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Recent Advancements in Electrospun Nanofibers for Wound Healing: Polymers, Clinical and Regulatory Perspective

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ABSTRACT: Wound management is an unmet therapeutic challenge and a global healthcare burden. Current treatment strategies provide limited efficiency in wound management, thus undergoing constant evolution in the treatment approaches. As wound healing is a complex physiological process involving precise synchronization of various phases like hemostasis, inflammation and remodelling, which necessitates innovative treatment strategies. Nanotechnology platforms like polymeric nanofibers (NFs) offer a promising solution for wound management. NFs contain a porous mesh-like structure that mimics the natural extracellular matrix and promote the cell adhesion and proliferation in the wound bed, thus displaying a great potential as a wound healing scaffold. Electrospinning is a simple, versatile and scalable technique for producing highly porous and tuneable NFs with a high surface area. Electrospun NFs are presenting extensive application in wound management, especially for burns and diabetic foot ulcers. This review briefly discusses the wound physiology and conventional treatment strategies. It also provides an overview of the electrospinning process and its principle, highlighting the application of electrospun polymeric NFs in wound management. The authors have made an attempt to emphasizes on the clinical challenges and future perspectives along with regulatory aspects of NFs as a wound dressing.

KEY WORDS: electrospinning, nanofibers, wound, diabetic foot ulcers, biomedical scaffold, regulatory aspects

ABBREVIATIONS: AgNPs, silver nanoparticles; **BIX**, bixin; **CA**, cellulose acetate; **CENP**, ceramic nanoparticles; **CEF**, chicken embryo fibroblasts; **CIP**, ciprofloxacin; **CNC**, cellulose nanocrystals; **CS**, chitosan; **CUR**, curcumin; **DSC**, differential scanning calorimetry; **DMF**, dimethyl formamide; **E-spun**, electrospun; **EnSCs**, endometrial stem cells; **EUNCL**, European nanomedicine characterisation laboratory; **FDA**, Food and Drug Administration; **GA**, gallic acid; **GEL**, gelatin; **GO**, graphene oxide; **GT**, gum tragacanth; **HA**, hyaluronic acid; **H & E**, hematoxylin and eosin; **HNT**, halloysite; **MH**, manuka honey; **MT**, Masson's Trichome; **NFs**, nanofibers; **NPs**, nanoparticles; **NT**, neurotensin; **PCL**, polycaprolactone; **PEO**, polyethylene oxide; **PLA**, poly lactic acid; **PLGA**, poly(lactic-co-glycolic acid); **PVA**, polyvinyl alcohol; **PU**, polyurethane; **QAS**, trimethoxysilylpropyl octadecyldimethyl ammonium chloride; **QCN**, quercetin; **REFINE**, regulatory science framework for nano(bio)material-based medical products and devices; **rhPDGF-BB**, recombinant human platelet-derived growth factor BB; **SSD**, silver sulfadiazine; **STZ**,

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streptozotocin; **TCH**, tetracycline HCL; **THF**, tetrahydrofuran; **WVTR**, water vapor transmission rate; **XRD**, X-ray diffraction; **ZSFC**, Zein-silk fibroin-chitosan

I. INTRODUCTION

Skin is the largest organ of the human body, acting as a protective barrier against the harmful external environment. It prevents pathogens from entering in our body along with maintaining homeostasis. Any injury on the skin surface, leading to breakage of the continuity of the skin is described as a wound.[1] Skin wounds can be categorized in two ways: (1) acute wounds caused by abrasion, burns, surgical procedures and (2) chronic wounds caused by specific disease which include diabetic foot ulcers, pressure wounds.[2] A retrospective study of Medicare, which analyzed both acute and chronic wounds, identified that 8.2 million Medicare beneficiaries had at least one type of wound or related infection.[3] It has been estimated that in developed countries, 1–2% of the population will experience chronic wounds during their lifetime. In the United States, 6.5 million people are affected by chronic wounds every year, incurring a $25 billion healthcare cost. According to an Indian community based epidemiological data, the prevalence of chronic wounds in the Indian population is approximately 45 per 10000 population, whereas the incidence of acute wounds is more than double at 105 per 10000 population.[4]

Chronic wounds such as diabetic foot ulcer is of major concern as they affect a large number of patients and disturb their quality of life. Negligence and improper treatment of acute wounds leads to the development of chronic wounds in patients. Globally, the impact of chronic wounds is adverse with an estimated prevalence of about 6%.[5] The prevalence of different chronic wounds depends on its etiology. For instance, diabetic foot ulcer occurs in about 15% of patients suffering from diabetes with an increasing prevalence each year. Chronic venous insufficiency leads to lower extremity ulcers with a prevalence of 1%. Chronic wounds being complex in nature is difficult to understand and treat leading to serious complications in patients. Overall, chronic wounds lead to increased hospitalizations along with serious effects on the patient's life. Therefore, skin wounds are a rapidly growing threat to public health as well as economy.

Standard wound care includes removal of non-viable tissue or debridement to promote cell proliferation, cleaning and swabbing the wound area to treat infection, and dressing of wound to protect from infections and improve the wound healing process.[6] Presently, dry gauze is the most commonly used dressing for wound healing as it is easily available and inexpensive. However, it has several disadvantages including high absorption capacity leading to wound bed dehydration and increase in infection rate along with re-injury of the wound epithelium on removal of gauze.[7] Therefore, more complex dressings with low absorption capacity and easy removal are developed. For example, dressings like hydrocolloids are developed, which allow moisture retention and gas exchange along with prevention of microbial penetration. These dressings may further possess biological properties that enhances local cell migration and matrix deposition. However, these dressing may not resemble the skin's extracellular matrix (ECM) altering the fate of the wound healing cells and further decreasing the wound healing

efficiency. The development of new wound dressing materials is hindered by a number of challenges. One of the major challenges is infection control as about 10% of wound dressings are predicted to develop infections. Wound etiology at molecular and cellular level along with above-mentioned challenges have hindered the development of effective wound therapies.[8]

The limitations of the available wound dressings led researchers to explore multiple options that could mimic various wound closure stages and provide a favourable atmosphere for wound healing. Recently, electrospun nanofibers (NFs) have gained interest among researchers as biomimetic scaffolds for wound healing applications. These scaffolds have a large surface area allowing easy incorporation of bioactive molecules to have an addendum to the wound healing effect.[9,10] Different wound environment requires wound dressings of a specific nature for good biocompatibility and faster recovery; therefore, NFs of specific polymers can easily be synthesized to meet specific wound requirements. Electrospun NFs have essential characteristics such as increased biocompatibility as it mimics the natural ECM, promotes faster restoration, allows exchange of gases and provides a moist environment around the wound to facilitate wound healing.[11] Although electrospun NFs have tremendous potential, the electrospinning process has certain limitations such as the need of a bulky and expensive setup. However, recent advancements have miniaturized the electrospinning setup. Small laboratory scale, battery operated, portable, light weight and small volume electrospinning devices are being designed for increasing practical applicability of NFs in biomedical applications such as wound healing.[8]

In this review, different polymers including natural and synthetic used for fabricating NFs along with their application have been discussed with a focus on wound healing. The conventional wound dressing material along with their properties are mentioned. Next, different techniques used for the NF production with special emphasis on electrospinning are discussed. Further, this review also highlights various polymers used to create electrospun NFs for wound healing and diabetic foot ulcers. A special emphasis is placed on multiple preclinical models used in wound healing using electrospun NFs, clinically used E-spun NFs and regulatory hurdles of E-spun NFs. Major breakthroughs observed with E-spun NFs in wound healing and their significance are discussed with research outcomes.

II. PHYSIOLOGY OF WOUND HEALING

A. Hemostasis

When any injury occurs, the initial events are designed so as to achieve hemostasis in the first few minutes to hours. This depends on a progression of serine protease occasions, which are intended to prevent blood loss. During this cascade, numerous zymogens (enzyme precursors) are converted into catalytically active, fully functional serine proteases, which cause the formation of a fibrin clot by platelet aggregation.[12] Besides platelet activation, hemostasis also release immune mediators and growth factors such

as platelet-derived growth factor.[13] The stages involved in the wound healing process are shown in Fig. 1.

