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Review Article

Nanofiber in Tissue Regeneration

Electrospun Nanofiber: Application in Tissue Regeneration

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Abstract: An injury to the human body is classified as a wound if it results in a cut or a break in the skin. Depending on the depth of the skin layer, a wound can either be limited to the epidermal layer, which heals via re-epithelialization without the need for skin grafts, or full-thickness wounds, which result in the loss of both the epidermis and dermis (FTW). A full-thickness wound cannot heal on its own and needs a skin graft or tissue regeneration product to heal quickly. This paper provides a comprehensive overview of the properties of electrospun nanofibers and their application as skin regeneration products rapid healing of the full-thickness wound. The paper first introduces the skin, its layers, and various problems associated with human skin. In the next part, a wound is discussed in terms of acute and chronic wounds. Primary, secondary and tertiary clinical wound healing has also been discussed. The next part briefly introduces the four different phases of healing, i.e. hemostasis, inflammation, proliferative and maturation of newly deposited collagen into tissues. The effect of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) on reactive oxygen species, reactive nitrogen species and reactive sulphur species, and their effect on healing time was discussed. The electrospinning process's evolution and setup, properties of electrospun nanofibers, a component of electrospinning solution, and various parameters affecting electrospinning were discussed. Application on nanofiber scaffold in terms of drug delivery and tissue regeneration was highlighted. In the end, improvement in the existing nanofibrous scaffold was briefly highlighted.

Keywords: Skin Regeneration, Wounds, Electrospun Nanofiber

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1. INTRODUCTION

With roughly 2 m^2 of surface area and about 15% of the adult body mass, the skin is the biggest organ in the human body [1]. It outlines the border between the body and the outer environment, enabling vital body functions to take place in a controlled physiological environment [2]. The human integumentary system provides general bodily protection and comprises the skin and its supporting organs, including hair, nails, and exocrine glands[1].

1.1 Different layers of skin

Figure 1 illustrates the two major layers of the skin, the epidermis and dermis, and the closely related hypodermis layer.

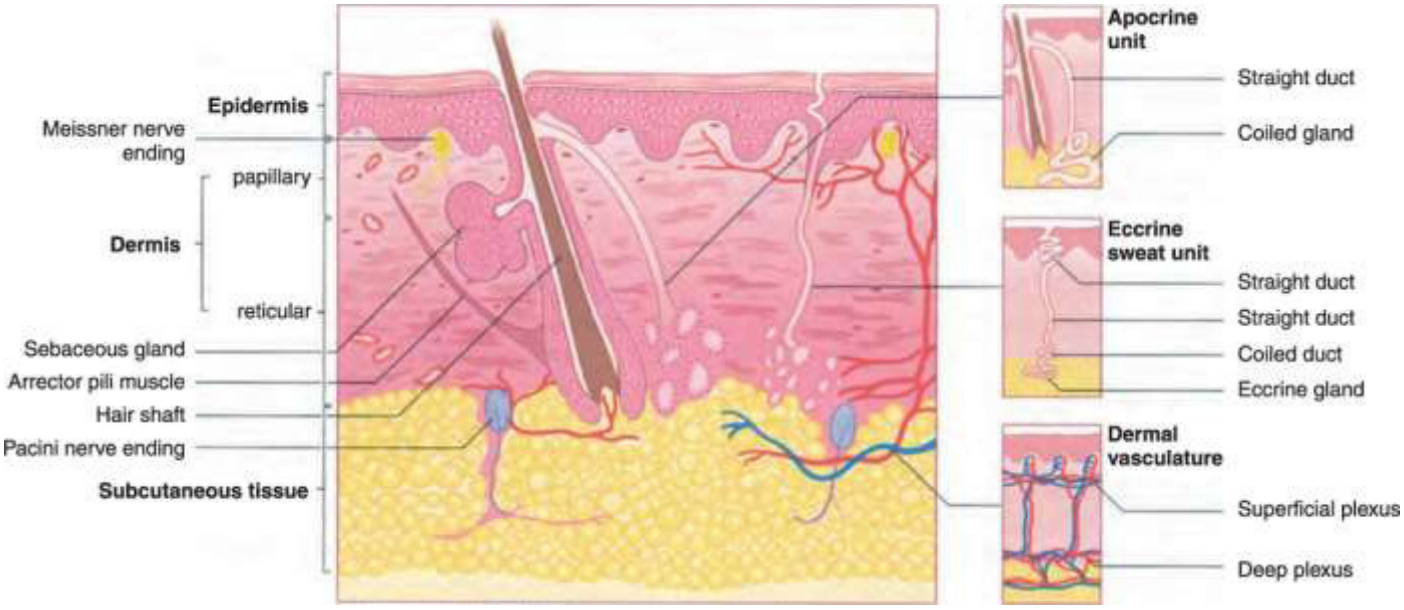

Fig 1: Different layers of skin[1].

1.2 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's location, it comprises four or five layers of epithelial cells, known as "thin skin" or "thick skin". The epidermis on the eyelid is the thinnest at less than 0.1 mm. Only the palms of the hands and the soles of the feet have "thick skin," which is only 1.5 mm thick there [3].

1.3 The Dermis (derma- = "skin")

As distinct from the epidermis and hypodermis, the dermis could be deemed the "core" of the integumentary system. It gives the skin strength and suppleness and is typically less than 1 mm thick. In addition to sebaceous glands, lymph vessels, sweat glands, sensory nerve endings, hair follicles, and blood vessels, it also has a variety of immune cells. It comprises an interconnected mesh of structural proteins, primarily elastin and collagen, produced by fibroblast. The upper papillary and lower reticular dermis of the dermis can be distinguished based on the arrangement of collagen fibres [2].

1.4 The Hypodermis (hypo- = "below")

The deepest layer of skin is called the hypodermis, sometimes referred to as the superficial fascia or subcutaneous layer. It links the skin to the fascia, or underlying fibrous tissue, that covers the muscles and bones. It is mostly made up of highly perfused, porous, areolar connective tissue, which stores fat and provides the integument with insulation and cushioning [2].

1.5 Problems associated with skin

The integumentary system is highly disposed to various disorders, diseases, and injuries. These can include severe burns and skin cancer, both of which have the potential to be fatal, as well as somewhat benign microbial infections that are classified as diseases. For example, a skin disorder can be a simple rash, an inflammation (dermatitis), an inflammation due to an overactive immune system (eczema), caused by a fungal infection (ringworm, tinea versicolor), a viral infection (herpes, shingles, viral exantham), an allergic reaction (hives), scabies (caused by tiny burrowing mite), lethal cancer (melanoma, basal cell and squamous cell carcinoma) or injuries (include burns, wounds, scars etc. As per the WHO report– "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".

1.6 Wound

A wound is the loss of the skin's protective function as a result of severe illness or damage (physical, chemical, thermal, or microbiological) [4]. This can cause damage to other tissues, such as tendons, muscles, arteries, nerves, parenchymal organs, and even bone. It can also cause a simple breach in the epithelial integrity of the skin.

1.7 Types of wounds

Depending on how quickly it heals, a wound can be categorized as acute or chronic.

1.8 Acute wound

An acute wound heals in the predicted time, and the entire process is complete within a few weeks. Typically, an acute wound goes through all the phases of normal wound healing for predictable tissue repair. Sudden loss of anatomical structure owing to surgery or trauma results in acute wounds. The acute wound usually occurs in normal or recently uninjured tissue [5, 6].

1.9 Chronic wound

When an acute wound fails to heal in the predicted time, it becomes chronic. Owing to underlying pathologies, for instance, pressure ulcer, diabetic ulcer, and vascular ulcers, dysregulation of normal healing mechanism occur, which results in prolonged and pathologic healing. Usually, a healing arrest occurs due to a prolonged inflammatory phase. Other reason for prolonged healing is the 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 [5].

