# CHAPTER II
# LITERATURE REVIEWS

## 1. Diabetes Mellitus and Complications

Diabetes mellitus, an important chronic non-communicating disease, can occur as the result of the inability to produce enough of insulin or as the result of the inability to use insulin effectively. It has been regarded as a major epidemic nowadays. Recent global status report on non-communicable diseases of the World Health Organization has estimated that the global prevalence of diabetes mellitus was about 9% among adults aged 18+ years (World Health Organization [WHO], 2014).

Diabetes has been reported to be associated with numerous complications. The diabetic complications are divided in to 2 groups the microvascular disorders which involve the damage of small blood vessels and macrovascular disorders which are associated with the damage of large blood vessels. The important microvascular complications are eye disease or "retinopathy", kidney disease termed "nephropathy," and neural damage or "neuropathy", whereas macrovascular complications are myocardial infarction and stroke (Forbes and Cooper, 2013).

It has been demonstrated that hyperglycemia can produce deleterious effects and can induce vascular complications by various mechanisms including activation of the polyol and hexosamine pathways, activation of protein kinase C, increased oxidative stress, increased production of advanced glycation end-products, increased synthesis of growth factors, cytokines and angiotensin II. These changes in turn induce a diffuse endothelial dysfunction and contribute to the progressive development of micro- and macrovascular complications and multi-organ damage (Chiarelli and Marcovecchio, 2013).

### 1.1 Polyol pathway and diabetic complications

When glucose concentration becomes too high, the unused glucose is increased giving rise to the increased activity of polyol pathway. This pathway comprises of two steps. The first step will convert nonphosphorylated glucose to sorbitol by aldose reductase (AR) and the second step is associated with the change of sorbitol to

fructose by sorbitol hydrogenase (SDH). Since AR has a high capacity and a low affinity for glucose while SDH has a high affinity and a low capacity for sorbitol, glucose flux from this pathway is low (Hotta, 1997). In addition, sorbitol oxidation is relatively independent of the sorbitol concentration within the physiological range (Gabbay, 1972; Travis et al., 1974). Therefore, sorbitol accumulates in complication-prone tissues which show high capacity for polyol pathway enzymes and in which glucose entry is not rate-limiting for glycolysis or mediated by insulin. Once sorbitol has been produced, it does not easily diffuse across cell membranes (Greene et al., 1985) and may contribute a role on the pathophysiology of diabetic complications. In addition, NADPH, an essential cofactor for regenerating a critical intracellular antioxidant such as reduced glutathione, is decreased due to the consumption of AR. Therefore, the amount of reduced glutathione is decreased resulting in the increased susceptibility to intracellular oxidative stress (Lee and Chung, 1999).

### 1.2 Advanced glycation end products (AGEs) and diabetic complications

AGEs are the heterogeneous group of molecules which are complex and often unstable. They can be produced from the non-enzymatic reaction of reducing sugars such as glucose with free amino groups of proteins giving rise to the generation of small amounts of stable Amadori products through Schiff base adducts. The production of AGEs is constant and slowly in the normal body, starting in early embryonic development, and accumulate with time. However, their productions are accelerated in diabetes due to the increased availability of glucose (Ahmed, 2005). It has been reported that the extent of protein binding by Amadori products which in turn resulting in AGEs production is proportional to the degree and duration of hyperglycemia (Vlassara, 1996). According to this binding, cells become stiffer, less pliable and more subject to damage and premature aging. In addition, AGEs will interact with their receptors (RAGE) on macrophages giving rise to the elevation of oxidative stress and activation of nuclear factor-$\kappa$B (NF-$\kappa$B) via mitogen-activated protein (MAP) kinase signalling pathway (Yan et al., 1994).

### 1.3 Protein kinase C (PKC) activation and diabetic complications

PKCs are widely distributed in mammalian tissues and responsible for the phosphorylation of various target proteins. The conventional PKC (cPKC) isoforms are activated by phosphatidylserine, calcium, and DAG or phorbol esters such as phorbol

12-myristate 13-acetate (PMA) (Geraldes and King, 2010), whereas novel PKCs (nPKCs) are activated by phosphatidylserine, DAG or PMA, but not by calcium. The atypical PKCs (aPKCs) are not activated by calcium, DAG or PMA. In addition, PKCs can also activated by oxidative stress such as $H_2O_2$ and mitochondrial superoxide induced by elevated glucose levels (Newton, 2003; Steinberg, 2008; Corbalan-Garcia and Gomez-Fernandez, 2006; Churchill et al., 2009; Konishi et al., 1997; Nishikawa et al., 2000). Evidence also suggests that the enhanced activity of PKC isoforms could also result from the interaction between AGEs and their cell-surface receptors (Derubertis and Craven, 1994). The increased activity of PKC can decrease NO production in smooth muscle cells (Ganz and Seftel,2000)and inhibit insulin-stimulated expression of eNOS in cultured endothelial cells (Kuboki et al.,2000) resulting in vascular dysfunction and finally leading to the formation of diabetic complications.

