Chapter: 2

Literature Review

2 Literature review

2.1 Structure of skin

Skin is largest organ of human body, with a complex and multifaceted structure that serves vital functions i.e. maintaining homeostasis, protection the body from external threats and thermoregulations [1]. Human skin composed of three main layers—the epidermis, dermis, and subcutaneous layer [43]. The epidermis, is a stratified squamous epithelium primarily composed of keratinocytes, melanocytes, langerhans cells, and merkel cells [44]. Keratinocytes cells undergo a process of keratinization and move from the basal layer to the surface and form a tough and protective and water proof protein called keratin [45]. Melanocytes are responsible for the melanin production which determine the skin colour and protect skin from harmful UV rays [46]. Langerhans cells are immune cells, primarily responsible for skin defence against pathogens [47]. Merkel cells, found in the selective part of body and responsible for the sensory functions.

Dermis is the connective tissue layer present under the epidermis layer and composed of collagen and elastic fibers which provide elasticity and mechanical strength to the skin [48]. The dermis layer also houses numerous blood vessels, nerves, hair follicles, sweat glands, and sebaceous glands. Blood vessels supply nutrients and oxygen to the skin, while nerve endings are responsible for touch, pressure, temperature, and pain sensations. Hair follicles are invaginations of the epidermis layer and that extended to the dermis. Hair follicle houses sebaceous glands, which produces sebum, an oily substance that helps lubricate the hair and skin. Sweat glands, including eccrine and apocrine glands, play a crucial role in thermoregulation by producing sweat, which evaporates and cools the skin [49].

The subcutaneous is the deepest layer of the skin and consists of loose connective tissue and adipose (fat) tissue [43]. It act as insulator helping regulate body temperature, and provides a cushioning effect that protects underlying structures like muscles and bones.

Larger blood vessels and nerves pass through the hypodermis, connecting the skin to deeper tissues [50].

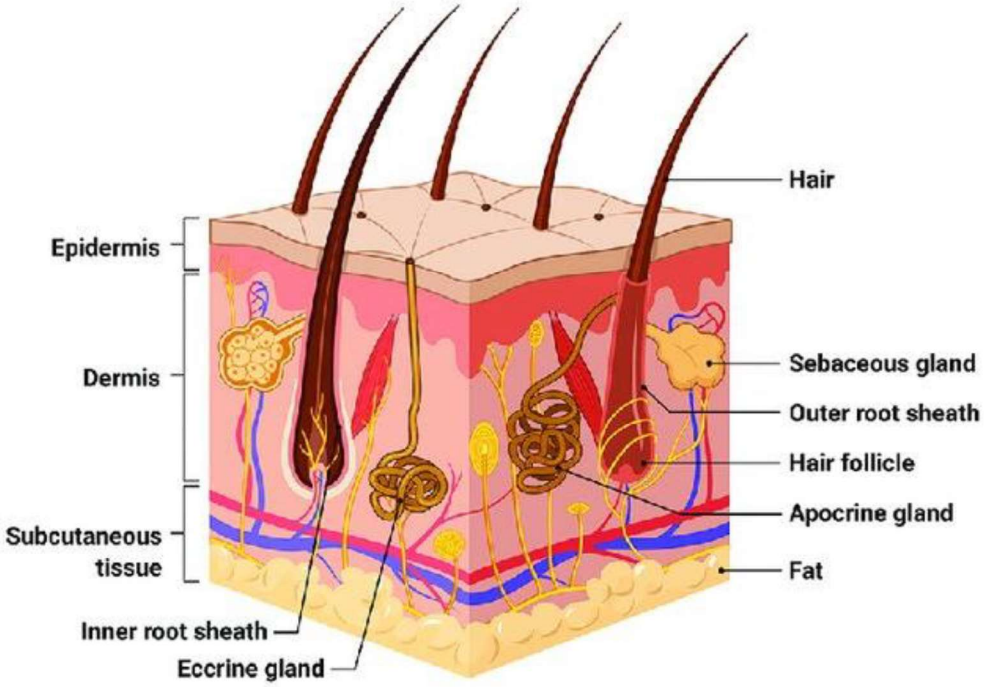

Figure 2.1 Structure of the skin [51]

2.2 Wounds

A wound refers to a disruption in the normal structure and function of the skin, often resulting from physical injury, surgery, or disease [52]. Wounds can vary in severity, ranging from minor cuts and abrasions to more complex injuries that involve damage to underlying tissues. These are classified based on various criteria, including the cause of injury, depth, and the nature of tissue involvement [53].

- Based on Cause:
    - **Traumatic wounds**: Result from physical injuries, accidents, or external forces [54] .
    - **Surgical wounds**: Occur as a result of planned surgical procedures
    - **Pressure wounds**: Due to continuous pressure over skin area.

- Based on Depth:
    - **Superficial wounds**: Superficial wounds involve only the outermost layer of the skin, known as the epidermis. These wounds are typically minor and limited to the skin's surface. Examples include scratches, minor cuts, and first-degree burns. Superficial wounds generally heal relatively quickly with minimal scarring, as they do not penetrate deep into the skin layers [55].
    - **Partial thickness wounds**: Partial thickness wounds extend beyond the epidermis into the deeper layer of the skin, known as the dermis. These wounds may result from more substantial trauma or injuries, involving damage to both the epidermis and the dermis. Examples include second-degree burns and abrasions that penetrate into the dermal layer. Healing of partial thickness wounds involves re-epithelialization, where new skin cells regenerate from the edges of the wound and migrate to cover the exposed area [56].
    - **Full thickness wounds**: Full thickness wounds extend through the entire skin, reaching the subcutaneous tissue and possibly deeper structures. These wounds are often the result of significant trauma, surgical procedures, or deep lacerations. Full thickness wounds expose underlying tissues, such as muscles, tendons, or bones. Healing of full thickness wounds is a complex process that involves granulation tissue formation, collagen deposition, and, in some cases, surgical interventions like sutures or skin grafts to facilitate closure [21]
- Based on characteristics
    - **Incised wounds**: These wounds result from clean, sharp-edged objects, leading to smooth cuts. Examples include knife or glass cuts. Incised

wounds typically have well-defined edges and may bleed more than other types of wounds [57].

Treatment: Immediate care involves controlling bleeding, cleaning the wound, and securing the edges through appropriate closure methods like sutures [58].

- **Lacerations:** These are irregular, torn wounds often caused by blunt trauma or sharp objects with jagged edges. The edges of lacerations may appear ragged, and the wound may be associated with significant bleeding.

  Treatment: Lacerations often require treatment thorough cleaning, careful alignment of tissue, and closure with stitches [59].