1.10 Types of clinical wound healing

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

1.11 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.

1.12 Secondary intention wound healing process.

Secondary wounds are characterized by the loss of a considerable amount of tissues, and their edges are so distant that they cannot be sutured. Usually, healing takes longer repair time with scar formation. Wounds are left open, and gaps are filled by exuberant granulation tissue deposition and epithelial cell migration. There is a risk of infection due to large-scale tissue loss or infection has already occurred.

1.13 Tertiary intention or delayed wound healing process.

A delayed closure involves both principles, i.e. primary and secondary healing. It occurs when healing needs to be delayed intentionally, for example, when blood perfusion is low or the wound is highly infected.

1.14 Different phases of wound healing

The restoration of tissue or bodily function occurs at the end of the wound-healing process, which is a normal recovery reaction to tissue injury. Extracellular matrix, soluble mediators, blood, and parenchymal cells all participate in the complicated physiological, dynamic, and interactive healing process [7, 8]. Hemostasis, inflammation, proliferation, and tissue remodelling are the three successive phases of healing that occur with different and overlapping durations depending on the wound's kind, severity, and aetiology [9-11]. A different phase of healing is shown in Figure 2 and summarized in Table 1.

1.15 Hemostasis and inflammatory phase

The hemostasis phase begins shortly after an injury to control bleeding and limit 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 development ends bleeding and creates a barrier to keep bacteria out [6]. In addition, the fibrin clot concentrates the growth factors and cytokines and serves as a scaffold for incoming cells such as monocytes, neutrophils, endothelial cells, and fibroblasts [12]. The inflammatory phase begins immediately with hemostasis and is characterized by chemotaxis and activation of inflammatory cells [12]. Additionally, it is characterized by producing several pro-inflammatory cytokines, reactive oxygen species, proteases, and growth factors that prevent infection in open wounds, enhance phagocytic activity, and aid in wound healing [13,14]. Neutrophils are the first respondents, which are drawn into the wound site, release caustic proteolytic enzymes, and begin the digestion of invading microbes and nonviable tissue. Next, monocytes will be attracted to the wound site from the adjacent tissue and blood and differentiate into macrophages, a key phagocytic cell in wound repair. Various cytokines and enzymes are released by the macrophage, comprising collagenases for debridement of the wound; tumour necrosis factor (TNF)-α and interleukin (IL)-1 for the activation of fibroblasts and angiogenesis; and transforming growth factor (TGF) for stimulation of keratinocytes[12, 13]. A lymphocyte is the last cell to infiltrate the wound site, attracted 72 h after injury or late inflammatory phase. It helps in tissue repair and the avoidance of immunosuppression [6, 15].

1.16 Proliferative phase

The proliferative phase is described by epithelialization, capillaries growth, collagen concentration and granulation tissue formation, and wound closure.

1.17 Epithelialization

Normally, the epithelial progenitor cells in the basement membrane migrate upward and restore the epidermis within 2-3 days. However, in case of full thickness wound or the absence of a basement membrane, the epithelial cells found on the skin edge start to increase and send projections to restore a protective barrier [12].

1.18 Angiogenesis

Angiogenesis is characterized by endothelial cell migration and capillary formation. TNF-α stimulates it. For the wound

site to receive enough oxygen and nutrients to heal correctly, capillaries must grow in the wound bed [12].

1.19 Granulation tissue formation

This concludes the proliferative phase. Fibroblasts, which are the main cells responsible for producing collagen, move from the surrounding tissue into the area of the wound during this phase, where they activate, begin depositing collagen and create a fresh extracellular matrix that is only temporary [12, 16]. **Neovascularization** leads to the growth of lymphatic and vessel capillaries from existing vessels present at the wound site, which results in granulation tissue formation [13].

1.20 Wound Contraction

In order to aid with wound contraction, "wound fibroblasts," or fibroblasts already present at the wound site, will begin producing collagen and differentiate into myofibroblasts [12].

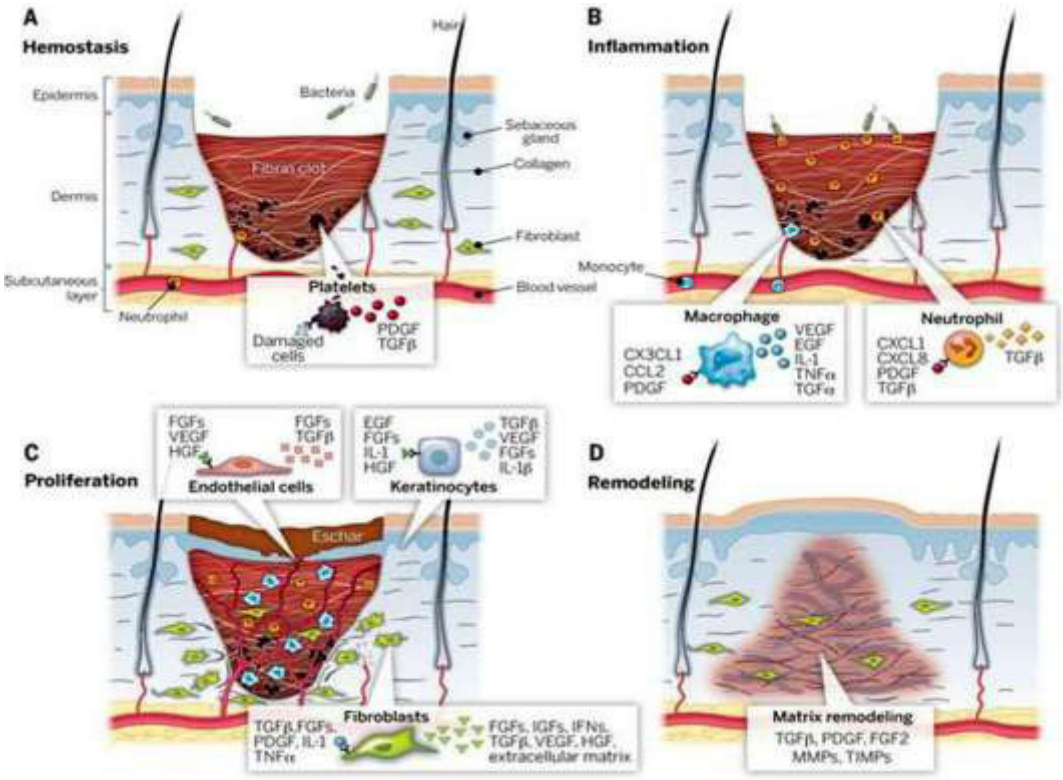

Fig 2: Different phases of wound healing.[63]

1.21 Maturation or remodelling phase

Clinically, the maturation or remodelling phase is crucial since it determines the durability and aesthetic quality of the repaired tissue [7]. Continuous collagen deposition in a structured network is the primary characteristic of this stage; however, 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 as in uninjured skin. Further, the strength of the wound also never restores to 100%. Even after three months and beyond, it will reach approximately 80% of uninjured skin [12].

Table 1: Phases of healing of a full-thickness wound [10]

Phase	Cellular and Bio-physiological Events
Hemostasis phase	Clotting factors are triggered as blood vessels constrict. Clot development stops bleeding and serves as a barrier to keep out microorganisms. Growth factors, which are released by platelets, signal different cells to begin the healing process at the site of the wound.
Inflammatory phase	Vasodilation, chemotaxis activation of inflammatory cells: - **Neutrophil infiltration:** begin the digestion of invading microbes and nonviable tissue - **Monocyte infiltration** and differentiation to macrophage principle phagocytic cells release numerous cytokines to activate fibroblast, angiogenesis and keratinocytes. - **Lymphocyte infiltration** helps in tissue repair.
Proliferative phase	In this stage, four significant processes take place: - **Re-epithelialization:** Granulation tissue and new epidermis formed. - **Angiogenesis:** New capillaries grow to supply the wound with nutrition and oxygen. - **Collagen deposition and Native ECM formation:** The wound is given strength and

	integrity. - **Wound Closure:** The wound starts to close.
Maturation (remodelling) phase	The wound grows stronger thanks to the collagen and develops into a scar. Vascular development and regress.

