2. Literature Review

2.1. Skin

The skin is largest organ of human body, covering nearly $2\,m^2$ of the body surface area and typically accounting for 15% of the adult body mass [Kolarsick et al. 2011, McGrath et al. 2004]. It outlines the border between the body and outer environment, enabling vital body function to take place in a controlled physiological environment. [Ng and Lau 2015]. The skin along with its accessory structures like hair, nails, and exocrine glands, make up the **integumentary system** of the human body, which offers the overall body protection [Kolarsick et al. 2011].

2.1.1. Different layers of skin

The skin is composed of two main layers, overlaying epidermis and dermis, and a closely associated hypodermis layer, as shown in Figure2.1.

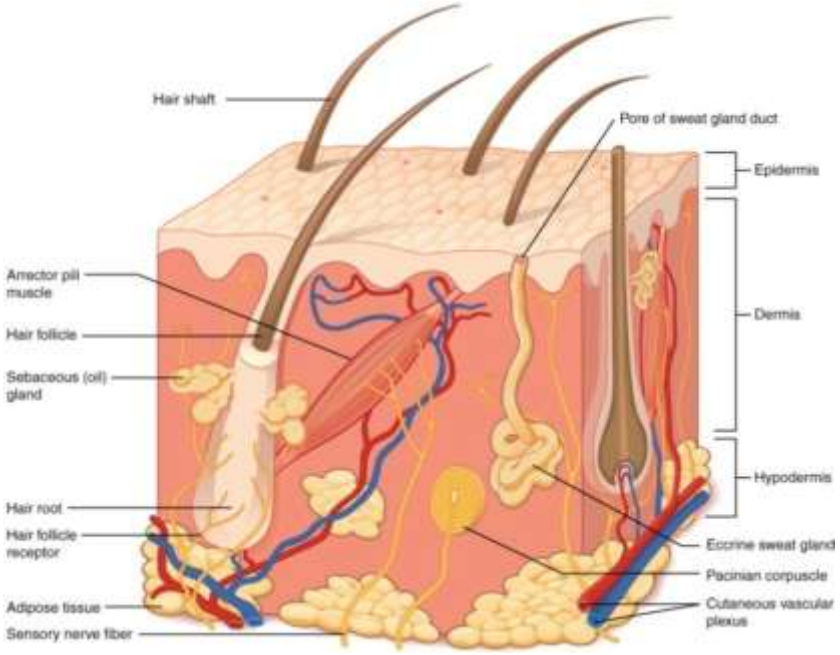

Figure 2.1: Different layers of skin. *Source: OpenStax 2013.*

2.1.1.1. The Epidermis (epi- = "upon" or "over")

The **epidermal layer** is a keratinized and stratified squamous epithelial layer. It is an avascular layer of the skin. Depending on the body location, it is composed of four or five layers of epithelial cells, and known as "thin skin" or "thick skin", respectively. The eyelid has the thinnest layer of the epidermis, measuring less than 0.1 mm. "Thick skin" is only found on the soles of the feet and the palms of the hands, where it is approximately 1.5 mm thick [Berger et al. 2006, OpenStax 2013].

2.1.1.2. The Dermis (derma- = "skin")

As distinct from the epidermis and hypodermis, the dermis could be deemed the "core" of the integumentary system. It is, generally $\geq$1 mm thick and responsible for the strength and elasticity of the skin. It contains a range of immune cells, lymph vessels, sensory nerves ending, sebaceous glands, sweat glands, hair follicles and blood vessels. It is composed of an interconnected mesh of structural proteins, primarily elastin and collagen, which is produced by fibroblast. Based on the packing of collagen fibers, the dermis can be divided into two layers, upper papillary dermis, and the lower reticular dermis [OpenStax 2013, Ng and Lau 2015].

2.1.1.3. The Hypodermis (hypo- = "below")

The hypodermis (also known as superficial fascia or subcutaneous layer) is the innermost layer of the skin. It connects the skin with the underlying fibrous tissue (or fascia) of the muscles and bones. It consists mainly of highly-vascularized, loose, areolar connective tissue and adipose tissue, which serves as a fat storage site and offers the cushioning and insulation for the integument [OpenStax 2013, Ng and Lau 2015].

2.1.2. Functions of the integumentary system

Skin performs a lot of vital functions, including protection of internal physiological environment from external chemical, physical, and biological assailants; preventing excess body water loss; act as sensory organ; help in regulation of body temperature; synthesize vitamin D; serve as excretory organ by dissolving minerals and other organic wastes in sweat; prevent microbial over-colonizing by secreting dermicidin in sweat; owing to Langerhans cells, dermal dendritic cells and macrophages in inner layer, defend the body against invading microorganisms; provide a "cushion" to inner organ by storing fats in underlaying hypodermis [OpenStax 2013].

2.1.3. Problems associated with skin

The integumentary system is highly disposed to a variety of disorders, diseases, and injuries. These range from moderately benign microbial infections that are characterized as disorders, to severe burns and skin cancer which can be lethal. A skin disorder can be a simple rash, an inflammation (dermatitis), an inflammation due to overactive immune system (eczema), caused by a fungal infection (ring worm, tinea versicolor), a viral infection (herpes, shingles, viral exantham), an allergic reaction (hives), scabies (caused by tiny burrowing mite), a lethal cancer (melanoma, basal cell and squamous cell carcinoma) or an injuries (include burns, wounds, scars etc) [OpenStax 2013].

As per WHO report– "an Injuries resulting from traffic collisions, drowning, poisoning, falls or burns - and violence - from assault , self-inflicted violence or acts of war–kill more than five million people worldwide annually and cause harm to millions more. They account for 9% of global mortality, and are a threat to health in every country of the world" [WHO report].

2.2. Wound

The loss of protective function of skin due to severe disease or trauma (physical, chemical, thermal or microbial) is known as a wound [Kant et al. 2017]. This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs and even bone [Velnar et al. 2009].

Wounds can arise from pathological processes that begin externally or internally within the involved organ. They can have an accidental or intentional aetiology or they can be the result of a disease process. Irrespective of the cause form, a wound damages the tissue and upsets the local environment within it.

2.2.1. Types of wounds

Depending on the healing time, a wound can be classified either acute or chronic wounds.

2.2.1.1. Acute wound

An acute wound heals in predicted time and entire process complete within few weeks. Typically, an acute wound goes through all the phases of normal wound healing for predictable tissue repair [Doherty 2012]. Sudden loss of anatomical structure owing to surgery or trauma results into acute wounds. Acute wound usually happen in normal or otherwise recently uninjured tissue. [Demidova-Rice et al. 2012, Velnar et al. 2009].

2.2.1.2. Chronic wound

When an acute wound fails to heal in predicted time, it becomes a chronic wound. Owing to underlying pathologies, for instance pressure ulcer, diabetic ulcer, and vascular ulcers, dysregulation of normal healing mechanism occur, which results into prolonged and

pathologic healing. Usually, healing arrest occurs due to prolonged inflammatory phase. Other reason for prolonged healing is development of drug-resistant bacterial biofilms, persistent infections, failure of epidermal or dermal cells to respond to reparative stimuli, tissue hypoxia, and failed re-epithelialization caused by repeated trauma [Demidova-Rice et al. 2012, Doherty 2012].

2.2.2. Types of clinical wound healing

Based on the abrasion, laceration, amount of skin and tissue loss, clinically a wound healing can be accomplished by one of the following ways:

2.2.2.1. Primary intention wound healing process

A wound heals by the primary process when it is aseptic and freshly created with minimum tissue loss, and its edges are in close proximity, smooth bordered and surgically closed by a suture. Primary wound healing e.g. after a surgical incision, generally occur within 6-8 days without any complication, and with scanty granulation tissues at the incised gap [Doherty 2010, Doyle and McCutcheon 2015].

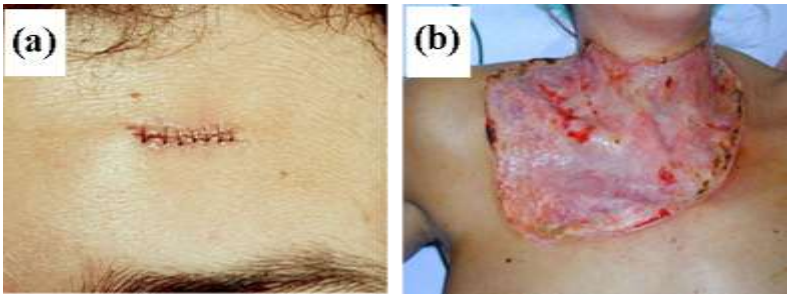

Figure 2.2: Clinical wound healing – **(a)** primary intention wound healing process, and **(b)** secondary intention wound healing process.
Source: *https://gmch.gov.in/e-study/e%20lectures/Pathology/L10%20wound%20healing.pdf*

2.2.2.2. Secondary intention wound healing process

In contrast to primary healing, secondary healing takes longer repair time with scar formation. Secondary healing occurs when the wound has lost a considerable amount of tissues and it edges are so distant that it cannot be sutured. Wounds left open and gaps are filled by exuberant granulation tissue deposition and epithelial cell migration. Owing to large-scale loss of tissue, there is a risk of infection, or an infection has already occurred. Secondary healing occurs either in acute wound with significant tissue loss or in chronic wounds [Doherty 2010, Doyle and McCutcheon 2015].