- **Contusions:** Contusions, commonly known as bruises, result from blunt force trauma damaging blood vessels beneath the skin. These wounds manifest as discoloration due to blood pooling beneath the skin.

  Treatment: In minor cases treatment of contusive wounds include supportive therapy (ice, rest, elevation) and severe contusions required medical attention [60].

- **Puncture wounds:** These wounds occur when a sharp, pointed object pierces the skin, creating a small, deep hole. Puncture wounds may not bleed profusely but can introduce bacteria deep into tissues, leading to infections.

  Treatment: Cleaning puncture wounds is crucial to prevent infections, and some may require medical evaluation [61].

- **Abrasions**: These wounds also termed as scrapes, are superficial wounds caused by friction against the skin's surface. The top layer of skin (epidermis) is affected, resulting in a raw, often painful area.

Treatment: Cleaning and protecting abrasions with bandages to prevent infection are essential components of care [62].

- **Avulsions:** Avulsions involve the tearing away of a portion of skin and underlying tissues. These wounds vary in severity, and the extent of tissue loss depends on the force of the injury.

  Treatment: Avulsions may require immediate medical attention, and surgical intervention may be necessary to repair and reconstruct the damaged area [63].

- **Penetrating wounds:** These wounds occur when objects enter and lodge into the body tissues. The depth and severity of damage depend on the nature of the penetrating object.

  Treatment: Urgent medical evaluation is crucial for assessing the extent of injury, removing foreign objects, and preventing complications [64].

2.3 Wound healing

Wound healing is a highly intricate and dynamic biological process that aims to restore the structural and functional integrity of damaged tissue [65]. It involves a series of complex cellular and molecular events orchestrated through a well-coordinated sequence of phases inflammation, proliferation, and remodelling [66]. These comprehensive processes are essential for the body's recovery from injuries, ranging from minor cuts and abrasions to more significant wounds.

The initial phase of wound healing is the inflammatory phase, which serves as the body's immediate response to injury. As soon as tissue damage occurs, blood vessels constrict to minimize bleeding. Simultaneously, platelets are activated, releasing clotting factors and forming a temporary clot to stop the flow of blood [67]. The inflammatory cells, predominantly neutrophils and later macrophages, are then recruited to the wound site to

clear away debris, bacteria, and damaged tissue. These immune cells play a crucial role in the early stages of the healing process by creating an environment conducive to tissue repair [68]. As the inflammatory phase progresses, the focus shifts to the proliferation phase [69]. The proliferation phase is characterized by a complex interplay of growth factors, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). This fibroblast growth factor (FGF) lead to activation of fibroblasts [70,71]. Fibroblasts migrate to the wound site and begin producing collagen, for creating a framework that supports tissue regeneration. The vascular endothelial growth factor (VEGF) results in angiogenesis to supply oxygen and nutrients to the healing tissue [72]. After proliferation phase, the wound healing process enters the remodelling phase. This stage is marked by the refinement of the newly formed tissue and the resolution of the scar tissue. Collagen fibers are reorganized and broken down by enzymes, leading to increased tissue strength and flexibility. Matrix metalloproteinase (MMPs) are enzymes that play a crucial role in collagen remodelling [73]. The balance between collagen synthesis and degradation determines the quality of the healed tissue and influences the formation of scars [74].

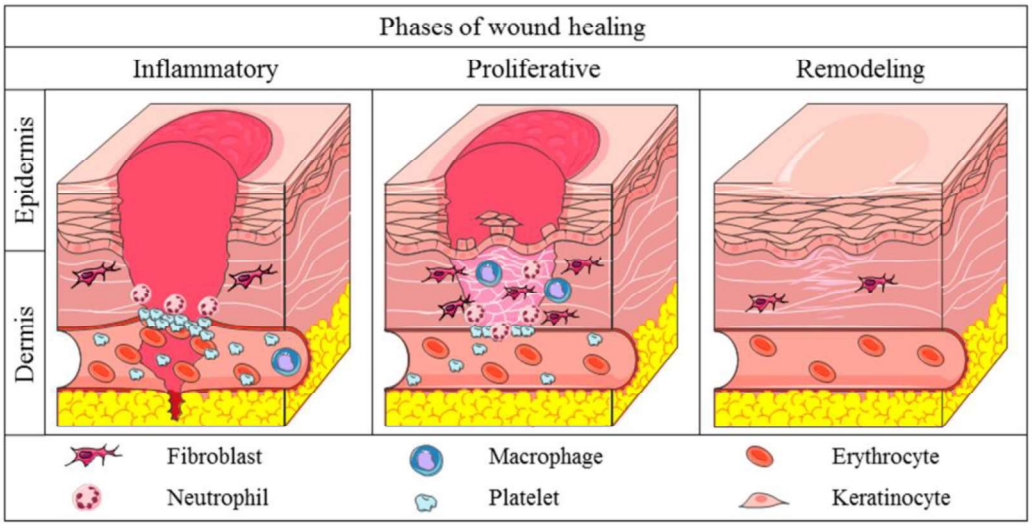

Figure 2.2 Phase of wound healing [75]

2.4 Wounds healing impairment in diabetic patient

2.4.1 Background

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from insufficient insulin production, ineffective insulin utilization, or a combination of both [6]. The escalating prevalence of diabetes has become a global health concern, representing a significant public health challenge in recent decades. According to the International Diabetes Federation, 536 million people between the ages of 20 and 79 suffered from diabetes worldwide in 2019 [76]. This surge is largely attributed to factors such as sedentary lifestyles, unhealthy dietary habits, and an aging population [77].

Complications arising from diabetes span a spectrum of systemic and localized effects, with some of the most pronounced impacts occurring in the vascular and nervous systems. Microvascular complications include retinopathy, nephropathy, and neuropathy, each contributing to vision impairment, kidney dysfunction, and peripheral nerve damage, respectively [7,78,79]. On a macrovascular level, diabetes significantly elevates the risk of cardiovascular diseases, such as coronary artery disease and stroke, further exacerbating the morbidity associated with the condition [79–81].

Another challenging aspect of diabetes is its propensity to compromise the body's natural wound healing processes. Diabetic individuals often face impaired immune responses, delayed tissue regeneration, and heightened susceptibility to infections [82]. This becomes particularly pertinent when considering the complications associated with wounds in diabetic patients, leading to chronic non-healing ulcers and an increased risk of microbial infections.

2.4.2 Healing impairment in diabetic

Wound care in diabetic patients represent a significant and challenging aspect of healthcare, requiring careful attention due to the unique characteristics of diabetes that can complicate the normal wound healing process [83]. One of the primary challenges in diabetic wound healing is the microvascular complications associated with diabetes. Hyperglycemia, a hallmark feature of diabetes, leads to damage and dysfunction of the small blood vessels, impairing blood flow to the affected tissues [84]. Reduced perfusion limits the supply of oxygen, nutrients, and immune cells to the wound site, hindering the normal healing cascade. This microangiopathy creates a hypoxic environment, exacerbating the impaired wound healing process [85].