1.22 *Factor influencing wound healing*

Factors that influence cellular function and physiologic responses may affect wound healing. Various factors that affect wound healing are shown in Table 2 [10].

Table 2: Factors affecting wound healing

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

1.23 Reactive oxygen species and their significance in wound healing

Free Due to the unpaired electrons in free radicals, they react with other molecules very quickly. These are known as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulphur species (RSS) and are produced in the biological system from molecules containing oxygen, nitrogen, and sulphur during cell metabolism and function [17]. Superoxide anion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), perhydroxyl radicals ($HO_2^{\bullet}$), and hydroxyl radicals ($HO^{\bullet}$) are all examples of reactive oxygen species (ROS). Low levels of intracellular ROS play significant roles in gene expression, ion transport, apoptosis, and cell signalling to support cell proliferation and survival pathways [18, 19]. In addition, Dunnill, Patton et al. discussed various roles of ROS in wound healing, as shown in Figure 3. 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, and wound heavily infiltrated with microorganism, the ROS excessively produced in the wound area (Figure 4). These excessive ROS trigger the activation of matrix metalloproteases, which change and degrade ECM proteins and can also affect the function of dermal fibroblasts and keratinocytes, as well as the persistent release of pro-inflammatory cytokines [20, 21]. Therefore, it is obvious that for proper wound healing, a precise equilibrium should be maintained between lower and high levels of ROS. A way of indirectly manipulating ROS can instead be to manipulate the local antioxidant system by increasing dietary antioxidant intake [20]. Exogenous or dietary antioxidants can reduce oxidative damage in one of three ways: (1) directly by scavenging free radicals, (2) indirectly by inhibiting the expression of enzymes that produce free radicals, or (3) by increasing the expression of endogenous antioxidant enzymes [18, 22.]

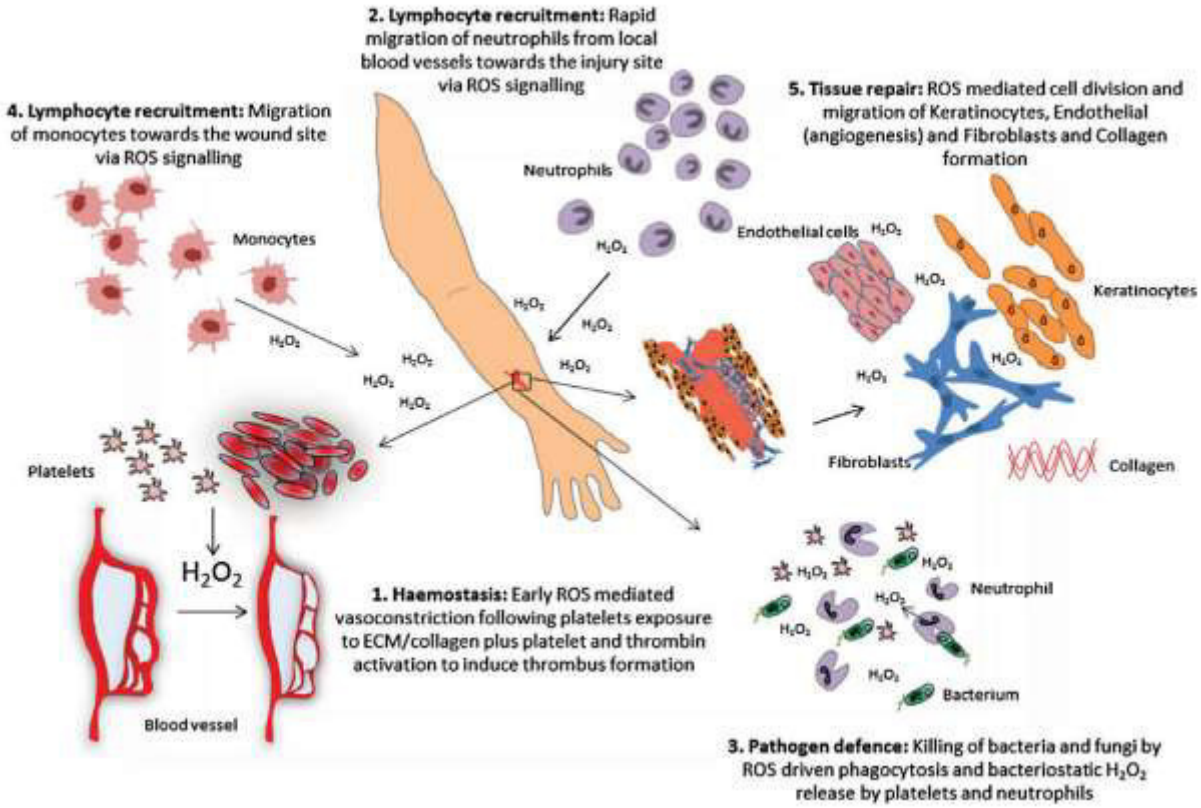

Figure 3: The schematic diagram represents the multiple roles of ROS in hemostasis state during acute wound healing [20].

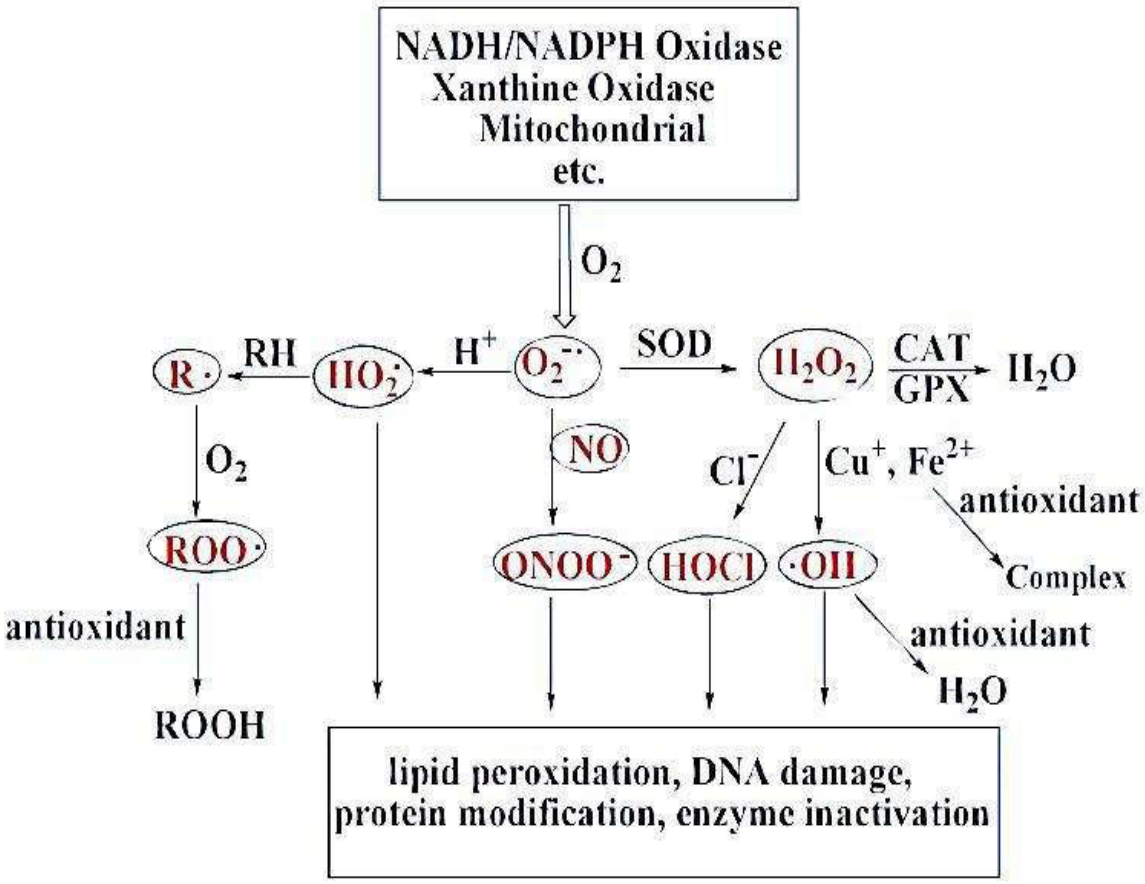

Fig 4: Summary of ROS types, sources, and action point of antioxidants [18].