B. Inflammation

This phase begins 72 hours after tissue injury. A complex series of molecular signals usually represents this phase. This ultimately facilitates monocyte and neutrophil infiltration of the wound bed, eliminating pathogenic organisms, foreign debris, and tissue damage. Pathogen associated molecular patterns are released when the wound site is infected with a pathogen. These danger molecules are then recognized by pattern recognition receptors, further leading to local cell activation.[14,15]

C. Proliferation

This phase involves forming vascular channels, granulation tissue generation, and re-epithelializing the surface of the wound. After 2–3 days of tissue injury, the proliferation of basal layer epidermal cells and the root sheath of the hair follicle is observed. Multiple chemical and physical signalling occur along with new blood vessel formation and re-epithelialization. These signals usually come from anti-inflammatory pro-repair macrophages along with immune cells. One of the important steps in the proliferative phase is vascular network regeneration. Angiogenesis or new blood vessel formation occurs in two steps vessel sprouting followed by vessel anastomosis.[1,16]

D. Remodelling

After the onset of a lesion, two to three weeks later remodelling phase occurs. This phase usually lasts for a year or more. Obtaining maximum tensile strength through reorganization, re-synthesis of ECM and degradation is the main aim of this stage. In this stage, an attempt occurs to recover the normal tissue structure, remodelling the normal tissue structure resulting in a less vascular and less cellular scar tissue. This phase is

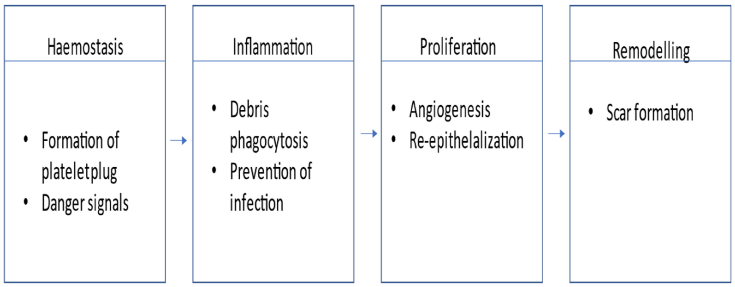

FIG. 1: Different stages of wound healing

further marked by deep changes in the ECM and improvement in the resolution of the initial inflammation.[16,17]

III. CONVENTIONAL TREATMENTS FOR WOUND HEALING

An ideal wound dressing should absorb exudates, act as a barrier to prevent microbial growth, allow gaseous exchange, and provide a moist environment. Further the wound dressing should be non-toxic, non-allergenic, and biocompatible.[18] The different types of dressings available, along with their limitations, are shown in Table 1.

A. NFs as Wound Dressings

NFs are promising nanomaterials that have shown immense application in healthcare, pharmaceutical, biotechnology, energy storage, environmental engineering, textile, defence, and security.[20,21] NFs are two-dimensional fibrous mesh-like structures with a fiber diameter ranging between 100 to 1000 nm.[22] These fibers have exceptional physicochemical properties and biocompatibility.[23] These fibers initiate cellular level programming by sensing the local biological environment and thus achieve the desired therapeutic effect. Compared with traditional wound dressings, these NF scaffolds provide promising wound care. These fibers resemble the ECM structure and thus favour attachment, migration and growth of fibroblast, which ultimately promotes skin tissue growth in the wounded area.[24,25] Several fabrication techniques for micro and NF production can be further classified as non-electrospinning techniques where the mechanical force is utilized.[26] These include phase separation,[20] template synthesis,[27] self-assembly,[28] and drawing,[29] whereas electrospinning techniques utilize electrostatic forces to form NFs.[30] Table 2 represents the advantages and limitations of various fabrication techniques. However, the usefulness of the non-electrospinning techniques is limited by combinations of restricted material properties, cost, production rate, and possible fiber assembly.[31] Electrospun (E-spun) NFs allows easy surface modifications and stretchability along with its simple, high production efficacy and cost-effective preparation. Electrospinning is the technique of choice for large scale production of NFs over the other available techniques, therefore, widely explored for the fabrication of non-woven, functional and continuous micro and nano-scale NFs.[32,33] The detailed principle and instrumentation of electrospinning are discussed in the later part of this article. E-spun NF scaffolds act as a surrogate for natural ECM, thereby acts as a substrate for cellular adhesion, proliferation, differentiation, and remodelling.[34] The E-spun scaffolds have found to have an edge over traditional approaches of drug encapsulation, which include large specific surface area, smaller diameter than the cells, narrow diameter distribution and tuneable porosity.[34,35] A wide range of raw materials, such as synthetic and natural polymers, composites, inorganic nanomaterials, ceramic, and biomolecules can be electrospun to form NF scaffolds.[36] As the diameter of the polymer fibers decreases from micrometers to sub-micron and nanometres, the surface area to volume ratio can increase up to 10^3 times to that of microfibers, which improves flexibility and mechanical

TABLE 1: Different types of conventional wound healing dressings[19]

Type and marketed products	Characteristics	Indications	Advantages	Disadvantages
Hydrogel -Opexa gel (Ajanta Pharma, India) -Hydroheal Am gel (Dr. Reddy's Laboratories Ltd., India)	Hydrogel sheet used for auxiliary and autolysis debridement	Full or partial thickness injury	Provide water to dry wounds keeping the wound moist	Poor absorptive capacity of exudates, no bacterial barrier
Alginate dressings -Activon® -Manuka Honey (Advancis medical, U.K.) - Hyalogran ® (Haemo pharma, Austria)	Extracted from algae, woven or non-woven materials	Full or partial thickness injury	No wound adherence, strong exudate absorption, promotes autolysis	Wound bed is dry and dehydrated as two layers of dressing is required, high cost, not easy to remove the residual dressing
Gauze - Kerlix™ Bandage Rolls (Cardinal health, Ireland) - ADAPTIC™ Non-Adhering Dressing (KCI USA, Inc.) - DermaGauze™ (DermaRite Industries LLC, US)	Woven or non-woven material, includes mostly cotton	For one time use on medical care, surgery or for hemostasis	Easily available, low cost, protects wounds and avoids bacterial invasion	One time use and thus frequent replacement of dressing is required, dressing removal damages new tissue causing pain
Foam - AQUACEL® (Convatech, U.K.) - LUOFUCON® (FORYOU Medical, China)	Good absorption property, provides a moist and warm wound environment	Full or partial thickness injury	Replacement frequency is low, no wound adherence Absorbs large amounts of exudates	Not suitable for eschar and dry wounds, opaque and thus the wound cannot be directly observed, high possibility of bacterial growth

TABLE 1: (*continued*)

Hydrocolloids - DuoDERM® (Convatech, UK) - Tegasorb™ (3 M, US)	Contains colloidal particles	Superficial, moderately deep wounds	Provides moist environment to wounds, promotes autolysis, provides hypoxic and wet healing environment comfortable, pain and friction free	Opaque and, thus not easy to observe the wound. Strong stickiness results in skin injury, not suitable for infectious wounds
Transparent membrane - Comfeel® Plus Transparent (Coloplast, Denmark)	Translucent, polymeric material, allows oxygen and water vapor passing	For superficial wounds, protects skin prone to abrasion	Prevents friction and bacterial infection, transparent thus easy to observe the wound, cheap price	Adheres to the wound, not suitable for severe exudative wounds

TABLE 2: Advantages and limitations of non-electrospinning techniques for fabrication of NFs

Technique	Procedure	Advantages	Limitations	Refs.
Phase separation	Involves the precipitation of polymers from a polymer-poor phase and a polymer-rich phase either thermally or through the use of a non-solvent to the polymer solution, to create a gel	Simple, inexpensive, continuous fibers can be produced	Applicable to few polymers, time consuming, lacks structural stability and porosity.	22,137
Template synthesis	Uses a nano porous membrane template containing cylindrical pores with uniform diameter to make solid NF and hollow NF	Uses nonporous membrane as a template	Long and continuous fibers cannot be fabricated.	138,139
Self-assembly	Used to generate peptide NFs/ amphiphiles similar to the natural folding process of amino acid residues to form unique three-dimensional protein structures. Driving forces include hydrophobic interactions, hydrogen bonding, electrostatic forces, and van der Waals forces, ionic strength and pH	Thinner and continuous NFs can be produced	Time-consuming, complicated processing, expensive, low productivity, poor mechanical strength, poor loading efficiency.	140,141
Drawing	Used to produce single strands that are lengthy through the pulling process which is later accompanied by solidification of dissolved spinning material into a solid fiber	Produces a long single NF	Limited to viscoelastic material, discontinuous process, diameter of NF is dependent on the orifice of the extrusion mold.	138,142

performance.[29] It has better air and moisture permeability; thereby provides a suitable atmosphere for repair, regeneration and wound healing.[37] Electrospun NF scaffold has been used in healthcare for a variety of biomedical applications such as catalysis,[38] tissue engineering,[39] drug delivery carrier system,[40] repair of meniscus,[41] sensors,[42] controlled drug release,[21] and wound healing.[33,43]

B. Electrospinning

Electrospinning is a direct extension of electrospraying. This technique was first patented by Cooley and Morton in 1902.[27] It is an efficient and most widely used method to produce homogenous and continuous NFs.[44]

1. Principle and Instrumentation

Electrospinning is an electrohydrodynamic process in which a liquid droplet or melt is electrified to produce a jet, followed by stretching and elongation to generate micro-scale to nano-scale fiber(s).[33,45] The NFs are formed due to the "electrostatic attraction" of charges between polymer solution at the needle tip and the grounded collector. An electrospinning instrument comprises of a feeding unit for containing and transporting the polymeric solution/melt, a blunt needle that serves as a nozzle, a DC high voltage source connected to the feeder, and a grounded or oppositely charged metallic collector.[46] On the application of DC high voltage (1–30 kV) to the nozzle, the electric charge of the same polarity develops on the polymeric solution particles; thereby repulsive Coulomb forces are created in between the nozzle and grounded or oppositely charged metallic collector (Fig. 2). On increasing the applied voltage, a bubble-like droplet forms at the nozzle tip, which on further increasing the voltage, transforms into a Taylor cone.[47] When applied voltage reaches a threshold value and repulsive forces are sufficient to overcome the surface tension, a

