrease in systolic function, and distortion of the mitral valve apparatus, leading to mitral regurgitation. (Reprinted with permission from Jessup M, Brozena S: *New Engl J Med* 348:2007–18, copyright 2003 Massachusetts Medical Society. All rights reserved.)

different systems and are caused by different pathological factors, the consequence of these disorders is the impairment of cardiac contractility, eventually leading to cardiac failure (Fig. 14.3).

At the molecular and cellular levels, cardiac disorders may induce apoptosis of cardiomyocytes and/or reduction in cardiac contractility due to the impairment of calcium transport and handling. Cell apoptosis results in reduction in the density of cardiomyocytes, which impairs cardiac performance. Cardiac ischemia and infarction are typical cardiac disorders that induce cell apoptosis. The impairment of calcium transport is a primary cause for nonapoptotic heart failure. Calcium plays a critical role in the regulation of muscular contraction. Calcium is primarily stored in a subcellular structure known as

sarcoplasmic reticulum (SR). In patients with heart failure and experimental heart failure models, the load of calcium in the sarcoplasmic reticulum is often reduced. Thus, calcium released to the cytoplasm, in response to an action potential, is insufficient to induce optimal actin–myosin interaction, resulting in impaired cardiac performance. The reduction in the load of sarcoplasmic reticulum calcium is related to malfunction of several molecules, including sarcoplasmic reticulum calcium ATPase (SERCA2a), phospholamban (PLB), and/or the Na^+–Ca^{2+}-exchanger (NCX), which participate in the regulation of calcium handling and transport.

In response to the stimulation of an action potential, cardiomyocytes undergo depolarization, which stimulates the cell membrane voltage-dependent calcium channels to induce calcium influx. The increase in the cytoplasmic calcium concentration activates the ryanodine receptor and induces the transport of additional calcium from the sarcoplasmic reticulum to the cytoplasm. Released calcium binds to troponin C, which in turn initiates actin–myosin interaction and muscle cell contraction. Immediately after the contraction process, calcium must be removed from the cytoplasm, allowing the relaxation of the muscular cells. The transport of calcium from the cytoplasm to the sarcoplasmic reticulum is controlled primarily by the sarcoplasmic reticulum Ca^{2+} ATPase, while calcium transport from the cytoplasm to the extracellular space is controlled by the Na^+–Ca^{2+} exchanger. A reduction in the activity of sarcoplasmic reticulum calcium ATPase is thought to contribute to the reduction in the sarcoplasmic reticulum calcium load and the impairment of cardiac contractility, leading to the development of heart failure.

Cardiac contractility and performance are also regulated by the β-adrenergic receptor signaling system. These receptors interact with catecholamines, including epinephrine and norepinephrine, and stimulate the contraction of cardiac muscle cells. The binding of catecholamine ligands to the β-adrenergic receptor activates the G_s proteins, which induce the activation of a cascade of signaling molecules, including adenylyl cyclase, cAMP, and protein kinase A (PKA). Activated protein kinase A can phosphorylate downstream proteins that regulate the contractile activity of the cardiac muscle cells. One of the substrate molecules for protein kinase A is the cardiac ryanodine receptor, which controls the gating the calcium channels in the sarcoplasmic reticulum. The phosphorylation of the ryanodine receptor promotes diastolic calcium release from the sarcoplasmic reticulum, initiating cardiomyocyte contraction.

Furthermore, activated protein kinase A by the β-adrenergic receptor can phosphorylate a molecule known as *phospholamban*. In its dephosphorylated form, phospholamban inhibits the activity of sarcoplasmic reticulum Ca^{2+} ATPase. The PKA-induced phosphorylation of phospholamban results in the deinhibition of the sarcoplasmic reticulum Ca^{2+} ATPase activity. Thus, protein kinase A-induced phosphorylation of phospholamban removes the inhibitory effect of phospholamban on sarcoplasmic reticulum Ca^{2+} ATPase and enhances the cardiac contractility. The accumulation of dephosphorylated phosholamban or a decrease in the phosphorylation of the molecule reduces the cardiac contractility and promotes the development of heart failure. The knockout of phospholamban in mice with transgenic heart failure can reduce ventricular dilation and cardiac fibrosis, and improves the ventricular performance (Fig. 14.4). Thus, phospholamban is a potential target molecule for the treatment of cardiac failure.

There are various types of heart failure. In terms of the rate of progression, heart failure can be classified into acute and chronic heart failure. Acute heart failure occurs suddenly, often due to large cardiac infarction, cardiac trauma, sudden cardiac valve rupture, or severe hemorrhage, resulting in a rapid reduction in cardiac output and arterial blood

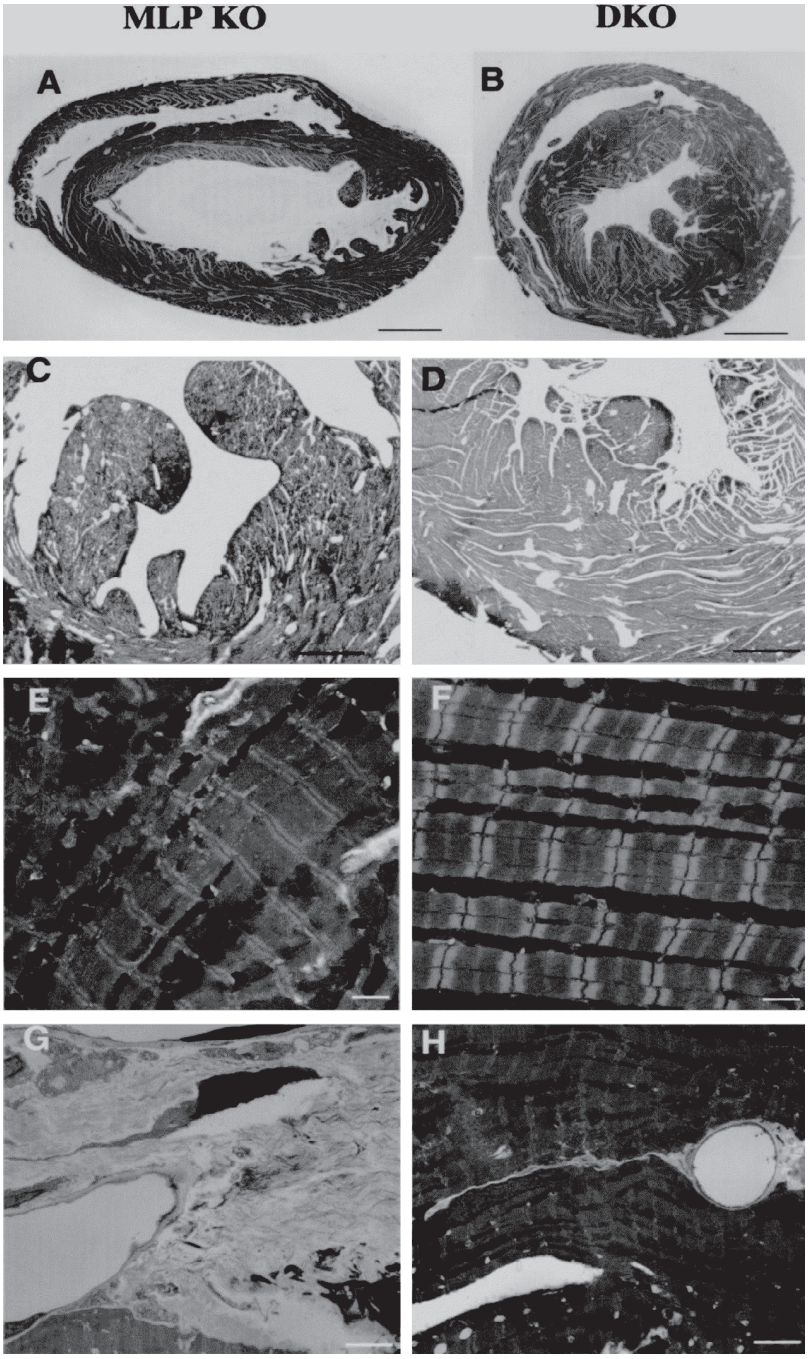


Figure 14.4. Rescue of ultrastructural defects and fibrosis in DKO hearts. Histological micrographs of transverse sections with hematoxylin-eosin (A, B) and trichrome (C, D) stain from cardiac specimens of muscle-specific LIM protein (MLP) knockout mice (MLPKO) and MLP/phospholamban (PLB) knockout mice (DKO). Marked chamber dilation (A) and massive fibrosis [red color, (C)] in MLPKO hearts were rescued in DKO hearts (B, D). Electron microscopic analysis documented that myofibrillar disarray, pronounced increases in nonmyofibrillar space (E), and massive fibrosis (G) in MLPKO hearts were rescued in DKO hearts (F and H). Bars: 1 mm (A, B), 0.5 mm (C, D), 1.0 μm (E, F), and 2.5 μm (G, H). (Reprinted from Minamisawa S et al: *Cell* 99:313–22, copyright 1999, with permission from Elsevier.)

pressure. Acute heart failure is followed with the failure of all peripheral organs within a short period. Changes in the function of peripheral organs depend on the degree of heart failure. A complete loss of arterial blood pressure due to acute heart failure may result in cell death within minutes in vital organs such as the brain, heart, and kidney. Chronic heart failure progresses slowly over months, years, or decades, and are often a result of hypertension, cardiomyopathy, and valvular diseases, such as aortic valve stenosis and mitral valve regurgitation. Hypertension and valvular disorders induce a gradual increase in the cardiac workload. The heart may initially adapt to the workload by increasing its muscle size, a process known as *hypertrophy*. Within a certain period (months, years, or even decades), the heart is still capable of providing sufficient bloodflow to the peripheral systems. However, when the heart reaches the limitation of its adaptive capacity, additional workload may lead to heart failure. Cardiomyopathy is a pathological disorder that gradually deteriorates the structure and function of the cardiac muscle cells, resulting in cardiac malfunction and eventually cardiac failure.

According to the structure of the heart, heart failure can be classified into left and right heart failure. Left heart failure is defined as the failure of the left ventricle, whereas right heart failure is the failure of the right ventricle. Left heart failure can be induced by left coronary arteriosclerosis and left ventricular infarction, systemic hypertension, mitral and aortic valvular diseases, and cardiomyopathy. Right heart failure is often a result of pulmonary hypertension, hypoxia, and tricuspid and pulmonary valvular diseases.

Pathological changes in heart failure often include enlargement of the heart, left and right ventricular dilation for left and right heart failure, respectively, and ventricular wall hypertrophy. Usually, before the onset of heart failure, the heart intends to compensate for the loss of its function by increasing the mass of cardiac muscle via cellular hypertrophy. Such a compensatory remodeling process results in cardiac hypertrophy. Other pathological changes may be dependent on the disease that causes heart failure.

Patients with heart failure often express a number of clinical symptoms and signs. These include dyspnea, orthopnea, paroxysmal dyspnea, cyclic respiration, fatigue, headache, anxiety, insomnia, memory impairment, an increase in heartbeat, and a reduction in blood pressure. All these symptoms and signs are due to the lack of blood supply in various organs such as the lung (dyspnea, orthopnea, paroxysmal dyspnea, and cyclic respiration) and brain (headache, anxiety, insomnia, memory impairment). The increase in heartbeat is a sign of cardiac adaptation. A decrease in blood pressure or blood supply in the peripheral tissue stimulates the sympathetic nerve system, while it suppresses the parasympathetic system, resulting in an increase in the cardiac activity.

Experimental Models of Heart Failure [14.3]. Experimental heart failure can be established by using three methods: cardiac infarction, aortic constriction, and gene knockout. Cardiac infarction can be induced by coronary arterial ligation. In an open-chest surgery, a selected coronary artery can be ligated with a suture, inducing acute myocardial infarction distal to the ligated artery. The severity of heart failure is dependent on the size or generation of the ligated coronary artery. Usually, the ligation of a major branch of the left coronary artery can induce a severe left ventricular failure. Left heart failure can also be induced by increasing the afterload (or arterial blood pressure) of the left ventricle. Such a condition can be achieved by partial constriction of the ascending aorta. The degree of change in aortic blood pressure proximal to the constriction is dependent on the level of constriction. Following the constriction surgery, left ventricular hypertrophy can be developed within days. Severe hypertension and ventricular hypertrophy can lead to heart

failure. Another method for inducing heart failure is to knock out genes involved in the regulation of cardiac performance. A typical gene is the sarcoplasmic reticulum Ca^{2+} ATPase gene. As discussed above, sarcoplasmic reticulum Ca^{2+} ATPase plays a critical role in calcium handling and cardiac muscle contraction. A decrease in the expression of the sarcoplasmic reticulum Ca^{2+} ATPase gene induces heart failure. For these models, the degree of heart failure can be assessed by measuring the movement of the ventricular wall and the ejection fraction of the left ventricle by magnetic resonance imaging or echocardiography.

Pathogenesis, Pathology, and Clinical Features of Cardiomyopathy [14.4]. *Cardiomyopathy* is a disorder that originates from the intrinsic properties of the cardiomyocytes, but not as a result of other diseases, such as coronary arteriosclerosis, congenital heart disease, valvular disease, or hypertension. This disorder is characterized by ventricular dilation and/or hypertrophy and a progressive reduction in cardiac contractility and performance, eventually resulting in heart failure. Cardiomyopathy can be divided into two different types on the basis of etiology: primary and secondary cardiomyopathy. Primary cardiomyopathy is a myocardial disorder of unknown cause. Major forms of primary cardiomyopathy include the idiopathic and hereditary cardiomyopathy. Secondary cardiomyopathy is a myocardial disorder of known cause, often induced by diseases involving other organs. Typical examples include cardiomyopathy resulting from viral, bacterial, and fungal myocarditis, metabolic disorders, electrolyte and nutritional deficiency, systemic lupus erythematosus, rheumatoid arthritis, amyloidosis, muscular dystrophy, and toxic reactions resulting from alcohol and drugs. In either primary or secondary cardiomyopathy, molecular changes described in the section on heart failure may also occur.