1.24 Microbial infection in an open wound

An open wound is highly prone to microbial infection and is a major reason for tissue regeneration product failure. Either pathogen penetration at the incision site or the implant material reacting to a foreign body causes infection [15, 23]. These persistent pathogens infection and released-endotoxin at the wound site extend the inflammatory phase and increases the level of MMPs, a protease that begins the degradation of ECM [12, 24]. Antibacterial biomaterials are thus one of the main targets in the fight against bacterial invasion and infection-related to-implant diseases. However, conventional dosage forms require a very high dose of antibiotics to deliver effective concentrations of antibiotics at the infected site, which also causes other systemic toxicity. Therefore, controlled local delivery of the drug is anticipated to minimize the systemic side effect and achieve an effective concentration of antibiotics inside deep tissue [25, 26]. Taking into consideration of mentioned issues faced by an open full-thickness wound, i.e., the requirement of a tissue regeneration product, an exogenous antioxidant to scavenge excess free radicals, and an antimicrobial to resist microbial infection at the wound site to promote rapid wound healing, we had suggested creating a polymer-based nanofiber membrane filled with an antibacterial and an antioxidant.

1.25 Nanofibers

The need for new skin regeneration products has grown significantly over the last ten years, and new ones are being developed from current components. Decellularized porcine dermal matrix, hydrogels, and freeze-dried or gas-foaming-produced scaffolds are often utilized for skin wound healing. However, these materials need to review the 3D architecture of the extracellular matrix (ECM) of skin [27]. Therefore, nanofibers have emerged as a potential candidate for skin regeneration due to their architectural resemblance with native ECM. A nanofiber-based scaffold also offers excellent dressing properties like absorbing extra wound exudate, exchanging gases, maintaining wound hydration, minimizing wound stress, and acting as a barrier to external microbial infiltration [13, 28]. Besides traditional functions, these nanofiber scaffolds can also diminish microbial infection and inflammation and support wound healing by incorporating antimicrobial and antioxidant agents in the nanofiber [27, 29]. Several methods, such as drawing, interfacial polymerization, force spinning, melt blowing, phase separation, template melt extrusion template synthesis, and electrospinning, can be used to create nanofibers. These methods are briefly summarized in Table 3 [30].

1.25 Electrospinning

Due to its adaptability, versatility, and relative ease of use for producing a range of biomimetic nanofibers from a wide variety of polymeric (natural and synthetic) and inorganic materials, the electrospinning technique has recently attracted significant attention in drug delivery and tissue engineering. Electrospinning is a relatively simple, cost-effective and adaptable technique compared to conventional fibre spinning techniques. An electrospinning setup also can produce dry nanofibers with adjustable size, shape and loading efficiency in a single step [31, 32]. Furthermore, the polymeric nanofibers created by electrospinning may successfully encapsulate a variety of biomolecules while maintaining their bioactivity, including antibiotics and protein medicines like growth factors [33, 34].

1.26 Properties of Electrospun Nanofibers

Various properties of electrospun nanofiber membranes which make them an ideal candidate for wound healing applications are as follows [35-37]:

(i) The Electrospinning technique produces randomly oriented, thin nanofibers (50-500nm), which imitate the structural and functional similarity of the natural ECM that promotes cell adhesion and growth.
(ii) Electrospun nanofibers have a high surface-to-volume ratio, stimulating cell signalling quickly and drawing fibroblasts to release extracellular matrix components.
(iii) Electrospun nanofiber membrane's highly porous structure (60–90% porosity) aids in preventing wound dehydration, gas penetration, and cell respiration.
(iv) Owing to extremely interconnected pores, electrospun nanofibers protect the wound from environmental contamination and microbial infiltration while allowing cellular ingrowth.
(v) Electrospun nanofibers function as a drug delivery system with a controlled and prolonged release profile and a high drug loading efficiency. The release profile and degradation rate of a drug-loaded nanofiber can be adjusted by altering the electrospinning parameter.

Table 3: Detailed explanation of the techniques most frequently used to produce nanofibers [30].

Method	Description	Advantages	Disadvantages
Drawing	Making fibre involves pulling a liquid fibre from a droplet of previously collected polymer solution, which is further solidified by solvent evaporation.	Simple process	Limited amount of product, discontinuous process
Electrospinning	Under high voltage, a viscoelastic polymer solution or its melt is converted into nanofibers in a single step.	Unlimited length, core-shell and simple nanofibers, and a wide range of solvents are all utilized.	High voltage, the requirement for solvents, and additional process-influencing factors
Forcespinning	A very concentrated polymer solution or melt is spun into fibres using centrifugal force.	Free from high voltage, simple method, high production yield	Thermal degradation of melts, fibres are usually much thicker than 1 µm in diameter
Phase	The polymer solution must first be cooled to	Simple, no special tools	Multiple parameters, few

separation	the gelation temperature to create a gel. The gel is then removed from the distilled water, blotted with filter paper, and transferred to freeze-drying for solvent exchange.	needed.	polymers only.
Self-assembly	The fundamental building blocks that self-associate to create nanofibers are amphiphilic molecules.	Suitable for making extremely thin nanofibers.	Limited control over the nanofibers' shape and orientation.
Template melt extrusion	In the head of extrusion machines, molten polymer is driven through a mould or spinning die and then cooled to solidify. Membranes made of anodic aluminium oxide (AAO) are utilized as a model.	No need for solvents. Homogenous fibre diameter	The short length of fibres, time-consuming
Template synthesis	An oxidative process causes nanofibers to develop inside the many cylindrical pores of a nonporous membrane.	Easily controlled fibre with homogeneous, aligned nanofibers.	Complex method

1.27 Electrospinning setups

Since John Francis Cooley pioneered it in 1900, the electrospinning technique has been one of the most widely utilized methods for producing nanofibers in the late 20th and early 21st centuries (US patent No. 692,631) [38]. Since its first use by Cooley, significant developments have been made in the instrumentation, diversity of materials used and application of nanofibers. As a result, electrospinning is receiving growing attention in science and business communities and is regarded as a crucial scientific and commercial project with worldwide economic benefit [39, 40]. A basic electrospinning setup, shown in Figure 5, consists of four main parts: an electrically conductive collector (a piece of aluminium foil or silicon wafer to collect the produced nanofibers), a high voltage (10–40 kV) power supply between two electrodes, a glass syringe fitted with a blunt end metallic needle acting as a nozzle, and a syringe pump to provide a steady flow of electrospinning solution. The electrically-conductive electrospinning solution is loaded into a five-cc glass syringe mounted on a pump. When a DC voltage was applied, the electrospinning solution acquired a stable shape due to the equilibrium between repulsive forces and surface tension. However, as the voltage increased, the charge repulsion started overcoming the surface tension, and at a critical potential, the solution acquired a conical shape known as Taylor Cone. Further potential increases will destroy the equilibrium of electric forces and surface tension. At this point, ultrafine nanofibers made of polymer solution emerge from the Taylor cone and move in the direction of the electric field. The stretched nanofibers are collected on the grounded metallic collector held at an ideal distance. The liquid jet is whipped during the electrospinning process, which decreases in diameter from several hundred micrometres to as thin as tens of nanometers due to the external and internal charge force. The polymer solution quickly evaporates solvents and solidifies into solids thanks to jet thinning [38, 41, 42].