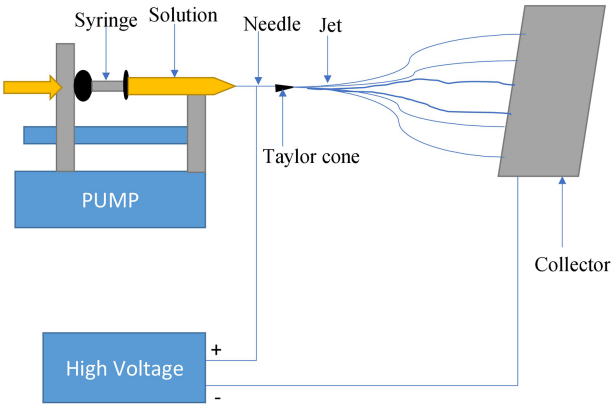

FIG. 2: Schematic diagram of the electrospinning process

thin stream of polymer solution or melt is created from the Taylor cone tip, wherein particles remains under the influence of repulsive Coulomb force, which leads to bending instability and the stream of polymer starts whirling (thus electrospinning) on its way to the collector.[33,48] However, e-spin has certain limitations, such as high electrical potential, electrically conductive targets, and the need for specialized equipment.[49]

IV. POLYMERS FOR WOUND HEALING

A. Chitosan (CS) Composite NFs

CS is a naturally occurring cationic, water-insoluble polysaccharide popular for its antimicrobial, biocompatible, biodegradable, non-toxicity, hemostatic, wound healing properties, and cost-effective.[50] The pros and cons of CS as a biomaterial are highlighted in Table 3. It is a linear polysaccharide produced by the alkaline deacetylation of chitin.[51] CS has the ability to interact with many cellular processes during wound healing. Minimal adverse reactions along with fibrous encapsulation are observed when CS dressings are applied. The cationic nature of CS also provides an anti-bacterial effect. Several studies revealed that CS when applied to wounds, promotes migration of neutrophils and macrophages and granulation by infiltration of dermal fibroblasts. CS also reduces scar tissue formation as a result of excess collagen production in the remodelling phase of wound healing thus resulting in good re-epithelialization. Growth factors like TGF-β1 expression are reduced by CS, which would otherwise promote scar formation.[52] These properties of CS are thought to modulate wound healing. Due to these properties, CS has been extensively utilized in NF dressings for wound healing. Nevertheless, it is difficult to electrospin CS alone due to its chemical structure and cationic nature. The amino groups in CS are protonated in acidic solvents. These ionic groups repel each other on the application of electric field during electrospinning to produce beads and restricts the formation of NFs. CS solutions are highly viscous in nature. The movement of polymeric chains is restricted when exposed to electrical field due to strong hydrogen bonds produced in the 3D network. To overcome this issue, CS can be paired with neutral synthetic polymer like polyvinyl alcohol (PVA) or polyethylene

TABLE 3: Pros and cons of CS as a biomaterial

ADVANTAGES	DISADVANTAGES
Anti-bacterial	Higher surface tension
Biocompatibility	Poor processability
Biodegradability	Reduced spinnability due to ionic nature
Soluble in most acids	Difficult to electrospin alone[144]
Non-toxicity	
Good bioactivity[143]	
Cost-effective	

oxide (PEO).[53,54] CS NFs produced along with other polymers have gained popularity in the development of biomaterials.

Alavarse et al. prepared tetracycline HCl (TCH) loaded PVA/CS NFs and evaluated its antibacterial and wound promotion ability. The antibacterial property of PVA/CS and PVA/CS/TCH NF scaffolds was evaluated against gram-positive *S. aureus*, *S. epidermis*, and gram-negative *E. coli.* In the case of TCH/PVA/CS NF scaffold, the zone of inhibition was broader than PVA/CS NF scaffold. The cell viability and wound scratch assay of TCH/PVA/CS NF scaffold was found to be more as compared with PVA/CS NF scaffold. Therefore, it was concluded that TCH/PVA/CS NF scaffold does not have a deleterious effect on cells and can be used as a wound dressing.[55]

Similar to tetracycline, Yang et al. loaded ciprofloxacin (CIP) in PVA/CS NFs. The wound healing ability of ciprofloxacin and ciprofloxacin hydrochloride (CIP HCl) loaded on PVA/CS NF and PVA/CS/graphene oxide (GO) NF was investigated. The swelling index and contact angle of CIP HCl-loaded NFs were found to be more than CIP NFs due to the hydrophilic nature of CIP HCl. *In vitro* release study revealed that NF membranes without GO had an initial "burst" release, followed by a steady-state. This difference in the release ratio was attributed to the hydrophilic nature of CIP HCl. With the addition of GO, the initial "burst" release slowed down. Moreover, GO increased the distance between NFs, promoting better drug encapsulation and drug release ratio. The cell viability of PVA/CS, PVA/CS/GO, CIP HCl/PVA/CS and CIP/PVA/CS/GO were similar, whereas CIP HCl/PVA/CS/GO and PVA/CS/CIP was over 110%. The high cell viability of melanoma cells showed that the CIP/CIP HCl loaded NFs were relatively non-toxic and had cell compatibility. Therefore, it can be suggested that drug-loaded PVA/CS/GO NF can be a potential candidate for wound dressings.[56] In a different study, a peptide-based antimicrobial, was loaded onto CS/PVA NFs by Zou and his colleagues. They developed PVA/CS NF loaded with carboxymethyl CS nanoparticles (CMC-NPs) encapsulating OH-CATH30 (antimicrobial peptide), i.e., NP-OH30-NFs. The NP-OH30-NFs exhibited excellent swelling ability compared with the control and the degree of swelling exceeded 100%. The antibacterial activities against *E. coli* and *S. aureus* of NFs doped with 3 mg/ml OH30 NPs had an inhibition rate of more than 80%. *In vivo* wound healing study revealed that re-epithelialization and collagen deposition were profound in groups treated with NP-OH30-NFs compared with the group treated with OH30-NPs. Therefore, it can be stated that NP-OH30-NFs has antibacterial as well as wound healing function.[57]

In 2016, Zhu et al. reported asiaticoside-loaded coaxial e-spun NFs of alginate, PVA and CS. The shell solution consisted of a mixture of alginate and PVA in acetic acid, whereas the core solution consisted of 1.5%, 2.5%, and 5% of asiaticoside and CS in acetic acid solution. The developed formulation showed dose-dependent healing activity. The decrease in tumor necrosis factor (TNF)-α and interleukin (IL)-6 at the end of the study in the NFs treated group indicted exhibits their potential to lower the inflammatory reaction. After the burn wound, levels of IL-6 and TNF-α increased sharply. Asiaticoside-loaded NFs down-regulated the levels of IL-6 and TNF-α to a level even lower than the normal levels.[58] CS NFs along with hyaluronic acid have also

been reported. However, fabrication of NFs from these two polymers is challenging due to the presence of opposite charges on the two polymers i.e., cationic charge on CS and anionic charge on HA. Therefore, different processes are employed for fabricating NFs from these polymers. Sun et al. reported NFs production from CS/HA complex coacervates. Coacervates were prepared by mixing NaCl along with methanol followed by the addition of CS/HA in the ratio of 1:1. The mixture was vortexed and finally centrifuged to obtain a dense coacervate. These coacervates were then loaded into a 5 ml syringe and electrospun to obtain NFs. The addition of alcohol was found to accelerate the electrospinning process. Therefore, complex coacervates could be further explored to form functional wound dressings from bio polyelectrolytes CS and HA.[59] In 2018, Chanda et al. also reported the formation of CS/HA NFs. However, they proposed the formation of bilayered scaffolds of CS/polycaprolactone (PCL)-HA. A layer of HA was fabricated on CS/PCL mesh for better mechanical strength, cytocompatibility and increased wound bed hydration. Initially, blending of CS/PCL solution is done and then electrospun to obtain fibers. A blend solution of HA/PEO is electrospun onto CS/PCL fibers to produce bilayered scaffolds. These are further cross-linked with glutaraldehyde vapours. Scanning electron microscope (SEM) studies of NF scaffolds reveal uniform diameter as compared with native ECM. An increase in swelling ability and degradation rate was observed with a reduction in the ability of bacterial adhesion. Overall results indicated improved physicochemical and biological properties of the fibers. Therefore, bilayered scaffolds provide a great platform for the development of CS/HA scaffolds.[60]