Cardiomyopathy is associated with a number of characteristic pathological changes. These include enlargement of the heart and dilation of the ventricles, hypertrophy of the ventricular wall, interstitial fibrosis, leukocyte infiltration, and occasionally myocardial cell death. In dilated ventricles, thrombi may be found on the wall near the apex. Ventricular dilation is induced by a decrease in the cardiomyocyte contractility, which renders the ventricle unable to pump sufficient blood into the arterial system. Excessive residual blood contributes to the dilation of the ventricles. Ventricular dilation induces extension of the sarcomeres, which enhances the cardiac performance within a certain range (see page 589 of this chapter). Cell death may be attributed to the effects of toxic, metabolic wastes and infectious factors. Interstitial fibrosis develops as a result of enhanced fibroblast proliferation and excess extracellular matrix generation in response to cell death. Ventricular hypertrophy is often a result of adaptation to reduction in cardiac contractility and performance. Cardiomyocytes increase their size to compensate the loss of cardiac performance. Leukocyte infiltration is induced by infectious factors, if any, or cell death.

The clinical manifestations of cardiomyopathy are dependent on the level of changes in cardiac function, regardless the causes of the disorder. In the early stage, the patient may not experience any noticeable symptoms. With progressive deterioration of the cardiac function, patients may show heart failure symptoms and signs, such as dyspnea, fatigue, orthopnea, lower-limb edema, and palpitation. Patients may also feel chest pain. Cardiomyopathy eventually develops to heart failure. Strategies for the treatment of cardiomyopathy include the removal of primary causes, protection of cardiomyocytes from injury, and enhancement of myocardial contractility. In the end stage, the treatment of cardiomyopathy is similar to that of heart failure.

Experimental Model of Cardiomyopathy [14.4]. Experimental cardiomyopathy can be established by modulating or knocking out genes encoding proteins that regulate the integrity and contractility of cardiomyocytes. Candidate genes for such a purpose include the muscle-specific lim protein (MLP), dystrophin, sarcoglycans, desmin, myosin heavy-chain, myosin-binding protein C genes. These genes are involved in the regulation of cardiac contractility. The modulation or knockout of these genes exhibits the phenotype of cardiomyopathy. These models are usually established in the mouse. Transgenic mice with a desired disorder may be acquired from investigators who developed the model or from commercial carriers such as the Jackson Laboratory.

Conventional Treatment of Cardiac Failure and Cardiomyopathy [14.5]. Heart failure is usually treated with several approaches, including the removal of primary causes, reduction of cardiac workload, enhancement of cardiac contractility, and control of salt and water intake. The first approach (removal of primary causes) is dependent on the original disease. Each disease should be treated with distinct methods. The reduction of the cardiac workload is often a priority for the treatment of heart failure. There are several methods that can be used for such a purpose. These include reduction in blood volume and arterial blood pressure as well as reduction in physical activities. Blood volume can be reduced by administration of diuretics, and arterial blood pressure can be reduced by administration of vasodilators. In addition, salt and water intake should be carefully controlled to reduce circulating blood volume and the workload of the heart. The enhancement of cardiac contractility can be achieved by the administration of digitalis glycosides. These compounds can act on cardiac muscle cells, increase muscular contractility, and reduce the conduction of action potentials, thus slowing down the heartbeat. A commonly used compound is digoxin. Chemical compounds that augment the sympathetic nerve function by acting on the β-adrenergic receptors, such as epinephrine and dopamine, can be used to improve the cardiac contractility.

Molecular Therapy for Cardiac Failure and Cardiomyopathy [14.6]. Although numbers of conventional approaches have been established for the treatment of heart failure, these approaches are primarily used for the relief of cardiac symptoms, but not for long-term improvement of the cardiac function. Molecular and cellular studies have demonstrated that cardiac contractile dysfunction in heart failure may be related to intrinsic defects in molecular signaling mechanisms that control the cardiac contractility and/or cell apoptosis. Thus, therapeutic modulation of the contractility-controlling molecular mechanisms and prevention of cell apoptosis may provide new approaches for the treatment of heart failure. Available experimental evidence indicates that cardiac contractility is controlled by calcium handling and transport, which are regulated by several molecules localized to the cell membrane and sarcoplasmic reticulum, including sarcoplasmic reticulum Ca^{2+} ATPase, phospholamban, and Na^{+}–Ca^{2+} exchanger. The engineering manipulation of the genes that encode these proteins may exert therapeutic effects on heart failure.

In cardiac failure, the expression and activity of sarcoplasmic reticulum Ca^{2+} ATPase are often reduced. In animal models of heart failure, the overexpression of the sarcoplasmic reticulum Ca^{2+} ATPase gene by gene transfer enhances the contractility of cardiomyocytes and improves the cardiac performance of failing hearts. As discussed above, phospholamban is a molecule that regulates the activity of sarcoplasmic reticulum Ca^{2+} ATPase. In the dephosphorylated form, phospholamban inhibits the activity of

sarcoplasmic reticulum Ca^{2+} ATPase, suppressing the sarcoplasmic reticulum calcium pump function. The phosphorylation of phospholamban, induced by the β-adrenergic receptor-activated protein kinase A, results in the deinhibition of the sarcoplasmic reticulum Ca^{2+} ATPase activity. In mouse models of heart failure, the knockout of the phospholamban gene enhances the cardiac contractility and improves cardiac performance, suggesting that dephosphorylated phospholamban may be dominant in failing hearts. In vitro investigations have shown that the application of antisense mRNA for phospholamban to rat and human cardiomyocytes reduces the translation of the phospholamban protein and improves the contractile performance of these cells. The transfer of a dominant-negative mutant phospholamban gene to hamsters with experimental cardiomyopathy enhances the performance of failing hearts. These observations suggest that the suppression of phospholamban expression may be a potential approach for the treatment of heart failure. Since the activation of the β-adrenergic receptor–protein kinase A signaling pathway induces the phosphorylation of phospholamban, which removes the inhibitory effect of phospholamban on sarcoplasmic reticulum Ca^{2+} ATPase, enhancement of the β-adrenergic receptor may serve as an alternative approach (see below). The Na^+–Ca^{2+} exchanger regulates the transport of calcium from the cytoplasm to the extracellular space. The overexpression of the Na^+–Ca^{2+} exchanger gene reduces the contractility of cardiac myocytes, suggesting that the suppression of the Na^+–Ca^{2+} exchanger may exert therapeutic effects on a failing heart.

Myocardial β-adrenergic receptors play an important role in the regulation of cardiac contractility. Myocytes contain two dominant types of adrenergic receptor: β_1-adrenergic receptor (accounting for 75–80%) and β_2-adrenergic receptor (20–25%). The two types of receptor possess different functions. The activation of the β_1-adrenergic receptors may induce cell apoptosis, whereas that of the β_2-adrenergic receptors exerts a protective effect against cell apoptosis, leading to enhanced cardiac function. The different roles of the two types of receptor have been demonstrated in transgenic mice with targeted overexpression of β_1- and β_2-adrenergic receptors. In these mice, the overexpression of the β_2-adrenergic receptor enhances the contractile performance of the cardiac muscle and prevents heart failure (Figs. 14.5 and 14.6), whereas the overexpression of the β_1-adrenergic receptor facilitates heart failure. It has been suggested that the selective involvement of G_i proteins may be responsible for the different functions of the two receptors. In vivo transfer of the β_2-adrenergic receptor to the animal heart has demonstrated an increase in the contractile activity of cardiac muscle cells in response to catecholamine and enhances the left ventricular function. Based on these investigations, it seems that the β_2-adrenergic receptor gene may be used for the treatment of heart failure.

Tissue Regenerative Engineering for Cardiac Failure and Cardiomyopathy [14.7]. Based on the pathogenic mechanisms and clinical manifestations of cardiac failure, tissue regenerative engineering approaches have been developed for treating cardiac failure. The principle of cardiac tissue regenerative engineering is to enhance the cardiac performance. One method for such a purpose is to construct cardiomyocyte-containing tissue scaffolds, implant the constructed cardiac scaffold around the heart, and induce synchronized cardiomyocyte beating between the heart and the cardiac scaffold, thus enhancing the performance of the heart. Alternatively, the constructed cardiomyocyte-containing scaffold can be implanted around the abdominal aorta. The cyclic contractile activity of the cardiac scaffold can enhance the circulation by dynamically altering the diameter of the aorta.

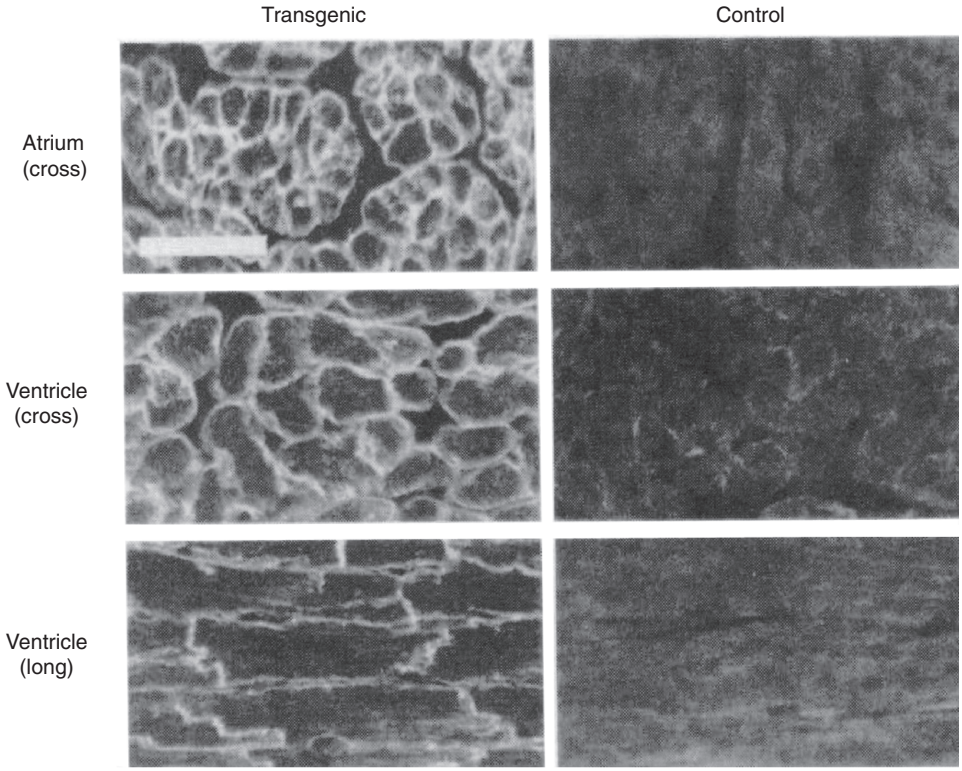


Figure 14.5. In situ demonstration of β2-adrenergic receptor transgene expression. Immunohistochemical labeling of the human β2-adrenergic receptor in TG4 (with transgenic overexpression of the β2-adrenergic receptor) and control myocardial frozen sections was done with rabbit antiserum to the COOH terminus of the human β2-adrenergic receptor. Atrium (cross), cross-sectioned atria; ventricle (cross), cross-sectioned ventricle; ventricle (long), longitudinal sectioned ventricle. Scale bar: 50 μm. (Reprinted with permission from Milano CA et al: *Science* 264:582–6, copyright 1994 AAAS.)

In an experimental model in the rat, researchers have constructed cardiac tissue scaffolds by seeding neonatal cardiomyocytes into a gel mixture containing collagen type I, matrigel, serum, and cell culture medium. The seeded cardiomyocytes can exhibit contractile activities. The matrix gel can be tailored into a desired shape according to the size and geometry of the host heart. The constructed cardiac tissue scaffold can be implanted around to host heart to cover a desired area. Following the implantation surgery, the implanted cardiac tissue scaffold can develop into a structure with organized cardiac muscle cells, which express mature cardiac protein markers such as actinin, connexin 43, and cadherins. More importantly, the implanted cardiac tissue scaffold demonstrates periodic contractile activities. Such activities can enhance the contractile performance of the host heart and reduce the pathological effect of heart failure. These investigations demonstrate that cell-based tissue regenerative engineering approaches can be used to improve the cardiac function in heart failure.

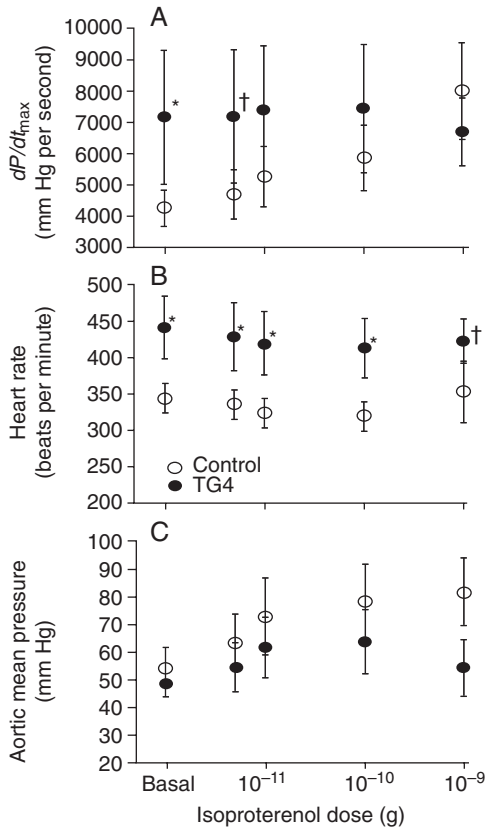


Figure 14.6. In vivo assessment of left ventricular function. Seven TG4 (transgenic) animals and seven controls were anesthetized and aortic and left ventricular catheters placed. Three measured parameters are shown at baseline and after doses of isoproterenol: (A) LV dP/dt_{max}; (B) heart rate; (C) mean aortic pressure. Data are reported as means plus minus SD and were analyzed with a two-way repeated measures analysis of variance with post hoc tests based on *t* test with Bonferroni correction for five comparisons (*, $P < 0.005$; dagger, $P < 0.05$). (Reprinted with permission from Milano CA et al: *Science* 264:582–6, copyright 1994 AAAS.)