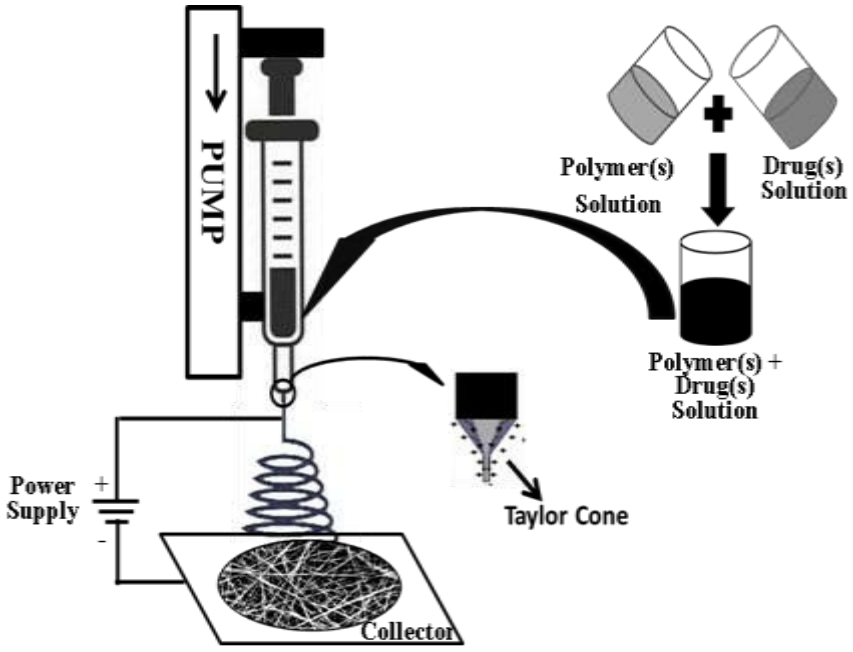

Fig 5: Schematic representation of the electrospinning setup [11].

1.28 Components of Electrospinning Material(s)

Due to the ease of producing nanofibers and their enormous promise for a wide range of applications, electrospinning technology underwent a remarkable development. Electrospun fibres can now be produced from various polymer sources, including blends, hybrid materials, and polymers containing metals, metal oxides, ceramics, carbon nanotubes, bacteria, viruses, and enzymes. Until recently, the sources of electrospun fibres were only limited to single-component polymers.

1.29 One component of electrospun nanofibers

Traditional early electrospun nanofibers were primarily made from a single-component polymer with a high enough molecular weight and easily soluble in suitable solvents. However, numerous commercially available polymers, such as nylons, polyethylene oxide (PEO), polyacrylonitrile (PAN), polyvinyl alcohol (PVA), poly-L-lactide (PLLA), PVDF, etc., have been effectively electrospun into nanofibers over the past ten years [43].

1.30 Two/multi-component electrospun nanofibers

Two and multi-component electrospun nanofibers offer a variety of compositions, topologies, and functionalities compared to single-component electrospun nanofibers. Electrospun nanofibers with two or more components are prepared using two different methods. The simplest method is to electro-spin polymer blend solutions using a single nozzle. Chemicals and fillers can be used to create hybrid and composite fibres. Another method is to use spinnerets or nozzles with two or more channels that can simultaneously deliver two or more solutions [43].

1.31 Recent biomaterials used for the electrospinning of nanofibers

Recently used biomaterials for electrospinning are discussed below:

1.32 Polymers

1.32.1 Natural Polymers

Natural polymers that have been electrospun are proteins and polysaccharides. One of the most commonly used is collagen. Other natural polymers in electrospinning are gelatin, hyaluronic acid, cellulose, pullulan, zein, etc. [44].

1.32 Synthetic polymer

Using naturally derived polymers has several drawbacks, such as batch-to-batch (or source-to-source) variation in materials isolated from tissues, limited flexibility in the range of material properties that are accessible, and safety concerns regarding the use of materials isolated from mammalian sources, artificial polymers have been created as substitute materials for biomedical applications. For tissue regeneration, synthetic biodegradable polymers like PLA, PGA, PLGA, and PCL are currently available [44]. The electrospun fibres of these polymers are listed in table 4.

1.33 Hydroxyapatite

Hydroxyapatite (HA), a significant mineral found in human hard tissues, has high in vitro and in vivo biocompatibility with bones, teeth, skin, and muscles. As a result, HA has many uses in orthopaedics and dentistry thanks to its bioactivity and osteoconductivity.

1.34 Carbon

The most well-known nanotechnology applications may be carbon nanomaterials, which include fullerenes, nanotubes, nanofibers, and a wide range of related forms. Although there are conflicting views on whether carbon nanoparticles are biocompatible, initial research on subcutaneous implantation and osteoblast co-culture experiments produced encouraging findings.

Table 4: Various electrospun nanofibers [44]

Polymer	Solvent	Concentration	Diameter (nm)
Poly(e-caprolactone)Mn = 80000	7:3 Dichloromethane:Methanol	8 wt%	810 ± 150
Poly(e-caprolactone)Mn = 80000	Tetrahydrofuran:*N*, N-dimethylformamide (1:1)	0.14 g ml^{-1}	700 ± 200
Poly(L-lactic acid)Mw = 300 000	Dichloromethane/*n*,*n*- dimethylformamide (70:30)	2 wt%	150-500
Poly(L-lactic acid)Mw = 450 000	Hexafluoropropanol or chloroform	5 wt%	800-3000
		5 wt%	290 (100-600)
Poly(glycolic acid)Mw = 14000-20000	Hexafluoropropanol or chloroform	15 wt%	310 (50-650)
PLGA (1-lactide/glycolide = 50/50)Mw = 108000	Chloroform	8 wt%	760 (200-1800)
PLGA (1-lactide/glycolide = 85/15	Tetrahydrofuran: *N*, N-dimethylformamide (1:1)	0.05 g ml^{-1}	500-800
PLGA (1-lactide/glycolide = 10/90	Hexafluoropropanol	5-7%	3900

1.35 Parameters affecting the electrospinning process

Although the electrospinning procedure is quite simple, it is affected by some different parameters, making it challenging to carry out. The variables that affect electrospinning can be divided into three categories: solution variables, process variables, and environmental variables [30].

1.36 Solution parameters

Solution parameters are the most widely studied and, contrarily, most erroneously concluded parameters. Often, a researcher concludes a polymer is inappropriate for electrospinning without adequately modifying the process and ambient parameters [30]. Polymer and solvent characteristics determine the solution parameters, which include:

1.37 Polymer characteristics

For electrospinning, high molecular weight polymers with high polymerization levels are chosen because they allow for a sufficient number of intermolecular entanglements. Generally, a low molecular weight polymer tends to form bead nanofiber rather than a smooth one. Furthermore, linear polymers are preferred over non-linear polymers since the latter form a viscous solution or sometimes gel, even at low concentrations [45, 46].