2.2.2.3. Tertiary intention wound healing process

A tertiary intention is also known as delayed closure. A delayed closure involves the principles of both i.e. primary and secondary healing. It occurs when healing needs to be delayed intentionally, for example when blood perfusion is low or wound is highly infected [Doherty 2010, Doyle and McCutcheon 2015].

2.2.3. Different phases of wound healing

The wound healing is a natural recovery response to tissue injury that commences with trauma and ends with the restoration of tissue or body function. Healing is a complex physiological, dynamic, interactive process that consists the participation of various components such as extracellular matrix, soluble mediators, blood and parenchymal cells [El-Ferjani et al. 2016]. The healing process is influenced by a series of factors ranging from wound severity, site, injury magnitude, and other external and internal variables that either impede or encourage wound healing.

Depending on the type, scale of injury and aetiology of wound, healing proceeds through three sequential phases of varying and overlapping duration, namely the hemostasis and inflammatory, proliferative, and tissue remodeling [Nafiu and Rahman 2015, Park et al. 2011]. These phases and their biophysiological functions need to take place at a specific time, in the proper sequence and proceed for a specific duration at an optimal intensity [Guo and DiPietro 2010]. Different phase of healing are shown in Figure 2.3 and summarized in Table 2.1.

2.2.3.1. Hemostasis and inflammatory phase

The **hemostasis phase** begins shortly after an injury with the aim of controlling bleeding and limiting the spread of pathogens in the body. Therefore, thromboxane A2 (TXA2) and prostaglandin 2-α mediated vasoconstriction, collagen activated clotting occurs at the wound location. Clot formation stops bleeding and acts as a barrier to avoid microbial contamination. During this phase, clotting factor such as collagen, thrombin, fibronectin and platelets release various cytokines and growth factors, which are responsible for the activation of inflammatory response [Velnar et al. 2009]. The fibrin clot act as scaffolding for arriving cells (such as monocytes, neutrophils, endothelial cells and fibroblasts), and concentrate the growth factors and cytokines [George Broughton et al. 2006].

The **inflammatory phase**, which begins immediately with the hemostasis, is characterized by chemotaxis and activation of inflammatory cells [George Broughton et al. 2006]. It is also marked by the release of various pro-inflammatory cytokines, reactive oxygen species, proteases, and growth factors to protect open wound from infection, to improve phagocytic activity, and to assist during wound repair [Pereira and Bartolo 2016, Rasik and Shukla 2000]. **Neutrophils** are the first respondent which drawn into the wound site and releases

caustic proteolytic enzymes and begin digestion of invading microbes and nonviable tissue. **Monocytes** will be attracted to wound site from the adjacent tissue and blood, and differentiate into macrophages, which is a key phagocytic cells in wound repair. Various cytokines and enzymes are released by the **macrophage**, comprising collagenases for debridement of wound; tumor necrosis factor (TNF)-α and interleukin (IL)-1 for the activation of fibroblasts and angiogenesis; and transforming growth factor (TGF) for stimulation of keratinocytes [George Broughton et al. 2006, Pereira and Bartolo 2016]. **Lymphocyte** is the last cell to infiltrate the wound site, attracted 72h after injury or late inflammatory phase. It helps in tissue repair and the avoidance of immunosuppression [Velnar et al. 2009].

2.2.3.2. Proliferative phase

The proliferative phase is described by **epithelialization**, **angiogenesis**, **collagen deposition** and **granulation tissue formation**, and **wound contraction**.

Epithelialization: Normally, the epithelial progenitor cells in the basement membrane migrate upward and restore the epidermis within 2-3days. However, in case of full thickness wound or absence of basement membrane, the epithelial cells found on the skin edge start to proliferate and send projections to restore a protective barrier. [George Broughton et al. 2006].

Angiogenesis: Angiogenesis is characterized by endothelial cell migration and capillary formation. It is stimulated by TNF-α. The formation of capillaries into the wound bed is vital for oxygen and nutrients supply to the wound site for proper wound healing [George Broughton et al. 2006].

Granulation tissue formation: It is the final part of proliferative phase. In this phase, fibroblasts, primary cells for collagen synthesis, move from the surrounding tissue into the wound site, become activated, start collagen deposition and form a new provisional extracellular matrix [George Broughton et al. 2006, Pang et al. 2017]. **Neovascularization** leads to growth of lymphatic and vessel capillaries from existing vessels present at the wound site, which results into granulation tissue formation [Pereira and Bartolo 2016].

Wound Contraction: **'**Wound fibroblasts', fibroblasts already situated in the wound site will start synthesizing collagen and differentiate into myofibroblasts which help in wound contraction [George Broughton et al. 2006].

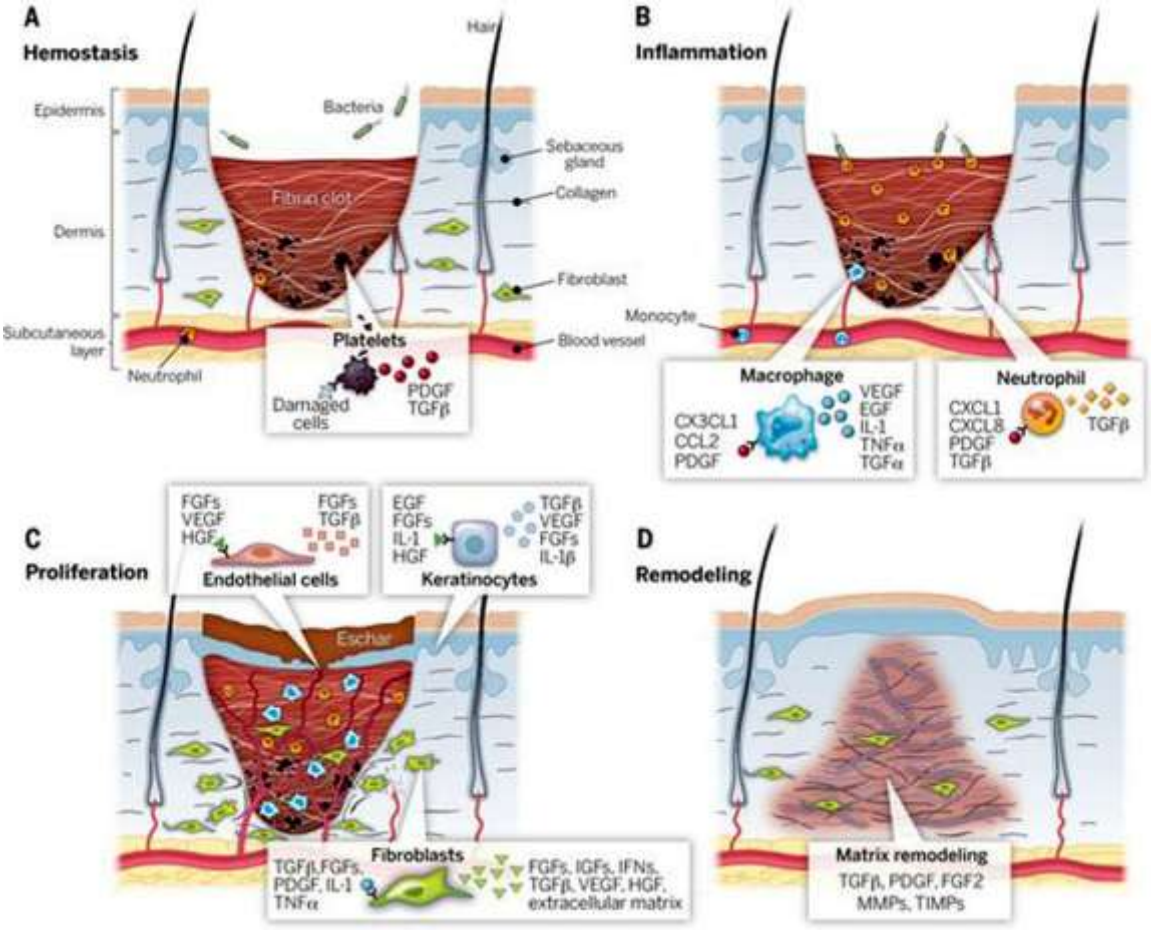

Figure 2.3: Different phases of wound healing.
Source: https://in.pinterest.com/pin/495607133977033157/

2.2.3.3. Maturation or remodeling phase

Clinically, the maturation or remodeling phase is the most important one as it controls the strength and appearance of the healed tissue [El-Ferjani et al. 2016]. Continuous collagen deposition in a structured network is the primary characteristic of this stage; however an excess collagen deposition results in a hypertrophic scar or keloid. Net collagen deposition will last for at least 4 to 5 weeks after wounding. Even after a year of maturation, the collagen in the scar would not become as arranged the collagen found in uninjured skin. Further, the strength of wound also never restores to 100%, even after three months and beyond it will reach approximately 80% of uninjured skin [George Broughton et al. 2006].