Ischemic Heart Disease

Pathogenesis, Pathology, and Clinical Features [14.8]. Ischemic heart disease is a disorder induced by inadequate blood supply to the cardiac tissue, a condition known as *ischemia*. This disorder is characterized by various levels of impairment of cardiomyocytes, ranging from reversible injury to acute death or infarction, depending on the degree of bloodflow deficiency. A complete lack of bloodflow or oxygen in the cardiac muscle induces acute cardiac infarction within 3–5 min. Cardiac infarction is seldom reversible. Dead cardiomyocytes are usually replaced with noncontractile fibrous tissue produced by fibroblasts (Fig. 14.3). The clinical consequence of cardiac infarction is the impairment of cardiac function. Severe cardiac infarction with a large volume of infracted cardiac tissue may result in acute heart failure or cardiac arrest. Cardiac ischemia and infarction are often found in the elder population and are among the leading causes of human death.

There are a number of vascular disorders that contribute to the pathogenesis of cardiac ischemia and infarction. These vascular disorders include coronary arteriosclerosis, thrombosis, and embolism. All these disorders block the coronary arterial bloodflow, resulting in blood and oxygen deficiency. The most common cause is coronary arteriosclerosis, a vascular disorder characterized by the presence of intimal atheroma, which grows continuously and partially or completely blocks bloodflow (see page 674 for details). Coronary arterial thrombosis is an acute, complex process involving endothelial cell injury, activation of the blood coagulation system, adhesion of platelets and leukocytes to injured endothelium, and formation of thrombi, which partially or completely block the lumen of an artery. Embolism is a condition with the arterial lumen blocked with loose thrombi detached from an upstream artery. All these vascular disorders cause similar changes in the heart.

Cardiac infarction is associated with apparent structural changes in the heart, while cardiac ischemia may not exhibit noticeable structural changes. When specimens are collected from an infracted area, one may find characteristic structural changes at different stages. In the acute stage, major structural changes include local edema, massive death of cardiac muscle cells, and leukocyte infiltration, in association with locally reduced contractile activities. If the patient survives, the dead cardiac muscles cannot self-regenerate, but are gradually replaced with proliferating fibroblasts and fibrous tissue composed of primarily collagen matrix and proteoglycans. In the late stage of cardiac infarction, a pathological examination often reveals changes seen in a typical scar tissue, including scattered fibroblasts and fibrous extracellular matrix.

The clinical manifestations of ischemic heart disease are dependent on the degree and location of arterial obstruction. When a coronary artery is partially blocked, the distal cardiac muscles may experience transient ischemia, but are not completely devoid of bloodflow. Such a condition often gives rise to a clinical syndrome known as *angina pectoris*. A major symptom of this syndrome is transient chest pain or discomfort. Often, pain is radiated to the left shoulder and both arms or to the back, neck, jaw, and teeth. Angina usually occurs after physical activities or emotional changes. An electrocardiographic examination often reveals ST-segment depression. In particular, a change in the ST segment after a defined level of exercise in a "stress test" provides further evidence for cardiac ischemia. A coronary angiographic test can provide convincing evidence ensuring the presence of partial arterial obstruction and cardiac ischemia.

In the case of acute cardiac infarction, patients often experience deep, heavy, crushing pain in the chest. Although the pain occurs at locations similar to those in angina pectoris, it is often more severe and lasts longer. The pain may radiate to the shoulder, neck, back, and jaw. The pain is usually associated with other symptoms and signs, such as sweating, weakness, nausea, vomiting, and sudden drop in arterial blood pressure. However, about 15% of patients with cardiac infarction may not experience any pain. Such infarction is referred to as *silent infarction*. This is a more dangerous situation, because patients with severe cardiac infarction can be easily ignored without prompt medical attention.

Conventional Treatment of Ischemic Heart Disease [14.8]. Acute cardiac infarction is often associated with two types of life-threatening disorder: electrical rhythmic disorder (arrhythmia) and mechanical pump failure. The principle of treating acute cardiac infarction is thus to prevent these disorders and minimize the size of cardiac infarction. Patients are always given additional oxygen to maintain an optimal level of blood oxygen to minimize the spread of cardiac infarction, in association with the administration of analgesics,

which keeps the patients calm, lowers physical activities and emotional stress, and reduces the heartbeat and oxygen consumption.

An electrocardiographic examination may reveal various forms of atrial and ventricular arrhythmia. The most serous life-threatening arrhythmias are ventricular tachycardia (heart rate >100 beats/min) and ventricular fibrillation. These forms of arrhythmia occur during the first 24 h following the onset of cardiac infarction. Often, patients are given a preventive treatment with an antiarrhythmia drug such as lidocaine. Ventricular tachycardia and fibrillation, if any, should be treated immediately by defibrillation. Another form of severe arrhythmia is sinus bradycardia (heart rate <45 beats/min). Patients may be administrated with atropine (note that atropine increases heart rate and should be used with caution). In a life-threatening case, patients should be given electrical pacing when blood pressure drops rapidly.

The impairment of the mechanical or contractile performance of an infracted heart is dependent on the size of the infract. Cardiac infarcts with a size larger than a critical level may result in heart failure. In the absence of heart failure, patients are often associated with tachycardia and an increase in arterial blood pressure as the heart intends to compensate for the lost function due to infarction. In such a case, α-adrenergic blocker should be administrated to lower the heart rate and arterial blood pressure. In the presence of heart failure, inotropic agents such as digitalis glycosides or catecholamines may be administrated to raise arterial blood pressure. However, these agents are not given for preventive purposes because they increase heart rate, cardiac contractility, and oxygen consumption, which may facilitate the spread of cardiac infarction. Other treatments described above should be applied in the presence of acute heart failure.

In a large fraction of patients with cardiac infarction, thrombus formation is a major cause of acute arterial obstruction near an atheromatic lesion. In such a case, thrombus dissolution should be carried out promptly with thrombolytic agents to reduce the arterial obstruction, introduce reperfusion, and minimize the size of cardiac infarcts. Common thrombolytic agents include streptokinase and tissue plasminogen activator. These agents can be used to effectively lyse freshly formed thrombi.

Molecular Regenerative Engineering for Ischemic Heart Disease. Molecular engineering approaches can be used to treat cardiac infarction and to improve cardiac function. The principle of cardiac molecular engineering for cardiac infarction is to prevent acute cell death, promote cell survival, protect cells from reperfusion injury, enhance angiogenesis, and improve the contractility of impaired myocardial cells. A number of molecules can be used to prevent cell death and promote cell survival. These include growth factors, such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and vascular endothelial growth factor or (VEGF), antiapoptotic proteins, such as Bcl2, protein kinase B, and Akt, and inhibitors of proinflammatory cytokines. Experimental investigations have demonstrated that these factors can be used effectively to protect impaired cells from death.

Growth Factors as Therapeutic Agents for Cardiac Regenerative Engineering

FIBROBLAST GROWTH FACTORS. Fibroblast growth factors (FGFs) are a family of growth factors, including about 22 known members, including FGF1–22. Among these members, FGF1 and FGF2, also known as acidic and basic FGF, respectively, have been intensively

studied and characterized. Other members are discovered more recently and have become the targets of increasing investigations. The members of the FGF family share about 30–70% identical amino acid sequence. Fibroblast growth factors play a critical role in the regulation of multiple biological processes, including cell proliferation, cell differentiation, cell migration, tissue morphogenesis, and organ formation during development and remodeling. Different FGFs exhibit distinct functions, depending on the structure of the individual members and the types of target cells. In this section, the two well-characterized FGFs, including FGF1 and FGF2, are discussed.

Fibroblast Growth Factor 1 (FGF1, Acidic FGF, or aFGF) [14.9]. Fibroblast growth factor 1 is a 155-amino acid protein with molecular weight of ~17 kDa. This growth factor is also known as *heparin-binding growth factor 1* (HBGF1), since heparin sulfate glycosaminoglycans can bind to FGF1 and facilitate the interaction of FGF1 with FGF receptor (FGFR). Fibroblast growth factor 1 shares the same gene with two growth factors known as endothelial cell growth factors α and β (ECGF α and ECGF β, respectively). The distinct forms of FGF1, ECGFα, and ECGFβ, result from different processes of posttranslational splicing. Fibroblast growth factor 1 is 14 amino acids shorter and ECGFα is 20 amino acid shorter at the *N*-terminus than ECGFβ. The gene for these growth factors is localized to chromosome 5 at gene locus 5q31. The amino acid sequence is highly conserved among mammals. Fibroblast growth factor 1 is expressed in several tissue types, including the central nervous system (primarily the cortex), kidney, pancreas, spleen, and skeletal muscle. This growth factor can interact with all four types of FGF receptors, including FGFR1, FGFR2, FGFR3, and FGFR4.

Fibroblast growth factor 1 exerts a mitogenic effect via the interaction with the fibroblast growth factor receptor (FGFR, see section on fibroblast growth factor receptors below for characterization of FGFR). Investigations by crystallography have demonstrated that FGF1 can interact with the extracellular immunoglobulin-like ligand-binding domain 2 and the linker between domains 2 and 3 of the FGFR. Indeed, this domain and the linker are general binding sites for all FGFs. The binding specificity of distinct FGFs is achieved via the interaction of the *N*-terminus of the FGFs and the immunoglobulin-like ligand-binding domain 3. This structural analysis provides a basis for understanding the interaction of FGF1 with its receptor.

Fibroblast growth factor 1 plays an important role in the regulation of cell proliferation, differentiation, and morphogenesis in a variety of tissue types during embryonic development and adult remodeling. FGF1 is expressed in the neuronal cells of the central nerve system and is responsible for the regeneration of neuronal cells in nerve injury. There exist three of the four types of FGF receptors in the central nerve system. These receptors can interact with FGF1 to induce mitogenic responses. Selective heparan proteoglycans (HSPGs) may mediate the interaction of FGF1 with its receptor. FGF1 also contributes to the formation of the liver from the foregut endoderm. During the embryonic stage, FGF1 is expressed in the mesoderm, which is close to the foregut endoderm, and released to the endoderm, where it stimulates the differentiation of stem and progenitor cells into liver cells. A treatment with FGF1 can induce the foregut endoderm to express genes that are required for liver generation.

Fibroblast Growth Factor 2 (FGF2, Basic FGF, or bFGF) [14.10]. Fibroblast growth factor 2 is protein of 288 amino acids with molecular weight approximately 23 kDa. This

growth factor is expressed widely and participates in the regulation of tissue and organ development, angiogenesis, wound healing, cell regeneration, and tumorigenesis. The level of FGF2 expression in the central nervous system is considerably higher than that in other systems, suggesting a critical role for FGF2 in regulating the development and survival of the neurons. Fibroblast growth factor 2 is encoded by a single gene, which is localized to chromosome 4 at locus 4q25-q27. The length of the human FGF2 is 288 amino acids, whereas that of the mouse FGF2 is 154 amino acids.

Fibroblast growth factor 2 can interact with all four types of FGF receptor, initiating intracellular signaling events. Studies with crystallography have demonstrated that FGF2 can bind to the extracellular immunoglobulin-like domains 2 and 3 of the FGF receptors. The binding of FGF2 induces dimerization of the receptors. The interaction of FGF2 with the immunoglobulin domain 2 is critical for stabilizing the dimerization of the FGF receptor. The interaction of FGF2's *N*-terminus with the immunoglobulin domain 3 of the FGF receptor plays a critical role in the specificity of the ligand–receptor interaction. These observations provide a structural basis for understanding the function of FGF2.

Fibroblast growth factor 2 plays a critical role in the regulation of multiple biological processes, including cell proliferation, differentiation, and morphogenesis during embryonic development and adult remodeling. This growth factor is highly expressed in the central nervous system during embryonic development. The interaction of FGF2 with FGF receptors activates intracellular signaling events, which control the differentiation of neuronal stem cells and the pattern formation of neurons. In the transgenic mouse model of FGF2 knockout induced by homologous recombination, histological abnormalities can be found in the frontal motor sensory area of the cortex with a significant reduction in the neuronal density, although the genetic modulation does not significantly influence the lifespan and fertility of the mouse. These observations suggest a role for FGF2 in regulating neurogenesis. Fibroblast growth factor 2 also participates in regulating the formation of other systems, including the limb, liver, and the Langerhans islets of the pancreas by controlling cell differentiation and proliferation.

Fibroblast growth factor 2 is known as a factor that regulates angiogenesis. In animal models of arterial ligation, a treatment with FGF2 significantly promotes arteriogenesis in regions distal to the ligation site. The application of platelet-derived growth factor (PDGF), together with FGF2, to the arterial ligation model exerts a synergistic effect on arteriogenesis. These growth factors upregulate the expression of PDGF receptors α and β, and activate intracellular mitogenic signaling pathways, leading to enhanced activity of angiogenesis. Furthermore, FGF2 has been shown to induce lymphangiogenesis in the mouse cornea.