1.38 Polymer concentration and solution viscosity

The optimum polymer concentration for efficient electrospinning depends on the polymer's characteristics and solvent used. The applied electric field and opposing surface tension lead the entangled polymer chains to break into fragments before reaching the collector at low polymer concentrations (low viscosity solutions), resulting in fractured and beaded nanofiber. Therefore, the polymer concentration (or viscosity of the solution) must be increased to overcome the surface tension and produce homogeneous, bead-free nanofibers. As the concentration of polymers rises, the viscosity of the solution also rises, making it more difficult for the solution to pass through the needle tip [47, 48].

1.39 Surface tension

Surface tension is the main force acting against the electric force of the surface charge, and it resists the Taylor cone formation. However, after numerous research types, a decisive link has yet to be established between surface tension value and fibre morphology. Usually, a low surface tension value solution produces bead-less nanofibers using a low-voltage power supply. Furthermore, the surface tension of an electrospinning solution can be manipulated by adding a surface active agent [30].

1.40 Solution conductivity

Solution conductivity affects the Taylor cone formation, controls fibre diameter, and enables the use of lower applied voltage. A polymer solution with low conductivity lacks sufficient surface charge to form a Taylor cone; hence no electrospinning will occur. When the conductivity is increased, the surface charge increases, contributing to the formation of Taylor cones and reducing the fibre diameter. Further increase in conductivity beyond a critical value causes a depletion of the tangential electric field along the drop surface, hindering Taylor cone formation [49, 50].

1.41 Dielectric constant

A few research studies have investigated how the dielectric constant affects the morphology of nanofibers. For example, it has been discovered that using solvents with high dielectric constants allows for efficient electrospinning of nanofibers with thin diameters [51, 52].

1.42 Process parameters

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

1.43 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 the fabrication of small-diameter nanofibers. A further increase in the applied voltage beyond a critical value results in Taylor cone stability and hence beaded nanofibers formation [53, 54].

1.44 Nozzle tip-to-collector distance

In most cases, fibre 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 in nanofiber fusion and polymer film formation. On increasing the distance, nanofibers with thin diameter produce; however, it should be accompanied by increasing the applied voltage and the flow rate otherwise beaded will produce [55, 56]. However, in some cases, no effect of distance was observed on nanofiber morphology.

1.45 Solution flow rate

The flow rate of the electrospinning solution determines the fibre morphology, and it depends chiefly on the volatility of the electrospinning solvent used. When a highly volatile solvent accompanied by a sufficiently high applied electric field is employed for electrospinning, smooth nanofiber nanofibers could be produced at a higher flow rate. However, some studies observed that a higher flow rate resulted in thicker nanofiber or beaded nanofiber or deposition of wet nanofibers [57, 58].

1.46 Nozzle design

Spinning nozzles have undergone numerous modifications to produce various types of nanofibers. A coaxial nozzle creates core-shell or even multilayered nanofibers, whereas a single-channel nozzle only allows for the creation of homogenous nanofibers [59]. When the polymer concentration in the solution flowing through the inner needle is very low, a coaxial nozzle can also create hollow nanofibers and nanofibers with coated inner walls. Core-shell nanofibers have an empty core with a thin film of polymer placed on it after the solvent is evaporated [60].

1.47 Collector

A conductive collector is required to produce a sufficient electric field and thus to initiate electrospinning—non-conductive collector results in charge accumulation and hence lower packing density of nanofibers [41]. The collector can be a rotating cylinder, a wheel-like disk, or a flat surface (patterned or continuous). While rotating collectors have been employed to collect aligned fibres, static planar collectors produce randomly arranged nanofibers [61].

1.48 Ambient parameters

Even though ambient factors like temperature and relative humidity aren't typically thought of as factors that affect electrospinning, they have a significant impact. Inversely,

higher temperatures lead to greater solvent evaporation and thicker nanofiber formation. Relative humidity impacts electrospinning depending on the makeup of the polymer solution. Water functions as a non-solvent in non-polar polymeric solutions, and increasing relative humidity creates porous nanofibers [30].

1.49 Application of electrospinning technique

The application range from filtration to tissue engineering scaffolds, cosmetic masks, military protective gear, nanosensors, energy-related applications, wound dressings, drug delivery, and enzyme immobilization [62].

1.50 Nanofibers in drug delivery

Numerous drugs, including antibiotics, analgesics, non-steroidal anti-inflammatory drugs, anticancer drugs, nucleic acids, and growth factors, have already been incorporated into nanofibers during the more than ten years that they have been studied as drug delivery systems for transdermal, oral, oromucosal, parenteral, and ocular application [30].

1.51 Nanofibers in tissue engineering

Regenerative medicine is a new, fascinating, and developing research area. It permits the production of functional tissue substitutes to restore or replace tissue or organ function lost due to ageing, damage, illness, or congenital impairments. Basic research in tissue engineering and regenerative medicine further attempts to investigate tissue deposition, development, and remodelling by utilizing knowledge from various fields. For example, electrospun nanofiber loaded can be used angiogenesis (blood capillaries regeneration), skin regeneration, cartilage growth and bone growth, etc.

1.52 Improvement of existing electrospun nanofibers

Although electrospinning technology has seen several advancements, in this part, we will address the future directions of electrospun nanofibers as they pertain to tissue regeneration applications.

1.53 Functionalized nanofibers

Using endothelial cell culture, Collagen-coated PLA-CL has shown greater cell adhesion, spreading, and survival than the unmodified nanofibers. This opens the door to composite materials that combine the benefits of synthetic polymers and natural proteins while retaining the nanofiber structure [44].

1.54 Three-dimensional extension

Extension in three dimensions: Based on in vitro and in vivo tests, earlier research on non-nanofibrous polymer scaffolds revealed that the 3D form reacted differently from the 2D materials. The 3D extension of nanofibers must be accomplished as soon as possible if we wish to continue using biomimetic nanofibers as the best scaffolds for tissue regeneration [44].

2. CONCLUSION

An open and hard-to-heal wound is very prone to microbial infection. It is heavily infiltrated with endogenous reactive oxygen species. Additionally, due to the loss of residual cells for regeneration, these types also require a skin regeneration scaffold. Electrospinning is a simple method for creating fibres with diameters ranging from tens of nanometers to several micrometres out of a variety of polymers and blends with various morphologies. The electrospun nanofibers have a large specific surface area and a high aspect ratio. The outstanding mechanical capabilities of the polymer are the result of the polymer molecules' strong orientation along the fibre axis. The mechanical properties of electrospun nanofibers are influenced by their size. Additionally, the electrospun nanofiber mats and membranes have very porous architectures. Electrospun nanofibers are a desirable option for composite reinforcement because of all these qualities. We suggested developing a polymer-based nanofiber membrane loaded with an antibacterial and an antioxidant for application at the wound site to achieve quicker wound healing, considering the abovementioned problems experienced by an open full-thickness wound.

3. AUTHORS CONTRIBUTION STATEMENT

Gufran Ajmal conceptualized and prepared the original draft. Narender Yadav designed the study and reviewed the manuscript. Mohammad Rashid Iqbal and Pooja Mittal discussed the methodology and provided the necessary inputs. The final paper was read and approved by all writers.

4. CONFLICT OF INTEREST

Conflict of interest declared none.

5. REFERENCES

1. Kolarsick PAJ, Kolarsick MA, Goodwin C. Anatomy and physiology of the skin. J Dermatol Nurs Assoc. 2011;3(4):203-13. doi: 10.1097/JDN.0b013e3182274a98.
2. Ng KW, Lau WM. Skin deep: the basics of human skin structure and drug penetration. In: Percutaneous penetration enhancers chemical methods in penetration. enhancement Book Company. Springer; 2015. p. 3-11.
3. Berger TG, Elston DM, James W. Andrews' diseases of the skin: clinical dermatology. Saunders Elsevier; 2006.
4. Kant V, Jangir BL, Nigam A, Kumar V, Sharma S. Dose regulated cutaneous wound healing potential of quercetin in male rats. Wound Med. 2017;19:82-7. doi: 10.1016/j.wndm.2017.10.004.
5. Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. Adv Skin Wound Care. 2012;25(7):304-14. doi: 10.1097/01.ASW.0000416006.55218.d0, PMID 22713781.
6. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528-42. doi: 10.1177/147323000903700531, PMID 19930861.