### 1.4 Oxidative stress and diabetic complications

It has been reported that hyperglycemia-induced the increase in electron transfer donors (NADH and FADH2) and increases electron flux through the mitochondrial electron transport chain leading to an increase of the ATP/ADP ratio and hyperpolarization of the mitochondrial membrane potential. This high electrochemical potential difference generated by the proton gradient giving rise to partial inhibition of the electron transport in complex III, resulting in an accumulation of electrons to coenzyme Q. This change in turn drives partial reduction of $O_2$ to generate the free radical anion superoxide (Nishikawa et al., 2000; Brownlee, 2001). It is this accelerated reduction of coenzyme Q and generation of reactive oxygen species (ROS). In addition, the over-expression and activity of mitochondrial inner membrane uncoupling proteins (UCPs) contribute to an increase in superoxide formation under diabetic conditions (Rolo and Palmeira, 2006). Since oxidative stress can produce deleterious effects to various substances and organelles, it is believed to be the fundamental source for mitochondrial dysfunction that plays a critical role in diabetes-related metabolic disorders and tissue histopathology (Matough et al., 2012).

# 2. Nerve Injury

## 2.1 Classification of nerve injury

Nerve injury classification is derived from the damage persistent by the nerve components, nerve function, and the ability for spontaneous recovery (Greenfield, 1997; Grant et al., 1999; Ristic, 2000). According to Seddon's 3-grade classification system, nerve injury is categorized into 3 grades (Grant et al., 1999).

Neurapraxia is an injury which decreases or completely blocks the conduction across a segment of a nerve while the continuity of axon is still preserved (Colohan, 1996; Grant et al., 1999; Trumble, 2000). This condition can induce dysfunction and/or paralysis without the loss of endoneurium, perimysium and epimysium (Schwartz, 1999; Ristic, 2000). This is no Wallerian degeneration in this type of nerve injury. However, sensory-motor problems are presented distal to the site of injury. The conduction is also intact in the distal segment and proximal segment, but no conduction occurs across the area of injury. The recovery of nerve conduction deficit is full, and requires days to weeks. (Grant et al., 1999).

The second type of nerve injury is axonotmesis which is more severe than neurapraxia. Axonotmesis occurs as a result of damage to the axons with preservation of the neural connective tissue sheath (endoneurium), epineurium, Schwann cell tubes, and other supporting structures (Colohan et al., 1996; Grant et al., 1999; Trumble, 2000). Therefore, the internal architecture is still preserved (Schwartz, 1999). This can direct proximal axonal regeneration to reinnervate distal target organs (Colohan, 1996; Greenfield, 1997). However, distal Wallerian degeneration can occur in axonotmesis (Ristic, 2000). Both sensory and motor deficits are also observed at the area distal to the site of lesion. In addition, no nerve conduction is observed around 3 to 4 days after injury at the area distal to the site of injury. The recovery and axonal regeneration occurs and recovery is possible without surgical treatment.

The most severe grade of peripheral nerve injury is neurotmesis which occurs when the axon, myelin, and connective tissue components are damaged and disrupted or transected (Greenfield, 1997; Schwartz, 1999; Ristic, 2000). The recovery through axonal regeneration cannot occur. Therefore, surgery is required for the recovery.

After nerve injury, all of the signals that relate to signal transduction are required to initiate nerve regeneration. When a nerve is transected, there are

several steps in the signal pathways that occur in the neurons, Schwann cells and the target tissues. Under the normal circumstance, signal transduction process occurs via the secondary messengers in the cells giving rise to the activation of proteins and can modify the cellular processes and gene transcription. After the transection of axon, various signal transduction pathways are activated to initiate the axon in the proximal stump to form growth cone for recovery process and the distal tip of the transected axons is sealed rapidly (Geddis and Rehder, 2003). The leftovers of the distal part of transected axons, including myelin debris, are digested by proliferating Schwann cells and invading macrophages (Hirata and Kawabuchi, 2002). It has been reported that high concentration of reactive oxygen species (ROS) such as superoxide, hydroxyl radicals and hydrogen peroxide are released by the oxidative burst of macrophages. The elevation of ROS can induce oxidative damage to proteins, lipids and nucleic acid. Recently, ROS has also been shown to induce DNA damage leading to neurodegeneration (Jerome et al., 2005).

### 2.2 Nerve recovery process

Following the severe crush injury, the first step of regeneration is the division of Schwann cells, and then they bridge the scar. In the second step, a nerve processes sprout from the proximal stump. At this step, the Schwann cell bridges act as guides for the regenerating axons to grow across the scar, hence maintaining the normal pathways of the growing axons. Even though many of the new nerve processes degenerate during regeneration process, their large number increases the chance of reestablishing sensory and motor connection. Axon regeneration occurs at a rate of 1-3 mm/d. This rate is corresponding with the slow rate of transport of the cytoskeletal material. After crossing the scar, neurites enter the surviving Schwann tubes in the distal stump. These tubes will guide the neurites to their destination as well as provide the microenvironment for continued growth (Michael, 2002).

