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Delivery of therapeutics from layer-by-layer electrospun nanofiber matrix for wound healing: An update

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Abstract

Increasing incidences of chronic wounds urge the development of effective therapeutic wound treatment. As the conventional wound dressings are found not to comply with all the requirements of an ideal wound dressing, the development of alternative and effective dressings is demanded. Over the past few years, electrospun nanofiber has been recognized as a better system for wound dressing and hence has been studied extensively. Most of the electrospun nanofiber dressings were fabricated as single-layer structure mats. However, this design is less favorable for the effective healing of wounds mainly due to its burst release effect. To address this problem and to simulate the organized skin layer's structure and function, a multilayer structure of wound dressing had been proposed. This design enables a sustained release of the therapeutic agent(s), and more resembles the natural skin extracellular matrix. Multilayer structure also referred to layer-by-layer (LbL), which has been established as an innovative method of drug incorporation and delivery, combines a high surface area of electrospun nanofibers with the multilayer structure mat. This review focused on LbL multilayer electrospun nanofiber as a superior strategy in designing an optimal wound dressing.

Keywords: *layer-by-layer; wound dressing; wound healing; nanofiber; electrospinning*

1. **Introduction**

Skin is a multi-layered structure, composed of epidermis, dermis and hypodermis (subcutaneous tissue), which possess an important task to protect the body from external trauma, and radiation, thermal, biological, chemical, physical and mechanical effects. This largest organ is also responsible for regulating the body's hydration state [1]. Fibroblast and keratinocytes are skin cells that are embedded in a three-dimensional, tissue-forming scaffold called extracellular matrix (ECM), which is thin fibers composed of collagen, elastin and fibrinogen. The secretion of signaling molecules and growth factors takes place in the ECM, which is crucial for tissue growth and regeneration [2,3].

Skin's structure and function can be affected by external physical damages; viz. cuts, burns, surgical incisions or physiological disorders such as diabetes. As a result, there will be disruption of the tissues at the wounded area or the cellular integrity will be compromised [4]. Ensuring of body homeostasis could be possible by the re-establishment of the damaged skin [5,6], by generating new tissue to restore its function within a short period of time. Generally, tissue regeneration is accomplished through a well-regulated wound healing process, which begins almost immediately after the skin injury to prevent infection by any foreign pathogen [7,8]. Thus, wound healing is a dynamic and highly complex process, which requires several weeks to be completed, involving the overlapping phases of haemostasis, inflammation, proliferation, and remodeling phase [7,9]. Following the injury of skin, several signaling cascades are initiated to begin the healing process, complemented by reactions of different cell types. For instance, growth factors and chemokines are secreted by the platelets during the first phage, haemostasis. These factors facilitate inflammatory cell recruitment, where early neutrophil responses are followed by the recruitment of macrophage and monocytes during the inflammatory phase. The neutrophils and formed fibrinolytic system release matrix metalloproteinase, which clears the fibrin clot and ECM of the wound area, facilitating migration of keratinocyte, leading to the

closure of the wound through proliferation stage. During the remodeling phase, macrophages and platelets produce transforming growth factor-β, which promotes differentiation and proliferation of myofibroblast, facilitating scar formation and wound contraction (Figure 1) [10–12]. The mode and extent naturally depending on the course and severity of the injury.

Figure 1.

Skin wounds can be classified as acute or chronic depending on the duration and nature of the healing process. An acute wound might occur abruptly to anyone because of punctures, lacerations, incisions, burns and abrasions [5]. These acute wounds heal in a short period of time and in an orderly manner of wound healing process. On the other hand, chronic wound occurs when the normal process of wound healing is hindered; thereby it fails to heal the wound in an orderly and timely manner. Chronic wounds usually impede in the inflammation phase of the healing process [13]. As a normal consequence, it was discussed that the skin exhibits self-regenerative capacity; however, for chronic wounds (e.g. diabetic foot ulcer) or wounds as a consequence of severe lesions, they are hard to heal or unable to heal. Various factors could affect the healing process, thereby leading to declining in wound healing potential. Local factors, those are affecting the healing process, include are venous insufficiency, foreign body reaction, infection and oxygenation, whereas the systemic factors include gender, age, diabetic condition, physiological stress, sex hormonal imbalance as well as inappropriate medication [9]. Chronic wounds can be categorized based on etiology according to the Wound Healing Society, which is diabetic, pressure, arterial and venous insufficiency ulcers [5].

Less effectiveness of the currently available treatments leads to wound infection and dehydration. In case of a severe infection due to the slow healing of the wound, amputation may need to be performed [7] to prevent any further spreading of the infection. Simultaneously, wound

infections have become an increasing cause of death in severely infected patients. An estimation of 4.5 million patients with chronic wounds was reported in the United States [14]. The rate of a chronic wound is higher in the elderly, which bears an estimated cost of $25 billion per year [15]. Thus, this disease has become an important economic burden to the healthcare system as the treatment of infected wounds is considerably difficult and costly [16]. Therefore, there is an urge to innovate a better and lower cost wound treatment and to accelerate the wound healing process, especially for chronic wounds.