Another function of FGF2 is the regulation of wound healing and cell regeneration in tissue and organ injury. For instance, in animal models of bone fracture, a treatment with FGF2 can significantly enhance the union of fractured bones. Such a treatment can also stimulate the mineralization and enhance the mechanical stiffness of the fractured bones. When the FGF2 gene is disrupted by genetic modulation, the rate of mineral deposition and bone formation are significantly reduced. Furthermore, FGF2 promotes cell survival and protects cells from apoptosis. In particular, FGF2 enhances the differentiation of transplanted cardiomyocyte progenitor cells into mature cardiomyocytes. FGF2-deficient progenitor cells exhibit reduced capability of differentiating to cardiomyocytes. These observations suggest that FGF2 is a critical factor for the regulation of wound healing and cell regeneration.

FIBROBLAST GROWTH FACTOR RECEPTORS. Fibroblast growth factor receptors (FGFRs) are a group of single-pass membrane proteins that interact with FGFs and can activate corresponding intracellular signaling pathways, resulting in the activation of mitogenic cellular processes such as cell proliferation, differentiation, migration, and pattern formation. There exist four known types of FGFR, designated as FGFR1, FGFR2, FGFR3, and FGFR4. These receptor types exhibit differential ligand binding affinity and tissue-specific expression. In general, each FGFR is composed of an extracellular region, a transmembrane region, and a cytoplasmic region. In the extracellular region, there are three distinct domains known as *immunoglobulin-like domains*, which are responsible for the binding of FGFs. A catalytic tyrosine kinase domain is found in the cytoplasmic region. This domain serves as a protein tyrosine kinase that phosphorylates downstream signaling molecules. Upon the binding of a FGF ligand, which is mediated by heparan sulfate glycosaminoglycans, the FGFRs are stimulated to form hetero- or homodimers, which induce autophosphorylation of the cytoplasmic domains of the receptors. This process induces the recruitment of adaptor and linker proteins to the receptor cytoplasmic domains, resulting in the activation of corresponding signaling pathways, such as the ras–mitogen-activated protein kinase pathway (see page 151), and activation of mitogenic cellular processes, such as cell proliferation and migration.

Fibroblast Growth Factor Receptor 1 [14.11]. Fibroblast growth factor receptor 1 (FGFR1) is also known as *FMS-like tyrosine kinase 2* or FLT2 (note that FMS is macrophage colony-stimulating factor receptor encoded by the *fms* oncogene). The human FGFR1 is composed of 820 amino acids with molecular weight ~92 kDa. The FGFR1 gene is localized to chromosome 8 at locus 8p11.1 and 8p11.2. Fibroblast growth factor receptor 1 is expressed in a variety of tissue types, including the central nerve system, kidney, lung, mammary gland, blood vessels, stomach, pancreas, thymus, uterus, and cornea. This growth factor receptor primarily interacts with FGF1, FGF2, and FGF5, resulting in the activation of the receptor. Activated FGFR1 can interact with adapter proteins, including growth factor receptor-bound protein 2 (Grb2) and Sos, which in turn activate intracellular signaling pathways and regulate mitogenic processes such as cell proliferation, differentiation, migration, and pattern formation. Mutation of FGFR1 contributes to the development of several disorders, including Pfeiffer syndrome (an anautosomal dominant craniosynostosis syndrome characterized by craniofacial anomalies and broad thumbs and large toes) and Kallmann syndrome (characterized by craniosynostosis).

Fibroblast Growth Factor Receptor 2 [14.12]. Fibroblast growth factor receptor 2 (FGFR2) is a 758-amino acid transmembrane receptor protein tyrosine kinase with molecular weight of approximately 92 kDa. It is also known as a *keratinocyte growth factor receptor*, *fibroblast growth factor receptor BEK*, *protein tyrosine kinase receptor-like 14* (TK14), or *BEK*. The receptor is composed of several domains, including three extracellular immunoglobulin-like domains, a transmembrane domain, and a tyrosine kinase domain. The FGFR2 gene is localized to chromosome 10 at locus 10q26. This receptor is expressed in a variety of tissues, including the brain, thymus, cornea, skin, pancreas, stomach, prostate gland and uterus. This receptor can interact with numbers of ligands, including FGF1, FGF2, FGF5, FGF7, FGF9, FGF10, and phospholipase C/γ1. The primary function of the FGFR2 is to regulate cell proliferation, differentiation, and pattern formation during embryonic development and adult remodeling. Mutation of the FGFR2 gene may induce several disorders, such as Pfeiffer syndrome, colorectal carcinoma, and gastric cancer.

Fibroblast Growth Factor Receptor 3 [14.13]. Fibroblast growth factor receptor 3 (FGFR3) is an 806 amino acid transmembrane protein tyrosine kinase with molecular weight ~88 kDa. This receptor is also known as *human tyrosine kinase JTK4*. It is expressed in the central nerve system, liver, intestine, cartilage, lung, and thymus. Fibroblast growth factor receptor 3 can interact with FGF1, FGF8, and FGF9, inducing the activation of the receptor. FGFR3 can activate intracellular adapter proteins, including Grb2 and SH2 domain-containing protein tyrosine phosphatase 2 (SHP2). These adaptor proteins induce activation of intracellular mitogenic signaling pathways. The mutation of FGFR3 induces several disorders, including thanatophoric dwarfism (sporadic lethal skeletal dysplasia with limb shortening, macrocephaly, platyspondyly, and reduced thoracic cavity), gastric and colorectal cancers, and hypochondroplasia (chondrodystrophy or abnormal development of cartilage).

Fibroblast Growth Factor Receptor 4 [14.14]. Fibroblast growth factor receptor 4 (FGFR4), also known as *TKF*, is a 802-amino acid transmembrane protein tyrosine kinase with molecular weight ~88 kDa. This receptor is expressed in the central nervous system, heart, lung, liver, intestine, adrenal gland, pancreas, spleen, thymus, retina, and cornea. Fibroblast growth factor receptor 4 can interact with FGF1, FGF2, FGF6, FGF8, and FGF19, inducing mitogenic cellular activities. The mutation of FGFR4 induces breast cancer and ovarian cancer.

EPIDERMAL GROWTH FACTOR [14.15]. Epidermal growth factor (EGF), also known as *urogastrone*, is synthesized first as a precursor of 1168 amino acids with molecular weight ~128 kDa. The EGF precursor os converted to mature EGF by protein cleavage. The mature EGF is a 53 polypeptide with molecular weight ~6 kDa. The EGF gene is localized to chromosome 4 at locus 4q25. Epidermal growth factor is expressed in the skin, intestine, ovary, pancreas, prostate gland, uterus, and blood vessels. Epidermal growth factor interacts with and activates EGF receptor (EGFR), leading to the activation of intracellular signaling pathways involving Grb2, PI3 kinase, Ras, and mitogen-activated protein kinase (see page 151). The activation of these signaling molecules results in cellular activities, such as cell proliferation and migration. The mutation of EGF gene may contribute to the development sporadic malignant melanoma.

EPIDERMAL GROWTH FACTOR RECEPTOR [14.16]. Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein kinase of 1210 amino acids with molecular weight ~134 kDa. The gene of EGFR is localized to chromosome 7 at locus 7p12.3–p12.1. This receptor is expressed ubiquitously. The EGFR is composed of two cheY-homologous receiver (REC) domains and three furin-like repeats in the extracellular region, a transmembrane domain, and a cytoplasmic protein tyrosine kinase. Note that the REC domain is a sequence homologous to that of the cheY protein, which regulates the rotation of *E. coli* flagellate motors, and the furin-like repeat is a cysteine-rich sequence found in receptors involved in signal transduction mediated by receptor tyrosine kinases. Epidermal growth factor receptor can interact with EGF, inducing autophosphorylation of the receptor. Phosphorylated EGFR can activate intracellular signaling molecules, including protein kinase A, Ras, focal adhesion kinase, integrins, Sos, protein kinase Cα, STAT, and SH2 domain-containing protein tyrosine kinase 2 (SHP2). The activation of these signaling molecules leads to mitogenic cellular processes such as cell proliferation, migration, and adhesion.

PLATELET-DERIVED GROWTH FACTOR A CHAIN [14.17]. Platelet-derived growth factor A chain (PDGF A), also known as *platelet-derived growth factor α peptide*, is a protein of 211 amino acids with molecular weight ~24 kDa. The PDGF A gene is localized to gene locus 7p22. Platelet-derived growth factor A is expressed in several tissues, including the uterus, lung, and blood vessels. Platelet-derived growth factor A chain can form a homodimer, known as *PDGF AA*, with another PDGF A molecule. PDGF A chain can interact with PDGF receptor α to induce mitogenic cellular activities, such as cell proliferation and migration. Platelet-derived growth factor A can bind to several extracellular matrix proteins, including collagen, laminin 1, and perlecan. The binding of PDGF A to collagen molecules requires the presence of calcium, whereas the binding to perlecan is not calcium-dependent. The formation of PDGF A–matrix protein complexes is an effective mechanism for the storage of PDGF A in the extracellular space. Platelet-derived growth factor A can be rapidly released in the case of cell injury, facilitating cell regeneration. The expression of PDGF A is upregulated in the vascular smooth muscle cells and endothelial cells in vascular disorders such as atherosclerosis, inducing smooth muscle cell proliferation and migration from the media to the intima, critical processes that contribute to atherogenesis. Furthermore, PDGF A plays a role in regulating spermatogenesis. The deficiency of PDGF A in a transgenic mouse model induces the arrest of spermatogenesis.

PLATELET-DERIVED GROWTH FACTOR B CHAIN [14.18]. Platelet-derived growth factor B chain (PDGF B) is also known as *PDGFβ polypeptide*, *V-SIS platelet-derived growth factor β polypeptide*, and *Simian sarcoma viral oncogene homolog*. It is a 241-amino acid protein with molecular weight ~27 kDa. The PDGF B gene, also known as the *sis* oncogene, is localized to chromosome 22 at locus 22q12.3–q13.1. Platelet-derived growth factor B is expressed in the heart, blood vessels, testis, kidney, eye, and ovary. This growth factor often forms a dimer with another PDGF B chain, known as *PDGF BB*. The dimeric complex can interact with PDGF receptor α and PDGF receptor β. The binding of PDGF BB to PDGFR induces dimerization of the receptors and autophosphorylation of the receptor cytoplasmic tyrosine kinase domains, a critical process for the action of mitogenic signaling pathways involving Ras, Raf1, and mitogen-activated protein kinases. The activation of these signaling molecules enhances cell proliferation and migration. PDGF B can bind to extracellular matrix proteins, including collagen and perlecan, a process for the storage of PDGF B.

PLATELET-DERIVED GROWTH FACTOR RECEPTOR α [14.19]. Platelet-derived growth factor receptor α (PDGFRα) is a transmembrane receptor protein tyrosine kinase (1089 amino acids, ~123 kDa). It is also known as *PDGFR A* or *PDGFR2*. The gene of PDGFRα is localized to chromosome 4 at locus 4q12. The receptor is composed of an extracellular region, a transmembrane region, and a cytoplasmic region. The extracellular region contains three immunoglobulin (Ig)-like domains and an immunoglobulin constant 2-type (IGC2) domain. PDGFRα is expressed in the brain, liver, pancreas, bone, platelets, and B cells. The extracellular region of the PDGFRα can interact with PDGF A and PDGF B, inducing dimerization of the receptors and autophosphorylation of the receptor cytoplasmic tyrosine kinase domains. Phosphorylated PDGFRα can activate several intracellular signaling molecules, including guanine nucleotide-releasing factor 2, growth factor receptor-bound protein 2 (Grb2), phospholipase Cγ and SH2 domain-containing protein tyrosine phosphatase 2 (SHP2). The activation of these signaling molecules results in

mitogenic cellular activities such as cell proliferation, adhesion, and migration. PDGFRα can also interact with integrins, including integrin αV and integrin 3, to mediate synergistically the transduction of signals from extracellular matrix proteins and thus augment mitogenic cellular processes.

PLATELET-DERIVED GROWTH FACTOR RECEPTOR β [14.20]. Platelet-derived growth factor receptor β (PDGFRβ), also known as *PDGFR1*, is a receptor protein tyrosine kinase of 1106 amino acids with molecular weight 124 kDa. The receptor is composed of an extracellular region, a transmembrane region, and a cytoplasmic region. The extracellular region contains several domains, including two Ig-like domains and an Ig constant 2 domain. The cytoplasmic region contains a protein tyrosine kinase domain. The gene of PDGFRβ is localized to chromosome 5 at locus 5q21–q32. PDGFRβ is expressed in the lung, kidney, blood vessels, bone, and prostate gland.

The extracellular region of PDGFR β can interact with primarily the PDGF B chain, inducing dimerization of the receptors and autophosphorylation of the receptor cytoplasmic domains. Phosphorylated PDGFR β can in turn activate a number of intracellular signaling molecules, including SH2 domain-containing protein tyrosine phosphatase (SHP)2, Raf1, focal adhesion kinase, growth factor receptor-bound protein 2 (Grb2), Grb4, and the Ras protein. These signaling molecules further induce activation of mitogenic processes such as cell proliferation and migration. PDGFRβ can also interact with integrins, including integrin αV and β3. This interaction synergistically enhances mitogenic activities induced by extracellular ligands and matrix components.

VASCULAR ENDOTHELIAL GROWTH FACTOR A [14.21]. Vascular endothelial growth factor (VEGF) A, also known as the *vascular permeability factor*, is a 189 amino acid protein of molecular weight approximately 22 kDa. This growth factor is encoded by the VEGF A gene, which contains 8 exons. Alternative splicing of the VEGF A gene may give rise to different sizes of the VEGF protein. The missing of the exon 6-encoded residues results in the formation of a VEGF variant of 165 amino acids, whereas the missing of exons 6 and 7 results in the formation of a VEGF variant of 121 amino acids. All these forms possess the normal biological activity of the growth factor. This growth factor may exist in the form of homodimer with molecular weight ~45 kDa.