Electrospinning of CS and poly(lactic-co-glycolic acid) (PLGA) is also a challenge due to the difference in properties of the two polymers. However, electrospinning of an emulsion prepared by adding CS and PVA into PLGA solution was found to produce NFs. Here, PVA was selected as an emulsifier and was later extracted from the scaffolds. The obtained homogenous emulsion is further electrospun to obtain NFs. The mechanical test indicated good mechanical strength of the scaffolds for biomedical application. CS/PLGA scaffold promoted fibroblast attachment and proliferation along with favourable interactions between cell-cell and cell-matrix. Also, differential scanning calorimeter (DSC) and SEM studies showed that the emulsion system was capable of introducing a compatible, homogeneously distributed mixture of PLGA and CS in PLGA/CS NFs. Therefore, emulsion electrospinning produced scaffolds of CS/PLGA which can be considered a good candidate for wound healing.[61] Another paper of CS/PLGA NFs reported incorporation of phenytoin-loaded PLGA nanoparticles into a CS/PEO solution for electrospinning. PLGA nanoparticles were coated with lecithin to study its effect on drug release. The nanoparticles were prepared by the nano-precipitation method. A part of the freeze-dried nanoparticles was coated with lecithin. The coated and uncoated NPs were separately added into the CS/PEO solution to produce NFs. Drug release studies indicated 100% release of phenytoin from NFs loaded with coated PLGA NPs. Noticeable wound healing effect was observed from NF patches reinforced with phenytoin-loaded PLGA/lecithin nanoparticles when applied on mice. This study concluded that CS/PLGA NFs could be explored for biomedical applications.[62]

Curcumin, an active ingredient obtained from turmeric, has anti-oxidant, wound healing, anti-bacterial, antiviral, and anti-cancer properties. Therefore, curcumin-loaded NFs have been investigated for wound healing. In 2020, curcumin-loaded electrospun Zein-silk fibroin-CS (ZSFC) NFs were developed for wound healing. Curcumin of different concentrations was loaded into the polymeric solution of ZSFC to form NFs. SEM images of NFs demonstrated uniform and smooth NF structure. Mechanical properties and thermal stability of NFs increased with increasing curcumin concentration. In-vitro test indicated that NFs are biocompatible and non-toxic. Overall, the properties indicated good degradation, biocompatibility and wound healing effects.[63] Similarly, curcumin-loaded CS/poly (lactic acid) (PLA) NFs were also reported. A base solution of CS/PLA was developed into which curcumin was added. This solution was further electrospun to form NFs. SEM images indicated uniform fibers without bead formation. In-vitro cytotoxicity studies conducted on L-929 fibroblast showed no cytotoxicity. Also, increased wound healing rate was observed in animal studies following treatment with curcumin loaded CS/PLA NFs. This suggests curcumin-loaded CS NFs can be developed and explored for wound management.[64]

B. Poly(ε-caprolactone) and Gelatin Composite NFs

Poly(ε-caprolactone) (PCL) is a synthetic, non-toxic, biodegradable and biocompatible polymer popular for biomedical applications to achieve long term drug delivery.[65] PCL being highly hydrophobic, degrades over several months; therefore, it is blended with hydrophilic polymers such as gelatin. PCL has been reported to promote wound healing by reducing the inflammatory cells in our body.[43] On the contrary, gelatin (GEL) is a natural polymer possessing excellent hydrophilicity and biocompatibility,[67] thus providing adequate attachment of the NF scaffold to the wound site.[68,69] Therefore, due to their physicochemical and biological properties PCL/GEL NFs are widely explored in wound healing studies. Table 4 enlists the pros of cons of using PCL and gelating composite NFs.

In 2019, Ajmal and his group prepared PCL/GEL NF scaffold enriched with CIP HCL and quercetin and evaluated its antibacterial and antioxidant activity on a full-thickness wound. The addition of GEL and quercetin (QCN) improved the hydrophilicity and biodegradability of NFs. The hemocompatibility study affirmed the protective action of QCN against erythrocyte lysis. Complete closure of the full-thickness wound was observed within 16 days of treatment with the drug-loaded NFs. These findings suggest the potential application of CIP HCL/ QCN/ PCL/ GEL NF scaffold in wound

TABLE 4: Pros and cons of PCL and gelatin as a biomaterial

ADVANTAGES	DISADVANTAGE
Biocompatible Mechanically competent Good wettability	Toxic solvents like trifluoro ethanol required for electrospinning[145]

dressing.[70] Another natural component similar to quercetin was electrospun into NFs. Zahiri et al. fabricated PCL/GEL NF scaffold containing curcumin(CUR)-loaded CS NPs. Different NF scaffolds such as PCL alone, PCL/GEL, PCL/GEL/CUR-CS NPs, and PCL/GEL/CUR-CS NPs/human endometrial stem cells (EnSCs) were fabricated and evaluated. The incorporation of GEL and CUR-CS NPs enhanced the hydrophilicity, wettability, and biodegradability while reducing the mechanical property. The cellular attachment and proliferation were maximum with PCL/GEL/CUR-CS NPs followed by PCL/GEL and PCL alone. Therefore, PCL/GEL/CUR-CS NPs with EnSCs might serve as a promising scaffold for the repair of injured skin tissue.[71] In another study, cerium oxide nanoparticles (CENPs) doped PCL/GEL NF mat was developed and the ability to protect cells against oxidative stress was studied. It was observed that the crystallinity of PCL alone reduced around 2.6 times on blending with GEL, which was necessary for the release of CeNPs from NF mat. The immediate release of CeNPs was due to the dissolution of uncrosslinked GEL, whereas PCL maintained the structural integrity for up to 14 days. CeNPs/PCL/GEL NFs showed better ROS (reactive oxygen species) scavenging and antioxidant potential, due to enhanced 3T3-L1 cell growth and cytoprotection observed. This study concluded that CeNPs/PCL/GEL NFs have potential in wound healing due to its antioxidant property.[72]

Rui Shi and colleagues developed a long-acting antibacterial PCL/GEL NF scaffold, containing trimethoxysilylpropyl octadecyldimethyl ammonium chloride (QAS). Varying concentrations in the range of 5 wt% to 20 wt% of QAS were incorporated in the PCL/GEL matrix. NF scaffold containing 15 wt% and 20 wt% of QAS had considerable bacteriostatic activity against *S. aureus* and *P. aeruginosa.* These NF scaffolds were found to have better cytocompatibility and low cytotoxicity when compared with other antibiotic-loaded NF scaffolds[73–75] and thus could be considered as a potential new generation antibacterial wound dressing.[76] Pavliňáková et al. prepared halloysite (HNT) reinforced PCL/GEL elastic NF scaffold using green chemistry. HNTs were added to the PCL/GEL solution in different concentrations ranging from 0.5 wt% to 9.0 wt% NF scaffold reinforced with 0.5 wt% HNTs was selected as it exhibited good mechanical property. Moreover, all HNTs containing the NF scaffold were confirmed to be non-cytotoxic based on the findings from *in vitro* cell line study on NIH-3T3 cells. Therefore, 0.5 wt% HNT-NZ reinforced PCL/GEL NF scaffold was reported as a good candidate for wound dressing.[77]

C. Polyurethane NFs

Polyurethane (PU) has been frequently used in wound dressings as it provides good barrier properties and oxygen permeability, which is essential for faster healing.[78] Table 5 mentions pros and cons of PU as NF polymer.

PU was studied for wound healing activity either alone or with other polymers such as gelatin. In 2009, Kim et al. studied the wound healing effect of GEL/polyurethane (PU) NF scaffold. The polymeric mixture consisted of 8 wt% GEL and 4 wt% PU in different ratios. As the ratio of PU concentration was gradually increased, the diameter

TABLE 5: Pros and cons of PU as a biomaterial

ADVANTAGES	DISADVANTAGES
Biocompatibility Good mechanical properties Oxygen permeability Flexibility to tailor polymer structure	Difficult to electrospin alone Highly polar organic solvents required for electrospinning[146]

of NFs decreased from micro-size to nano-size and NF surface roughness increased. Moreover, GEL is a natural polymer that has biological characteristics similar to collagen, therefore it supports cell proliferation and viability. Therefore, GEL/PU NF scaffolds would be a suitable candidate for enhanced wound healing.[79] In 2013, Heo et al. investigated the burn-wound healing effect of GEL/PU NF scaffold containing silver sulfadiazine (SSD). The SSD was incorporated into strands of electrospun NFs and was further evaluated. In-vitro SSD release, the rate of wound closure, and histopathological evaluation showed that SSD-loaded NFs released the drug in a controlled manner compared with gauze. The rate of wound closure and burn-wound healing ability of NFSSD-2 was more when compared with other groups based on the histopathological evaluation.[80] In 2019, Sheikh et al. investigated the wound healing activity of PU NFs composite consisting of the reduced form of silver nanoparticles (Ag NPs) and lavender oil. NFs were produced by electrospinning with reduced form of Ag NPs (5% w/w) in dimethylformamide (DMF) and 15% w/w lavender oil along with 12% w/w PU in tetrahydrofuran (THF). The biocompatibility of PU mats and drug-loaded composites with chicken embryo fibroblasts (CEFs) was evaluated using MTT assay, which suggested that lavender oil and Ag NPs beyond concentrations of 15% and 5% was toxic to the CEFs.[81] Manikandan and his group prepared PU NF mats reinforced with murivenna oil for wound dressings. The incorporation of murivenna oil increased the wettability and hydrophilicity of PU NFs, which was confirmed by a decrease in the contact angle. Prothrombin time and activated partial thromboplastin time was more in the case of murivenna oil/PU NF than the control. A delayed blood clotting was reported, maybe due to the surface roughness and smaller fiber diameter. Murivenna oil/PU NF was highly non-hemolytic, thus it could be considered as a potential candidate for wound dressing application.[82]