7. El-Ferjani RM, Ahmad M, Dhiyaaldeen SM, Harun FW, Ibrahim MY, Adam H, et al. In vivo assessment of antioxidant and wound healing improvement of a new Schiff base derived Co (II) complex in rats. Sci Rep. 2016;6:38748. doi: 10.1038/srep38748, PMID 27958299.
8. Ajmal G, Bonde GV, Mittal P, Khan G, Pandey VK, Bakade BV, et al. Biomimetic PCL-gelatin based nanofibers loaded with ciprofloxacin hydrochloride and quercetin: A potential antibacterial and antioxidant dressing material for accelerated healing of a full thickness wound. Int J Pharm. 2019;567:118480. doi: 10.1016/j.ijpharm.2019.118480, PMID 31255776.
9. Nafiu AB, Rahman MT. Anti-inflammatory and antioxidant properties of unripe papaya extract in an excision wound model. Pharm Biol. 2015;53(5):662-71. doi: 10.3109/13880209.2014.936470, PMID 25431122.
10. Guo Sa, DiPietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3):219-29. doi: 10.1177/0022034509359125, PMID 20139336.
11. Ajmal G, Bonde GV, Thokala S, Mittal P, Khan G, Singh J, et al. Ciprofloxacin HCl and quercetin functionalized electrospun nanofiber membrane: fabrication and its evaluation in full thickness wound healing. Artif Cells Nanomed Biotechnol. 2019;47(1):228-40. doi: 10.1080/21691401.2018.1548475, PMID 30688107.
12. George Broughton I, Janis JE, Attinger CE. Wound healing: an overview. Plast Reconstr Surg. 2006;117;Suppl:(7S): p. 1e-s-32e-s.
13. Pereira RF, Bártolo PJ. Traditional therapies for skin wound healing. Adv Wound Care. 2016;5(5):208-29. doi: 10.1089/wound.2013.0506, PMID 27134765.
14. Rasik AM, Shukla A. Antioxidant status in delayed healing type of wounds. Int J Exp Pathol. 2000;81(4):257-63. doi: 10.1046/j.1365-2613.2000.00158.x, PMID 10971747.
15. Bonde GV, et al. Lapatinib-loaded nanocolloidal polymeric micelles for the efficient treatment of breast cancer. J Appl Pharm Sci. 2020;10(9):023-9.
16. Pang Y, Zhang Y, Huang L, Xu L, Wang K, Wang D, et al. Effects and mechanisms of total flavonoids from Blumea balsamifera (L.) DC. on Skin Wound in Rats. Int J Mol Sci. 2017;18(12):2766. doi: 10.3390/ijms18122766, PMID 29257119.
17. Bonde GV, et al. Polyethylene glycol block polymeric micelle: A promising delivery vehicle for lymphatic targeting; 2018.
18. Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J Cell Mol Med. 2010;14(4):840-60. doi: 10.1111/j.1582-4934.2009.00897.x, PMID 19754673.
19. Xu L, Ji X, Zhao N, Song C, Wang F, Liu C. The conjugation of Cu/Zn superoxide dismutase (SOD) to O-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (O-HTCC) enhances its therapeutic potential against radiation-induced oxidative damage. Polym Chem. 2016;7(9):1826-35. doi: 10.1039/C5PY02025E.
20. Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int Wound J. 2017;14(1):89-96. doi: 10.1111/iwj.12557, PMID 26688157.
21. Singh J, Mittal P, Vasant Bonde G, Ajmal G, Mishra B. Design, optimization, characterization and in-vivo evaluation of quercetin enveloped Soluplus®/P407 micelles in diabetes treatment. Artif Cells Nanomed Biotechnol. 2018;46(sup3);Suppl 3:S546-55. doi: 10.1080/21691401.2018.1501379, PMID 30322273.
22. Mittal P, Vardhan H, Ajmal G, Bonde GV, Kapoor R, Mittal A, et al. Formulation, optimization, hemocompatibility and pharmacokinetic evaluation of PLGA nanoparticles containing paclitaxel. Drug Dev Ind Pharm. 2019;45(3):365-78. doi: 10.1080/03639045.2018.1542706, PMID 30394795.
23. Campoccia D, Montanaro L, Arciola CR. A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces. Biomaterials. 2013;34(33):8018-29. doi: 10.1016/j.biomaterials.2013.07.048, PMID 23932292.
24. Madhukiran D, Jha A, Kumar M, Ajmal G, Bonde GV, Mishra B. Electrospun nanofiber-based drug delivery platform: advances in diabetic foot ulcer management. Expert Opin Drug Deliv. 2021;18(1):25-42. doi: 10.1080/17425247.2021.1823966, PMID 32924638.
25. Feng K, Sun H, Bradley MA, Dupler EJ, Giannobile WV, Ma PX. Novel antibacterial nanofibrous PLLA scaffolds. J Control Release. 2010;146(3):363-9. doi: 10.1016/j.jconrel.2010.05.035, PMID 20570700.
26. Xue J, He M, Niu Y, Liu H, Crawford A, Coates P, et al. Preparation and in vivo efficient anti-infection property of GTR/GBR implant made by metronidazole loaded electrospun polycaprolactone nanofiber membrane. Int J Pharm. 2014;475(1-2):566-77. doi: 10.1016/j.ijpharm.2014.09.026, PMID 25240438.
27. Chen S, Liu B, Carlson MA, Gombart AF, Reilly DA, Xie J. Recent advances in electrospun nanofibers for wound healing. Nanomedicine (Lond). 2017;12(11):1335-52. doi: 10.2217/nnm-2017-0017, PMID 28520509.
28. Kim TG, Park TG. Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly (D, L-lactic-co-glycolic acid) nanofiber mesh. Tissue Eng. 2006;12(2):221-33. doi: 10.1089/ten.2006.12.221, PMID 16548681.
29. Bonde GV, Yadav SK, Chauhan S, Mittal P, Ajmal G, Thokala S, et al. Lapatinib nano-delivery systems: a promising future for breast cancer treatment. Expert