Further, the immune dysfunction observed in diabetes further complicates wound healing. Chronic hyperglycemia impairs the function of immune cells, such as neutrophils and macrophages, leading to decreased microbial clearance and an exaggerated inflammatory response [86]. The inflammatory phase of wound healing is a critical stage involving the recruitment of immune cells, release of cytokines, and removal of debris [87]. However, in diabetic wounds, this phase tends to be prolonged, creating a chronic inflammatory state. The dysregulation of pro-inflammatory and anti-inflammatory signals contributes to the persistence of inflammation, hindering the transition to the subsequent stages of wound healing, such as proliferation and remodelling [88].

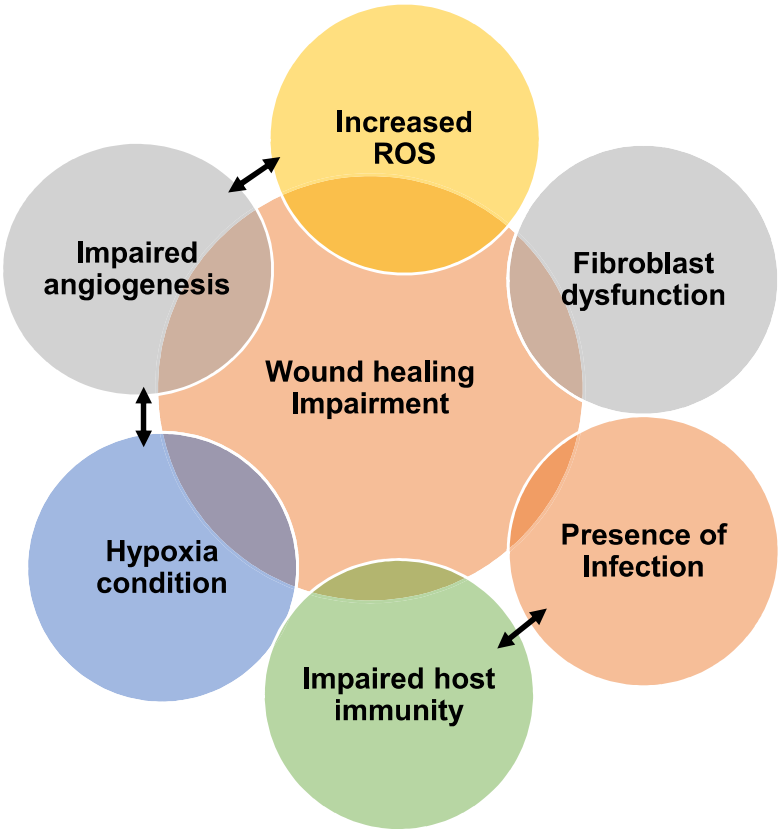

Figure 2.3 Wound impairment in diabetic mellitus

2.4.3 Fungal infection in diabetic

Fungal infections, pose a distinctive set of challenges in the context of diabetic wounds. The altered microenvironment created by diabetes, characterized by poor blood circulation and reduced oxygen supply to tissues, provides an ideal breeding ground for fungi [89]. Fungal pathogens thrive in warm, moist environments, making the chronically moist diabetic wounds an inviting habitat for these microorganisms [90].

Sometimes, challenges posed by fungal infections in diabetic wounds extend beyond the superficial aspects of wound management. Fungi can infiltrate deep tissues, exacerbating the severity of the wound and impeding the natural healing process [15]. Moreover, the presence of fungi can lead to persistent inflammation, delaying the closure of wounds and increasing the risk of secondary infections [91].

The significance of addressing these challenges becomes evident when considering the potential consequences of untreated or poorly managed diabetic wounds. Chronic non-healing ulcers, exacerbated by fungal infections, can lead to severe complications, including cellulitis, osteomyelitis, and, in extreme cases, limb amputation [92].

2.5 Conventional treatment of fungal wounds

The conventional treatment of diabetic wounds often involves the use of dressings and antimicrobial creams to create a conducive environment for wound healing and prevent infections [93]. However, these approaches come with certain disadvantages. Traditional dressings, while essential for maintaining a moist wound environment, may require frequent changes, causing discomfort and disruption to the healing process [94]. Moreover, the risk of infection remains a concern despite the use of antimicrobial creams, and the precise control over the release of therapeutic agents is limited [95]. To address these drawbacks, there is need of novel dressing method that combines the benefits of conventional dressings with controlled-release capabilities of nanoformulation, to present a compelling solution.

2.6 Use of biopolymer based nanofiber dressings

The biopolymer-based nanofiber dressings emerging as a cutting-edge solution, offering a distinctive advantage over conventional approaches, these dressings facilitate a sustained and controlled release of therapeutic agents, minimizing the need for frequent dressing changes [96]. The nanofibrous structure, created through electrospinning techniques, not only enables efficient drug loading due to a high surface area-to-volume ratio but also provides a prolonged release of therapeutic agents, including antimicrobial and antioxidant [97].

The biomimetic nature of the nanofiber structure is a notable feature of biopolymer-based dressings [98]. The nanofiber interconnected architecture promotes cell adhesion,

migration, and proliferation, and these dressings enhance integration with the wound site, fostering an optimal milieu for tissue regeneration [32]. Furthermore, the nanofiber architecture aids in moisture retention, a critical factor supporting the maintenance of a moist wound healing environment, which is instrumental in promoting efficient tissue repair and minimizing the risk of complications [22].

Biopolymer-based nanofiber dressings boast an inherent adaptability that allows for customization to exhibit specific functionalities. This adaptability addresses the diverse requirements of different types of wounds, providing enhanced characteristics to wound dressings [99]. The incorporation of biopolymers, sourced from natural materials like chitosan, gelatin, collagen, or alginate, not only imparts these dressings with enhanced biocompatibility but also reduces the risk of adverse reactions, promoting tissue compatibility and acceptance by the patient [100].

In addition to their adaptability, biopolymer-based nanofiber dressings play a pivotal role in controlled therapeutic agent release, making them particularly relevant in the context of diabetic wounds [101]. These dressings can be engineered to release antimicrobial agents, providing a sustained defence against pathogens that commonly complicate diabetic wounds. Simultaneously, the incorporation of antifungal and antioxidant agents will reduce the infection and accelerates tissue regeneration, addressing the intricate challenges posed by diabetes [102]. This innovative approach to wound care holds great promise in revolutionizing the management of diabetic wounds and other complex wound scenarios, marking a significant leap forward in the quest for more effective and patient-friendly solutions.