D. Cellulose Acetate NFs

Cellulose acetate (CA) is a hydrophilic polymer with great biocompatibility, considerable biodegradability, and high mechanical strength. The hydrophilic nature of polymer enables good moisture management, thereby making CA more desirable for the fabrication of NF and its application in wound dressings.[83–86] Various research groups have worked on CA NFs for improving the antibacterial activity of wound dressings. Different NF scaffolds containing active therapeutic agents such as silver sulfadiazine, gallic acid, Manuka honey and silver(Ag) NPs have been developed. Khan et al. fabricated silver

sulfadiazine (SSD) reinforced CA composites (SSD/CA) for burn wound-related infections. Four different concentrations of SSD that were incorporated in CA NFs were 0.125 wt%, 0.25 wt%, 0.37 wt%, and 0.5 wt%. The addition of SSD did not affect the morphology and diameter of CA NFs. Antibacterial property of these fabricated NF composites was confirmed by the agar diffusion disc method. Activity against gram-positive *B. subtilis* and gram-negative *E. coli* was studied. SSD/CA was found to be more effective against gram-negative bacteria in comparison to gram-positive bacteria. A dose-dependent zone of inhibition was observed and the authors concluded that 0.5 wt% SSD/CA can be a great alternative for existing wound dressings.[87] In 2018, Jatoi et al. reported AgNPs and titanium dioxide (TiO_2) immobilized CA NF composites as a novel biomaterial for delivering Ag NPs with a reduced propensity of silver leaching from the composite. Both AgNPs and TiO_2 are known for their antibacterial property, therefore can be used together for a longer antibacterial action. The SEM images showed that inclusion AgNPs. TiO_2 increased the solution conductivity leading to regular and bead free NFs. Based on the antibacterial test, it was concluded that NF composites showed excellent activity against gram-positive and gram-negative bacteria for up to 36 hours, after which the activity reduced. However, NF composites successfully inhibited the growth for up to 72 hours. Therefore, these NF composites can be used for long term antibacterial wound dressing.[88]

Another study in 2019 developed gallic acid (GA)-loaded CA NF mats and tested its antibacterial capacity on wounds. In this study, GA-loaded CA cast film (CF) and NF mats were compared at two different concentrations of GA, i.e. 20 wt% and 40 wt%. Mechanical strength of NF containing 20 wt% GA was greater than the NF mats containing 40 wt% GA and neat NFs. GA released from NF20GA and NF40GA mats exhibited maximum antioxidant activity of 78.6% and 84.4%, respectively. The antibacterial property of GA containing NFs was carried out by the diffusion disc method against gram-positive bacteria *S. aureus*, a dose-dependent inhibition was observed. These findings indicated that GA-loaded CA NF mats can be used for wound healing.[89] Ullah et al. reported CA-Manuka honey (MH) NF composites as antimicrobial and biocompatible wound dressings. The inclusion of MH enhanced the antibacterial activity and antioxidant abilities of CA NF composite. NF mats were highly porous (~ 85–90%) and water vapor transmission rates (WVTR) was 2600 to 1950 g/m^2/day, making it breathable and thus suitable for wound dressing.[90]

V. POLYMERS FOR DIABETIC FOOT ULCERS

A. Poly(Lactic-co-Glycolic Acid) NFs

PLGA is a biocompatible and biodegradable polymer, exhibiting a wide range of erosion time. PLGA is extensively used for the controlled delivery of small molecules, proteins and other macromolecules.[91] It is used for various biomedical applications including wound healing. Being hydrophobic in nature, it remains undissolved when exposed to biological media. PLGA NFs are widely used as they speed up the wound healing

process after tissue damage. However, being hydrophobic PLGA shows the incompatibility with hydrophilc biomolecules. Hence uniform mixing of biomolecules with the polymeric solution is not possible. Therefore, the polymer is functionalized prior to electrospinning and a composite polymeric solution is chosen for electrospinning. This helps in achieving electrospinning of therapeutic biomolecules with PLGA.

In 2015, Lee et al. fabricated a biodegradable PLGA NF membrane for the sustained release of human platelet-derived growth factor (rhPDGF-BB) to repair the diabetic wounds. *In vivo* animal study was carried out on STZ-induced diabetic rat, wherein three groups were compared, namely, rhPDGF-BB /PLGA NF, only PLGA NF, and conventional gauze sponge group. rhPDGF-BB/PLGA membrane released rhPDGF-BB for more than 21 days and showed better proliferative and angiogenetic property in diabetic rats, owing to increased MMP-9 levels. rhPDGF-BB/PLGA NFs were functional and effective in treating diabetic rats through the initial phases of the wound healing process.[92] In 2016, Lee et al. carried out a similar study by including collagen to above developed NFs. It was observed that the water absorbency and hydrophilicity of rhPDGF-BB/PLGA-collagen hybrid NFs were significantly high. rhPDGF-BB/PLGA-collagen hybrid NFs treated diabetic rats showed faster and denser re-epithelialization, collagen deposition, and increased expression of matrix MMP-9 as compared with the other groups. These findings suggest that collagen composite scaffold caused potent cell infiltration and epithelialization process.[93] Zheng et al. combined PLGA, cellulose nanocrystals (CNC) and neurotensin (NT) to fabricate NT-doped PLGA/ CNC NF membrane and studied the diabetic wound healing ability of NT. The biomolecule was found to improve wound healing by downregulating the pro-inflammatory skin fibroblasts,[94] dendritic cells, and increased epidermal growth factor expression.[95] The fabricated NF membrane had a smooth surface. *In vivo* wound closure was evaluated in female diabetic rats for 14 days on groups, namely; control (untreated) group and another group with loaded NT NFs. On day 14, the group treated with NT NFs showed the fastest rate of epithelialization while the control group had the slowest rate. The group treated with NT NFs reduced the levels of IL-1β and IL-6 significantly as compared with other groups. From the above results, it can be concluded that NT had potent anti-inflammatory activity by inhibiting IL-1β and IL-6, accelerating collagen deposition, and re-epithelialization in diabetic wounds and can be considered as an effective treatment for diabetic foot ulcers.[96]

B. Poly(ε-Caprolactone) NFs

PCL is a biodegradable and biocompatible synthetic polymer. PCL fabricated NFs outcast a unique structure for effective use in medical application. PCL electrospinning is employed easily by using a single or combination of solvents, the most common solvent being chloroform. It can be electrospun alone; however, when electropsun with other polymers, an improvement in PCL properties have been observed.[97] PCL has also been widely applied for wound healing today. Due to its elastic nature, it covers a large area of the wound. Also, PCL degrades at a slower rate than other polymers; therefore, it

is suitable for biomedical application. It also exhibits other properties such as ease of manufacturing and manipulation, making it a suitable polymer to render into various shapes like fibers and spheres.[98]

Merrell et al. (2009) investigated the potential of PCL NFs loaded with curcumin for diabetic wound healing with varying concentrations of curcumin. The fabricated NFs were found to possess antioxidant and anti-inflammatory properties. In the *in vivo* animal model, the group treated with curcumin-loaded PCL NFs showed about 80% wound closure on day 10, whereas PCL NFs showed around 60% closure. Based on these findings curcumin-loaded PCL NFs were found to show better wound healing property.[99] Similarly, in 2015, Ranjbar-Mohammadi et al. produced 3 wt% curcumin-doped NFs from PCL/gum tragacanth (GT) for sustained and efficient delivery of curcumin. The tensile strength of curcumin/PCL/GT NFs was relatively high when compared with PCL/GT NFs. Addition of GT and curcumin forms a hydrophilic surface, thereby decreasing the contact angle and making it suitable for cell attachment and proliferation. *In vitro* release of curcumin NFs showed a sustained release of 65% over 20 days with less burst release. Therefore, it could be considered a potential candidate for further evaluation of dressing in diabetic wound healing.[100] In 2017, Pinzón-García[67] reported an efficient cutaneous wound healing in diabetic mice using PCL NFs loaded with bixin. Bixin (BIX) is a carotenoid pigment extracted from the seeds of *Bixa orellana* L, known for its anti-inflammatory,[101] antioxidant,[102] and hypoglycemic[103] activity. BIX-PCL1 and BIX-PCL 2 NFs showed an initial burst release of 30% and 40%, respectively, in the first 10 hours, whereas for both NFs, almost 100% of release was observed by the 14th day. In-vivo wound healing activity of BIX-PCL1, BIX-PCL2, and control was assessed on diabetic mice for 14 days. On day three and five of post-wounding, BIX-PCL1 treated diabetic mice exhibited a significant increase in percent wound closure and reduced scar tissue, collagen deposition compared with the control group, which indicates good remodeling activity of BIX. Increasing concentration of BIX on hydrophobic PCL NFs produced an unfavorable environment for healing; therefore, BIX-PCL1 with 2.5% BIX was found to be suitable for accelerated wound closure.[69]