The VEGF gene is localized to chromosome 6 at locus 6p12. VEGF A is expressed in a number of tissues, including the heart, blood vessels, kidney, adrenal gland, lung, liver, stomach, pancreas, uterus, retina, and skin. VEGF A can interact with VEGF receptor 1 (VEGFR1) and VEGFR2. Such interaction activates these receptors, leading to mitogenic cellular responses such as cell proliferation, differentiation, and migration. In particular, VEGF A is involved in the regulation of endothelial cell differentiation and angiogenesis, or the formation of blood vessels on the basis of an established vascular network. Another mitogenic factor, angiopoietin 2, acts synergistically with VEGF A in the regulation of angiogenesis. Under conditions with reduced oxygen and nutrient supply, VEGF plays a critical role for the survival of vascular endothelial cells and the formation of blood vessels, which is an adaptive process in response to hypoxia and nutrient depletion.

VASCULAR ENDOTHELIAL GROWTH FACTOR B [14.22]. Vascular endothelial growth factor B (VEGF B), also known as *vascular endothelial growth factor-related factor* (VRF), is a 22 kDa protein, which exists in two different isoforms: one with 186 amino acids and

the other with 167 amino acids. The two VEGF B isoforms differ at their carboxyl ends due to a shift in the open reading frame. Both VEGF B isoforms possess similar function. VEGF B exhibits strong homology to VEGF A. The VEGF B gene is localized to chromosome 11 at locus 11q13. The human VEGF B gene shares about 88% amino acid sequence identity with the mouse VEGF B gene. VEGF B is expressed in the heart, skeletal muscle, pancreas, and prostate gland. VEGF B can bind to VEGF receptor (VEGFR) 1 to induce phosphorylation of the receptor protein tyrosine kinase, leading to activation of intracellular mitogenic signaling cascades, involving Grb2, Ras, mitogen-activated protein kinases, and mitogenic cellular activities, such as cell proliferation, migration, and angiogenesis. The deficiency of VEGF B in transgenic mice is associated with abnormal vascular structure and impaired recovery from cardiac injury.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 [14.23]. Vascular endothelial growth factor 1 (VEGF1) is a protein tyrosine kinase-containing transmembrane receptor. It is also known as vascular permeability factor receptor, FLT, and FLT1. The length of the receptor is 1338 amino acids and the molecular weight is approximately 151 kDa. The receptor is composed of five immunoglobulin-like domains and two immunoglobulin constant 2 domains in the extracellular region, a transmembrane region, and a cytoplasmic region, which contains a protein tyrosine kinase domain. VEGFR1 is encoded by the oncogene flt1, which belongs to the src oncogene family and is localized to chromosome 13 at locus 13q12. VEGFR1 is expressed primarily in vascular endothelial cells in several tissues and organs, including the bone marrow, testis, intestine, pancreas, ovary, prostate gland, and placenta. VEGFR1 can interact with VEGF A and VEGF B at the extracellular domains 2 and 3, inducing autophosphorylation of the cytoplasmic protein tyrosine kinase domain. Phosphorylated VEGFR1 activates intracellular signaling molecules, including phospholipase Cγ2 and SHC. An important function of VEGFR1 is to regulate endothelial cell proliferation and angiogenesis. Furthermore, VEGFR1 can interact with placental growth factor (PGF), which mediates the crosstalk between VEGFR1 and VEGFR2. VEGF and PGF can form heterodimers, which stimulate the formation of VEGFR1 and VEGFR2 heterodimers. The VEGFR1/PGF combination synergistically enhances the activity of VEGFR1 and VEGFR2. A soluble form of VEGFR1 can be generated by alternative splicing of the VEGFR1 pre-mRNA and is termed *sFLT1*. This form of VEGFR1 can bind to VEGF with high affinity. Thus, sFLT1 sequesters VEGF and competitively inhibits the activity of VEGF.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 [14.24]. Vascular endothelial growth factor receptor 2 (VEGFR2) is a transmembrane receptor with a cytoplasmic protein tyrosine kinase domain. VEGFR2 is also known as *fetal liver kinase 1* (Flk1), *kinase insert domain receptor* (KDR), and *tyrosine kinase growth factor receptor*. The length of the receptor is 1356 amino acids, and the molecular weight is approximately 151 kDa. This receptor is encoded by the oncogene kdr, which is localized to the gene locus 4q12. VEGFR2 is composed of five immunoglobulin-like domains in the extracellular region, a transmembrane region, and a cytoplasmic region with a catalytic protein tyrosine kinase domain. The structure of the protein tyrosine kinase domain is similar to that of the platelet-derived growth factor receptor, colony-stimulating factor 1 receptor, fibroblast growth factor receptor, and KIT. The receptor is primarily expressed in the vascular endothelial cells and is often used as a marker for the identification of the endo-

thelial cells. VEGFR2 can interact with VEGF A. The binding of VEGF A to VEGFR2 induces autophosphorylation of the cytoplasmic protein tyrosine kinase domain, which further activates intracellular signaling molecules, including Grb2, Grb10, phospholipase Cγ2, Src-homology collagen protein (Shc), and Shc-like protein (Sck). These signaling molecules participate in the regulation of endothelial cell division and angiogenesis. VEGFR2 and associated signaling molecules also regulate the differentiation and development of vascular endothelial cells as well as vasculogenesis during the embryonic stage.

VEGFR2 is found in embryonic hematopoietic stem cells. About 0.1–0.5% of $CD34^+$ embryonic hematopoietic stem cells co-express VEGFR2. The $CD34^+VEGFR2^+$ cell population contains pluripotent hematopoietic stem cells that can differentiate into hematopoietic progenitor cells and vascular endothelial cells. The adult bone marrow also contains $CD34^+VEGFR2^+$ cells. These cells can potentially differentiate into vascular endothelial cells. VEGFR2 is considered a marker for the identification of endothelial cell progenitor cells. Furthermore, $VEGFR2^+$ progenitor cells contribute to the formation of blood vessels and regulate the organization of the vasculature. It is interesting to note that the growth factor VEGF stimulates the transformation of $VEGFR2^+$ progenitor cells to endothelial cells, whereas PDGF induces the formation of the vascular smooth muscle cells.

HEPATOCYTE GROWTH FACTOR [14.25]. Hepatocyte growth factor (HGF) is a protein of 728 amino acids with molecular weight ~83 kDa. HGF is also known as hepatopoeitin A, lung fibroblast-derived mitogen, and scatter factor (SF). This growth factor is composed of a heavy and light chain. The heavy chain is about 50–60-kDa in molecular weight, and the light chain is about 30–35 kDa. The two chains are linked by disulfide bonds. The HGF gene is localized to the gene locus 7q21.1 of chromosome 7. The structure of HGF is apparently different from that of other growth factors as described above. HGF is composed of an apple-like domain, four Kringle domains, and a trypsin-like serine protease domain. The apple-like domain is a fourfold repeat apple-like structure found in plasma kallikrein and coagulation factor XI. This domain mediates the binding of factor XI to platelets. The Kringle domain is a triple-loop, three-disulphide bridged structure found in several serine proteases such as prothrombin and urokinase-type plasminogen activator. This domain mediates the binding of molecules. HGF is expressed in a number of tissues, including the liver, blood vessels, brain, bone marrow, and placenta. This growth factor can interact with HGF receptor (HGFR), inducing mitogenic responses. HGF plays a critical role in regulating the development and morphogenesis of embryonic tissues and organs. The deficiency of HGF in transgenic mice is associated with incomplete liver and placental development, resulting in premature death of the animal.

HGF exerts a protective effect on injured cardiomyocytes (Fig. 14.7). HGF is upregulated in cardiac injury and stimulates the survival and proliferation of injured cardiomyocytes. In particular, this growth factor protects injured cardiac tissue from fibrosis (note that fibrosis reduces cardiac contractility). These effects render HGF a potential therapeutic factor for the treatment of cardiac injury.

HEPATOCYTE GROWTH FACTOR RECEPTOR [14.26]. Hepatocyte growth factor receptor (HGFR), also known as *MET* and *RCCP2*, is a transmembrane receptor with a cytoplasmic protein tyrosine kinase. The length of the receptor is 1400 amino acids, and the molecular

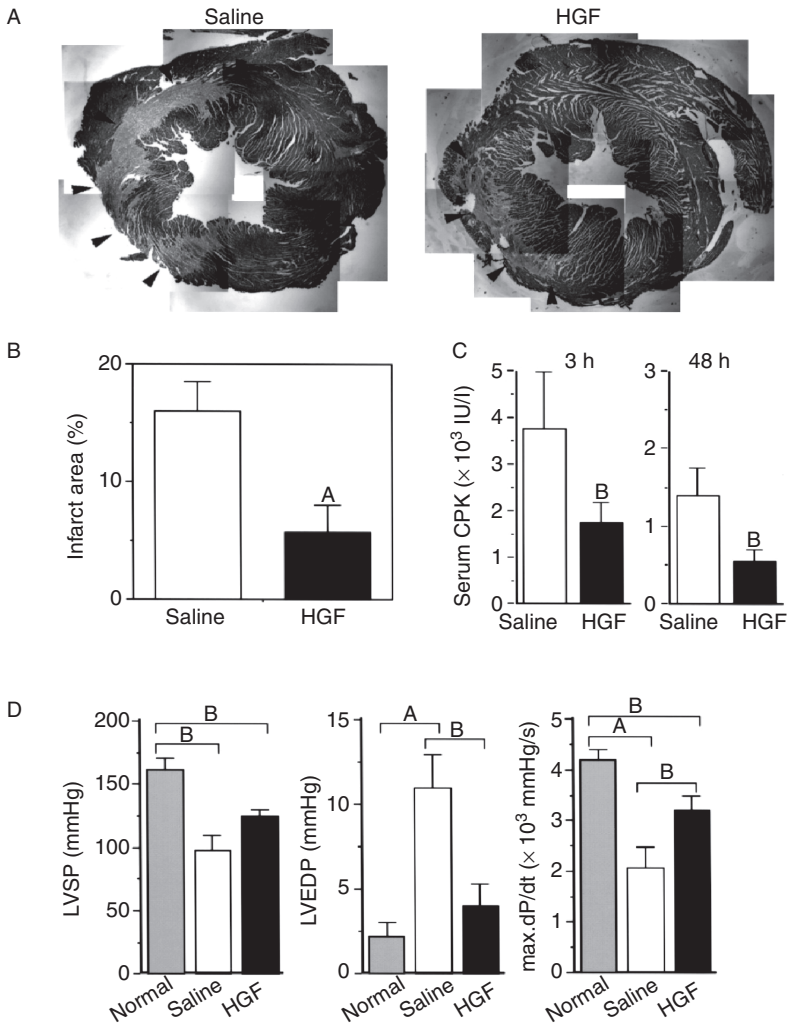


Figure 14.7. Amelioration of ischemia/reperfusion injury by hepatocyte growth factor (HGF). Recombinant human HGF ($n = 8$) or saline ($n = 8$) was injected immediately after and every 12 h after reperfusion. After 48 h, specimens were collected for observation. (A) α-Sarcomeric actin staining done to depict the infarct area (original magnification, ×40). Arrowheads indicate the alpha-actin–negative infarct area. (B, C) Changes in infarct area (B) and serum CPK activity (C). $^{A}P < 0.01$, $^{B}P < 0.05$. (D) Change in cardiac functions after ischemia/reperfusion injury. $^{A}P < 0.01$, $^{B}P < 0.05$. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; max *dP/dt*, maximal rate of left ventricular pressure rise. (Reprinted with permission from Nakamura T et al: Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF, *J Clin Invest* 106:1511–9, copyright 2000.)

weight is 157 kDa. This receptor is composed of a semaphorin (SEMA) domain, a PSI domain, and four IPT domains in the extracellular region, a transmembrane domain, and cytoplasmic protein tyrosine kinase domain. The SEMA domain is a structure found in semaphorins (proteins that induce the collapse and paralysis of neuronal growth cones

during development) and is characterized by the presence of conserved cysteine residues. These residues form disulfide bonds and stabilize the protein structure. The PSI domain is found in plexins (proteins involved in the development of neural and epithelial tissues), semaphorins, and integrins. The IPT domain is an immunoglobulin-like structure found in plexins and certain transcription factors. HGFR is encoded by the oncogene *met*, which is localized to the gene locus 7q31. The protein product of the *met* oncogene is a dimer composed of an α subunit and a β subunit linked by disulfide bonds. The α subunit contains only an extracellular region, whereas the β subunit consists of extracellular, transmembrane, and cytoplasmic regions. The β subunit of the MET protein is the primary subunit that interacts with HGF. HGFR is expressed in the liver, brain, placenta, and skeletal muscle. The deficiency of the HGFR gene in transgenic mouse models induces liver and limb muscle defects, resulting in embryonic death of the animal. HGFR is often upregulated in human cancer cells. The expression of HGFR is enhanced during metastasis. This receptor may contribute to the metastatic properties to nontumorigenic and tumorigenic cells.

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Prevention of Cardiac Injury [14.27]. Blood reperfusion following cardiac infarction may introduce additional harmful stress to the heart, exacerbating the damage induced by the original ischemia and infarction. The reoxygenation of ischemic myocardial cells may induce an in increase in the formation of reactive-oxygen species (ROS). The reactive oxygen species may react with endogenous antioxidant factors, potentially depleting the buffering capacity of the endogenous antioxidant system. Excessive reactive oxygen species may activate inflammatory reactions, inducing cell membrane injury, contractile dysfunction, and cell death, which increase the propensity of heart failure. Thus, the prevention of reperfusion injury is a critical step for the treatment of cardiac infarction.