Table 2.1 Literature survey reporting the applications of nanofibers in wound healing

S.No.	Overview	Application	Reference
1.	Parathyroid hormone loaded cellulose acetate nanofiber	Wound healing	[103]
2.	Antimicrobial nanofiber dressing	Wound healing	[104]
3.	Clove oil encapsulated chitosan /PEO nanofiber	Wound healing	[105]
4.	PCL/Gelatin core shell nanofiber with herbal components	For burn injuries	[106]
5.	Injectable silk nanofiber hydrogels	For wound healing	[107]
6.	Curcumin loaded cellulose and PVA nanofiber	For wound healing	[108]
7.	3D nanofiber aerogel of zinc oxide	Wound healing	[109]
8.	Bilayer nanofiber based scaffold of chitosan/gelatin and alginate	For full thickness wound healing	[110]
9.	Layer nanofiber sponge loaded with silver metal organic frameworks	For wound healing	[111]
10.	Chitosan/PVA biofibers loaded with honey	For wound healing	[112]
11.	Polydopamine loaded composite nanofibers	Diabetic wound healing	[113]
12.	Graphene oxide and curcumin loaded cellulose nanofibers	Complex wound treatment	[114]
13.	α-Lactalbumin loaded polycaprolactone nanofiber	For burn wounds	[115]
14.	Nanofiber reinforced hydrogels for wound healing	Wound healing	[116]

15.	PLCL/Gelatin nanofiber composites dressing	For wound healing	[117]
16.	Silver curcumin nanofiber dresses	For wound healing	[118]
17.	Mesoporous silica loaded PCL/curcumin nanofibers	Wound healing	[119]
18.	Polygalacturonic/Hyaluronic acid embedded silver nanoparticles loaded nanofiber dressings	For wound healings	[120]
19.	Multifunctional janus nanofiber based antibacterial dressings	For complex wounds	[121]
20.	Curcumin nanoparticles loaded PCL/Chitosan nanofibers	For complex wounds	[122]
21.	Nanofiber reinforced self-healing hydrogels	Tissue engineering and wound healing	[123]

2.6.1 Preparation of biopolymer nanofibers

Electrospinning is a technique used to prepare fibers in nanosize range, though uniaxial stretching of the viscoelastic polymeric solution-driven through a process governed by electrodynamics phenomenon. A typical electrospinning setup consists of three components viz. a metallic spinneret through which solution is fed through, a high voltage power system (voltage above 5 kV) to create the necessary pull to stretch the visco-elastic solution to elongate, and a metal collector (connected to ground), which facilitates the deposition of the resultant fibers in the form of nonwoven mat/mesh.

Electrospinning elongation takes place only when the repulsive force within the charged solution exceeds the polymeric solution's surface tension. At first, the jet erupts from spinneret tip which while travelling towards the collector plate undergoes bending instability which causes the elongation of the solution jet and subsequent evaporation the solvent, and ultimately result in fibers in the range of submicron to nanometre [124,125].

Table 2.2 Different type of nanofiber and their applications

S.No	Nanofibers	Description	Characteristics	Applications
1.	Aligned Nanofibers:	Nanofibers with a well-defined orientation in a specific direction	Enhanced mechanical properties along the alignment direction	Tissue engineering scaffolds, filtration membranes, and reinforced composites.
2.	Randomly Oriented Nanofibers	Nanofibers without a specific alignment, exhibiting a random arrangement.	High surface area, isotropic properties	Filtration, wound dressings, and drug delivery systems.
3.	Core-Shell Nanofibers	Nanofibers with a well-defined orientation in a specific direction	Enhanced mechanical properties along the alignment direction.	Tissue engineering scaffolds, filtration membranes, and reinforced composites.
4.	Coaxial Nanofibers	Nanofibers with a concentric arrangement of materials, often produced through coaxial electrospinning	Allows for precise control over the core and shell components, enabling dual functionalities.	Controlled drug delivery, encapsulation of sensitive materials, and dual-functional materials.
5.	Cross-linked Nanofibers	Nanofibers with covalent or physical crosslinks between polymer chains.	Improved stability, durability, and resistance to environmental factors.	High-performance materials, biomedical implants, and stable membranes.

6.	Hollow Nanofibers	Nanofibers with a hollow structure or nanotubes.	Low density, high surface area.	Catalysis, drug delivery, and sensors
7.	Porous Nanofibers:	Nanofibers with an interconnected porous structure.	Enhanced surface area for improved adsorption and permeability	Tissue engineering, filtration, and controlled release systems.
8.	Janus Nanofibers	Nanofibers with two distinct regions, often with different properties	Asymmetry in structure and properties	Catalysis, sensors, and asymmetric membranes
9.	Yarn-Like Nanofibers	Nanofibers arranged in a yarn-like structure	Flexibility and textile-like properties	Wearable electronics, smart textiles, and flexible sensors
10.	Nanofiber Bundles	Aggregated nanofibers forming bundles or bundles with aligned structures.	Enhanced mechanical strength and specific functionalities	Reinforced composites, biomimetic structures, and filtration membranes.

2.6.2 Factors affecting nanofiber preparations

The size of nanofibers produce through electrospinning depends on different parameters which including solution viscosity [126,127], conductivity, applied voltage, spinneret tip-to-collector distance and humidity [128–132]. For example, reduction in spinneret tip-to-

collector distance results in mesh with inter-connected fibres [133], similarly lower polymeric concentration results in reduced polymer chain entanglement, ultimately affecting fiber diameter [134]. Similarly process parameter, such as changes in the supplied electric voltage, and variations in the instruments configuration i.e. changing the spinneret design or use of a dynamic collector affects the fiber morphology and process output.

2.6.2.1 Solution conductivity

The solvent conductivity plays a crucial role in the process of formation of nanofibers by directly influencing the tylor cone formation and diameter of the nanofiber so formed. During nanofiber formation, electric charge gets transferred from the electrode to the droplet on the needle tip to generate the necessary coulomb repulsion force against the surface tension of the liquid. And, it has been evidenced that with increase in conductivity favours the formation of thinner nanofiber. After that limit further increase in the conductivity beads formation occur in the nanofiber [135,136].