Table 2.1: Phases of healing of a full thickness wound – [Doyle and McCutcheon 2015, Guo and DiPietro 2010]

Phase	Cellular and Bio-physiological Events
Hemostasis phase	Blood vessels constrict and clotting factors are activated. Clot formation blocks the bleeding and acts as a barrier to prevent bacterial contamination. Platelets release growth factors, which alert various cells to start the repair process at the wound location.
Inflammatory phase	Vasodilation, chemotaxis activation of inflammatory cells: - **Neutrophil infiltration:** begin digestion of invading microbes and nonviable tissue - **Monocyte infiltration** and differentiation to macrophages, principle phagocytic cells, which also releases numerous cytokines for activation of fibroblast, angiogenesis and keratinocytes - **Lymphocytes infiltration** helps in tissue repair
Proliferative phase	Four important processes occur in this phase: - **Re-epithelialization:** new epidermis and granulation tissue are developed - **Angiogenesis:** New capillaries formation occurs to bring oxygen and nutrients to the wound - **Collagen deposition and Native ECM formation:** this provides strength and integrity to the wound - **Wound Contraction:** the wound begins to reduce in size
Maturation (remodelling) phase	Collagen continues to strengthen the wound, and the wound becomes a scar. Vascular maturation and regression

2.2.4. Factor influencing wound healing

Factors that influence cellular function and physiologic responses may affect wound healing. Various factors that effect a wound healing are shown in Table 2.2 [Guo and DiPietro 2010].

Table 2.2: Factors affecting wound healing

Local Factors	Systemic Factors
Infection	Stress
Oxygenation	Age and Gender
Venous Sufficiency	Sex Hormones
Foreign Body	Obesity
	Ischemia
	Nutrition
	Alcoholism & Smoking
	Medications: Non-steroidal Anti-inflammatory drugs, Glucocorticoid Steroid, Chemotherapy
	Disease: Jaundice, Diabetes, Fibrosis, Keloid, Uremia, Hereditary, Healing Disorder
	Immuno-compromised Conditions: Cancer, AIDS, Radiation Therapy

2.2.5. Reactive oxygen species and its significance in wound healing

Free radicals are highly active to react with other molecules due to their unpaired electrons. These are generated in the biological system from the oxygen, nitrogen and sulfur containing molecules, during cell metabolism and function, and known as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS). RNS are derived from nitric oxide (NO) through the reaction with superoxide anion ($O_2^{-\bullet}$) to form peroxynitrite ($ONOO^-$). RSS are easily formed from thiols by reaction with ROS [Lü et al. 2010]. Reactive oxygen species (ROS) commonly refer to the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), perhydroxyl radical ($HO_2^{\bullet}$) and hydroxyl radicals ($HO^{\bullet}$). Intracellular

ROS at low levels have important roles in cell signalling to support cell proliferation and survival pathways, apoptosis, gene expression and ion transportation [Lü et al. 2010, Xu et al. 2016]. In addition, Dunnill, Patton et al. discussed various roles of ROS in wound healing as shown in Figure 2.4.

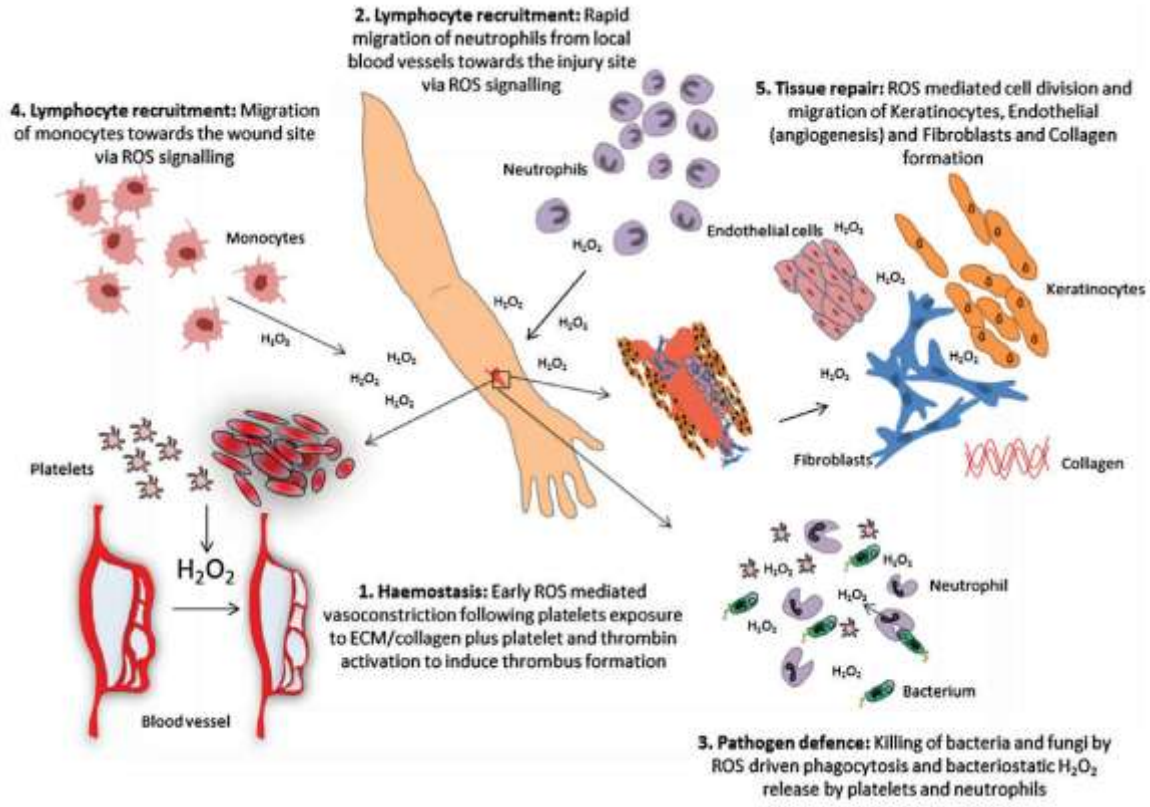

Figure 2.4: The schematic diagram represents the multiple roles of ROS in hemostasis state during acute wound healing (Note- this diagram does not represent the role of excessive levels of ROS) [Dunnill et al. 2017].

Under normal conditions, body homeostasis balances the level of ROS using the endogenous antioxidant capacity [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] of the human body. However under adverse conditions, such as impaired wound healing associated with full thickness wound, chronic wound, wound heavily infiltrated with microorganism, the ROS excessively produced in the wound area. These excessive ROS causes sustained release of pro-inflammatory cytokine and activation

of matrix metalloproteases, which modify and/or degrade ECM proteins and can also impair dermal fibroblast and keratinocyte function [Dunnill et al. 2017, Lü et al. 2010].

It is, therefore, obvious that for proper wound healing, a precise equilibrium should be maintained between lower and high level of ROS; low ROS values are crucial to stimulate healing mechanism, whereas excessive ROS causes cellular damage and impaired wound repair. A way of indirectly manipulating ROS can instead be to manipulate the local antioxidant system by increasing the intake of dietary antioxidant [Dunnill et al. 2017]. An dietary/exogenous antioxidants can minimize the oxidative damage either; (1) directly by scavenging the free radicals, or (2) indirectly by suppressing the expression of free radical generating enzymes or (3) augmenting the expression of endogenous antioxidant enzymes [Lü et al. 2010].

2.2.5.1. Endogenous antioxidants

The homeostatic state of intracellular ROS depends on its production during cell metabolism, and elimination by the endogenous antioxidant system. Cells contain several antioxidants system to eradicate the deleterious effects of ROS, and they do so by donating their own electrons, thus preventing them from capturing electrons from other important molecules, such as DNA, proteins and lipids [Dunnill et al. 2017, Lü et al. 2010]. Endogenous antioxidant systems can be proteinaceous, non-enzymatic, and certain metal ion. **Primary proteinaceous antioxidants** confined in mammalian cells that are SOD, CAT and GPx. The SOD catalyzes the conversion of $O_2^{-\bullet}$ into H_2O_2 and molecular oxygen. The catalase enzyme converts H_2O_2 into water and molecular oxygen, whereas GPx catalyzes the conversion of hydrogen peroxide into water only (Figure 2.5) [Weydert and Cullen 2010]. Along with proteinaceous antioxidants, cells also contain **non-enzymatic**

antioxidants, such as vitamin C, vitamin E, Glutathione, β-carotene, bilirubin, Q-coenzyme, nicotinamide adenine dinucleotide phosphate (NADPH), α-tocopherol, and urate, to control the deleterious effect of ROS. Moieties with a **metal ion capable of redox reactions**, such as transferrin and ferritin, possess an enhanced ROS-scavenging capability [Dunnill et al. 2017].