C. Polyvinyl Alcohol and CS Composite NFs

As mentioned in 3.1.1, CS, being polycationic in nature, is difficult to electropsun alone. Therefore, for electrospinning CS, a composite polymeric solution is used. CS is combined with neutral polymers such as PVA, PEO or PVP. PVA is biocompatible, biodegradable and comparatively cheap. It is mainly used for electrospinning along with CS. PVA is added to the CS solution to improve the mechanical, hydrophilic, and biodegradable properties enhance the uniformity of CS NFs[104] proliferation, gene expression, and viability of fibroblasts.[105,106]

Majd et al. studied the potential of PVA/CS NFs as a diabetic wound dressing. Here, a PVA/CS ratio of 75:25 was considered for the NF fabrication. The formulated fibers were evaluated on STZ induced Wistar rats for 14 days. All the animals were randomly divided into three experimental groups (n = 6), including: nondiabetic control, diabetic

control and treatment group i.e., the group treated with CS/PVA NF. On the 14th day, animals treated with PVA/CS NFs were completely healed when compared with diabetic and non-diabetic control groups.[107] In 2018, Ahmed et al. investigated the diabetic wound healing capacity of CS/PVA NFs mats doped with ZnO NPs of 400 nm.[108] In vivo wound healing assay was performed on subcutaneous wounds in diabetes induced rabbits for 12 days. On day 12, wound contraction for CS/PVA/ZnO NFs was found to be maximum with complete epidermal regeneration and mature granulation tissue. CS/PVA/ZnO NFs showed a significant amount of collagen deposition as compared with CS/ PVA NFsand control. Therefore, CS/PVA/ZnO NFs can be predicted to serve as a useful wound dressing for diabetic patients based on clinical studies on human subjects and genotoxicity studies.[109] PVA was added to the CS solution to improve the mechanical, hydrophilic, and biodegradable properties, enhancing the uniformity of CS NFs[104] proliferation, gene expression, and viability of fibroblasts.[105,106] Overall, copolymers are shown to have attractive characteristics in electrospinning when compared with homopolymers. Further, using copolymers, it is possible to tailor the morphological, mechanical, thermal, and biodegradability properties of electrospun NFs.

VI. PRECLINICAL STUDIES OF NFS FOR WOUND HEALING

Among all the developed NF scaffolds, some were tested on animals as a part of preclinical testing. The pre-clinical studies examined the efficacy, cell adhesion, proliferation, viability as well as compatibility of the scaffolds. Information about toxicity, safety and efficacy of the scaffolds are also revealed. Animal models provide clinically relevant information such as the pathophysiology of wound healing in real time.[110] The most commonly used animals in wound healing studies include rodents, rabbits and pigs. They provide several advantages over in vitro studies. The information from animal studies is critical and mandatory for all new therapeutics before moving on to clinical trials. A majority of studies published in the literature used rodent models as they are easy to handle, inexpensive, and provide quicker results due to accelerated healing compared with humans.[111] In this section we will highlight some of the most commonly used animal models in wound healing studies using electrospun NF scaffolds.

A. Diabetic Foot Ulcer Model

Diabetic wound healing models are usually developed in type 1 diabetic mice. Wounds are generally created on the back of the rat/mouse, the plantar skin of the paw or at the foot dorsal.[112] In one study, electrospun NF scaffolds of cellulose acetate/gelatin were fabricated with berberine for diabetic foot ulcer healing in 24 male Wistar rats. Diabetes was induced by giving a single dose of 55 mg/kg STZ via intraperitoneal injection. Wounds were created on the dorsal surface of the foot in rectangular patterns. A transparent plastic template was used to create wounds. A layer of skin of standard 2 mm × 5 mm was removed. The sterilized dressings were then applied to the wounds. After 16 days, animals were sacrificed, and the wound tissue was harvested. The harvested tissue was

then fixed by using 10% formalin. Hematoxylin-eosin (H&E) and Masson's trichrome (MT) stain were used to stain the wounds. Histopathological evaluation revealed that the CA/Gel NF dressing showed complete epithelialization and less polymorphonuclear inflammatory cells (PMNs) infiltration when compared with the negative control. Skin appendix rejuvenation was observed and resemblance to healthy skin was noted. Therefore, the developed scaffolds were proved for their efficacy and cell proliferation. Further, berberine incorporation enhanced the wound healing process.[113]

B. Burn Wound Model

A burn healing process involves proliferation, granulation, epithelialization, and collagenation. A burn wound model is typically used to understand the healing process of burn wounds. In a study conducted by Bayat et al., bromelain-loaded CS NFs were prepared by electrospinning process for burn wound healing. A CS-2% w/v bromelain and CS-4% w/v bromelain NF scaffold were prepared and studied on burns induced in rats. The burn was induced using a metal coin which was previously immersed in boiling water (100°C) for 3 min. The animals were divided into two groups control and treated. Treated groups were treated with scaffolds at different times (0, 1, 7, 14, and 21 days). Every two days, the dressing materials were changed till the end of the study. During the study it was noted that in all groups density of the fibroblast cells reduced whereas the collagen fiber density increased. After 21 days, the wounds treated with CS-2% bromelain showed a decrease in the burn area and were almost cured with skin and hair regeneration, whereas in the other two groups (CS-4% bromelain, CS only) covering tissue along with necrosis tissue was observed. In the CS-2% bromelain, after a 21-day period no necrosis residual tissue was observed. Therefore, the study concluded the efficacy of 2% bromelain-CS containing NFs as well as its safety could be seen with less toxicity as compared with 4% bromelain-CS NFs.[114]

C. Excisional Wound Model

This model accommodates the assessment of several mechanisms involved in wound healing such as epithelialization, granulation, and angiogenesis. This is the most commonly used model in wound healing studies. Xie et al. fabricated CS/PEO NFs loaded with PLGA NPs containing vascular epidermal growth factor (VEGF). Sprague-Dawley rats were used for wound healing studies. Four wounds were inflicted on each of the animals using a 5-mm-diameter biopsy puncher. Samples of NF meshes without growth factor and with growth factor were placed on the wound. The wound area diameter was measured using a Vernier calliper. After a week, the wound area of both the groups reduced; however, after 4 weeks, NFs loaded with NPs had smaller scar tissue as compared with other groups. Histological studies using H&E stain revealed that more new capillaries were formed for NFs loaded with NPs. Masson's trichome staining indicated more collagen deposition and myofibroblast formation for NFs loaded with NPs as compared with commercial wound dressing. Therefore, it was found that for NFs containing

NPs the wound area regained normal skin functionalities in 4 weeks proving it to be an ideal candidate for wound healing.[115]

Though animal skin is not close to the human skin, they are utilized to study the complexity involved in the wound healing process, especially in chronic wounds. Most preclinical studies published in the literature used rodent models for examining the efficacy of electrospun NFs in wound healing. Similar to the human skin, a mouse skin contains the same layers such as epidermis and dermis. Nevertheless, the healing process is different in rodents and humans. In rodents healing occurs through wound contraction, while in humans, it is mainly re-epithelialization and granulation.[116] Porcine skin is considered to be closest to the human skin due to the similarities in cutaneous wound healing such as inflammation, proliferation and re-epithelialization.[117] However, in contrast to the human skin, porcine skin has poor dermis vascularization and a porcine model is expensive for large-scale experiments. Considering the pros and cons of all wound healing models in animals, rodents will remain as preferred models for pharmacological testing NFs before heading to human trials.