For electrospinning, the solvent is expected to have good conductivity, but commonly used solvents in the electrospinning have a lower conductivity for example, dichloromethane has a conductivity of 0.03 mS/m which is much lower than the conductivity of the water [136]. The dissolved polymers also contribute to the conductivity of electrospinning solutions which eases the process. But it's not a rule that all solvent with lower conductivity can be electrospun, its only found in some of the cases where polymer dissolved into the solvent gained the enough conductivity that can achieve the critical value of the conductivity. If it not possible to gain conductivity up to a critical value just by dissolving the polymer (uncharged polymer), in that cases various type of additives are used in minute concentration or the different solvents mixtures are used. Additives used in electrospinning are of two type organic and inorganic; Inorganic salts commonly used is NaCl (0.01 M)

[137] and charged organic compounds [pyridinium formate (PF)] [136], palladium diacetate, trialkyl benzyl ammonium chloride in L-polylactide (PLLA) [138] or in poly(3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV) solution [139] are used in the electrospinning

2.6.2.2 Viscosity and polymer concentration

The process of electrospinning is based on the uniaxial stretching of the charged polymeric jet. And the stretching of the charged jet is affected by concentration and viscosity of the polymeric solution. A low polymer concentration and viscosity results in lower entanglement between the polymeric monomers which results in short nanofibers fragments or beads [140,141]. At optimized polymer concentration and viscosity, monomer entanglement is strong enough overcome the surface tension of the solution which leads to the continuous, uniform and beadless nanofiber formation. Further, increase in the polymer concentration enables very strong entanglement between the polymeric chain which leads to the high viscosity of the polymeric solution which result in the reduced flow of the polymeric solution through the needle which cause the blockage of the needle (solution dry up), ultimately resulting non-continuous, non-uniform and beaded nanofiber [141]. Fong and co-worker electrospun the Polyethylene oxide by varying the viscosity of the solution and found that with increase in the viscosity of the solution the shaped of the beads change and finally beadles nanofiber form at a critical value of the electrospinning [142]. Doshi *et al.* defined this viscosity critical range in their paper for the PEO in the range of the 800–4000 cp for generation of the optimum nanofiber. Further, he reported that smooth nanofibers of polyacrylonitrile (PAN) can be electrospun when the solution viscosity was in the range between 117–215 cp [143].

2.6.2.3 Surface tension

The role of the surface tension in the electrospinnability of the polymeric solution can't be overstated as it's the primary force which oppose the coulomb repulsion force. The region where this occur is called region of the instability (From this region nanofiber originates through the taylor cone). The angle of the cone is depend on the instability between the surface tension and coulomb force [142,144]. Surface tension have the direct effect in determining the minimum critical voltage (V_C) required for the electrospinning, as surface tension of the liquid is increase the minimum critical voltage value is also increase (not necessary linearly). The solvent surface tension is dependent on the nature of the liquid and different solvent can contribute differently in the polymeric solution. Surface tension of the system is affected by the polymer concentration, polymer nature [145] [146]. The surface tension of the liquid define the minimum critical voltage for a process. So, its desirable to have a low surface tension, but it's not always essential that solution with low surface tension is best for process [147].

2.6.2.4 Dielectric constant (ε)

Dielectric constant is the capacity of solvents to hold the electric charge. Dielectric constant directly affect the charge density a key parameter of electrospinning. Generally, high dielectric constant of solvents maintain uniform charge density which results in the formation of good quality nanofibers. Wannatong reported that number of webs per unit area of polymer solution depend on the type of solvent used for electrospinning [148]. Similarly, Min and his co-worker compared the effect of solvent dielectric constant (polarity) on fiber morphology of poly (lactide-co-glycolide) (PLGA) (LA:GA 50:50) electrospun from 15% w/v solutions of either hexa-fluoro-isopropanol (HFIP) ($\varepsilon \simeq 16.7$) or chloroform ($\varepsilon \simeq 4.81$) at 25°C. The average fiber diameter formed from the less polar chloroform was around 760 nm, while in HFIP the fiber diameter was around 310 nm [149].

2.6.2.5 Volatility and elasticity

For the collection of solid nanofibers, the solvent from the emerging stream of jet has to evaporate, leaving behind dry and porous nanofibers. Ideally, the nanofiber should be devoid of any trace of the solvent before reaching the collector, which otherwise cause the wet fibers to fuse together forming a reticular or melded mat [150]. The inadequate evaporation of solvent may lead to formation of a flat ribbon-like nanofibers solvent [151]. Thus, it is advised to use volatile solvents to avoid the formation of irregular nanofibrous mat. One particular disadvantage associated with volatile solvents is that the needle tends to clog when the solvent dries out prematurely in the needle/capillary [152].

Solvent volatility is also known to influence the phase separation kinetics thus, altering the morphology of the fibers so obtained. Fine fibers of polycarbonate and polybutadiene were obtained from chloroform solution, compared to the tetrahydrofuran (THF) solvent mixture which gave coarser nanofibers. This might be due to the high volatility of chloroform (vapour pressure 21.2 kPa), as compared to that of THF (vapor pressure 17.9 kPa) which forbids the coarsening of the fibers [153].

2.6.2.6 Distance between collector and needle.

The morphology of nanofibers is also controlled by the needle tip-collector distance as it affects the deposition time, evaporation time, and instability and whipping interval. The distance has to be optimised for each of the polymeric solution being used in order to produce smooth and uniform nanofibers [154,155]. Many research groups have explored the effect of the needle tip-collector distance. It has been reported that defective and large-diameter are a result of small distance between the needle tip and collector. Conversely, the fiber diameter had decreased when the distance was increased [155–157]. However, there are even some cases wherein the distance had no effect on the nanofiber morphology [158].

2.6.2.7 Effect of solution flow rate

The solution flow rate also affects the morphology of nanofibers because uniform and beadless nanofibers could be spun if the flow rate is optimised and controlled. Higher flow rates may lead to incomplete evaporation of solvent thereby causing bead defect in the nanofibers. During electrospinning of polystyrene, it was observed that smooth and uniform nanofibers were produced at flow rate of 0.07 mL/min, but beads appeared with an increase in flow rate to 0.10 mL/min. Along with bead defect, the fiber diameter and pore size of the mat increases at higher flow rates [152].

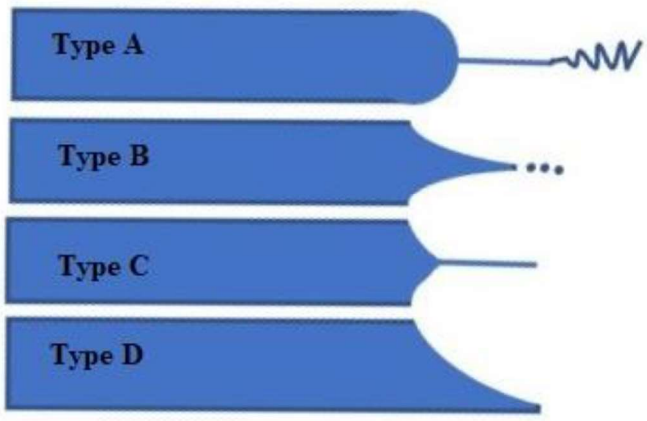

Figure 2.4 Formation of various type of jets with flow rates (A) Aggregated fluid, (B) Cone jet with droplets, (C) Cone Jet and (D) Receded jet [159,160]

Theron *et al*. revealed the relation between electric current and flow rate by studying the effect of solution flow rate and surface charge density by electrospinning various polymers such as PEO, Polyvinyl alcohol (PVA), polyacrylic acid (PAA), polycaprolactone (PCL), and polyurethane (PU) [131]. The Reneker and co-worker observed that electric current had increased with increasing flow rate in electrospinning, but the surface charge density decreased which allows the nanofiber merging during their flight towards the collector. This merging of nanofibers leads to the formation of garlands [161].