During recovery process after nerve injury, nerve growth factor (NGF) has played an important role in the survival and maintenance of neuron as well as to stimulate the outgrowth of neurites. Consistent with the nerve growth factor production, the macrophages that remove myelin after nerve injury may also secrete Schwann cell mitogens, such as platelet derived growth factor and fibroblast growth factor to elicit growth cone formation of axon. Then, the axon processes sprout into

the Schwann cell tubes of proximal stump to reinnervate the target organs again (Sun and Zigmond, 1996). During nerve regeneration process endogenous antioxidants or oxidative stress response proteins like catalase, metallothioneins, uric acid, SOD and hemoxygenase were released to protect against neuroinflammation (Jerome et al., 2005). Not only oxygen free radicals just mentioned, but also reactive nitrogen species such as nitrogen oxide (NO) that can induce neuronal apoptosis and neurodegeneration (Estevez et al., 1998).

## 3. Sciatic Nerve

Sciatic nerve, the largest and longest nerve in the body, originates from the lumbo-sacral plexus, arising from the ventral rami of L4 through S3 (Figure 2-1). The sciatic nerve passes through the inferior part of the greater sciatic foramen and is the most lateral structure emerging inferior to the piriformis muscle. The sciatic nerve runs inferolaterally under the cover of the gluteus maximus, midway between the greater trochanter and ischial tuberosity. It descends along posterior aspect of thigh. Unfortunately, sciatic nerve supplies no structure in the gluteal region. It supplies the skin of the foot, most of the leg, posterior thigh muscles, and all leg and foot muscles. It also supplies articular branches to all joints of the lower limb. The sciatic nerve is resulted from the binding tibial and common peroneal nerves that are in the same connective tissue sheath. The tibial nerve supplies flexor muscles and the common peroneal nerve supplies extensor and abductor muscles.

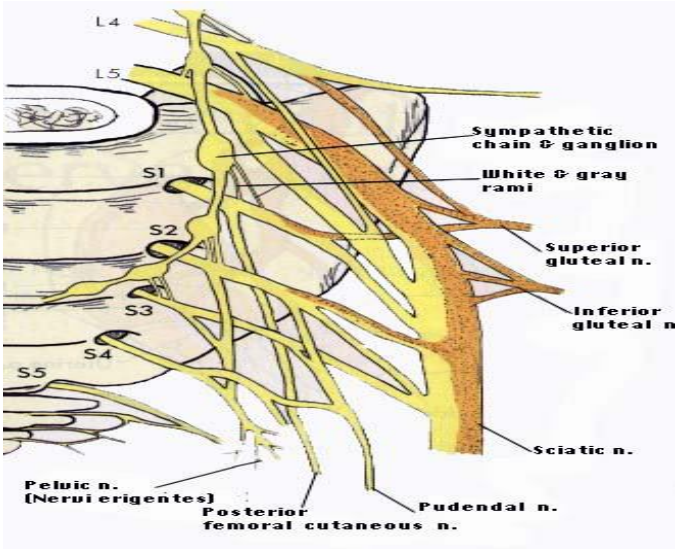


**Figure 2-1** Sciatic nerve

## 4. Free Radicals and Antioxidants

A free radical is referred to any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily produced when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron (Karlsson, 1997). Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). There are numerous types of free radicals that can be formed within the body. The most common ROS include: the superoxide anion ($O^{2-}$), the hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide ($H_2O_2$). Hydroxyl radical is short-lived, but it is the most deleterious radical within the body. Free radicals have been implicated to play a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease, and Parkinson's disease. Previous study showed that high concentration of reactive oxygen species usually retarded the functional recovery of injured nerve. They also have capabilities to damage protein, lipids, DNA and induce nerve degeneration (Jerome et al., 2005). Not only reactive oxygen species but nitrogen oxide is also a free radical that can induce neuronal apoptosis and nerve degeneration (Estevez et al., 1998). The free radicals also include hydrogen atom (one unpaired electron), most transition metals and the oxygen molecule. The transfer to an oxygen molecule yields the superoxide anion ($O^{2-}$). Superoxide dismutase (SOD) has a central role in the defense against oxidative stress (Beyer, et al., 1991; Bowler et al., 1992; Scandalias, 1993). SOD is now known to catalyse the dismutation of superoxide to hydrogen peroxide and oxygen.

$$2O^{2-} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

Hydrogen peroxide is converted to water by the catalase enzyme

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

Superoxide dismutase can transform superoxide anion into hydrogen peroxide that could be converted to water by glutathione peroxidase and using the co-enzyme glutathione (GSH)

$$2GSH + H_2O_2 \xrightarrow{GSH} GSSG + 2H_2O$$

Free radicals and oxidative stresses that have been generated can be scavenged by antioxidant system either from endogenous or from exogenous sources such as from fruits and vegetables. Two lines of antioxidant defense including enzymatic and non-enzymatic systems are presented in the cell. During nerve regeneration, the body released endogenous antioxidants such as catalase, metallothioneins, uric acid, superoxide dismutase and hemoxygenase to buffer the level of free radicals. These can reduce inflammation and improve nerve regeneration and recovery capacity (Jerome et al., 2005).