VII. CLINICAL TRIALS AND COMMERCIALIZATION OF NF SCAFFOLDS

In recent times, although many studies have been performed for the development of nanofibrous scaffolds, the clinical studies are limited. A few products of electrospun fibers are commercially available for use in wound treating and wound dressings. Surgiclot® (St. Theresa Medical Inc., Eagan, MN, USA) consists of electrospun dextran NFs infused with human-sourced fibrinogen and thrombin. It is rapidly dissolvable in body fluids and releases thrombin to convert fibrinogen into fibrin to form a natural clot at the injury site and stops bleeding. Phoenix Wound Matrix® (Nanofiber Solutions™, Dublin, OH, USA) is a bioabsorbable advanced wound care device that has the ability to use the innate inflammatory response and resulting in progression into the proliferative phase, and promotes the regeneration of functional skin in the wound bed. Phoenix Wound Matrix® is used to treat both partial- and full-thickness wounds such as diabetic ulcers, venous ulcers, pressure ulcers, arterial/ischemic ulcers, tunneled/undermined wounds, surgical wounds trauma wounds and draining wounds. HealSmart™ (PolyRemedy®, Inc, Concord, MA, USA) is an antimicrobial dressing designed to assist in wound healing. It contains hyaluronic acid, which aids in cell proliferation and migration to promote wound healing. Other than wound healing, electrospun NFs are also used as surgical implants. Nicast (Lod, HaMerkaz, Israel) was successful in obtaining a certification for AVflo™ from Conformitè Europèenne (CE) for use as a vascular access graft. Also, PK Papyrus® is an electrospun polyurethane fibers on the stent surface approved for the treatment of acute perforations of native coronary arteries and coronary bypass grafts in vessels 2.5 to 5.0 mm in diameter. Similarly, electrospun PTFE NFs (Zeus Bioweb™, Orangeburg SC, USA) are used as biocompatible coverings for stents used in small vasculatures. ReDura™ (Medprin, Guangzhou, China) is a biodegradable/bioabsorbable implant designed to prevent CSF leakage and tissue adhesion, which in turn promote regeneration of dural defect.[118] A few products of electrospun NFs are currently undergoing clinical

trials. Kossovich et al. conducted clinical trials on burn patients using electrospun nanofibrous material as a novel wound dressing for burns. CS/polyethylene oxide (PEO) NFs were prepared using electrospinning technique and tested on patients having II, IIIa, and IIIb degree burns. Nanofibrous dressing of thickness 200 micrometres was applied on the burns. The clinical study revealed that the CS nanofibrous dressing provided ventilation of the wound, lesser pain after dressing removal, protection from infection along with absorption of exudate, and stimulated the process of skin tissue regeneration. It was also observed that degradation of NF dressing prevented the mechanical damage of the wound while dressing removal.[119] Other than the above-mentioned study, only a few other studies have been reported. The clinical studies reported on the official website of www.ClinicalTrials.gov are summarized in Table 6.

Despite the vast literature concerning the advantages of electrospun NFs in wound healing, only a few products are translated into clinics for human trials. This low success rate could be attributed to the scaling-up issues in the manufacturing process of electrospun NFs and the difficulty to achieve the required standards set by the regulatory bodies of these newer technologies.[120] Although NFs are easily produced in small-scale, it becomes highly challenging to replicate the same on an industrial-scale. The large-scale production is a time-consuming process that is difficult to fit the capacity of pharmaceutical industries. The electrospinning technique is fairly simple, easy to handle; however, achieving fibers of high quality with consistent properties between different batches is difficult.[118] Nanospider™ by Elmarco (Liberec, Czechia) is claimed to be the first commercialized instrument capable of scaling-up of electrospun NFs for pharmaceutical applications. Further development of more such commercial instruments is needed based on the research findings. In addition to the scaling-up issue, the toxicity problem due to residual organic solvents and the drug stability at elevated temperatures during electrospinning are also considered as challenges in the production of electrospun NFs.[118] The vast potential of electrospun NFs in wound healing may be translated into clinical outcomes by addressing the above-mentioned hurdles.

A. Regulatory Aspects of NFs

NFs have a stimulating effect on tissue regeneration, making them suitable for clinical applications such as wound healing, absorbable dressings, grafts, stents, sclerotherapy, and diagnostic sensors. The small size of NFs permits them to flow in the body without disrupting normal blood flow and avoid elimination by renal and hepatic pathways.[122] NFs are considered to be an attractive field in nanomedicines due to ease of manufacturing and use in a broad variety of applications. However, similar to other nanomedicines, there is a lack of guidance with respect to regulations in this field. One of the major hurdles associated with nanomedicines' translation into clinics is the lack of knowledge on nano-bio interaction.[123] Thus, the FDA requires all nanomedicines to be submitted as an investigational new drug application (IND) followed by a new drug application (NDA). Nanomedicines are considered as new entities that are non-bioequivalent to the original drugs because of enhanced properties such as higher absorption, bioavailability,

TABLE 6: Clinical studies on NF scaffolds used in wound healing[121]

Clinical trials number and sponsor	Year	Study title	Condition	Methods	Results	Status
NCT00428727 Fundación Cardiovascular de Colombia	2012	Clinical trial for the treatment of diabetic foot ulcers using a releasing patch of nitric oxide: PATHON	Diabetic foot	A phase 3, double-blind, placebo-controlled clinical trial on 100 diabetic patients were randomly assigned to one of two groups and treated with active and placebo patches for over 90. Patient's health status and the presence of adverse events were assessed during healing process of the ulcer. A multilayer polymeric transdermal patch containing nitrous oxide was produced by electrospinning technique.	No results posted	Completed[147]
NCT00317629 Fundación Cardiovascular de Colombia	2010	Controlled nitric oxide releasing patch versus meglumine antimoniate in the treatment of cutaneous leishmaniasis	Cutaneous leishmaniasis	A phase 3, double-blind, randomized, double-masked, placebo-controlled clinical trial on 620 patients suffering from cutaneous leishmaniasis. Group 1 is treated with meglumine antimoniate and placebo of nitric oxide patches, while Group 2 is treated with placebo of meglumine antimoniate and active nitric oxide patches.	Terminated as an interim analysis revealed that nitric oxide patches are not enough effective	Terminated[148]

TABLE 6: (*continued*)

Clinical trials number and sponsor	Year	Study title	Condition	Methods	Results	Status
NCT02237287 University Hospital, Basel, Switzerland	2015	Combination of taliderm and vacuum-assisted closure (VAC) for treatment of pressure ulcers	Pressure ulcer wounds	An early phase 1, prospective randomized clinical trial to examine the wound healing promoting effect of negative wound pressure therapies (NWPT) with and without the implementation of a thin interface of poly-N-acetyl glucosamine NFs (sNAG).	Terminated because of insufficient number of patients under anti-aggregation	Terminated[149]
NCT02680106 Nicast Ltd., Israel	2017	Evaluation of the SPINNER device for the application of wound dressing: treatment of split skin graft donor sites (SPINNER01)	Skin wounds	A prospective, safety and efficacy, open labelled, two arms, randomized, multicentre, controlled study to evaluate Spinner™ device for the application of wound dressing in treatment of split skin-graft donor sites. The Spinner™ device is compared with Jelonet™. A nano-fibrous dressing is thus created. The Spinner™ device is aimed at creating a nano-fibrous dressing at donor-site wound.		Ongoing[150]

dose-reduction and reduced toxicity.[124] Compared with conventional formulations, clinical translation of nanomedicines is considered as a complex, time-consuming, and expensive process. A thorough understanding is required on the potential toxicity (immune reactions and inflammation) of nanomedicines upon interaction with biological tissues. These toxic effects also depend on various parameters such as shape, size and zeta-potential of nanomedicines. In order to see increased market penetration of nanomedicines in a broad range of diseases, a deeper understanding of product characterization and the relation between physicochemical properties and biological effect is required. This knowledge helps in preventing the unforeseen immune reactivity effects in patients. Unlike conventional formulations, adverse effects of nanomedicines may not show up during clinical trials. Most clinical trials may or may not include enough subjects to detect rare side events some and might require more prolonged exposure to develop. Pharmaceutical companies should take initiative in conducting post-marketing studies for nanomedicine products as long-term studies (5–10 years in duration) provide critical information on long-term drug safety.

There are several hurdles polymeric NFs should overcome before entering Phase I clinical trials (Fig. 3). The robustness of the synthesis and scale-up should be explained in the chemistry, manufacturing and controls (CMC) as per the USFDA's regulations and guidance documents. Nanomedicines including NFs should comply with the regulations listed in the CFR - Code of Federal Regulations Title 21 and International Conference on Harmonization (ICH) guidelines. This could be very challenging for nanomedicines as there are high chances of unexpected interaction with other molecules during the manufacturing process. Further, polymeric NF scaffolds are complex structures which require more sophisticated analytical tests to fully characterize physical, mechanical, biological and chemical properties, also known as critical quality attributes.[125] The physical properties include morphology of the fibers, diameter, and surface area. The surface area and pore size of NFs promotes cell attachment and migration in tissue engineering applications. The orientation of fibers