2.6.2.8 Effect of the applied voltage

The electric voltage supplied to the needle is an important parameter that directly influences the fluid dynamics and the fiber morphology. The minimum voltage required is proportional to the polymer concentration and the needle tip-collector distance. The high voltage facilitates the formation of taylor cone and ejection of charged polymeric jet to form ultrafine nanofibers only at a threshold voltage [162]. The value of voltage to be applied varies among different polymers. At high voltages, the charge repulsion is greater which facilitates the stretching of polymer solution thus forming smaller diameter fibers [163]. Although, subsequent increase in voltage beyond the threshold results in bead defect in the nanofiber with increased fiber diameter. This behaviour can be attributed to the formation of smaller taylor cone and increased jet velocity when voltage is increased at a given flow rate. Deitzel *et al.* prepared nanofibers using PEO/water system and reported formation of beads at higher voltage [145]. Furthermore, it was evident that fiber diameter increased with an increase in the voltage due to increase in jet length [156,158].

2.7 Polymer profiles

2.7.1 Polycaprolactone

Molecular structure:

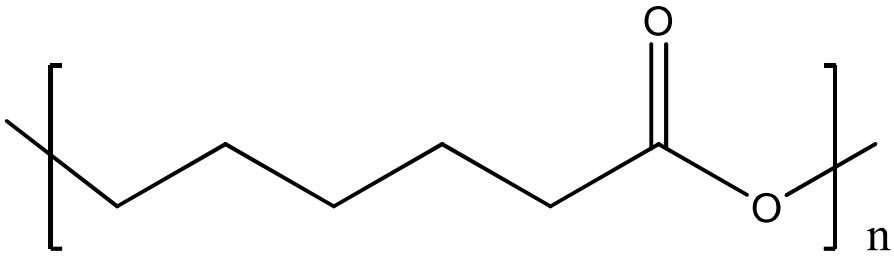

Figure 2.5 Molecular Structure of Polycaprolactone.

Molecular formula: $(C6H10O2)_n$

IUPAC Name: 1,7-Polyoxepan-2-one

Molecular weight: ~120,000 Dalton.

Appearance (Colour): White to Off White.

Appearance (Form): Pellets (~3mm).

Solubility: Good solubility: Dichloromethane, Chloroform, benzene, carbon tetrachloride, toluene, cyclohexanone, and 2-nitropropane.

Low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide, and acetonitrile and is insoluble in alcohol, petroleum ether, and diethyl ether [164].

Glass transition temperature Tg (°C): -60°C.

Storage temperature: 2-8°C.

Melting point: 60°C.

Physiochemical properties: Polycaprolactone is a synthetic, semi-crystalline, biodegradable polyester with a melting point of about 60 °C and a glass transition temperature of about −60 °C. It is a hydrophobic polymer and semi-crystalline characteristics. It is synthesized from ring-opening polymerization (ROP) of a lactone (ε-caprolactone) [165].

Regulatory status: Polycaprolactone has been approved by the Food and Drug Administration (FDA) for specific applications used in the human body, such as a drug delivery device, suture, or adhesion barrier [31].

Application in drug delivery: Polycaprolactone has been widely used in long-term implants and controlled drug release applications. It is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) and has therefore received a great deal of attention for use as an implantable biomaterial [161,166–168].

2.7.2 Gelatin

Gelatin is a protein substance derived from collagen, a natural protein present in the tendons, ligaments, and tissues of mammals. It is produced by boiling the connective tissues, bones, and skins of animals, usually cows and pigs. It's mainly of two types Type A and Type B. Type A gelatin is derived from acid treatment and an isoelectric point between pH 7 and 9. The type B gelatin is prepared from alkali pre-treatment and have an isoelectric point between pH 4.7 to 5.2 [169–171].

Molecular structure: The polypeptide structure of gelatin, composed primarily of glycine, proline, and hydroxyproline, contributes to its gelation property, allowing it to form a three-dimensional network [172].

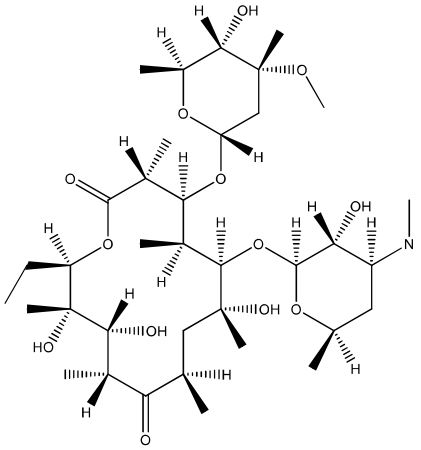

Figure 2.6 Molecular Structure of gelatin.

IUPAC: (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-(((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-14-ethyl-7,12,13-trihydroxy-4-(((2R,4R,5S,-6S)-5-hyd-roxy-4-methoxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-3,5,7,9,11,13-hexam-ethyloxacy-clotetradecane-2,10-dione

Physiochemical properties: Gelatin is nearly tasteless and odourless polymer with a colourless or slightly yellow appearance [173]. It exhibit biocompatibility and biodegradability with excellent water-holding capacity and film-forming ability, with

bloom strength of 30-300g. It is soluble in polar solvents like hot water, glycerol, and acetic acid can, but it insoluble in organic solvents like alcohol [174,175].

Molecular weight: 50kD to 250kD.

Storage temperature: 2-8 °C.

Water solubility: - 67mg/ml at 50°C, slightly hazy, slightly yellow

Physical form: Powder.

pH: 4.0-6.0 (25°C, 67mg/ml).

Stability: Stable, hygroscopic and also incompatible with strong oxidizing agents

Category: Pharmaceutical aid (encapsulation agent; suspending agent; tablet binding and Coating agent).

Description: Light amber to faintly yellow, translucent flakes, sheets, powder or granules; has slight odour. It's is stable in air but is subjected to microbial decomposition when moist or in solution.