## 5. Blood-Nerve Barrier (BNB)

Blood–tissue barriers play an essential role in the maintenance and homeostasis of organs or tissue environments. Barriers such as the blood–brain barrier (BBB), blood–cerebrospinal fluid barriers, blood–nerve barrier (BNB), and blood–retinal barrier (BRB) consist of endothelial or epithelial cells are sealed by tight junctions. A selective transport system localized in the cells of the barrier provides substances needed by the cells inside the barrier (Takata, 1997).

The axonal environment in the endoneurium is isolated from the general extracellular space of the body by a diffusion barrier called the blood–nerve barrier (BNB) (Ortiz-Hidalgo, 1997). BNB is the barrier between the perineurium of peripheral nerves and the endothelium (endothelium, vascular) of endoneurial cappillaries. Tight junctions between the endothelial cells in the endoneurial blood vessels, and the junction between the perineurial cells in the perineurium are responsible for barrier functions. Tight junctions are intercellular junctions that play two distinct roles in selective permeability barrier functions (Anderson, 1999). A barrier seals neighboring cells together in a sheet to prevent the leakage of molecules between them, and to prevent the diffusion of membrane proteins between the apical and basolateral regions of the

plasma membrane. The perineurium also acts as a diffusion barrier, which limits entry of blood-borne, water-soluble substances into the endoneurial compartment and maintains the homeostasis of the endoneurial environment (Waksman, 1961). It has been found that ion permeability at the BNB is still greater than at the BBB (Virtualhealthlibrary, 2006).

The defect of the BNB induces edema in the endoneurium can increase the vulnerability of the peripheral nervous system to harmful agents. Crush injury has been shown to induce BNB impairment (Motte, 1976). The breakdown of BNB occurred simultaneously with the introduction of substances needed for nerve regeneration in the entire endoneural space of the degenerating nerve. The arrival of regenerating axons allows the recovery of BNB. This recovery parallels the growth of the axons but occurs with a delay of one week. BNB breaks simultaneously and the restoration of BNB begins from proximal to distal.

## 6. Diabetic Wound

Diabetics have a higher risk for amputation than the general population which greater than 15% due to chronic ulcers (Robert et al., 2005). In DM systemic illnesses, poor circulation, neuropathy, difficulty moving, age, and repeated trauma are factors that contribute to chronic wounds. Diabetes can induce neuropathy which in turn disturbs nociception and the perception of pain (Robert et al., 2005). Thus patients may not initially notice to a small wound at leg or foot, and may therefore fail to prevent infection or repeated injury (Kathleen, 2005). Moreover, diabetes causes immune compromise and damage to small blood vessels, preventing adequate oxygenation of tissue, which delay healing process and can cause chronic wounds (Kathleen, 2005). The insensate, poorly perfused foot is at risk for ulcers from pressure necrosis (Mustoe et al., 2004) or inflammation from repeated skin stress and unnoticed minor trauma. These can evolve into cellulitis, osteomyelitis, or nonclostridial gangrene and end in amputation.

Ischemia can also induce inflammation and triggers the release factors that attract neutrophils such as interleukins, chemokines, leukotrienes, and complement factors (Mustoe et al., 2004). During fighting with the pathogens, neutrophils also release inflammatory cytokines and enzymes that damage cells (Robert et al., 2005;

Mustoe et al., 2004). One of their important jobs is to produce ROS to kill bacteria, for which they use an enzyme called myeloperoxidase (Mustoe et al., 2004). The enzymes and ROS produced by neutrophils and other leukocytes damage cells and prevent cell proliferation and wound closure by damaging DNA, lipids, proteins, (Alleva et al., 2005) the ECM, and cytokines that speed healing (Mustoe et al., 2004). Neutrophils remain in chronic wounds for longer than they do in acute wounds, and contribute to the fact that chronic wounds have higher levels of inflammatory cytokines and ROS. (Taylor et al., 2005; Schönfelder et al., 2005). Since wound fluid from chronic wounds has an excess of proteases and ROS, the fluid itself can inhibit healing by inhibiting cell growth and breaking down growth factors and proteins in the ECM. (Robert et al., 2005).

Infections can induce considerable morbidity and mortality in patients with diabetes. Infections may precipitate metabolic derangements and, conversely, the metabolic derangements of diabetes may facilitate infection. Hyperglycemia and acidemia exacerbate impairments in humoral immunity and polymorphonuclear leukocyte and lymphocyte functions but are substantially reversed when pH and blood glucose levels return to normal. Although the exact level above impaired leukocyte function is not defined, in vitro evidence suggests that glucose levels >250 mg/dL impair leukocytic function.