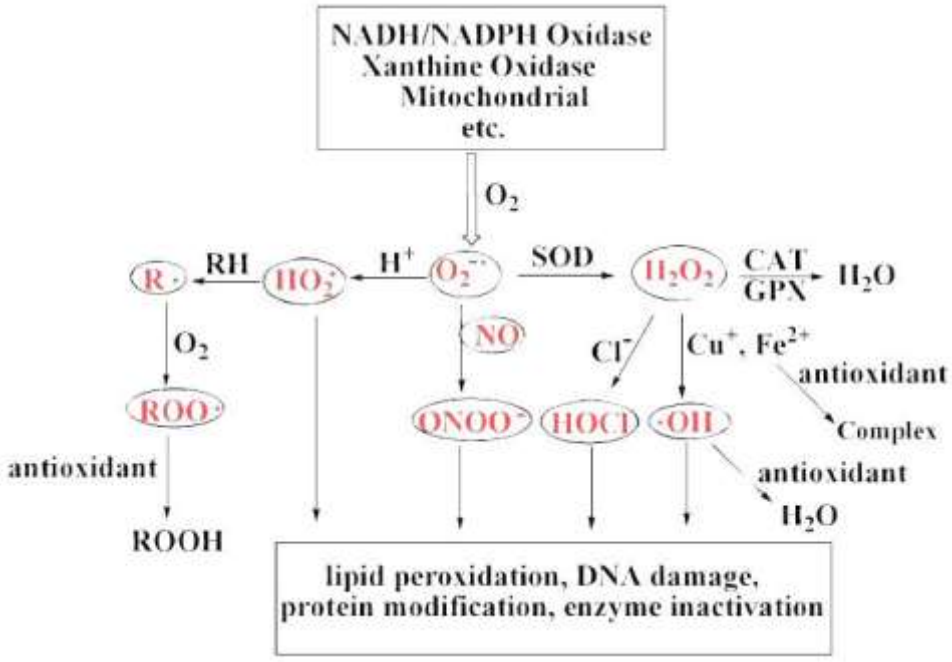

Figure 2.5: Summary of ROS types, sources, and action point of antioxidants [Lü et al. 2010].

2.3. Nanofibers

In the past decade, there has been significant increase in demands and development of new skin regeneration products from existing materials. Commonly used materials for skin wound healing include decellularized porcine dermal matrix, hydrogels and freeze-dried or gas-foaming formed scaffold. However, these materials lack the capability to review the 3D architecture of extracellular matrix (ECM) of skin [Chen et al. 2017]. Nanofibers have emerged as potential candidate for skin regeneration due to architectural resemblance with

native ECM. In addition, a nanofiber-based scaffold also provide ideal characteristics of a dressing material like absorption of excess wound exudate, exchange of gases, preservation of wound hydration, reduction of wound trauma and as a barrier to external microbial infiltration [Kim and Park 2006, Pereira and Bartolo 2016]. Besides to traditional functions, these nanofiber scaffolds can also be meant to diminish microbial infection, inflammation and support wound healing by incorporating antimicrobial and antioxidant agents in the nanofiber [Chen et al. 2017].

Nanofibers can be fabricated using several techniques for example drawing, interfacial polymerization, force spinning, melt blowing, phase separation, template melt extrusion template synthesis and electrospinning, as briefly represented in Table 2.3 [Pelipenko et al. 2015]. Among the various available techniques for nanofiber fabrication, electrospinning is the most widely used technique and has attracted incredible research and commercial interest due to its simplicity, versatility and cost effective set-up [Szentivanyi et al. 2011].

2.3.1. Electrospinning

Recently, electrospinning technique has received widespread attention in drug delivery and tissue engineering due to its relative ease of use and adaptability, versatility for producing a range of biomimetic nanofibers from wide variety of polymeric (natural and synthetic) and inorganic materials. Electrospinning is relatively simple, cost-effective and adaptable technique in comparison to other conventional fiberspinning techniques such as wet spinning, melt-blown, etc. or advanced techniques for example lithography, self-assembly, etc. for fabricating nanofibers for various applications. Electrospinning set up also has distinct capability to produce dry nanofibers with adjustable size, shape and loading efficiency in a single step [Celebioglu et al. 2014, Szentivanyi et al. 2011]. A wide range of

Table 2.3: Brief description of the most commonly used methods for nanofiber production [Pelipenko et al. 2015].

Method	Description	Advantages	Disadvantages
Drawing	The fabrication of fiber is done by contacting a previously collected polymer solution droplet with a sharp tip and then drawing it as a liquid fiber, which is further solidified by solvent evaporation.	Simple process	Limited amount of product, discontinuous process
Electrospinning	Nanofibers are produced in one-step method from a viscoelastic solution of a polymer or its melt under applied high voltage.	Unlimited length, simple and core-shell nanofibers, great diversity of polymers and solvents used	High voltage, solvents required, numerous parameters affecting the process
Forcespinning	Fibers are produced using a very highly concentrated polymer solution or melt under applied centrifugal force.	Free from high voltage, simple method, high production yield	Thermal degradation of melts, fibers are usually much thicker than 1 μm in diameter
Phase separation	Firstly, a gel is formed by cooling the polymer solution to the gelation temperature. Afterwards, the gel is immersed in distilled water for solvent exchange, followed by removal from the distilled water, blotting with filter paper, and transfer to freeze-drying	Simple, no special equipment required	Numerous parameters, limited to a few polymers
Self-assembly	Amphiphilic molecules are used as basic building blocks that self-associate to produce nanofibers	Suitable for production of very thin nanofibers	Poor control over morphology and orientation of nanofibers
Template melt extrusion	The molten polymer is forced by an extruder screw through a mold or spinning die in the head of extrusion devices and then cooled to solidify. Anodic aluminum oxide (AAO) membranes are used as a template.	No need for solvents, homogenous fiber diameter	Short length of fibers, time-consuming
Template synthesis	Nanofibers are formed within the numerous cylindrical pores of a nonporous membrane by oxidative	Aligned nanofibers, homogenous and easily controlled fiber	Complex method

biomolecules from antibiotics to protein drugs like growth factors can be successfully encapsulated in the polymeric nanofibers produced by electrospining while retaining the bioactivity of the biomolecules [Ranganath and Wang 2008, Xie and Wang 2006]. Various properties of electrospun nanofiber membrane which make ideal candidate for wound healing application are as follows [Kataria et al. 2014, Kim et al. 2009, Liu et al. 2010a, Liu et al. 2010b]:

(i) Electrospining technique produces randomly oriented, thin nanofibers (50-500 nm) which imitate the structural and functional similarity of the natural ECM that support cell attachment and its proliferation.

(ii) Electrospun nanofibers offer high surface area to volume ratio which quickly start cell signaling and attract fibroblast for extracellular matrix components secretion.

(iii) Highly porous strucutre (60-90% porosity) offered by electrospun nanofiber membrane helps in cell respiration, gas permeation and wound dehydration prevention.

(iv) Owing to extremely interconnected pores, electrospun nanofibers protect the wound from environmental contamination and microbial infiltration while allowing the cellular ingrowth.

(v) Electrospun nanofibers act as drug delivery device with controlled and sustained release profile, and with high drug loading efficieny. The release profile and degradation rate drug loaded nanofiber can be adjusted by altering the electrospuninning parameter.

2.3.2. Electrospinning setups

Electrospinning technique has been one of most commonly used method for nanofiber production in the late 20th and early 21st century , since it was patented by John Francis Cooley in 1900 (US patent No. 692,631) [Reneker and Yarin 2008]. Since its first use by Cooley, significant developments have been done in the instrumentation, diversity of materials used and application of nanofibers. Electrospinning is receiving growing attention in science and business communities, and is regarded as a crucial scientific and commercial project with worldwide economic benefit [LF Nascimento et al. 2015]. A basic electrospinning setup, displayed in Figure 2.6, mainly consists of four primary components: a glass syringe fitted with a blunt end metallic needle acting as a nozzle, a high voltage (10-40 kV) power supply between two electrodes, an electrically conductive collector (a piece of aluminum foil or silicon wafer) to collect the fabricated nanofibers, and a syringe pump to provide a constant flow of electrospinning solution. The electrically-conductive electrospinning solution is loaded into a 5 cc glass syringe mounted on a pump. When a DC voltage applied, electrospinning solution acquired a stable shape due to equilibrium between repulsive forces and surface tension. As the voltage increased, the charge repulsion started overcoming the surface tension and at a critical potential, solution acquired a conical shape, known as Taylor Cone. Further increase in potential will destroy the equilibrium of electric forces and surface tension. At this stage, polymer solution emerges from the Taylor cone in the form of ultrafine nanofibers and flow in the direction of electric field. The stretched nanofibers are collected on the grounded metallic collector held at an optimized distance. During the electrospinning process, the external and internal charge forces causes the whipping of the liquid jet, which results into the reduction of diameter from several hundred micrometers to as thin as tens of nanometers. The jet thinning simultaneously

enables the polymer solution quickly to evaporate solvents and solidify into solid. [Coelho et al. 2018, Reneker et al. 2000, Wang et al. 2009].