There exist a number of antioxidant enzymes, such as superoxide dismutase (an enzyme dismutating the oxygen free radical superoxide O_2^-) and heme oxygenase (an enzyme

involved in the catabolism of heme), which reduce the activities of reactive oxygen species. These protein enzymes or their genes can be delivered to the infarct area of the heart for therapeutic purposes. The overexpression of heme oxygenase and superoxide dismutase in cardiac infarction has been shown to significantly reduce the size of infarcts, in association with a decrease in oxidative stress and inflammatory reactions. These changes are associated with improvement of the contractile function of impaired cardiac tissue. Other proteins that exert antioxidant effects include glutathione peroxidase, stress-induced heat-shock proteins, survival genes (Bcl2, Akt), immunosuppressive cytokines, adenosine A_1 and A_3 receptors, and hepatocyte growth factor. These molecules can be used to protect the heart from reperfusion injury.

Antioxidant Molecules as Therapeutic Agents

SUPEROXIDE DISMUTASES [14.28]. Superoxide dismutases (SODs) are a family of enzymes, including SOD1 (CuZn-SOD), SOD2 (Mn-SOD), and SOD3 (EC-SOD), which remove the reactive superoxide radical O_2^-. The superoxide radical is produced by oxygen reduction and exerts a highly toxic effect on molecular and cellular functions and activities. This radical has been implicated in degenerative processes including amyotrophic lateral sclerosis, ischemic heart disease, Alzheimer's disease, Parkinson's disease, and aging. Superoxide dismutase can catalyze the dismutation of the toxic superoxide anion O_2^- to O_2 and H_2O_2, thus reducing the toxicity of the superoxide radical. Although the three superoxide dismutases exert a similar function, the structure and gene locus of these enzymes are different. SOD1 is a 154-amino acid protein with molecular weight about 16-kDa. This enzyme is also known as *soluble superoxide dismutase*, *copper–zinc superoxide dismutase*, and *indophenoloxidase A* (IPOA). SOD1 is a copper- and zinc-containing homodimer found primarily in the cytoplasm. The SOD1 gene is localized to gene locus 21q22.1. SOD1 is expressed ubiquitously. The mutation of SOD1 can induce amyotrophic lateral sclerosis, a degenerative motor neuron disorder.

Superoxide dismutase 2 (SOD2) is a manganese-containing enzyme of 222 amino acids with molecular weight ~25 kDa. This enzyme is also known as *mitochondrial superoxide dismutase*, *indophenoloxidase B* (IPO-B), and *manganese superoxide dismutase*. It exists in the form of tetramer and is found primarily in the mitochondria. The SOD2 gene is localized to the gene locus 6q25.3. SOD2 is expressed ubiquitously. The function of SOD2 is similar to that of SOD1 as described above. SOD2 helps to stabilize the activity of mitochondrial enzymes, which are susceptible to superoxide-induced toxicity. The knockout of the SOD2 gene in mice results in cardiomyopathy, lipid accumulation in the liver and skeletal muscle, metabolic acidosis, and premature animal death. At the molecular level, the deficiency of the SOD2 gene induces the suppression of the respiratory chain enzymes NADH-dehydrogenase (complex I) and succinate dehydrogenase (complex II), the inhibition of the tricarboxylic acid cycle enzyme aconitase, functional defect in the 3-hydroxy-3-methylglutaryl-CoA lyase in association with aciduria, and oxidative damage of DNA. Mutation in the SOD2 gene causes idiopathic dilated cardiomyopathy.

Superoxide dismutase 3 (SOD3) is a copper- and zinc-containing enzyme (240 amino acids, 26-kDa) and exists in the form of tetramer. It is also known as extracellular *superoxide dismutase* [Cu–Zn] and extracellular superoxide dismutase. The gene is localized to gene locus 4p15.3-p15.1. SOD3 is found primarily in the extracellular space of several systems, including the heart, lung, liver, kidney, pancreas, thyroid, uterus, and skeletal muscle.

HEME OXYGENASE [14.29]. Heme oxygenase, also known as HO and HMOX, is an enzyme that cleaves heme to form biliverdin, a molecule subsequently converted to bilirubin by biliverdin reductase. This process consumes oxygen and electrons donated by the NADPH-cytochrome p450 reductase. Activated HO exerts an antioxidative effect. Such an effect is exerted through the production of bilirubin. Whereas heme is known as a prooxidant, bilirubin is an antioxidant. The accumulation of bilirubin contributes to the antioxidative effect. There are two isoforms for the heme oxygenase: HO1 and HO2. HO1 is the inducible form of HO. This enzyme is 288 amino acids in length and about 33 kDa in molecular weight. The gene of HO1 is localized to the gene locus 22q12. HO is expressed in a number of tissue and organ systems, including the central nervous system (cerebral cortex and hippocampus), lung, blood vessels, kidney, and prostate gland. HO1 plays a critical role for the prevention of oxidant-induced cell injury and death. For instance, the expression of HO1 is upregulated in skin wounds in response to heme release. Enhanced HO1 exerts an antagonistic effect on oxidative stresses and inflammation induced by heme.

Heme oxygenase 2, or HO2, is the constitutive form of heme oxygenase (316 amino acids, 36 kDa). HO2 exerts an antioxidative effect similar to that of HO1. The HO2 gene is localized to the gene locus 16p13.3. HO2 is expressed in the brain, skin, and placenta. In HO2-null mice, exposure to hyperoxia induces more severe oxidative injury and cell death compared to control animals. Furthermore, HO2 is expressed primarily in the endothelial cells of blood vessels and the neurons of several autonomic ganglia, including the petrosal, superior cervical, and nodose ganglia. HO2 catalyzes the formation of carbon monoxide (CO), which potentially induces endothelial relaxation. Thus, HO1 is thought to serve as a vasodilator.

GLUTATHIONE PEROXIDASE [14.30]. Glutathione peroxidase (GPX) is an enzyme that reduces hydrogen peroxide H_2O_2. The enzyme reduces H_2O_2 to H_2O by oxidizing glutathione ($H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$, where GSH is glutathione and GSSG is oxidized glutathione). A further reduction of oxidized glutathione is catalyzed by glutathione reductase ($GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$). Since H_2O_2 induces cell injury and death, GPX plays a critical role for protecting cells from injury and death. The action of glutathione peroxidase requires a metal cofactor selenium. Transgenic mice with GPX deficiency exhibit increased sensitivity to oxidative stress. Cells derived from GPX-deficient mice are committed to apoptosis when exposed to hydrogen peroxide, whereas cells from wildtype control mice exhibit a significant decrease in susceptibility to hydrogen peroxide. There exist at least six GPX isoforms: GPX1–6, which are encoded by different genes and expressed in different tissues and organs. The characteristics of these isoforms are presented in Table 14.1.

STRESS-INDUCED HEATSHOCK PROTEINS [14.31–14.35]. Heatshock proteins (HSPs) are a family of cytoplasmic proteins that are upregulated in response to environmental stress conditions, such as an alteration in temperature, inflammation, viral and bacterial infection, hypoxia, starvation, and exposure to toxins and ultraviolet light. These proteins are also called *stress proteins*. The HSP family contains a number of members with various protein structure and molecular weight. These members are grouped on the basis of their moelcular weights. For example, HSPs with molecular weight ~10 kDa are classified as HSP10, and those with molecualr weight ~40 kDa HSP40, and so on. The primary functions of HSPs are to protect cells from stress-induced injury and present signals of infection and inflammation to the immune defense system in resposne to altered environmetnal

stress condictions. Under physiological conditions, HSPs are expressed at a basal level. These proteins serve as chaperones that mediate protein folding and assembly, intracellular protein sorting and transport, protein–protein interactions, and disposal of diordered and aged proteins. Release HSPs from necrotic or apoptotic cells, such as infarcted cardiac cells, may serve as signals which activate the defense system to remove disintegrated cells and repair injured cells. These functions are well conserved among most organisms and mammals ranging from bacteria to humans. Several common HSPs are listed in Table 14.2.

BCL2 [14.36]. Bcl2 (B-cell lymphoma 2) is a protein of 239 amino acids with molecular weight ~26 kDa. This protein is also known as *B-cell lymphoma protein 2*α and *apoptosis regulator Bcl2*. The molecule is composed of a Bcl2 homology (BH)1, BH2, and BH4 domain as well as a transmembrane domain. The Bcl2 gene is localized to gene locus 19q21.3. Bcl2 is expressed ubiquitously and is primarily found in the mitochondria.

The primary function of Bcl2 is to inhibit cell apoptosis. The anti-apoptotic effect of Bcl2 was initially found in studies with pro-B-lymphocyte cells. Overexpression of Bcl2 blocks the apoptosis of these cells. Overexpressed Bcl2 can also protect neurons from apoptosis by enhacing the expression of choline acetyltransferase during development, resulting in hypertrophy of the central nervous system. Transgenic mice with Bcl2 overexpression exhibit enhanced resistance to ischemic injury induced by cerebral artery occlusion as well as increased proliferation of monocytes. In contrast, the deficiency of Bcl2 in transgenic mice induces a marked decrease in the number of $CD4^+$ and $CD8^+$ lymphocytes shortly after birth, retardation of tissue and organ growth, and animal death within several weeks after birth. These observations suggest that Bcl2 plays a role in the regulation of not only cell survival but also organ tissue and organ development.

There are a number of proteins, including Bcl2, Bcl-x(L), Bcl-w, Bcl-x(S), Bad, and Bak, which exhibit a structure similar to that of Bcl2 and participate in the regulation of cell apoptosis. These proteins are defined as members of the Bcl2 family. A unique feature is that all Bcl2 family proteins contain BH1 and BH2 domains. Among the Bcl2 family members, some exert an antiapoptotic effect, such as Bcl2, Bcl-x(L), and Bcl-w, whereas others exerts a proapoptotic effect. It is interesting to note that the antiaoptotic proteins usually contain an N-terminal BH4 domain. In contrast, proapoptotic proteins usually contain a BH3 domain except for Bad.

AKT1 [14.37]. Akt1 is a serine/threonine protein kinase of 480 amino acids with molecular weight ~56 kDa and participates in the regulation of inflammatory and mitogenic activities. Akt1 is also classified as v-Akt murine thymoma viral oncogene homolog 1, oncogene Akt1, protein kinase B-alpha, and Rac serine/threonine protein kinase. The Akt1 protein is characterized by the presence of a pleckstrin homology (PH) domain, which is a sequence about 100 residues found in a wide range of proteins involved in intracellular signaling or as a constituent of the cytoskeleton, and a serine/threonine protein kinase (ST kinase) domain, which is a signature of serine/threonine kinases. Akt1 resides in the cytoplasm in quiescent cells. On stimulation by mitogenic factors, such as platelet-derived growth factor and insulin-like growth factor, Akt1 is activated and recruited to cell membrane by the phosphoinositide 3 kinase (PI3K), which is activated by platelet-derived growth factor receptor β and insulin-like growth factor receptor. The Akt1 gene is localized to the gene locus 14q32.3. Akt1 is expressed in the brain, heart, lung, thymus, liver, pancreas, kidney, intestine, placenta, ovary, prostate gland, spleen, and testis. The Akt1

TABLE 14.1. Characteristics of Selected Glutathione Peroxidase Isoforms*

Isoforms	Amino Acids	Molecular Weight (kDa)	Expression
GPX1	203	22	Sperm, alveolar macrophage, monocyte, red blood cells
GPX2	189	22	Liver, intestine, mammary gland
GPX3	225	25	Heart, lung, liver, kidney, eye, placenta, mammary gland
GPX4	196	22	Sperm
GPX5	221	25	Testis
GPX6	221	25	

*Based on bibliography 14.30.

TABLE 14.2. Characteristics of Selected Heatshock Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression
Heatshock 10-kDa protein (HSP10)	HSPE1, chaperonin 10 homolog, CPN10, GroES	102	11	Heart, uterus
Heatshock 27-kDa protein (HSP27)	HSPB2	182	20	Heart, skeletal muscle, skin
Heatshock protein (HSP40)	Dna J homolog subfamily B member 1, HSPF1, HDJ1, DNAJ1	340	38	Brain, heart, lung, liver, kidney, pancreas, skeletal muscle, placenta
Heatshock 60-kDa protein (HSP60)	Chaperonin, chaperonin 60 homolog, cpn60 homolog, CPN60, 60-kDa chaperonin, mitochondrial 60-kDa heatshock protein, GroEL homolog, mitochondrial matrix protein P1, P60 lymphocyte protein, and HuCHA60	573	61	Heart, adrenal gland, liver, kidney, pancreas, intestine, placenta, thyroid gland, retina, skeletal muscle, blood cells, macrophages
Heatshock 70-kDa protein 2 (HSP70-2)	Heatshock-related 70-kDa protein 2	633	70	Ubiquitous

*Based on bibliography 14.31–14.35.

protein kinase exhibits about 70% similarity in amino acid sequence to protein kinase A (PKA) and protein kinase C (PKC).

Akt1 is capable of regulating the activity of downstream molecules through phosphorylation. An example is Akt1-mediated activation of nuclear factor κB (NFκB). NFκB can be activated by inflammatory cytokines via the mediation of the IκB-kinase (IKK) complex composed of IKKα and IKKβ. Akt1 can phosphorylate IKKα, which in turn phosphorylates IκB and activates NFκB (see page 222). NFκB mediates cytokine-initiated immune and inflammatory responses. Akt1-induced activation of IκB-kinase has also been implicated in antiapoptotic events initiated by platelet-derived growth factor. Akt1 plays a critical role in the protection of cells from apoptosis. Furthermore, Akt1 can directly phosphorylate and activate nitric oxide synthase (NOS), which catalyzes the synthesis of nitric oxide (NO). Activated Akt1 induces an increase in basal NO release. Mutation of the NOS gene at Akt1 phosphorylation site results in a reduction in the responsiveness of NOS to Akt1 stimulation and a decrease in NO production. PIK3 is involved in the activation of Akt1 and NOS. In transgenic mouse models, the deficiency of Akt1 gene induces an increase in spontaneous apoptosis in the testis and thymus, in association with reduced spermatogenesis and enhanced thymocyte susceptibility to cell injury in response to irradiation, toxins, apoptotic ligands, and serum withdrawal. In contrast, the transfection of cells with an active form of Akt1 gene promotes cell proliferation and tumor development. These observations suggest that Akt1 is a mitogenic factor that mediates the signaling process of cell survival and proliferation.