Application in drug delivery: Gelatin is a biopolymer used for the development of numerous drug delivery systems due to its applicability to several physiochemical properties [170]. It serves as a versatile carrier system for delivering anti-inflammatory, anticancer, antibacterial, and, more recently, nucleic acid and hydrophobic drugs for topical to systemic applications [169,176–178].

2.7.3 Polyvinyl alcohol

Synthesis: Polyvinyl alcohol (PVA) is synthesized through hydrolysis of polyvinyl acetate (PVAc) using strong alkaline solution, typically sodium hydroxide (NaOH), which breaks

the ester bonds in PVAc and replaces the acetate groups with hydroxyl groups (-OH), while retaining the same backbone structure [179].

Physiochemical properties: PVA is a water-soluble, flammable, and crystalline polymer. It has high flexibility and tensile strength, is miscible in water and is odourless. PVA offer several physiochemical advantages which make it attractive for tissue engineering application with other polymers. PVA is generally considered biocompatible, as it is non-toxic and does not induce significant immune responses or cytotoxic effects. This makes it suitable for various tissue engineering applications. It also possesses hydrophilic properties, enabling it to absorb and retain large amounts of water [179].

Polyvinyl alcohol (PVA) offers attractive physicochemical properties; however, it does have certain limitations in tissue engineering applications. PVA exhibit relatively low cell adhesion properties compared to natural or other synthetic polymers. The absence of cell-adhesive motifs or specific binding sites within the nanofiber matrix can restrict cell interactions, hindering cell attachment and spreading [180]. Furthermore, PVA alone nanofiber may undergo physical and chemical instability over time, resulting in changes in their structure, mechanical properties, and degradation kinetics [181]. This instability can affect their long-term functionality and limit their use in certain tissue engineering applications.

To overcome these limitations, different polymer composites were made by blending PVA with other polymers, such as natural polymers like gelatin, collagen or synthetic polymers like polyethylene glycol (PEG) to provide properties i.e. enhance cell adhesion and tissue integration. The matrix made by combining PVA and natural polymer can improve their mechanical properties and stability [182]. The incorporation of nanoparticles, such as hydroxyapatite, silica, or magnetic nanoparticles, into PVA matrix also provides additional

functionality and improves their mechanical properties, bioactivity, and drug delivery capabilities [180].

Molecular structure:

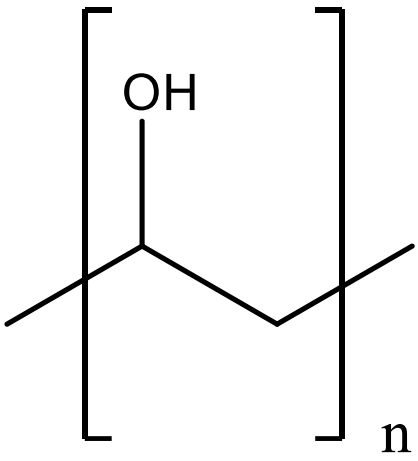

Figure 2.7 Molecular Structure of Polyvinyl Alcohol.

IUPAC Name: Poly(1-hydroxyethylene).

Molecular Formula: $[CH_2CH(OH)]n$.

Application in drug delivery: PVA is used in various drug delivery systems due to its biocompatibility, high hydrophilicity, and low toxicity [183,184]. PVA is deemed safe for food packaging by the FDA, recognized as a GRAS (Generally recognized as safe) ingredient PVA is widely used in drug delivery systems due to its excellent properties [185–187]. It also act as stabilizer in nanoparticle preparation and enhances bioavailability of drugs by forming solid amorphous dispersions [188].

2.7.4 Chitosan

Chitosan is a biopolymer derived from chitin found in crustacean exoskeletons. Its biodegradability ensures it breaks down into non-toxic components, reducing the risk of accumulation in the body [189]. With excellent biocompatibility, chitosan is well-suited for use in biological systems without causing adverse reactions. Notably, its mucoadhesive

properties make it adept at adhering to mucosal surfaces, enhancing drug residence time and absorption in tissues such as the gastrointestinal tract and nasal or ocular mucosa [190]. Chitosan's versatility allows it to be formulated into nanoparticles, microspheres, hydrogels, and films, enabling controlled drug release for sustained therapeutic effects [191]. Additionally, chitosan's ability to open tight junctions between epithelial cells improves drug absorption, particularly for poorly soluble drugs. Its antimicrobial properties contribute to infection prevention in drug delivery applications, such as wound healing [192]. Chitosan have rich applications in oral, transdermal, ocular drug delivery, and gene delivery, making it a versatile and valuable component in the development of innovative drug delivery systems [189,190,193].

Molecular structure

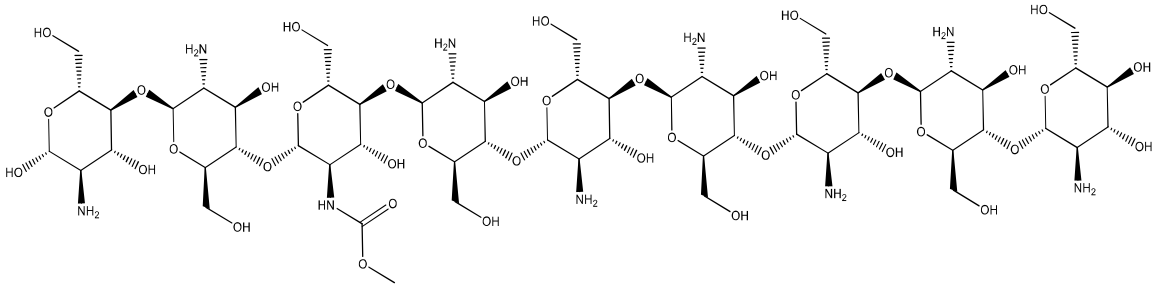

Figure 2.8 Molecular Structure of Chitosan

IUPAC name:

methyl ((2S,3R,4R,5S,6R)-5-(((2S,3R,4R,5S,6R)-3-amino-5-(((2S,3R,4R,5S,6R)-3-amino-5-(((2S,3R,4R,5S,6R)-3-amino-5-(((2S,3R,4R,5S,6R)-3-amino-5-(((2S,3R,4R,5S,

6R)-3-amino-5-(((2S,3R,4R,5S,6R)-3-amino-4,5-dihydroxy-6(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4-hydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)-4-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-2-(((2R,3S,4R,5R,

6S)-5-amino-6-(((2R,3S,4R,5R,6R)-5-amino-4,6-dihydroxy-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-4-hydroxy-2-(hydroxymet-hyl)tetrahydro-2H-pyran-3-yl)oxy)-4-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl) carbamate

Degree of acetylation: >80%

Molecular weight: 200-350kDa

Viscosity: 300-1000cps

Glass transition temperature: ~150 ^{o}C.