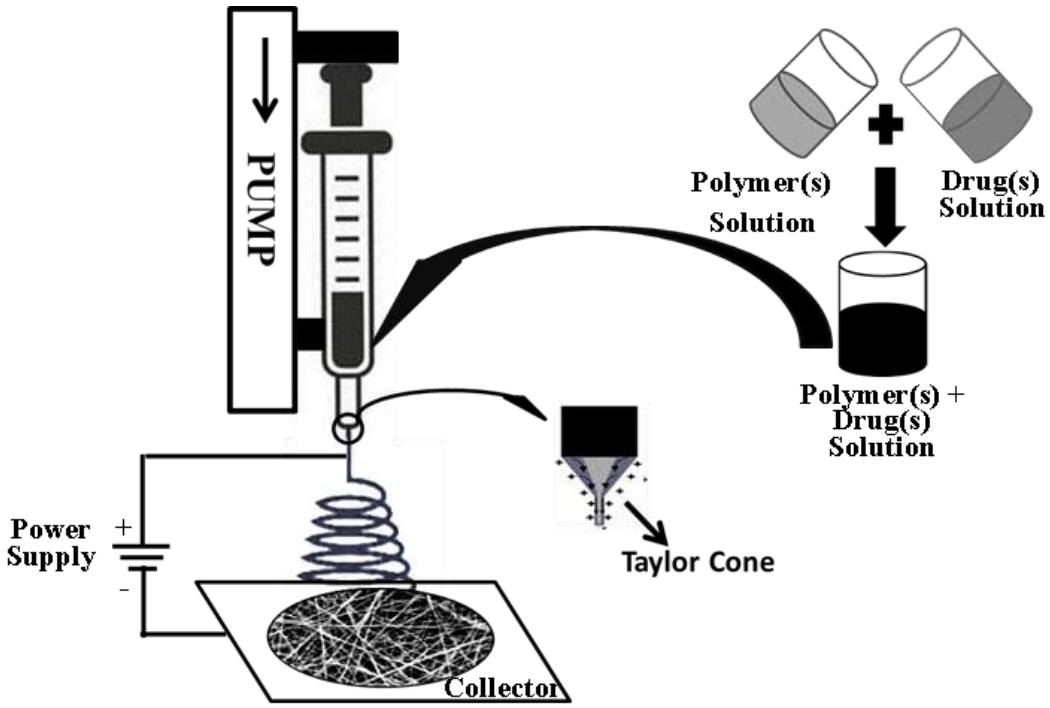

Figure 2.6: Schematic representation of the electrospinning set up.

2.3.3. Parameters affecting the electrospinning process

Although electrospinning technique is quite simple, it is affected by various parameters, making the electrospinning process challenging to operate. The parameters that influence the electrospinning can be grouped under three major heading: solution parameters, process parameters and ambient parameters [Pelipenko et al. 2015].

2.3.3.1. Solution parameters

Solution parameters are the most widely studied, and contrarily most erroneously concluded parameters. Often, a researcher concludes a polymer inappropriate for electrospinning without adequately modifying process and ambient parameters [Pelipenko

et al. 2015]. Polymer and solvent characteristics determines the solution parameters, which include:

(a) Polymer characteristics

High molecular weights polymers which provide high degree of polymerization are preferred for electrospinning in order to enable a sufficient number of intermolecular entanglements. Generally, a low molecular weight polymer has a propensity to form bead nanofiber rather than a smooth one. Furthermore, linear polymers are preferred over the non-linear polymers, since the latter form a viscous solution or sometimes gel even at low concentration [Gupta et al. 2005, McKee et al. 2006].

(b) Polymer concentration and solution viscosity

Optimum polymer concentration for efficient electrospinning is dependent on characteristics of polymer and solvent used. At low polymer concentration (low viscosity solution), the applied electric field and opposing surface tension causes the entangled polymer chains to break into fragments before reaching the collector, which results into fragmented and beaded nanofiber. Increasing the polymer concentration (or viscosity of solution), increases the polymer chain entanglement, which overcome the surface tension and results in uniform bead-free nanofibers production. Further increase in polymer concentration, increases the solution viscosity that will hinder the flow of the solution through the needle tip. [Haider et al. 2013, Rošic et al. 2012].

(c) Surface tension

Surface tension is the main force acting against the electric force of surface charge, and it resists the Taylor cone formation. However, after numerous researches, still a decisive link

has not been established between surface tension value and fiber morphology. Usually, a solution with low surface tension value produces bead-less nanofibers, by using low voltage power supply. Surface tension of a electrospinning solution can be manipulated by adding a surface active agent [Pelipenko et al. 2015].

(d) Solution conductivity

Solution conductivity affects the Taylor cone formation, control fiber diameter, and enables the use of lower applied voltage. A polymer solution with low conductivity lack sufficient surface charge to form a Taylor cone, hence no electrospinning will occur. When the conductivity is increased, the surfaces charge increases that contribute to the formation of Taylor cones and also to the reduction of the fiber diameter. Further increase in conductivity beyond a critical value causes a depletion of tangential electric field along the drop surface which hinder Taylor cone formation [Angammana and Jayaram 2011, Sun et al. 2014].

(e) Dielectric constant

A few studies have explored the effect of dielectric constant on nanofiber morphology, and it has been found that when solvents with high dielectric constants are used, effective electrospinning takes place with thin diameter of fabricated nanofiber [Jarusuwannapoom et al. 2005, Pelipenko et al. 2015].

2.3.3.2. Process parameters

Different process parameters which affect the electrospinning comprise applied voltage, flow rate of polymer solution, nozzle tip-to-collector distance, nozzle design, collector composition and its geometry, and rotation speed.

(a) Applied voltage

The critical voltage value differs from polymer to polymer. Generally, a voltage value between 5-40kV is applied for productive electrospinning. An electrospinning solution with high surface tension, low conductivity, and high viscosity need higher voltages, and vice versa. A high voltage causes more extensive stretching of polymer solution due to increased charge repulsion within the polymer jet, which results in fabrication of small-diameter nanofibers. A further increase in the applied voltage beyond a critical value results into Taylor cone stability and hence beaded nanofibers formation [Pham et al. 2006, Sill and von Recum 2008].

(b) Nozzle tip-to-collector distance

In most the cases fiber morphology can be readily affected by the distance between needle tip to collector distance since it affects the evaporation rate, deposition time, and instability or whipping interval. A too short distance results into nanofiber fusion and polymer film formation. On increasing the distance, nanofibers with thin diameter produces, however, it should be accompanied by increasing the applied voltage and the flow rate otherwise beaded will produced [Bhardwaj and Kundu 2010, Matabola and Moutloali 2013]. However, in some cases no effect of distance was observed on nanofiber morphology.

(c) Solution flow rate

The flow rate of electrospinning solution determines the fiber morphology and it depends chiefly on volatility of electrospinning solvent used. When a highly volatile solvent accompanied by a sufficiently high applied electric field is employed for electrospinning, smooth nanofiber smooth nanofibers could be produced at higher flow rate. However, some

studies observed that a higher flow rate resulted into thicker nanofiber or beaded nanofiber or deposition of wet nanofibers [Cramariuc et al. 2013, Pelipenko et al. 2015].

(d) Nozzle design

Many modifications of spinning nozzles have been made for producing different kinds of nanofibers. A single-channel nozzle allows formation of uniform nanofibers, where as a coaxial nozzle enables formation of core–shell or even multilayered nanofibers [Maleki et al. 2013]. In addition, hollow nanofibers and inner wall coated nanofibers can be produced by a coaxial nozzle when the polymer concentration in solution flowing through inner needle is very low. After solvent evaporation the polymer is deposited on the inner wall of core–shell nanofibers as a thin film, while the rest is empty core [Pelipenko et al. 2015].

(e) Collector

A conductive collector is required to produce sufficient electric field and thus to initiate electrospinning. A non-conductive collector results into charge accumulation and hence lower packing density of nanofibers [Coelho et al. 2018]. The collector can be a rotating cylinder, or a wheel-like disk, a flat surface (patterned or continuous). While rotating collectors have been employed to collect aligned fibers, static planar collector used to produce randomly arrange nanofibers [Pelipenko et al. 2015].