2. Wound dressing

A proper dressing on the wound provides a protective barrier used to contribute to several aspects of the healing process, which functions to promote healing of wounds and prevents any infection. There are several wound dressings from herbal origin, animal origin or synthetic origin that have been introduced; however, those conventional wound dressings are no longer applicable for effective healing, especially for heavy or chronic skin wounds [17,18]. Traditional gauze dressing, for instance, does not exhibit occlusive properties, where the gauze dries on the wound and results in tissue damage when it is removed [19]. A few of the wound dressings with their limitations have been represented in Table 1. Recent advances in wound dressing, such as hydrocolloid [20], hydrogel [21] and film [22], are able to provide a moist environment to the wound. However, these dressings are also lacking ideal properties of a perfect wound dressing. Thus, there is an increasing number of research focusing on developing alternative dressings to accelerate wound healing process with simultaneous prevention of bacterial infections [23–25].

Progressing research in the field of nanotechnology has brought several delivery systems in the medical application [26–28]. Simultaneously, these nanotechnology-based products are also introduced in the complete and effective healing of chronic wounds. Unique properties of nanocarriers exhibiting superiority in application over each other [29]. Definitely, the nanocarriers are somehow overcoming the limitations of conventional wound dressings, thereby providing sustained drug release to carry and protect the wound surface for a longer period [30]. Progress in research has brought polymeric nanoparticles, gold nanoparticles, carbon nanotubes, quantum dots, silver nanoparticles, solid-lipid nanoparticles, liposome, dendrimer and nanofibers in the advancement of wound dressings (Figure 2) [30].

Figure 2

2.1 Properties of ideal wound dressings

Currently, wound dressing fabrication has achieved a higher standard based on the concept of creating and maintaining an optimal environment, which is appropriate for wound healing process [17,31]. It is based on the principle to prevent the wound from dehydration and promote or facilitate the healing [32]. These kinds of dressings are able to moisten the wound environment under the occlusive dressing, where initial presence of low oxygen is responsible to promote the inflammatory phase and increases the rate of epithelialization [19]. Thus, a moist environment is important for re-epithelialization and a porous structure able to manage fluid equilibrium, in a way avoiding either dehydration or exudate accumulation of wound bed [33]. Modern wound dressings have been developed to be multifunctional, to facilitate the healing of wounds rather than just to cover it like a traditional dressing.

Several features of a multifunctional wound dressing make it an ideal wound dressing. This includes; able to provide a moist environment, able to manage wound exudate, allows gaseous exchange, has good haemostasis, able to prevent wound infection, controls wound odour, provides mechanical protection and able to promote cell proliferation and migration for the healing process [17,32]. Trinca *et al.* had reported the characteristics of modern dressings, which provide a suitable cell regeneration environment (e.g., transportation of moisture, gases and liquids with maintenance of temperature), and the materials used should be able to stimulate cell migration, proliferation and reorganization of tissues [33]. Furthermore, the materials used also should be non-toxic, non-adherent and not allergenic to the human body. Patient compliance such as ease of application, painless removal, less frequency of dressing changing and cost-effective also should be met in designing an ideal wound dressing [34].

Recently, nanofiber scaffolds with loaded drugs have tempted attention for the development of wound dressings particularly in skin tissue engineering because of its several characteristics,

such as biocompatibility, controlled drug release efficiency, tailoring ability, which improved tissue repairmen capacity [35].

2.2. Nanofibers as ideal candidate for wound dressing

Nanofiber delivery is gaining researchers' interest over the years due to its outstanding properties; extremely high surface to volume ratio, greatly porous assembly and small pore size. Special properties of nanofiber are mainly due to its high surface to weight ratio as compared to conventional fibers. Being the volume is proportional to the square of the diameter and the surface area is proportional to the fiber diameter, the specific surface area is inversely proportional to the fiber diameter. This relation explained the extremely high specific surface areas and surface area-to-volume ratio for small-diameter fibers [36]. Additionally, the nanofiber is able to form highly porous and interconnected pores [37]. Different areas in the medical field have benefited from the employment of nanofibers; viz. tissues engineering, drug delivery, wound dressing, local chemotherapy, vascular grafts, and enzyme immobilization. Among the potential applications of nanofiber, one of the most promising applications is for drug delivery systems due to its features of high loading capacity, high encapsulation efficiency, and delivery of multiple therapies simultaneously [38].