Opin Drug Deliv. 2018;15(5):495-507. doi: 10.1080/17425247.2018.1449832, PMID 29521126.
30. Pelipenko J, Kocbek P, Kristl J. Critical attributes of nanofibers: preparation, drug loading, and tissue regeneration. Int J Pharm. 2015;484(1-2):57-74. doi: 10.1016/j.ijpharm.2015.02.043, PMID 25701683.
31. Celebioglu A, Umu OC, Tekinay T, Uyar T. Antibacterial electrospun nanofibers from triclosan/cyclodextrin inclusion complexes. Colloids Surf B Biointerfaces. 2014;116:612-9. doi: 10.1016/j.colsurfb.2013.10.029, PMID 24262865.
32. Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B. Electrospun cellular microenvironments: understanding controlled release and scaffold structure. Adv Drug Deliv Rev. 2011;63(4-5):209-20. doi: 10.1016/j.addr.2010.12.002, PMID 21145932.
33. Ranganath SH, Wang CH. Biodegradable microfiber implants delivering paclitaxel for post-surgical chemotherapy against malignant glioma. Biomaterials. 2008;29(20):2996-3003. doi: 10.1016/j.biomaterials.2008.04.002, PMID 18423584.
34. Xie J, Wang CH. Electrospun micro-and nanofibers for sustained delivery of paclitaxel to treat C6 glioma in vitro. Pharm Res. 2006;23(8):1817-26. doi: 10.1007/s11095-006-9036-z, PMID 16841195.
35. Kataria K, Gupta A, Rath G, Mathur RB, Dhakate SR. In vivo wound healing performance of drug loaded electrospun composite nanofibers transdermal patch. Int J Pharm. 2014;469(1):102-10. doi: 10.1016/j.ijpharm.2014.04.047, PMID 24751731.
36. Liu S-J, Kau Y, Chou C, Chen J, Wu R, Yeh W. Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing. J Membr Sci. 2010;355(1-2):53-9. doi: 10.1016/j.memsci.2010.03.012.
37. Pandey VK, Ajmal G, Upadhyay SN, Mishra PK. Nanofibrous scaffold with curcumin for anti-scar wound healing. Int J Pharm. 2020;589:119858. doi: 10.1016/j.ijpharm.2020.119858, PMID 32911047.
38. Reneker DH, Yarin AL. Electrospinning jets and polymer nanofibers. Polymer. 2008;49(10):2387-425. doi: 10.1016/j.polymer.2008.02.002.
39. Nascimento ML, Araújo ES, Cordeiro ER, de Oliveira AH, de Oliveira HP. A literature investigation about electrospinning and nanofibers: historical trends, current status and future challenges. Recent Pat Nanotechnol. 2015;9(2):76-85. doi: 10.2174/1872210509021508191515 32, PMID 27009122.
40. Dhedage N, Khan G, Ajmal G, Kumar M, Jha A, Mishra B. Metronidazole loaded polycaprolactone-Carbopol blends based biodegradable intrapocket dental film for local treatment of periodontitis. Drug Deliv Lett. 2021;11(1):34-43. doi: 10.2174/2210303110999200910104334.
41. Coelho DS, et al. Electrospinning technology: designing nanofibers toward wound healing application. In: Nanofibers-from preparation to applications. IntechOpen; 2018.
42. Wang HS, Fu GD, Li XS. Functional polymeric nanofibers from electrospinning. Recent Pat Nanotechnol. 2009;3(1):21-31. doi: 10.2174/187221009787003285, PMID 19149752.
43. Jiang S, Chen Y, Duan G, Mei C, Greiner A, Agarwal S. Electrospun nanofiber reinforced composites: a review. Polym Chem. 2018;9(20):2685-720. doi: 10.1039/C8PY00378E.
44. Liao S, Li B, Ma Z, Wei H, Chan C, Ramakrishna S. Biomimetic electrospun nanofibers for tissue regeneration. Biomed Mater. 2006;1(3):R45-53. doi: 10.1088/1748-6041/1/3/R01, PMID 18458387.
45. Gupta P, Elkins C, Long TE, Wilkes GL. Electrospinning of linear homopolymers of poly (methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. Polymer. 2005;46(13):4799-810. doi: 10.1016/j.polymer.2005.04.021.
46. McKee MG, Layman JM, Cashion MP, Long TE. Phospholipid nonwoven electrospun membranes. Science. 2006;311(5759):353-5. doi: 10.1126/science.1119790, PMID 16424332.
47. Haider S, Al-Zeghayer Y, Ahmed Ali FA, Haider A, Mahmood A, Al-Masry WA, et al. Highly aligned narrow diameter chitosan electrospun nanofibers. J Polym Res. 2013;20(4):105. doi: 10.1007/s10965-013-0105-9.
48. Rošic R, Pelipenko J, Kocbek P, Baumgartner S, Bešter-Rogač M, Kristl J. The role of rheology of polymer solutions in predicting nanofiber formation by electrospinning. Eur Polym J. 2012;48(8):1374-84. doi: 10.1016/j.eurpolymj.2012.05.001.
49. Angammana CJ, Jayaram SH. Analysis of the effects of solution conductivity on electrospinning process and fiber morphology. IEEE Trans Ind Appl. 2011;47(3):1109-17. doi: 10.1109/TIA.2011.2127431.
50. Sun B, Long YZ, Zhang HD, Li MM, Duvail JL, Jiang XY, et al. Advances in three-dimensional nanofibrous macrostructures via electrospinning. Prog Polym Sci. 2014;39(5):862-90. doi: 10.1016/j.progpolymsci.2013.06.002.
51. Jarusuwannapoom T, Hongrojjanawiwat W, Jitjaicham S, Wannatong L, Nithitanakul M, Pattamaprom C, et al. Effect of solvents on electro-spinnability of polystyrene solutions and morphological appearance of resulting electrospun polystyrene fibers. Eur Polym J. 2005;41(3):409-21. doi: 10.1016/j.eurpolymj.2004.10.010.
52. Mittal P, Vrdhan H, Ajmal G, Bonde G, Kapoor R, Mishra B. Formulation and characterization of genistein-loaded nanostructured lipid carriers: pharmacokinetic, biodistribution and in vitro cytotoxicity studies. Curr Drug Deliv. 2019;16(3):215-

25. doi: 10.2174/1567201816666181120170137, PMID 30465502.
53. Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. Tissue Eng. 2006;12(5):1197-211. doi: 10.1089/ten.2006.12.1197, PMID 16771634.
54. Sill TJ, von Recum HA. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials. 2008;29(13):1989-2006. doi: 10.1016/j.biomaterials.2008.01.011, PMID 18281090.
55. Bhardwaj N, Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv. 2010;28(3):325-47. doi: 10.1016/j.biotechadv.2010.01.004, PMID 20100560.
56. Ajmal G, Mishra B. Advanced drug delivery systems in the management of Gaucher disease. In: Drug delivery systems for metabolic disorders. Elsevier; 2022. p. 137-48.
57. Cramariuc B, Cramariuc R, Scarlet R, Manea LR, Lupu IG, Cramariuc O. Fiber diameter in electrospinning process. J Electrostat. 2013;71(3):189-98. doi: 10.1016/j.elstat.2012.12.018.
58. Khan G, Patel RR, Yadav SK, Kumar N, Chaurasia S, Ajmal G, et al. Development, optimization and evaluation of tinidazole functionalized electrospun poly (ε-caprolactone) nanofiber membranes for the treatment of periodontitis. RSC Adv. 2016;6(102):100214-29. doi: 10.1039/C6RA22072J.
59. Maleki M, Latifi M, Amani-Tehran M, Mathur S. Electrospun core–shell nanofibers for drug encapsulation and sustained release. Polym Eng Sci. 2013;53(8):1770-9. doi: 10.1002/pen.23426.
60. Bonde GV, Ajmal G, Yadav SK, Mittal P, Singh J, Bakde BV, et al. Assessing the viability of Soluplus® self-assembled nanocolloids for sustained delivery of highly hydrophobic lapatinib (anticanceranticancer agent): optimization and in-vitro characterization. Colloids Surf B Biointerfaces. 2020;185:110611. doi: 10.1016/j.colsurfb.2019.110611, PMID 31704609.
61. Pandey VK, Srivastava KR, Ajmal G, Thakur VK, Gupta VK, Upadhyay SN, et al. Differential susceptibility of catheter biomaterials to biofilm-associated infections and their remedy by drug-encapsulated Eudragit RL100 nanoparticles. Int J Mol Sci. 2019;20(20):5110. doi: 10.3390/ijms20205110, PMID 31618903.
62. Hu X, Liu S, Zhou G, Huang Y, Xie Z, Jing X. Electrospinning of polymeric nanofibers for drug delivery applications. J Control Release. 2014;185:12-21. doi: 10.1016/j.jconrel.2014.04.018, PMID 24768792.
63. Benson, H. A. (2012). Skin structure, function, and permeation. *Topical and Transdermal Drug Delivery: Principles and Practice, 1st ed.; Benson, HAE, Watkinson, AC, Eds*, 1-22.