2.3.3.3. Ambient parameters

Although, ambient parameters (**temperature** and **relative humidity**) are least considered parameters affecting electrospinning, but they play an important role. The laboratory temperature affects the solvent evaporation rate. Higher temperatures result in higher evaporation of solvents and the production of thicker nanofibers, vice versa. The effect of

relative humidity on electrospinning depends on composition of polymer solution. In case of non-polar polymeric solution, water acts as a non-solvent and higher relative humidity causes the production of porous nanofibers. In case of polymer dissolved in water, relative humidity can be employed to manipulate nanofibers diameter and their mechanical properties [Pelipenko et al. 2015].

2.3.4. Application of electrospinning technique

The applications include filtration, cosmetic mask, military protective clothing, nano-sensor, energy-related applications, wound dressings, drug delivery, enzyme immobilization, and tissue engineering scaffolds [Hu et al. 2014].

Nanofibers in drug delivery

Nanofibers have been investigated for over a decade as drug delivery systems for transdermal, oral, oromucosal, parenteral, and ocular application and numerous drugs have already been incorporated into nanofibers, namely antibiotics, analgesics, non-steroidal anti-inflammatory drugs, anti-cancer drugs, nucleic acids, and growth factors [Pelipenko et al. 2015].

Nanofibers in tissue engineering

Regenerative medicine is a comparatively new, exciting, and continuously progressing research field. It enables the formation of functional tissue substitutes to repair or replace tissue or organ function lost due to aging, damage, disease, or congenital disabilities. Basic science in tissue engineering and regenerative medicine further aims to investigate the deposition, growth, and remodeling of tissues by exploiting the knowledge from a range of different disciplines.

2.4. Drugs Profile

2.4.1. Ciprofloxacin hydrochloride

Ciprofloxacin hydrochloride is a most frequently used second generation fluoroquinolone antibiotic for a variety of systemic such as joint infection, intra-abdominal infections, typhoid fever, lower respiratory tract infection, urinary tract infection etc., as well as local bacterial infections, such as ear, nose, eye, skin, etc. Low minimal inhibitory concentration (MIC) (<0.1 µg/mL) against Gram-negative and relatively higher (1-2 µg/mL) against Gram-positive microorganism and lower frequency of microbial resistance makes it a promising antimicrobial for wound infection [Jannesari et al. 2011, Kevadiya et al. 2014, Tripathi 2013].

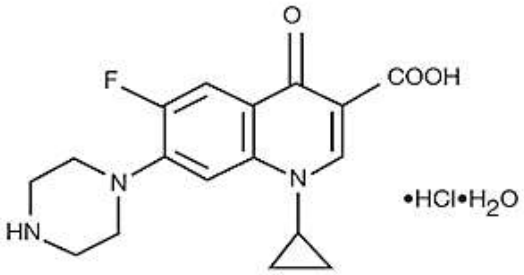

Figure 2.7: Chemical structure of ciprofloxacin hydrochloride monohydrate

IUPAC Name : l-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(l-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate

Molecular Weight : 385.8 g/mol

Chemical Formula : $C_{17}H_{18}FN_3O_3,HCl,H_2O$

Source: Indian Pharmacopoeia 2014, page no-1401

Physical and Pharmacokinetic Properties of Ciprofloxacin hydrochloride

Identification : Infrared Spectrum, UV Spectroscopy (λ_{max} = 275 nm in Water)

Description : A pale yellow, crystalline powder

Solubility : Water: 35 mg/mL; Methanol: 2.1mg/mL

LogP ($K_{o/w}$) : 0.28

pka : 6.09 (carboxylic acid group); 8.74 (nitrogen on piperazinyl ring)

Half-life ($t_{1/2}$) **:** 3-5h
Bioavailability **:** 70%
Source: Indian Pharmacopoeia 2014, page no-1401; Drug Bank and Pubchem.

Mechanism of Action: All quinolones restrict the bacterial DNA replication by inhibiting DNA gyrase (topoisomerase II) and topoisomerase IV. DNA gyrase is required for introducing negative supercoils and stabilizing the same during DNA replication. Thus, blockage of DNA gyrase causes the inhibition of the relaxation of positively supercoiled DNA and replication of bacteria. Inhibition of topoisomerase IV interferes with separation of replicated chromosomal DNA into the respective daughter cells during cell division [Brunton et al. 2011, Tripathi 2013].

Antimicrobial Spectrum: Ciprofloxacin hydrochloride is a potent anti-microbial agent having a broad spectrum against variety of Gram-negative and Gram-positive bacteria. The highly susceptible species of microorganism are *E. coli, N. gonorrhoeae, N. meningitides, Salmonella typhi, Vibrio cholera* etc. The drug also exhibits moderate activity against *Pseudomonas aeruginosa*, *Staphylococcus* species including methicillin-resistant Staphylococcus aureus (MRSA), *M. tuberculosis* etc. [LeBel 1988, Tripathi 2013].

Pharmacokinetics: After oral administration, the ciprofloxacin hydrochloride is well absorbed and shows around 70% absolute bioavailability with little or no first pass metabolism. After a single oral dose, it shows 20-40% plasma protein binding, and a varied range of distribution coefficient (2.48-5.93 L/kg), indicating that the drug is widely distributed in the extravascular body fluid and tissues. Tubular secretion and glomerular filtration is the major excretion route [LeBel 1988, Tripathi 2013].

Adverse effects: Ciprofloxacin has good safety record; generally mild side effects occur in ~10% patients. The most common gastro-intestinal adverse reactions include mild nausea, vomiting, and/or abdominal discomfort. Achilles tendon rupture or tendinitis is a recognized adverse effect, especially in those >60 years old, patients taking corticosteroids, and in solid organ transplant recipients. Ciprofloxacin may destroy growing cartilage and induce an arthropathy. Thus the routine use in patients below the age of 18 is not suggested [Brunton et al. 2011, Tripathi 2013].

Recent studies on ciprofloxacin application in wound healing

Sripriya et al. had developed a collagen bilayer dressing loaded with ciprofloxacin to reduce the bacterial infection at wound site. They had examined the succinylation of collagen and ionic binding of ciprofloxacin to succinylated collagen by FT-IR, surface morphology by SEM, antimicrobial activity on agar plate, *in-vitro* drug release profile and *in-vivo* wound healing efficacy of the dressing materials. Ciprofloxacin loaded collagen bilayer dressing material was effective to heal a full thickness 21 days [Sripriya et al. 2007].

Unnithan et al. dextran/polyurethane based electrospun nanofiber loaded with ciprofloxacin hydrochloride and characterized for various *in-vitro* properties. Proliferation of cell on its surface indicated that nanofiber membrane supported cell growth. The scaffold exhibited excellent anti-bactericidal activity against both of Gram-negative and Gram-positive bacteria. Overall, results conclude that scaffold might be an ideal candidate for wound dressing applications [Unnithan et al. 2012].

A novel chitosan/poly ethylene glycol (PEG) composite scaffold loaded with ciprofloxacin hydrochloride was prepared by Sinha et al. as wound healing dressing. Incorporation of

PEG in chitosan scaffold had increased the drug loading and improved the cumulative release. The developed scaffold was characterized for antibacterial activity, swelling property, haemocompatibility, moisture vapour transmission rate, biodegradation profile and *in-vivo* wound healing efficacies [Sinha et al. 2013].

To achieve fast delivery of antibiotic at acute injury site, Kataria et al. developed a ciprofloxacin loaded hydrophilic biodegradable sodium alginate (NaAlg) and poly vinyl alcohol (PVA) based electrospun nanofiber for transdermal application. They found that the scaffold provided a sustained and controlled release of drug. The *in-vivo* wound healing studies demonstrate that, healing takes place in less time as compared drug unloaded patch [Kataria et al. 2014].

Okoye and Okolie prepared a ciprofloxacin incorporated gelatin & sodium carboxymethyl cellulose (Na-CMC) based film by solvent casting method and characterized it for thickness weight uniformity, sorption capacity, surface pH, bending endurance, physic-chemical stability by FT-IR and DSC, antibacterial activities and *in-vitro* drug release. Results shown that polymer blends at 2:1 or 1:1 (gelatin : Na-CMC) stood out as the most promising combination for the formulation of ciprofloxacin wound dressing films [Okoye and Okolie 2015].

Esrafilzadeh et al. developed a ciprofloxacin hydrochloride loaded multifunctional scaffold consisting of a core-sheath structure. Here PLGA sheath (nanofiber) was coated onto conducting poly(3,4-ethylenedioxythiophene) poly(styrene sulfonate) (PEDOT:PSS) and Chitosan (CHI) (PEDOT:PSS–CHI) wet-spunfibers which was of micron size. The scaffold provide controlled release of ciprofloxacin hydrochloride with excellent electrochemical

performance, mechanical properties and cytocompatibility, which hold great potential for the application of conductive electrospun scaffolds in regenerative medicine [Esrafilzadeh et al. 2016].