Solubility: The solubility of chitosan depends on the degree of acetylation and molecular weight. Chitosan oligomers are soluble over a wide pH range, from acidic to basic ones (i.e., physiological pH 7.4). On the contrary, chitosan samples with higher molecular weight are only soluble in acidic aqueous media even at high deacetylation degrees [194].

Application in drug delivery: Chitosan has been used in drug delivery systems due to its properties like biocompatibility, biodegradability, muco-adhesiveness, antimicrobial activity and non-toxic nature [190,193]. It can form hydrogenic and ionic bonds with drug molecules, which is one of its most useful properties. There are various mechanisms for chitosan nanoparticle/ nanofibers synthesis. The fibers in composites with PVA improve the mechanical properties of PVA and impart bioadhesivity [195,196].

2.8 Drug profile

2.8.1 Luliconazole

IUPAC Name:

(R,E)-2-(4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene)-2-(1H-imidazol-1-yl)acetonitrile

Molecular weight: 354.27g/mol

Molecular formula: C14H9CL2N3S2

Molecular structure:

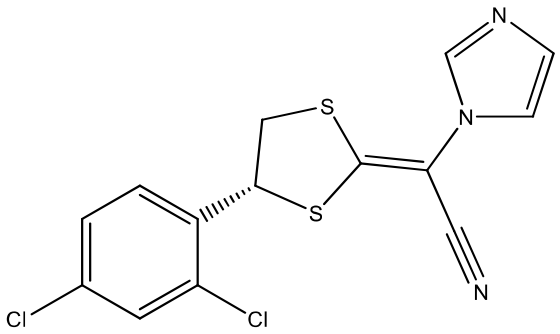

Figure 2.9 Molecular structure of luliconazole.

Physical appearance: Off white to pale yellow powder

Nature: Crystalline.

Density: 1.52 g/cm^3.

Melting points: 152 ºC.

Boiling point: 499.1 ºC.

pKa: 6.34.

Plasma protein binding: >99%.

Half-life: >25 hr.

Solubility: DMSO 50mg/mL, Water 0.0659mg/mL.

Mechanism of action Luliconazole inhibits the lanosterol demethylase synthesis an enzyme responsible for ergosterol synthesis (A major component of fungus cell membrane) [197].

Indications: Luliconazole used to treat *Tinea pedis*, *Tinea cruris* and *Tinea corporis*.

Marketed formulation: LulicanXL™ cream, Luliford™ lotion, Lulifresh™ cream, Lulifin™ cream, Lulisign™ cream, and Luligel™ gel.

Table 2.3 Literature work published in recent decades for the luliconazole formulation development.

S.No.	Formulation	Applications	Reference
1	Polycaprolactone nanofibers loaded with luliconazole	For vaginal candidiasis	[198]
2	Luliconazole nanocrystal loaded hydrogel	Improving antifungal activity	[199]
3	Spray-Dried Alginate/Gelatin microparticles	For mucoadhesive delivery	[200]
4	Luliconazole nanosponge	Enhanced permeation rate and skin retention time	[201]
5	Polymeric nanoparticles and nanogels	Improved efficacy	[202]
6	Luliconazole liposomes formlation	Dermatophytes treatment	[203]
7	Luliconazole nanocrystals	Dermatological applications	[204]
8	Luliconzole role in fungal infections	*Candida* infection	[205]
9	Luliconazole role in fungal infections	Topical treatment of onychomycosis	[206]

2.8.2 Naringenin

IUPAC: 5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one.

Molecular Formula: C15H12O5.

Molecular Weight: 272.2528 g/mol.

Use: Naringenin is used for skin healing and airway inflammatory diseases.

Molecular Structure:

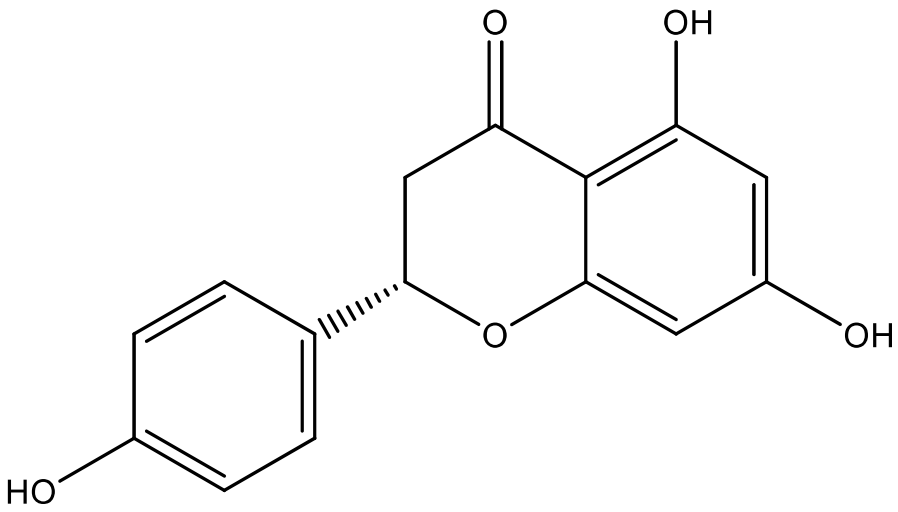

Figure 2.10 Molecular structure of naringenin.

Physical appearance: White powder.

Nature: Crystalline.

Boiling point: 577.5°C.

Melting point: 251°C.

Solubility: Ethanol 2.5mg/mL, DMF 10 mg/mL.

Log P: 2.2513.

Half-life: 1.5-2.2hr.

Oral Bioavailability: <25%.

Plasma protein binding: >95%.

pKa: 7.0514.

Biological activity: Naringenin possesses excellent anti-inflammatory, antioxidant, antihypertensive, hypo-lipidemic and hepatoprotective activity.

Table 2.4 Published literature work for naringenin and its application in wound healing.

S.No.	Overview	References
1	Role of naringenin in wound healing applications	[207]
2	Antimicrobial and wound healing properties of naringenin	[41]
3	Wound healing activity of naringenin	[208]
4	Anti-inflammatory and wound healing activity of naringenin	[209]
5	Antioxidant activity of naringenin in diabetic wounds	[210]
6	Anti-inflammatory and antifibrotic effect of naringenin	[211]
7	Antidiabetic effect of naringenin	[40]
8	Antioxidant and hypoglycemic effect of naringenin	[212]
9	Regulation of lipid and glucose metabolism by naringenin	[213]
10	Antioxidant activity of the naringenin	[214]
11	Antioxidant and neuroprotection	[215]
12	Antioxidant and anti-genotoxic properties of naringenin	[216]