CYTOKINES [14.38–14.52]. Cytokines are proteins produced and secreted by lymphocytes, monocytes, macrophages, and mast cells in response to immune and inflammatory stimuli. Fibroblasts and endothelial cells can also produce cytokines. These molecules are responsible for the regulation of immune responses, inflammatory reactions, and/or hematopoiesis via interacting with corresponding cell membrane receptors. There are several alternative names for cytokines. Cytokines produced by lymphocytes and monocytes are referred to as *lymphokines* and *monokines*, respectively. Cytokines that mediate cell chemotactic activities are known as *chemokines*. Cytokines produced by one cell type and acting on other cell types are known as interleukins.

There are several common features for cytokines:

1. A cytokine may be produced by multiple cell types. For instance, tumor necrosis factor (TNF)α can be produced by macrophages, mast cells, and natural killer (NK) cells. This mechanism ensures the production of sufficient cytokines for the regulation of immune or inflammatory reactions.
2. Different cytokines may exert a similar function. For instance, granulocyte–monocyte colony-stimulating factor (GM-CSF), interleukin (IL)1, IL2, IL3, IL4, and IL5 all initiate and promote cell proliferation and differentiation. This functional redundancy is another mechanism to provide sufficient cytokine activity in response to immune and inflammatory stimuli.
3. One type of cytokine, once secreted and activated, may stimulate the production of another cytokine, an effective mechanism for amplifying cytokine production and cell activation in response to the stimulation of immune and inflammatory factors.
4. Certain types of cytokine can act synergistically. For instance, IL2 and IL4 can coordinately regulate the proliferation of lymphocytes. On the other hand, certain

types can exert opposing effects. For instance, tumor necrosis factor induces cell apoptosis, whereas IL3 promote cell survival and proliferation. The characteristics of common cytokines are presented in Table 14.3.

Cytokines exert their effects via interacting with corresponding cell membrane receptors, which are functionally linked to the Janus tyrosine kinase (JAK)—signal transducers and activators of transduction (STAT) signaling pathways. The binding of a cytokine molecule to a cytokine receptor induces the activation of JAKs, which in turn phosphorylate STATs. Phosphorylated STATs serve as transcriptional factors and translocate to the nucleus, inducing gene expression and cellular activities. See Chapter 5 for signaling pathways and mechanisms of cytokines.

Enhancement of Angiogenesis [14.53]. The enhancement of angiogenesis is another major task for the treatment of cardiac infarction. Several proangiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) have been used for such a purpose (see page 600–610 for these factors). These factors have been shown to effectively promote neovascularization and improve the function of ischemic myocardium. In addition, it is beneficial to enhance the contractility of impaired myocardial cells in the infarct area. Methods described on page 595 can be used for such a purpose.

The therapeutic proteins described above can be directly delivered to the infarct area of the heart. Consecutive deliveries are usually required because growth factors are degraded rapidly. Alternatively, genes encoding selected growth factors can be delivered to the target sites of an infracted heart. The effectiveness of the transferred genes can last longer than that of injected proteins. For the past several years, a number of clinical trials have been carried out for treatment of cardiac infarction with gene transfer. Growth factor genes, including VEGF and FGF genes, have been used in these trials. Promising results have been obtained from these clinical trials. It is important to note that, for acute cardiac infarction, direct protein delivery may be more effective than gene transfer, because proteins can exert therapeutic effect immediately following delivery, whereas it requires 1–2 days for gene expression.

There are potential problems for the application of molecular engineering approaches to the heart. One of the problems is the promotion of cell proliferation and migration in atherosclerotic coronary arteries following the delivery of growth factor genes. Such an influence may enhance the progression of atherosclerosis, a major cause of cardiac infarction. A potential approach for solving such a problem is to use local condition-sensitive gene promoters, such as a promoter that can be controlled by local hypoxia or reduced oxygen concentration, a condition often associated with cardiac infarction. As shown in previous studies, the erythropoietin-responsive element (HRE) is a hypoxia-sensitive gene promoter. The incorporation of this promoter to the VEGF gene, carried by an adenovirus-associated viral vector, renders the VEGF gene hypoxia-sensitive; that is, it can be activated only under a reduced oxygen condition as seen in cardiac infarction. With such a controlling approach, the VEGF gene is not expressed in normal cardiac tissue with a physiological level of oxygen.

Cell Regenerative Engineering for Ischemic Heart Disease [14.54]. Cardiac infarction can be potentially treated with cellular engineering approaches, specifically, transplanting appropriate cell types into an infracted heart to replace dead or injured cells. Cellular

TABLE 14.3. Characteristics of Selected Cytokines*

Cytokines	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Colony-stimulating factor 1	CSF1, macrophage colony-stimulating factor 1, MCSF1, macrophage granulocyte inducer IM, MGI-IM	554	60	Lymphocytes, osteoclasts, microglia, astrocytes, bone marrow stromal cells, liver, and placenta	Promoting proliferation and differentiation
Erythropoietin	EPO	193	21	Bone marrow, spleen, liver, kidney, nervous system	Promoting erythroid differentiation and hemoglobin synthesis, protecting nervous system from injuries, and preventing apoptosis
IL1	IL1α, hematopoietin 1, IL1F1	271	31	Monocytes, macrophages, brain, skin, lung	Regulating inflammatory and immune processes, mediating hematopoiesis, inducing apoptosis, mediating osteoclastogenesis and gene polymorphisms, and inducing rheumatoid arthritis and Alzheimer's disease
IL2	T cell growth factor (TCGF), and aldesleukin	153	18	Leukocytes, bone marrow, brain, colon, thymus, kidney	Regulating T- and B-cell proliferation and mediating immune and inflammatory reactions
IL3	Multipotential colony-stimulating factor (multiCSF), hematopoietic growth factor, mast cell growth factor (MCGF), P-cell-stimulating factor	151	17	Eosinophils, brain, intestine	Promoting proliferation of hematopoietic cells, regulating cell differentiaon, preventing apoptosis, and mediating nerve development

TABLE 14.3. *Continued*

Cytokines	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
IL4	B-cell stimulatory factor 1 (BSF1), lymphocyte stimulatory factor 1, B-cell growth factor (BCGF), B-cell differentiation factor γ (BCDF γ)	153	17	T cells, basophils, eosinophils, mast cells	Regulating immune and inflammatory reactions and recruiting myoblasts during mammalian muscle growth
IL5	T-cell-replacing factor (TRF), eosinophil differentiation factor (EDF), B-cell differentiation factor 1	134	15	T cells	Regulating growth and differentiation of B cells and eosinophils, stimulating eosinophil maturation and activation, and contributing to asthma or hypereosinophilic syndrome
IL6	Interferon β2, IFNB2, B-cell differentiation factor, BSF2, hepatocyte stimulatory factor (HSF), hybridoma growth factor (HGF), B-cell stimulatory factor 2, 26-kDa protein	212	24	Monocytes, fibroblasts, B cells, brain, kidney, placenta, thymus, adipocytes	Mediating inflammatory reactions, regulating T-cell development and activity, inducing neuroendocrine cell proliferation and differentiation, and mediating hepatocyte proliferation and regeneration

IL7	Lymphopoietin 1, LP1, pre-B-cell factor	177	20	Thymus, bone marrow, intestine, skin, dendritic cells	Regulating B/T-cell development, promoting lymphocyte survival
IL8	Small inducible cytokine subfamily B member 8 (SCYB8), monocyte-derived neutrophil chemotactic factor (MDNCF), monocyte-derived neutrophil-activating protein, neutrophil-activating peptide 1 (NAP1), Granulocyte chemotactic protein 1 (GCP1), CXC chemokine ligand 8 (CXCL8), T-cell chemotactic factor, lymphocyte-derived neutrophil-activating factor (LYNAP), protein 3-10C, neutrophil-activating factor (NAF), emoctakin	99	11	Monocytes, macrophages, lymphocytes, bone marrow, intestine, kidney, placenta	Mediating inflammatory response, stimulating angiogenesis, and inducing chemotaxis

TABLE 14.3. *Continued*

Cytokines	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
IL9	T-cell/mast cell growth factor P40, P40 T-cell and mast cell growth factor, P40 cytokine, TCGF III, MEA	144	16	T cells	Stimulating cell proliferation, preventing apoptosis, inducing inflammatory reactions, and contributing to pathogenesis of asthma
IL10	Cytokine synthesis inhibitory factor (CSIF), mast cell growth factor III, B-cell-derived T-cell growth factor (B TCGF)	178	21	Manocytes, T cells, macrophages, epidermal cells.	Downregulation of cytokine expression in Th1 cells and macrophages, enhancement of B-cell survival, proliferation, and antibody production; also inhibition of NFκB activity
IFNα	α interferon, Interferon α1, IFN, interferon leukocytic, IFN leukocytic, interferon, αD, LeIF D	189	22	Leukocytes	Activating natural killer cells, exerting antiviral inflammatory reactions, and suppressing tumor cell growth
IFNβ	IFN β1, interferon fibroblast (IFF), β interferon, IFB, IFNB, fibroblast interferon	187	22	Leukocytes	Exerting antiviral and antiproliferative activites, regulating immune and inflammatory reactions, and suppressing tumor cell proliferation

*Based on bibliography 14.38–14.52.

engineering approaches are used to achieve three goals: replacement of malfunctioned myocardiocytes, induction or stimulation of angiogenesis, and delivery of therapeutic agents for the prevention of myocardiocyte death.

Replacement of Malfunctioned Cardiomyocytes. A number of cell types have been tested and used for the replacement of dead and severely injured myocardial cells. These cell types include embryonic stem cells, fetal cadiomyocyte progenitor cells, bone marrow myocyte progenitor cells (chapter-opening figure), and adult cardiomyocyte stem cells. Embryonic stem cells from blastocyst, as described on page 381, are capable of differentiating into all specialized cell types, including cardiomyocytes, under appropriate developmental conditions. These cells are primary candidates for cardiac cell transplantation. Fetal cadiomyocyte progenitor cells can be collected from the mesoderm, where the heart is generated. These are committed cells that are to be differentiated into cardiomyocytes and are excellent candidates for cardiac cell transplantation. The bone marrow contains various types of progenitor cell, including mesenchymal stem cells, which contain cardiomyocyte progenitor cells. These cells can be enriched based on cell surface markers, such as Sca1 and c-Kit, by fluorescence-activated cell sorting (FACS) or magnetic bead-mediated cell sorting. The bone marrow progenitor cells can differentiate into cardiomyocytes in vitro and in vivo (Fig. 14.8). It is interesting to note that, under appropriate culture conditions, mesenchymal stem cells can transform into beating cardiomyocytes. The transplantation of bone marrow cells into models of cardiac infarction significantly improves the performance of the injured heart. A number of clinical trials have been conducted for the treatment of myocardial infarction by transplantation of bone marrow cells. These studies have shown that such a cellular therapy results in a reduction in the infarct size and improvement of cardiac perfusion and function in patients with late-stage ischemic heart disease.

The adult heart contains undifferentiated adult stem cells and progenitor cells, which are capable of proliferating and differentiating into cardiomyocytes, endothelial cells, and

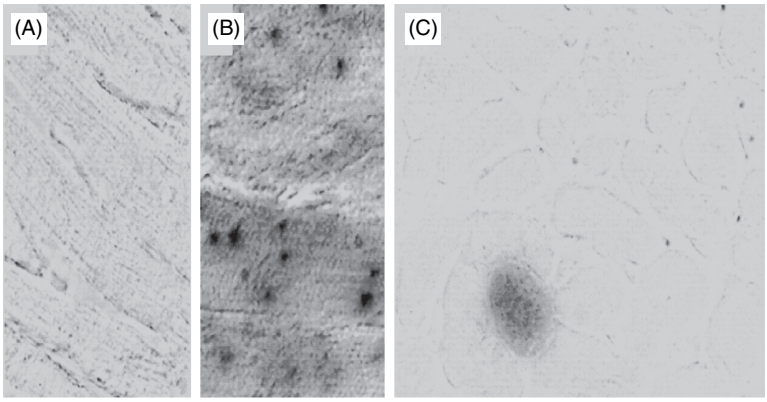

Figure 14.8. Incorporation of bone marrow side population (SP) cells into cardiomyocytes. (A) Negative control: C57Bl/6 cardiac tissue stained for lacZ expression. (B) Positive control: C57Bl/6-Rosa26 cardiac tissue stained for lacZ expression. (C) Cross section of a heart from an SP cell transplant recipient, which received an infarct. (Reprinted by permission from Macmillan Publishers Ltd.: Orlic D et al: Bone marrow cells regenerate infarcted myocardium, *Nature* 410:701–5, copyright 2001.)

vascular smooth muscle cells, although the density of these cells is extremely low. These cells can be enriched by FACS or magnetic bead-mediated cell sorting based on cell surface markers as described above for bone marrow cell enrichment. The transplantation of adult cardiomyocyte stem and progeniotor cells into infarcted heart in animal models significantly improves the cardiac performance. The discovery of adult cardiac stem cells has changed the traditional view that the heart contains finally differentiated cells, which cannot regenerate, and will greatly facilitate the development of cardiac cell regenerative engineering.