A deep wound, infiltrated with bacteria does not heal spontaneously and it often requires skin regeneration product loaded with antibiotics. For the same purpose, Suhaeri et al. prepared a PVA hydrogel based skin patch loaded with ciprofloxacin and decellularized human lung fibroblast-derived matrix (hFDM) for prompt wound healing. The results supported that skin patch successfully reduced bacterial infection and thus achieved wound closure within 21 days [Suhaeri et al. 2018].

2.4.2. Quercetin

Quercetin is a naturally occurring flavonoid belonging to flavanol class, commonly found in fruits and vegetables, especially onions, broccoli, apples, tea and red wine [Zheng et al. 2017]. High propensity for electron transfers due to phenolic group proves it as strong free radical scavenger and potential anti-oxidant. Quercetin checks tissue damage by attenuating free radicals that predispose tissue to oxidative damage. Additionally, it also shows other health-beneficial effect like cardiovascular protective effect, anti-carcinogenic, antiviral, anti-allergic and anti-inflammatory properties, anti-diabetic, gastro-protective effects. [Aceituno-Medina et al. 2015, Arvand et al. 2015, Li et al. 2016b].

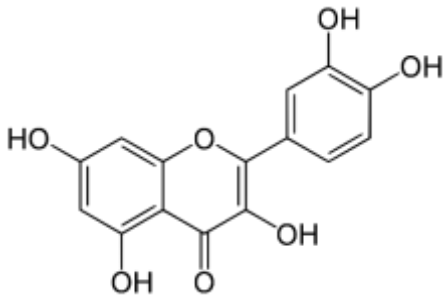

Figure 2.8: Chemical structure of quercetin

IUPAC Name	**:**	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one
Molecular Weight	**:**	302.23 g/mol
Chemical Formula	**:**	$C_{15}H_{10}O_7$

Source: Pubchem

Physical and Pharmacokinetic Properties of Quercetin

Identification	**:**	UV-Visible Spectroscopy (λ_{max} at 343nm in chloroform)
Description	**:**	Yellow needles or yellow powder.
Solubility	**:**	Very soluble in ether, methanol; soluble in ethanol, acetone, pyridine, acetic acid. Insoluble in water (less than 0.06 mg/mL at room temperature)
LogP ($K_{o/w}$)	**:**	1.81
pka	**:**	6.44
Half-life ($t_{1/2}$)	**:**	11.9 ± 4.0 h
Oral Bioavailability	**:**	3-17% after receiving 100mg oral dose

Source: [Pubchem, Li et al. 2016a]

Mechanism of Action: Multiple mechanisms have been discussed in literature for the antioxidant progress of flavonoids (ArOH). Three main antioxidant mechanisms are follows [Zheng et al. 2017]:

1. Hydrogen Atom Transfer (HAT):

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$$

2. Single Electron Transfer followed by Proton Transfer (SET-PT):

$$R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{+\bullet}$$
$$R^{-\bullet} + ArOH^{+\bullet} \rightarrow RH + ArO^{\bullet}$$

3. Sequential Proton Loss Electron Transfer (SPLET):

$$ArOH \rightarrow ArO^{-} + H^{+}$$
$$ArO^{-} + R^{\bullet} \rightarrow ArO^{\bullet} + R^{-}$$
$$R^{-} + H^{+} \rightarrow RH$$

Pharmacokinetics: Owing to highly hydrophobic nature, quercetin show poor and variable oral bioavailability, while its glycosidic form showed enhanced absorption from GIT due to their increased hydrophilicity imparted from the attached sugar moiety. After absorption, the drug is widely and quickly metabolised in various organs such as liver, small intestine, colon (colonic bacteria), and kidney. Methylated form is the main metabolite following metabolism in the liver. The large protein binding of about 99.4% is another reason for low bioavailability. Due to extensive protein binding, its biological half-life varies from 11-28 h, causing very slow elimination from human body. Renal excretion is major route of drug removal [Li et al. 2016a].

Recent studies on quercetin application in wound healing

Quercetin incorporated collagen matrix was developed by Gomathi et al. as a novel dressing material for skin wound healing application. Researchers examined the *in-vitro* antioxidant activity of matrix, and various biochemical parameters such as hydroxyproline, uronic acid and protein content in the granulation tissues. The results revealed the quercetin incorporated collagen matrix as better healing material in comparison to pure collagen treatment [Gomathi et al. 2003].

Considering the importance of quercetin in angiogenesis and fibroblasts proliferation of, Kant et al. applied the four concentration of quercetin (0.1, 1.0 and 10.0% w/v) in full thickness wound and observed healing efficiency and histological changes in granulation tissues. They also examined the biochemical estimation of SOD, malondialdehyde levels, superoxide radicals, total thiols and proteins. Treated groups did not show any significant difference in terms of biochemical parameters, however, 0.1% quercetin treated group caused fastest wound closure, as compared to other treatments groups [Kant et al. 2017].

Ma et al. used astrocyte migration model to investigate the effect of quercetin-loaded solid lipid nanoparticles on traumatic repair and scar inhibition and related mechanism. They removed brain external skin of rats and used Sirius Red staining, Masson staining and scanning electron microscopy for the assessment of the effect of quercetin-loaded solid lipid nanoparticles. Western blotting technique was used to determine the expression of various Tumor growth factor (TGF) and SMADs proteins in traumatic skin and astrocytes. The finding revealed that Quercetin-loaded SLN can obstruct the activation of TGF-β/Smad pathway and hence accelerating the wound healing and preventing the scar formation [Ma et al. 2018].

The effect of quercetin in the recovery of focal entrapment neuropathy was examined by Thipkaew et al. Researchers fabricated quercetin-loaded zein-based nanofibers by electrospinning technique. Right sciatic nerve was crushed to cause mononeuropathy in streptozotocin-induced diabetic rats. Functional recovery was measured by measurements of foot withdrawal reflex, nerve conduction velocity, walking track analysis and morphological analysis. The Results revealed that quercetin-loaded zein-based nanofibers slightly improved functional recovery from neuropathy in STZ-induced diabetic rats [Thipkaew et al. 2017].

Li et al. developed quercetin-loaded zein nanoribbons using single fluid and coaxial process electrospinning process. They examined the fabricated scaffolds by FT-IR and XRD and found that drug was encapsulated in amorphous form. *In-vitro* dissolution confirmed that nanoribbons offer sustained release of quercetin via a classic Fickian diffusion mechanism, and the coaxial process displayed better performance than the single fluid process in terms of short initial burst effect and leveling-off release [Li et al. 2014].

2.5. Polymers Profile

2.5.1. Poly(ε-caprolactone) (PCL)

Poly(ε-caprolactone) (PCL) is one of the extensively examined, and USFDA approved polymer for controlled drug delivery. Biodegradable**,** bioresorbable, biocompatible and sound mechanical properties of PCL find its significant use for medical and drug delivery devices. Chemical compatible nature of PCL with a wide range of drugs and slow degradation rate paved the way for designing the controlled and sustained delivery device [Chong et al. 2007, Xue et al. 2014]. Comparatively low cost of PCL along with mentioned properties render it a suitable candidate for biomedical applications.

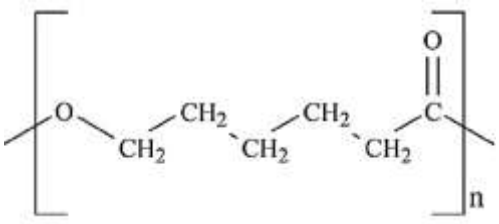

Figure 2.9: Chemical structure of monomer of poly(ε-caprolactone)

Synonym	**:**	2-Oxepanone homopolymer, 6-Caprolactone polymer
Number Average Molecular weights (Mn):	**:**	~ 80000 Da
Empirical Formula	**:**	$(C_6H_{10}O_2)_n$

Source: Merk (https://www.sigmaaldrich.com/catalog/product/aldrich/440744?lang=en&region=IN)

Description: PCL is a synthetic homopolymer of ε-caprolactone. The ring-opening polymerization of ϵ-caprolactone yields a semi-crystalline, hydrophobic polymer with a low melting point (58-63 °C) and glass transition temperature (−60 °C) with a degradation time on the order of two years [Saad and Suter 2001]. The polymer is commercially available in varying molecular weights, either in powder form (low molecular weight grade) or as pellets (high molecular grade; ~3 mm).

Solubility: Practically insoluble in water; soluble in chloroform, acetone, methanol, tetrahydrofuran, ethyl acetate, dichloromethane, and hexafluoroisopropanol.

Regulatory Status: Listed as GRAS, and included in Canadian list of Non-medicinal ingredients [Health Canada]. Its medicinal devices are FDA approved [Yang et al. 2011].