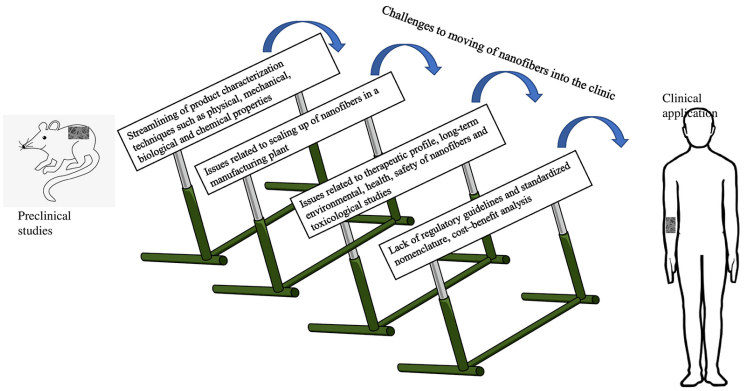

FIG. 3: Major challenges in the progression of NFs into the clinic

also plays an important role in attaching to various tissues including skin and bone. The mechanical properties of electrospun NFs explain their durability and structural integrity. Good mechanical properties of electrospun NF scaffolds play a vital role in support of cell growth and stability.[126] The biological properties mainly include assessment of cell attachment to NFs as they have a higher surface area to absorb proteins and promote binding sites. Under chemical properties it is important to study biocompatibility issues of NFs after they are broken down in a biological environment with the help of lysozymes.[127,128] The chemical properties of electrospun NFs mainly depend on chemical composition and hydrophilicity. In addition to conducting tests on critical quality attributes, it is also important to study pharmacokinetics, efficacy, safety, and toxicological studies of NF scaffolds to understand risk versus benefit ratio. There are no firm regulatory guidelines worldwide for nanomedicines due to various limitations such as insufficient knowledge regarding nanomedicine properties, testing protocols and lack of standardized nomenclature. Regulatory agencies are cautious while reviewing nanomedicines for approval due to various safety and toxicity issues associated with them. There have been cases in which nanomedicines are withdrawn from the market after approval due to the occurrence of adverse events. For instance, a magnetic imaging resonance contrast agent Sinerem, containing small magnetic particles, was withdrawn from the market by the European Medicines Agency (EMA) due to adverse events. This product resulted in muscle pains, mainly in the lower back, leading to the patient's death. The risk with the product outweighed the benefits and thus was denied marketing authorization (CHMP, 2008).[129] Since 2008, there has been a steady progress in the development of a regulatory framework for nanotechnology-based products (Fig. 4). In 2017, the European Commission's Joint Research Centre arranged a workshop to connect various expert communities from regulatory bodies, research institutions and industry who are working in the field of nanomedicine to discuss on specific topics related to the regulation on nanomedicines. Two ongoing projects were funded by Horizon 2020 Research and Innovation programme to improve and advance the regulatory guidelines on nanomedicines and assist in the availability of apt test protocols for characterizing nanomedicines. These include the European Nanomedicine Characterisation Laboratory (EUNCL) and the Regulatory Science Framework for Nano(bio)material-based Medical Products and Devices (REFINE).[130]

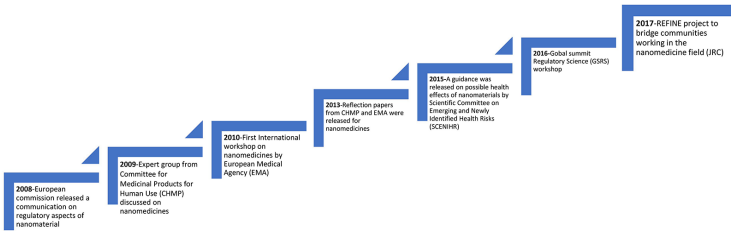

FIG. 4: Major events in the advancements on the regulatory framework for nanotechnology-based products[134]

EUNCL is working on streamlining and developing assays for reliable testing of next generation nanomedicines, whereas REFINE is aimed at setting up a scientific regulatory framework for nano biomaterial based medical products.[131]

Possible environmental impact after use, during manufacturing and disposal, is another important factor to contemplate regarding nanomedicines. Pharmaceuticals that are conventional are recovered in the environment. Therefore, nanomedicines are also considered to behave in a similar manner, i.e., negatively impact the environment. The lack of data is a reason cited by the FDA to determine the safety of nanomedicines to humans and the environment. Thus, FDA is struggling to formulate a criterion to ensure safe and efficacious development of nano-products. There is a guidance report released by the FDA in June 2011 as a response to regulation on nanomedicines. However, a final response is yet to be generated. The environmental assessment and pre-screening of nanomedicines for environmental impact in the USA and EU includes estimation of the environmental concentration for surface water with the acceptable limit being 0.01 ppb. If the concentration of the product is assessed to be below the above-mentioned concentration, no further questions are raised about the environmental safety of the product. Usually, the data collected by the FDA is representative of safety data of bulk materials, which does not display pharmacokinetic and pharmacodynamic data of nanomedicines. Therefore, the safety data collected will not be a true representation of the actual clinical process. This leads to problems in the regulations of nanomedicines. Therefore, all the factors are to be considered while approving a nano-product. A standardized procedure followed worldwide for regulating nano-products are thus required, which will further strengthen the field of nanomedicines and help in exploring its true potential.[132] To summarize, the safety of nanomedicines in humans remains as a major concern. Regulatory authorities are currently working towards improving the framework for controlling various aspects of nanomedicines such as manufacturing processes, product quality, and safety.[133] Most academic and industry researchers often exaggerate the early research and development studies in the field of nanomedicine as potentially revolutionary advances and claim them as novel products in the very near future. However, such claims should be backed by long term studies wherein patients undergoing treatment with nanomedicines are continuously monitored. Regulatory aspects of nanomedicines will remain as a key issue that will drive faster and safer development of nanomedicines in the very near future. Figure 3 shows major challenges of moving NFs to clinics.

B. Future Perspectives and Challenges in Electrospinning

Electrospun NFs provide great care in wound healing. Excellent results have been obtained by different researchers in the field of wound healing. However, some of the elementary processes of NF production are yet to be understood. Better NFs with smooth surfaces are researched today by exploring the process of electrospinning. Substantial progress in the field of electrospinning has been made in the last decade along with improvement in its technological development. Significant breakthrough have been

reported in the last few years; however, numerous problems need to be tackled in the future.

Large-scale production of NFs, along with specific morphologies and chemical and mechanical properties for specific applications remains a challenge. NF production by electrospinning needs to be further optimized by understanding how solution parameters can affect the electrospinning process. Interaction of different electrospinning parameters makes the process complicated, so future investigations are required for understanding interaction between different parameters. Different electrospinning processes such as co-axial electrospinning has been demonstrated as an attractive process to tailor physical properties of NFs. However, the process control and mechanism for core-shell structures still needs to be explored in a systemic manner. Productivity complication as well as environmental and health complications include some existing drawbacks of solution electrospinning, so substitute methods to fabricate NF has become crucial. Alternate electrospinning techniques such as melt electrospinning which involves absence of toxic solvents needs to be further explored.

Different natural as well as synthetic polymers have been fabricated into NFs. However, limited studies have been found on macromolecular orientation and crystalline structure of fibers. Role of molecular orientation and crystalline phase on mechanical properties is still not clear. Focus needs to be made on characterisation tools such as atomic force microscopy – infrared (AFM-IR) spectroscopy, nano-focus XRD to confirm structural changes at the single fiber level which will help in investigating fiber properties at a deeper level. In fields such as tissue engineering the limiting factor that hinders NF application is scaffold thickness and pore size. Therefore, fundamental research is required for studying physics of electrospinning. In case of drug loaded NFs, there are good research studies undertaken to understand drug release behavior and kinetics. However, most of the studies only report *in vitro* results and exclude studying *in vivo* behavior of NF scaffold. Therefore, extensive *in vivo* studies at pre-clinical and clinical stage needs to be undertaken to prove efficacy and safety of NFs. Moreover, long term stability studies needs to be reported for NF according to ICH guidelines for new drug substances and drug products.[135]

In recent research work, electrospinning has been accepted as a versatile technique for NF production, however, its true potential is yet to be realized. A better fundamental understanding of the electrospinning process is required. Different variables govern the electrospinning process and a better control of these variables to produce smooth, bead-free NFs is desired. For fabricating NFs with all the required properties, many parameters need to be optimized depending on the polymers selected and the biomolecules to be loaded. Advanced electrospinning set-up along with innovative research involving a combination of different polymer materials can help overcome these challenges. Eventually, it will be possible to predict polymer behavior during electrospinning and have control over the final fiber properties, particularly with new synthetic analogs whose solution behavior (viscosity, concentration, and conductivity) will need to be predicted and determined experimentally.[136]

VIII. CONCLUSION

Electrospinning is a versatile technique developed for NF production. This review summarizes different polymeric scaffolds developed by electrospinning and their applications. Electrospun NFs have a great potential for the treatment of wounds and diabetic foot ulcers. Different polymers such as CS, PVA, PLGA, and PCL have been explored for the fabrication of NFs. However, good polymeric scaffolds for wound healing are produced when composite solutions of polymers such as CS/PVA and PCL/gelatin are used. Bioactive small molecules and cells can be loaded in NFs to optimize the wound healing process. The research work conducted in the past decade resulted only in a handful of clinical studies with only a few commercialized products. Such low success in the commercialization of NFs could be attributed to lack of reproducibility, robustness, regulatory requirements, scale-up issues, and environmental impact of NF production. Futuristic studies should mainly focus on developing electrospun NF products that are easy to manufacture, scale-up and further combine electrospun NFs with electrical stimulation for enhanced wound healing. In summary, although many challenges remain in NF production, electrospinning appears to be an attractive method for the fabrication of NF scaffolds encouraging researchers of different fields to design and produce NFs exhibiting novel properties.

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