Although cardiac cell regenerative engineering is successful in experimental investigations and certain clinical applications, there are still problems. A major problem is immune rejection reactions in response to the transplantation of allogenic embryonic stem cells, fetal progenitor cells, and adult stem cells. This problem may be overcome by using autogenous bone marrow-derived progenitor cells. Another problem is cell death after transplantation. Many transplanted cells die within a short period. The maintenance of cell survival remains a challenge in cell regenerative engineering. A potential approach is to transfer growth factor genes into cell candidates for transplantation. Such an approach has been shown to reduce the rate of cell death.

Enhancement of Angiogenesis. Another strategy for treating myocardial infarction is to stimulate angiogenesis in the ischemic areas by transplant angiogenic cells. Potential cell types include the bone marrow endothelial progenitor cells. These cells originate from the bone marrow and are characterized by the expression of a number of endothelial lineage markers, including von Willebrand factor, VE-cadherin, Flk-1 (vascular endothelial growth factor receptor 2), PECAM1, CD34, and E-selectin. These cells can be isolated from the bone morrow or peripheral blood and transplanted into an ischemic heart via direct injection or intravenous delivery for therapeutic purposes. The transplanted cells may participate in the process of angiogenesis in injured and infarcted areas, contributing to the neovascularization and recovery from cardiac infarction (Fig. 14.9).

As a general strategy, cells selected for therapeutic purposes can be engineered for augmentation of specified properties by gene transfer. Cytoprotective genes (e.g., Bcl2, Akt1, and growth factor genes) and angiogenic genes (e.g., VEGF and Flk-1) can be transferred into candidate cells in vitro. At the time of maximal gene expression (usually 2–3 days), the cells can be transplanted to the target tissues. For myocardial infarction, such an approach may be more important than direct delivery of therapeutic genes into injured cardiomyocytes. The reason is that cardiomyocytes are either injured or dead in cardiac infarction. Even though therapeutic genes are delivered to the infarction site, the injured cardiomyocytes may not be able to express the delivered genes efficiently. Genetically engineered cells can release proteins encoded by the transferred genes, such as anti-apoptotic factors and angiogenic factors. These factors in turn promote the survival of cardiomyocytes and angiogenesis, respectively.

Tissue Regenerative Engineering for Ischemic Heart Disease [14.55]. Ischemic heart disease is characterized by the presence of regional cardiac injury and/or infarction. In severe cases, cardiomyocyte death occurs in large areas, often resulting in acute heart failure. To reduce the effect of cardiac injury and infarction, a tissue engineering strategy is to construct a cardiac tissue scaffold and implant the scaffold to the inury site of the heart. The cardiac scaffold can be constructed with the integration of various cell types, such as cardiac stem cells, neonatal cardiomyocytes, fibroblasts, or vascualr smooth

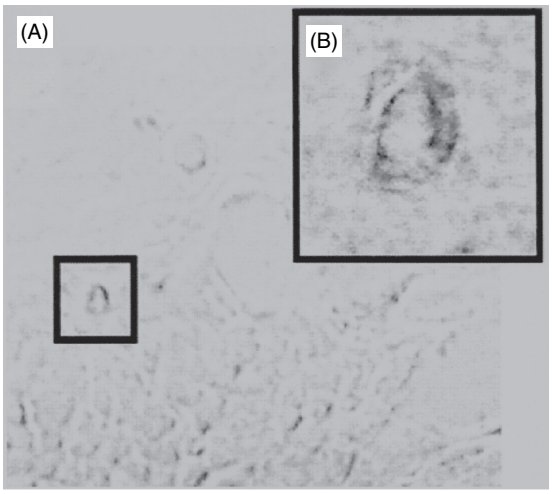

Figure 14.9. Incorporation of bone marrow side population (SP) cells into vascular endothelial cells. (A) X-gal-stained section of cardiac tissue from an infarcted SP cell transplant recipient. Panel B shows magnification of the indicated capillaries from panel A. (Reprinted with permission from Jackson KA et al: Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells, *J Clin Invest* 107:1395–402, copyright 2001.)

muscle cells. The cardiac stem and progenitor cells can potentially transform to cardiomyocytes, whereas the fibroblasts and smooth muscle cells can produce mitogenic factors, which enhance the survival of injured cardiac cells and stimulate the transformation of stem/progenitor cells into cardiomyocytes. Furtherore, various mitogenic factors, such as fibroblast growth factor and vascular endothelial growth factor, can be integrated into the constructed cardiac scaffold. These mitogenic factors can directly promote the survivcal of injured cardiomyocytes and enhance the differentiation of cardiac stem and progector cells into functional cardiomyocytes. Alternatively, the integrated cells can be transfected with growth factor genes to enhance the expression of growth factors. These approaches have been tested in experimental models. These investigations have demonstrated the potential of applying tissue regenerative engineering approaches to cardiac therapy.

Valvular Diseases

Pathogenesis, Pathological Changes, and Clinical Features. Valvular diseases are a group of disorders that occur in the mitral, tricuspid, aortic, and pulmonary valves, are often caused by inflammatory reactions, and are characterized by structural distortion, calcification, and mechanical stiffening of the valves in association with altered hemodynamics in the atrial and ventricular chambers and remodeling of the atrial and ventricular structure and function. The pathogenesis of the valvular diseases is closely related to rheumatic fever, which is possibly a result of group A streptococcal infection. Rheumatic fever often occurs several days after acute streptococcal infection and involves the heart, joints, and nervous system. Antibodies developed in response to the stimulation of antigens from streptococci have been shown to cross-react with components of cardiac valves. Thus, autoimmune reactions are a potential cause for the inflammatory responses in the

cardiac valves. Although all valves are susceptible to rheumatic fever, the mitral valves are most frequently involved. Several pathological changes can be found in involved valves, including fibrosis, thickening, calcification, fusion, distortion, and shortening of the valves.

Valvular diseases can be classified into two types according to the form of the disease: valvular stenosis and regurgitation. Each type may occur in either of the valves. The most common types are mitral valve stenosis and regurgitation. While all types of valvular disease influence the performance and hemodynamics of the heart, the type and severity of cardiac malfunction and associated clinical consequences are dependent on the location of the involved valves and the degree of the structural and geometric changes.

Mitral stenosis is a form of valvular disease characterized by the narrowing of the mitral orifice resulting from mitral valve fibrosis, fusion, and stiffening. Mitral stenosis exhibits a sequence of pathophysiological changes. The resistance of the mitral orifice to bloodflow increases and the rate of bloodflow from the left atrium to the left ventricle decreases during the diastole. When bloodflow is reduced to a rate below the critical level required for filling the left ventricle, the left ventricle receives insufficient blood volume during the diastole and is thus unable to pump sufficient blood into the arterial system. The physical activities of patients are often limited due to the lack of arterial bloodflow. In severe cases, patients often exhibit orthopnea and paroxysmal dyspnea. In regions above the mitral valves, an excessive amount of blood accumulates in the left atrium and the pulmonary veins, raising pulmonary venous and capillary pressure. To a certain level, pulmonary edema occurs due to an increase in the capillary transmural pressure. Pathophysiological changes in tricuspid stenosis are similar to those described above except that the changes occur in the right heart and blood accumulation occurs in the right atrium and the systemic veins.

Mitral regurgitation is a valvular disease characterized by incomplete closure of the mitral valves during systole due to fibrosis, distortion, and stiffening of the mitral valves. As a result, blood flows back from the left ventricle to the left atrium during the systole, leading to a reduction in blood ejection into the arterial system and excessive expansion of the left atrium. The reduction in systemic arterial bloodflow results in limited physical activities. To compensate the reduction in arterial bloodflow, the sympathetic system is usually activated, inducing an increase in the heart rate and contractility. The heart also undergoes adaptive remodeling in structure, resulting in an increase in its myocardial mass, a process referred to as cardiac hypertrophy. Long-term hypertrophy may lead to left heart failure. The increase in atrial blood volume induces an elevation in pulmonary capillary blood pressure, which is a common cause of pulmonary edema. Similar changes can be found in tricuspid valve regurgitation in the right heart.

Aortic valve stenosis is characterized by the narrowing of the aortic orifice and is induced by the fibrosis, fusion, and stiffening of the aortic valves. These changes result in an increase in the resistance of the aortic orifice to bloodflow, a decrease in aortic bloodflow, and thus an increase in workload for the left ventricle during the systole. The left ventricle adapts to these changes by increasing its contractile strength and mass, which often leads to ventricular hypertrophy. When the left ventricle is capable of compensating for the decrease in arterial bloodflow, there may be no apparent clinical symptoms. However, in a severe case of aortic stenosis, the left ventricle is not capable of overcoming the aortic orifice resistance. Left heart failure occurs due to left ventricular hypertrophy and/or excessive workload. Similar changes are observed in pulmonary valve stenosis in the right heart.

Aortic regurgitation is characterized by incomplete closure of the aortic valves during the diastole due to fibrosis, distortion, shortening, and stiffening of the aortic valves. As a result, there is backward bloodflow from the aorta to the left ventricle during the diastole. Pathophysiological changes include a reduction in arterial bloodflow toward the peripheral systems during the diastole, an increase in diastolic filling volume in the left ventricle, and an increase in workload for the left ventricle. The left ventricle adapts to these changes by increasing its contractile strength, cardiac muscle mass, and chamber size, resulting in daptive ventricular hypertrophy and dilation. During the end-stage, left heart failure and shortage of arterial bloodflow are often observed. Pulmonary regurgitation exhibits similar changes in the right heart.

Treatment. Surgical correction and replacement of malfunctioned cardiac valves are the most effective approaches for the treatment of valvular diseases. For mitral stenosis, the narrowed mitral orifice can be widened surgically via a procedure known as *mitral valvotomy*. Stenosis in the tricuspid, aortic, and pulmonary valves can be treated with a similar approach. For valvular regurgitation, including mitral, tricuspid, aortic, and pulmonary regurgitation, valve replacement with a prosthesis is usually required, especially when malfunction occurs in the left or right ventricle. In special cases with ruptured chordae or flail leaflets, it is usually necessary to conduct valve reconstruction.

Artificial Cardiac Valves [14.56]. Cardiac valve prostheses have been developed for the replacement of malfunctioned mitral, tricuspid, aortic, and pulmonary valves. Since the first case of aortic valve replacement in 1952 by Dr. Charles Hufnagel and colleagues, cardiac valve replacement has become a common treatment for severe valvular diseases. There are two major types of cardiac valve prostheses: mechanical and tissue valves. A *mechanical valve* is composed a frame and a ball- or disk-shaped valve. The ball or disk opens and closes depending on the pressure gradient across the device during a cardiac period (systole or diastole). According to the shape of the valve, mechanical valves are classified into several subtypes: ball-and-cage valves (such as the Star-Edwards ball-and-cage valve, Smeloff–Cutter valve, and Magovern valve), caged disk valves (the Kay–Shiley and Beall valves), tilting disk valves (the Bjork–Shiley tilting disk valve, Medtronic-Hall valves), and bileaflet valves (the St. Jude bileaflet valve, CarboMedics bileaflet valve, and parallel bileaflet valve). The ball, tilting disk, and bileaflet valves have been used for replacing all types of valves, and the disk valves have been used primarily for replacing the mitral and tricuspid valves.

The mechanical valves, although strong in material, are problematic in several aspects: (1) these devices stimulate blood coagulation and thrombogenesis—it is necessary to administrate anticoagulants following valve replacement, (2) mechanical valves induce blood flow disturbance, and (3) these devices may induce blood cell damage. To overcome these problems, natural tissue-based cardiac valves have been developed and tested. Major types include autogenous valves (using the pulmonary valves for replacing the aortic valves of the same patient), allogenic valves (collected from human cadavers), and glutaraldehyde-treated zenogeneic tissue valves (porcine valves and calf pericardium-based valves). Overall, these valves exhibit improved performance and hemodynamics compared with mechanical valves. However, there are potential problems. While the autogenous valves are ideal, the source of the valves is limited and the removal of the pulmonary valves obviously influences the function of the right heart. Allogenic valves often cause immune responses and undergo progressive degradation and leaf wear, reducing the

lifespan of the valves. These valves also cause blood coagulation and thrombosis. Glutaraldehyde-treated zenogeneic valves, although exhibiting improved material strength, cause blood coagulation, thrombosis, and valve calcification. These problems remain to be resolved.

Tissue-Engineered Cardiac Valves [14.57]. Tissue-engineered valves are valves constructed with synthetic polymers or extracellular matrix constituents with seeded stem or somatic cells. Because of the presence of living cells, it is expected that these valves may adapt to the physiological environment, integrate into the host tissue, and maintain functions for a longer time than mechanical and tissue-based valves. Because cardiac valves are subject to dynamic movements and fluid shear stress, there are several issues that ought to be considered specially: (1) the material for constructing the valve frame must be mechanically strong, flexible, and durable; (2) the construction material should be antiinflammatory and thrombosis-resistant; (3) the cell type selected for seeding should be able to survive under dynamically moving conditions and withstand the influence of fluid shear stress; and (4) the material and cells should be non-immunogenic.

Several tissue-engineered cardiac valve designs have been developed and tested in experimental models. These include biodegradable polymer valves based on polyglycolic acid and polyhydroxyoctanoates, as well as zenogeneic valve matrix seeded with cells. Preliminary studies have provided promising results. Cells seeded in the valve frame are able to grow under flow conditions in vitro. When grafted into the heart of host animals, these valves can carry out normal valve functions and maintain pressure gradients across the valves. However, results from a small clinical trial are disappointing. Tissue-engineered porcine heart valves grafted into the human heart induce inflammatory reactions and thrombosis. Valve failure occurs due to valve rupture and degeneration. These human trial studies provide insights into future design and improvement of cardiac valves.

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