Applications in Pharmaceutical Formulation: Being a biocompatible and biodegradable carrier, PCL is used in many types of implantable or injectable drug-delivery systems and also a broad application in tissue engineering.

2.5.2. Gelatin

Gelatin (GE) is a hydrophilic biopolymer obtained from partial hydrolysis of collagen, the principal ECM building protein. Gelatin also contains cell-recognition site (Arg-Gly-Asp amino acid sequences), which is recognized by integrins and thus aids in cell attachment and it's spreading. Hence, in this way, GE increased the cellular attachment and enhanced spreading to the surface of the material [Dulnik et al. 2016, Xue et al. 2014].

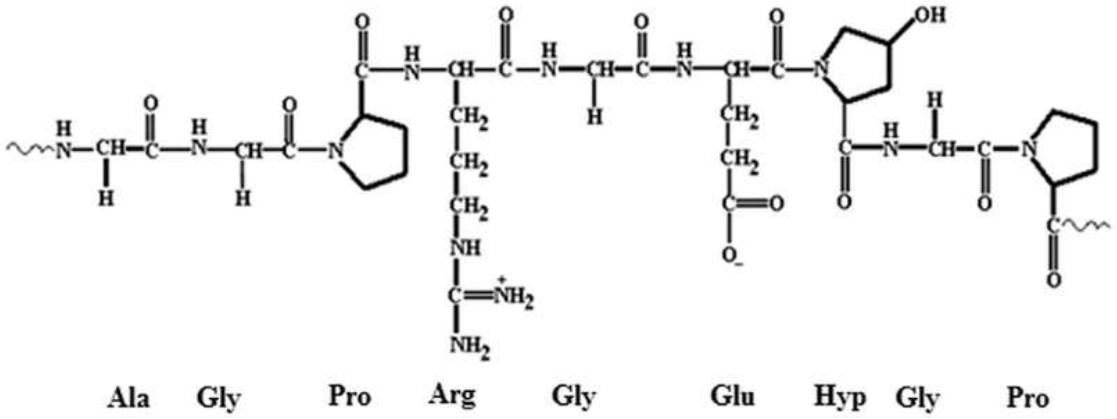

Figure 2.10: A typical structure of gelatin

Properties of Gelatin

Appearance	**:**	White to beige powder or granule
Solubility	**:**	33.3 mg soluble in 1 mL of water
Gel Strength	**:**	~300 g Bloom
Average Molecular weight	**:**	15,000 to 2,50,000 Da
Storage	**:**	Store below 30°C
Stability	**:**	Stable, hygroscopic & also incompatible with strong oxidizing agents

Source: himedialabs.com

Description: Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules with a slight odour. It is stable in air but is subjected to microbial decomposition when moist or in solution (IP 2014, page no-1847).

Chemical Composition and Structure: Gelatin is a heterogeneous mixture of single-stranded or multi-stranded polypeptides, containing about 50-1000 amino acids. GE molecules contain repeating sequences of glycine, proline and hydroxyproline triplets, which are responsible for the triple helical structure of GE [Harrington and von Hippel 1961].

There are two types of GE are available namely A and B which are obtained from acid (hydrochloric acid or sulfuric acid) and alkali treatment of collagen with a distinct difference in isoelectric points. Type B GE has more carboxyl groups and lowers isoelectric point (4.8-5.0) as compared to Type A (7.0-9.0) [Peng et al. 2008, Zhang et al. 2006]. Alkaline pre-treatment of collagen leads to conversion of asparagine and glutamine residues to their respective acids and results in higher viscosity. Table 2.4 shows the list of different amino acids that are a part of GE.

Table 2.4: Approximate amino acid composition of a typical Gelatin

Components	Content	Components	Content
Glycine	26-28%	Lysine	4-6%
Proline	14-18%	Serine	3-4%
Hydroxyproline	14-16%	Threonine	2-3%
Glutamic acid	10-12%	Valine	2-3%
Alanine	9-11%	Tyrosine	1%
Arginine	8-9%	Histidine	1%
Aspartic acid	5-7%	Hydroxylysine	1%
Leucine	5-6%	Methionine	1%
Isoleucine	5-6%	Phenylalanine	1%

Applications of gelatin: Because of its distinctive mechanical and technological characteristics, gelatin is commonly used in food, pharmaceutical, cosmetic and medical field. In the medical and pharmaceutical areas, gelatin is currently used as device coatings, matrix for implants and as a stabilizer in vaccines. It is also used in hard and soft capsules, intravenous infusions, wound dressings, plasma expanders, hemostats, tissue bioadhesives and in drug delivery systems [Foox and Zilberman 2015]. Gelatin was blended with PCL and loaded with drug for wound healing application [Bakhsheshi-Rad et al. 2019, Rather et al. 2018].

2.5.3. Poly (D,L-lactide-co-glycolide) (PLGA)

PLGA is one of the most successfully used biodegradable polymer and FDA approved for the development of multiple therapeutics. It undergoes hydrolytic degradation in the body to produce the biodegradable metabolite monomers, namely lactic acid and glycolic acid (Figure 2.12). Since the body effectively deals with these two monomers, there is very minimal systemic toxicity associated by using PLGA for drug delivery or biomaterial applications. PLGA provide a consistent degradation profile [Kumari et al. 2010].

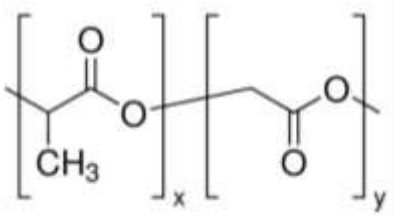

Figure 2.11: Chemical structure of monomer of PLGA, x is the number of lactic acid units and y is number of glycolic acid units.

Molecular Weight	**:**	66,000-107,000
Linear Formula	**:**	$[C_3H_4O_2]_x[C_2H_2O_2]_y$
Polymer Composition	**:**	Lactide:glycolide (75:25)
Appearance	**:**	White to yellow, Pellets
Storage Temperature	**:**	−20°C
Inherent Viscosity (dL/g)	**:**	0.55-0.75
Solubility	**:**	It is soluble in most organic solvents such as methylene chloride, tetrahydrofuran, ethyl acetate, acetone, chloroform, and hexafluoroispropanol.

Source: Merck (https://www.sigmaaldrich.com/Graphics/COfAInfo/SigmaSAPQM/SPEC/P1/P1941/P1941-BULK_____SIGMA____.pdf)

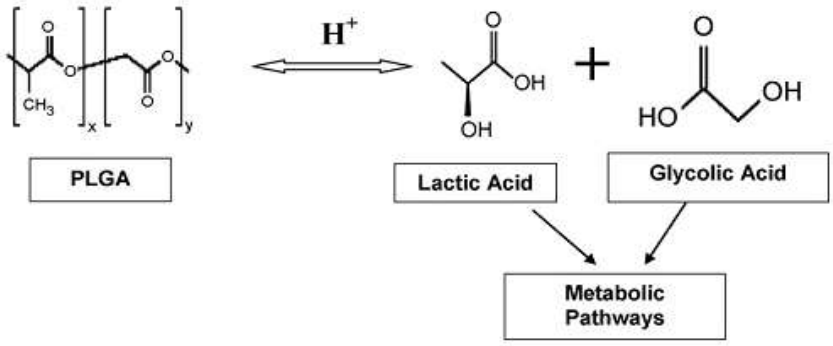

Figure 2.12: Hydrolysis of PLGA polymer in body fluid [Kumari et al. 2010].

Polyester PLGA is a copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA). Poly lactic acid contains an asymmetric α-carbon which is typically described as the D or L form in classical stereochemical terms. The enantiomeric forms of the polymer PLA are poly D-lactic acid (PDLA) and poly L-lactic acid (PLLA). PLGA is generally an acronym for poly D,L-lactic-co-glycolic acid where D- and L- lactic acid forms are in equal ratio

[Makadia and Siegel 2011]. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding linear, aliphatic polyester as a product [Astete and Sabliov 2006]

Different forms of PLGA can be identified in regard to the monomers' ratio used (e.g. PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid). All PLGAs are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60 °C. Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate [Makadia and Siegel 2011].

Application of PLGA in drug delivery: Despite having extreme long-term storage temperature (−20°C), PLGA can be used for short time period at room temperature for developing a variety of drug delivery system. It ranges from microparticles (by double emulsification followed by solvent evaporation method), microspheres (by solvent evaporation, single and double emulsification methods), nanoparticles (by nanoprecipitation, double emulsification, modified solvent diffusion technique), implants, PLGA rods, inhalable dry powders, nanofibers (by electrospinning technique) and loaded with numerous drugs such as analgesics, antibiotics, hormone, anti-cancer drugs, non-steroidal anti-inflammatory drugs, nucleic acids, growth factors and minerals for bone substitute [Alnuman et al. 2018, Garcia-Orue et al. 2019, Kapoor et al. 2015, Zheng et al. 2018].