Chronic wounds differ from acute wounds in their levels of proteolytic enzymes such as elastase (Edwards et al., 2004) and matrix metalloproteinases (MMPs) which are higher, while their concentrations of growth factors such as Platelet-derived growth factor and Keratinocyte Growth Factor are lower (Schönfelder et al., 2005; Crovetti et al 2004). Since growth factors (GFs) are crucial in timely wound healing, inadequate GF levels may be an important factor in chronic wound formation (Crovetti et al., 2004). In chronic wounds, the formation and release of growth factors may be prevented, the factors may be sequestered and unable to perform their metabolic roles, or degraded in excess by cellular or bacterial proteases. (Crovetti et al., 2004). Chronic wounds are also caused by a failure of fibroblasts to produce adequate ECM proteins and by keratinocytes to epithelialize the wound (Foy et al., 2004).

Though all wounds require a certain level of elastase and proteases for proper healing, too high a concentration is damaging (Edwards et al., 2004). Leukocytes in

the wound area release elastase, which increases inflammation, destroys tissue, proteoglycans, and collagen, (Kanda et al., 2005) and damages growth factors, fibronectin, and factors that inhibit proteases (Edwards et al., 2004). The activity of elastase is increased by human serum albumin, which is the most abundant protein found in chronic wounds (Edwards et al., 2004). However, chronic wounds with inadequate albumin are especially unlikely to heal, so regulating the wound's levels of that protein may in the future prove helpful in healing chronic wounds (Edwards et al., 2004). Excess matrix metalloproteinases, which are released by leukocytes, may also cause wounds to become chronic. MMPs break down ECM molecules, growth factors, and protease inhibitors, and thus increase degradation while reducing construction, throwing the delicate compromise between production and degradation out of balance.

## 7. Wound Healing or Wound Repair

Wound healing, or wound repair, an intricate process in which the skin repairs itself after injury, is divided into various phases: (1) hemostasis (not considered a phase by some authors), (2) inflammatory, (3) proliferative and (4) remodeling. (Singer and Clark, 1999)

After injury as tissue is disrupted and blood vessels are severed, releasing blood plasma and peripheral blood cells into the wound site, the wound healing process begins immediately. A clot is formed that acts as a temporary barrier that prevents excess bleeding and limits the spread of pathogens into the blood stream.

In the inflammatory phase, bacteria and debris are phagocytosed and removed. In addition, the released factors induce the migration and division of cells involved in the proliferative phase. It has been shown that in a proliferative phase, angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction are observed. The wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells.

In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. However, this process is not only complex but fragile, and susceptible to interruption or

failure leading to the formation of chronic non-healing wounds. Factors which may contribute to this include diabetes, venous or arterial disease, old age, and infection.

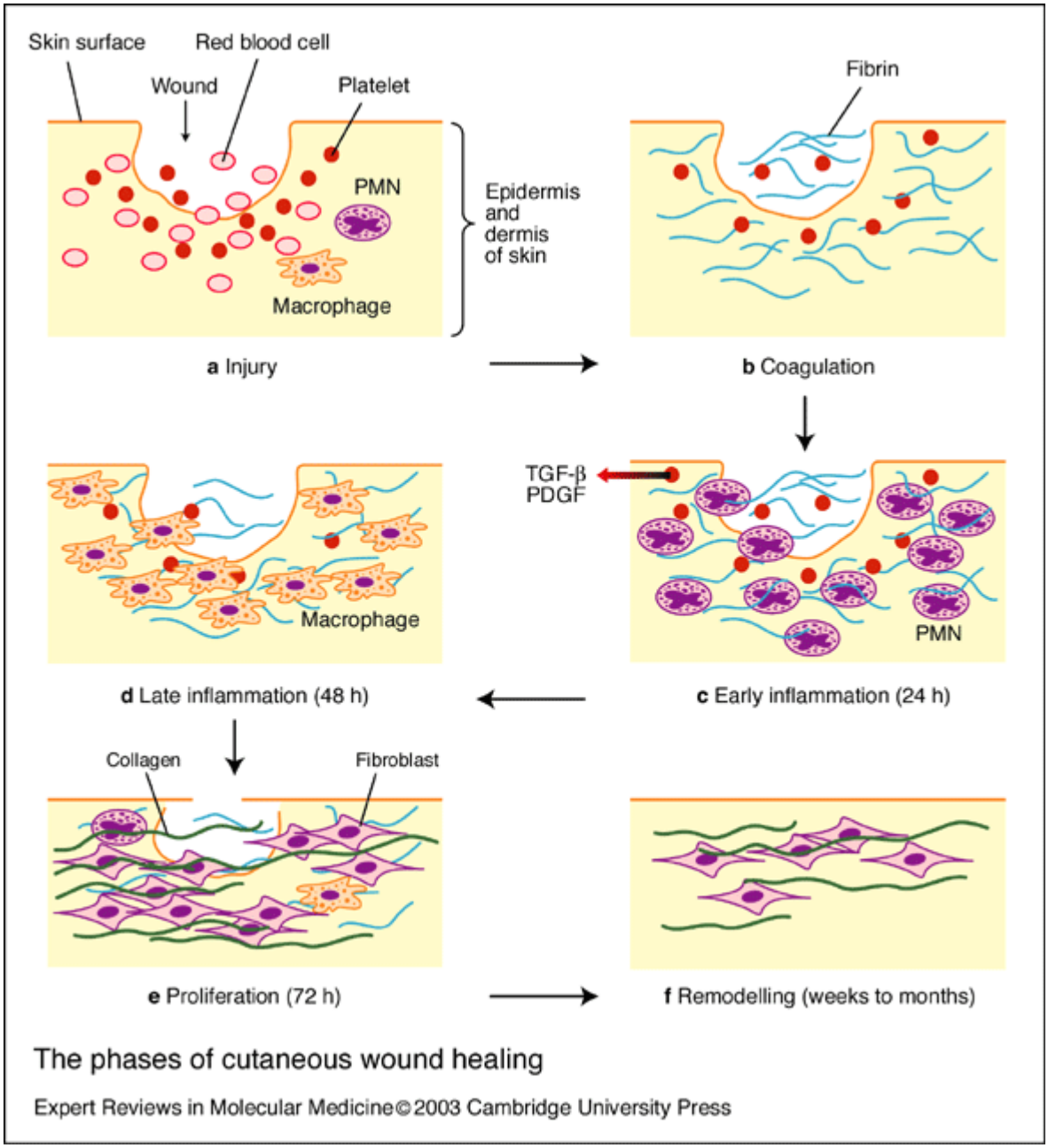


**Figure 2-2** The phases of coetaneous wound healing

## 8. Hydroxyproline

Hydroxyproline is produced by hydroxylation of the amino acid proline, as a post-translational modification following protein synthesis, by the enzyme prolyl hydroxylase. The processes occur in the lumen of the endoplasmic reticulum. Hydroxyproline, a major component of the protein collagen, plays a major role for collagen stability (Szpak and Paul, 2011). Since hydroxyproline is found mainly in collagen hydroxyproline content has been used as an indicator to determine collagen conteent.

## 9. Transdermal Route Administration

Transdermal application is usually designed to offer a slow, sustained release of drug over long periods of time (Naik et al., 2000; Thomas and Finnin, 2004). In addition, transdermal application is also bypassing the gastrointestinal tract. Therefore, it can prevent the irritation and avoid partial first-pass inactivation by the liver. However, due to its barrier properties, the skin membrane is equally capable at limiting the molecular transport from and into the body. Therefore, overcoming this barrier is the main purpose of transdermal drug delivery. Therefore, an essential physicochemical property of drugs that will be able to develop for passive transdermal delivery should be aqueous solubility 1 mg/ml, lipophilicity 10< Ko/w <1000 (oil-water partition coefficient), molecular weight less than 500 Da, melting point less than 200 °C, pH of saturated aqueous solution pH 5-9, and the dose deliverable less than 10 mg/day (Naik et al., 2000).

Under the normal conditions, there are three pathways postulated for the absorption of substances through the stratum corneum; transcellular, intercellular (paracellular) and transappendageal (Roberts and Cross, 2002). The predominant route of transdermal penetration occurs via intercellular spaces. Therefore, the drug must possess both lipoidal and aqueous solubility, which promote its permeation through the domains of the stratum corneum, the drug mobility must be high i.e. molecular weight and volume must be appropriate to facilitate its diffusion through the lipid bilayer. The permeation through the skin will also depend on the ionization degree of the drug at physiological and formulation pH, influencing as well as its solubility and partition behavior (Naik et al., 2000; Naik et al., 2004; Cevc, 2004; Hadgraft, 2004). The good drug should provide enough amount of drug to overcome the skin barrier without skin irritation

and drug will not be inactivated on the skin's surface or during the permeation process (Langer, 2004).

Transdermal absorption is a result of a slow diffusion driven by the gradient between the high concentration in the delivery system and the zero concentration prevailing in the skin. Thus, the delivery system must be kept in continuously contact with the skin for a considerable time. The criteria that merit consideration in transdermal delivery of drugs are: the nature of the barrier (skin), the balance between physicochemical properties of the membrane and the drug, and the technology available to facilitate the transdermal transport.

Transdermal drug absorption can alter drug kinetics and depends on a variety of factors including site of application, thickness and integrity of the SC, size of molecule, permeability of the membrane of the transdermal drug delivery system, state of skin hydration, pH of the drug, drug metabolism by skin flora, lipid solubility, depot of drug in skin, and alteration of blood flow in the skin by additives and body temperature (Singh and Singh, 1993; Stevenson, Crook et al., 1993; Berner and John, 1994).

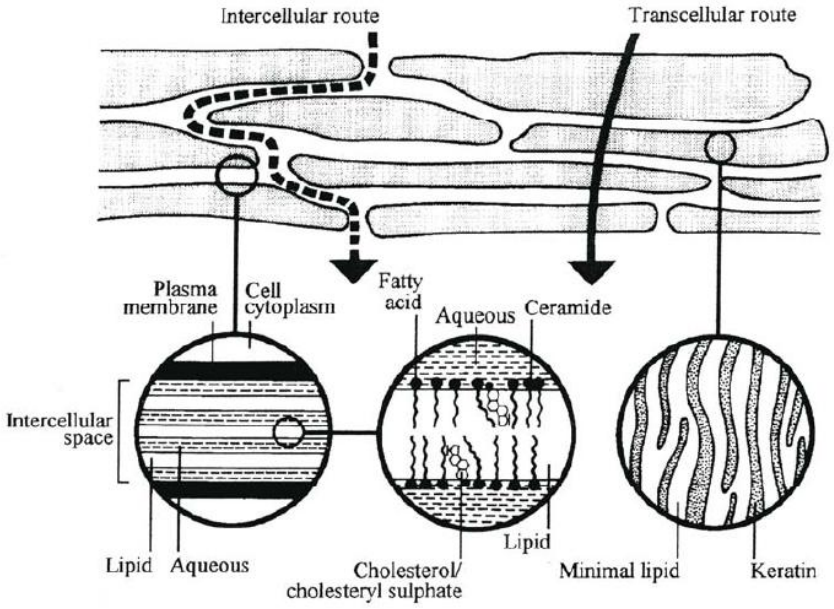


**Figure 2-3** Schematic representation of the "brick and motar" model of the stratum corneum, lipid bilayer organization and possible pathways (Moghimi et al., 1996).

## 10. Rodent Model of Diabetes

DM animal models have been developed for the experiment in pre-clinical trial phase of novel drug discovery to treat DM and its complication as shown in Table 2-2.

**Table 2-1** Rodent models that have been used to study diabetes type I

| Model | Mechanism DM | Glucose | Insulin |
|---|---|---|---|
| STZ diabetes | β-Cell toxicity | ↑↑ | ↓↓ |
| BB/Wor rat | β-Cell auto immunity | ↑↑ | ↓↓↓ |
| NOD mouse | β-Cell auto immunity | ↑↑ | ↓↓↓ |
| Zucker fa/fa rat | Defective leptin receptor | =/↑ | ↑↑ |
| Diabetic Zucker rat | fa/fa rat, inbred for hyperglycaemia | ↑↑ | ↑↑ |
| db/db mouse | Defective leptin receptor | =; ↑↑ with age | ↑↑; ↓ with age |
| GKrat | Inbred for glucose intolerance | ↑ | ↑ |
| OLETF rat | Defective CCK-A receptor | =; ↑ with age | ↑; ↓ with age |
| Akita mouse | Pro insulin misfolding, β-cell destruction | ↑; ↑↑ with age | ↓; ↓↓ with age |

(Biessels, 2005)

=: normal; ↑: moderately increased; ↑↑: markedly increased.

Intravenous or intraperitoneal injection of the glucosamine–nitrosourea compound streptozotocin (STZ) is a well-characterised experimental model for insulinopenic Type I diabetes mellitus that has been widely used to study about the pathophysiology of diabetes and its complications. STZ-diabetic rodents are hypoinsulinaemic, but do not require insulin treatment to survive. Blood glucose levels are 20–25 mmol/1 (normal 5 mmol/1). Like diabetic patients, STZ-diabetic rats develop end-organ damage affecting the eyes, kidneys, heart, blood vessels, and nervous

system (Birrell et al, 1999; Sima and Sugimoto, 1999). The main advantages of the STZ-model are that it is well characterized and that diabetes can be induced at any given age.

STZ is responsible for its cellular toxicity, which is probably mediated through a decrease in NAD levels and the formation of intracellular free radicals (Schnedl, 1994; Shafrir, 1997). The deoxyglucose moiety of STZ facilitates its transport across the cell membrane, in which the GLUT-2 glucose-transporter appears to play an essential role (Schnedl, 1994) The insulin-producing β cells of the islets of Langerhans not only express high levels of GLUT-2 transporters, but also have a relatively low NAD content, making them are particularly vulnerable to STZ toxicity (Schnedl, 1994; Shafrir, 1997). The mechanism of STZ induces diabetes has been well documented that STZ induces diabetes through damaging DNA in the nuclei of pancreatic b-cells by alkylation, leading to an increase in poly (ADP-ribose) synthase (Okamoto, 1985). The increase in this enzyme activity results in a drastic decrease in nicotinamide adenine dinu-cleotide (NAD) concentrations of the cells, then a decrease in the number of b-cells and finally death of the cells. All these changes may induce the impairment of the pancreatic function (Okamoto, 1985).

STZ transported into β-cells through glucose transporter GLUT-2 located on their cell membranes, and then it is activated inside the cells and injures their mitochondria. This unavoidably leads to a reduction of ATP generation through electron transport system and an increase in ADP concentrations. Subsequent degradation of ADP provides hypoxanthine, a substrate of xanthine oxidase (XOD). When XOD reaction takes place in the b-cells where XOD activity is intrinsically very high, $O_2$ radicals are produced, resulting in cell damage and the onset of diabetes. STZ also activates XOD directly and augments $0_2$" generation (Kawada, 1992).

## 11. Quercetin

Quercetin is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin. Quercetin is a plant-derived flavonoid found in citrus fruit, apples, buckwheat, onions; especially red onion which contains high concentrations of quercetin in the outermost rings, black and green tea, capers, lovage, tomato, broccoli and other

leafy green vegetables, and a number of berries including cherry, raspberry etc (Tranchimand et al., 2008; Juergenliemk et al., 2003; Zi et al., 2011; Mullen et al., 2008).

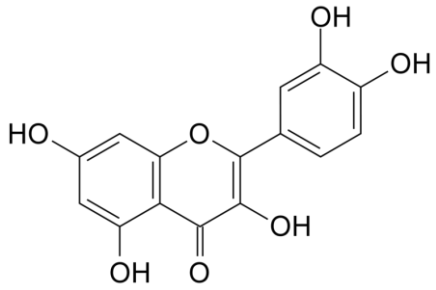


**Figure 2-4** Structure of Quercetin 2-(3, 4-dihydroxyphenyl)- 3,5,7- trihydroxy- 4*H*-chromen- 4-one

Quercetin possesses anti-inflammatory and antioxidant activities. It may also have positive effects in combating or helping to prevent cancer, prostatitis, heart disease, cataracts, allergies/inflammations, and respiratory diseases such as bronchitis and asthma (Verschoyle et al., 2007; Rietjens et al., 2005; van der Woude et al., 2005; Neuhouser, 2004; Murakami et al., 2008; Nöthlings et al., 2007). In addition, it is also claimed for antidepressant properties (Saaby et al., 2009), anti-hypertension effect (Edwards et al., 2007; Egert et al., 2009). It had been reported that administration of quercetin at a dosage range of 12.5 to 25 mg/kg via oral route to mice increased gene expression of mitochondrial biomarkers and improved exercise endurance (Davis et al., 2009). A bioavailability study in rats showed that ingested quercetin is extensively metabolized into non-active phenolic acids, with more than 96% of the ingested amount excreted within 72 hours, indicating actual physiological roles, if they exist, involve quercetin in only minute amounts (Mullen et al., 2008). It has been metabolized mainly by CYP3A4. CYP2C9 and CPY3A4, which are members of the cytochrome $P_{450}$ and mixed-function oxidase system (Hsiu et al., 2002; Raucy, 2003; Dayong et al, 2009).

## 12. Tomato

**Figure 2-5** Tomato (*Solanum lycopersicum* Linn.)

Tomato (*Solanum lycopersicum* Linn.), a plant in the family of Solanaceae, is a biennial plant that can grow throughout the year. However, the best tomato season is July through September. Tomato fruit has high nutritional value including lycopene and vitamins A, K, E, B1, B2, B3, B5, B6 and folate addition to the minerals, including Molybdecum, Potassium, Manganese, Chromium, Magnesium, Iron, Phosphate, fiber, protein and amino acids including glutamate.

Lycopene, an active ingredient in tomato, is an antioxidant and can protect against many cancers, including prostate and colon cancer, etc. (Polívková et al., 2010; Zhang et al., 2009; Freedman et al., 2008) and neurodegenerative diseases (Rao et al., 2002; Fall et al., 1999; Suganuma et al., 2002).Tomatoes also decreases the risk of heart disease and improves lipid profile, reduces blood clotting and inflammation (Shidfar et al., 2011). Recently, Wattanathorn and coworkers (2012) found that tomatoes can decrease the body weight in the high fat diet induced obese rats and also facilitated the wound healing in diabetic wound as well as prevent and reduce the neuronal cell death in stroke animal model (Wattanathorn et al., 2012).

## 13. Zein Based Nanofibers

Nanofibers are defined as fibers with diameters on the order of 100 nanometers. They can be produced by interfacial polymerization and electrospinning. However, electrospinning is become popular as a convenient method for producing fibers of high surface area such as high performance filters, protective textiles, sensors, composites, photovoltalic cells, wound dressings and scaffolds in tissue engineering.

In electrospinning, a high voltage is applied to create electrically charged jets of a polymer solution when these jets dry, they form fibers, which are collected on a target as a nonwoven fabric. Zein, a major protein of corn, has been successfully prepared as nanofiber by using electrospinning technique and served as delivery system.In addition; it also has high potential to be applied in various aspects as described following;

- Medical: artificial organ components, tissue engineering, implant material, drug delivery, wound dressing, medical textile materials.
- Protective materials: sound absorption materials, protective clothing's against chemical and biological warfare agents, sensor applications for detecting chemical agents.
- Textile: sport apparels, sport shoes, climbing, rainwear, outerwear garments, baby aiapers.
- Filtration: HVAC system filters, HEPA, ULPA high efficient filters, air, oil, fuel filters for automotive, filters for beverage, pharmacy, and medical applications.
- Dye-sensitized solar cell
- Pigments for cosmetics
- Energy: Li-ion batteries, photovoltaic cells, membrane fuel cells.
- Carrier materials for various catalysts
- Photocatalytic air/water purification
- Micropower to operate personal electronic devices via piezoelectric nanofibers woven into clothing.