These nanofibers are formulated using various methods, such as template synthesis, self-assembly, electrospinning, phase separation, freeze-drying, interfacial polymerization etc. [39]. While there are a number of methods to fabricate nanofiber, electrospinning appears to be of interest to many researchers because of several advantages of this method of preparation. In addition to efficiently producing fiber in nanoscale, this method also has simple operation, tunability of fiber diameter, porosity, as well as surface characteristics [40]. Electrospinning often produces a uniform and continuous nanofibers, where a wide range of materials can be introduced to prepare it. This makes it a preferred method to fabricate nanofiber for specific application [41]. In the connecting section, attention on electrospun nanofiber has been made with

special emphasis on electrospinning technique and application of electrospun dressing on wound dressing.

3. Electrospun nanofiber

3.1 Electrospinning technique

Electrospinning is a process that utilizes electrostatic force to the polymeric solution or melted polymer to generate ultrathin fibers. The main setup of electrospinning consists of high voltage electrical supply, needle/spinneret and grounded conductive collector. The polymer solution will be filled in the syringe and the tip of the needle will hold fluid droplets by means of surface tension. Electrical fields will be applied to the needle, causing the solution to be electrified. The electrified polymer solution results in electrostatic repulsion between the same charges in that solution [42]. As the repulsive forces are greater than the surface tension, the droplet elongates to form a cone referred to as 'Taylor cone', which then will be extruded in the form of fiber jet [40,42]. The jet will travel towards the collector and undergo stretching or elongation. The solvent will evaporate during its journey to the collector. Eventually, a solid non-woven fiber mat is formed at the collector. In electrospinning, the presence of sufficient chain entanglements of an electrospinnable solution is crucial to prevent jet breakup due to electrostatic repulsions. Insufficient entanglements and low viscosity of polymer solution lead to droplet formation, which is undesirable in nanofiber fabrication [43].

The electrospinning method is considered as a simple and effective for fabricating nanofibrous structures similar to the ECM. Polymeric fiber fabricated by electrospinning technique results in ultra-fine fibers with diameters ranging from few nanometres up to several micrometers [44]. The resulting electrospun scaffolds are found to imitate the fibrillar structure and functions of skin's ECM with their interconnecting pores and large surface area to volume ratio [45]. Due to the ability to mimic natural nanometer scales of skin tissue, nanofiber membranes have been explored in wound therapy. Their surface can be orchestrated with bioactive molecules and cell recognizable ligands, as those in the natural ECM [46] and thus possess functional surface for cell growth [47]. Previously, materials utilized for skin wound healing such as hydrogels,

decellularized porcine dermal matrix and freeze-dried or gas-foaming formed scaffold lack capability of mimicking the architecture of human skin ECM [48]. Thus, electrospun polymeric nanofibers is a promising tool for skin regeneration because of the structural similarity with the skin's ECM.

3.2. Factors influencing nanofiber development

There are numbers of factors that influence the electrospinning technique in the process of nanofiber development, namely solution parameters (i.e., viscosity, conductivity, concentration, molecular weight, and surface tension), processing parameters (i.e., voltage supply, flow rates, and needle to collector distance), and ambient parameters (i.e., temperature and humidity). The above-mentioned parameters play a significant role in determining the features of the resulted electrospun nanofibers and their application. These parameters can be controlled to maintain the fiber thickness and morphology [49]. By manipulation of these parameters properly, nanofibers of desired morphology and diameters can be obtained [50]. In addition, the surface area generated by nanofiber directly depends on fiber diameter. Nanofiber with smaller fiber diameter generates higher surface area and *vice versa* [51,52]. Furthermore, a decrease in electrospun fiber diameter will also reduce mean pore size, due to an increase in fiber to fiber contact per unit length. This could affect the application of nanofiber as a scaffold for different biological applications, including tissue engineering. Small pores will limit the ability of the larger size of cell to migrate and proliferate within the scaffold [50].

3.2.1 Polymer solution parameters

The solution concentration, viscosity, conductivity, molecular weight as well as surface tension are known to affect the final nanofiber morphology and its ability to be successfully electrospun. At low polymer concentration, morphology obtained is the mixture of beads and fibers. By increasing the concentration, more uniform fibers are produced but have increased in diameter [50]. An increase in solution concentration also means increases its viscosity, which results in

formation of bead-less fiber. For polymer solution with very low viscosity, continuous fibers are unable to be formed. While a very high viscosity solution may cause difficulty in the ejection of jets from polymer solution [50]. Thus, an optimum viscosity of a particular polymer solution must be identified for a successful formation of fibers. The conductivity of polymer solution also affects the produced fiber, where high solution conductivity leads to an increase in the charge of the solution and results in finer fiber diameter. Thus, the high electrical conductivity of a polymer solution possibly results in the smallest fiber diameter. While at low solution conductivity, there is insufficient jet elongation to produce uniform fibers and beaded fibers may also be formed [50]. Next, low surface tension of solution results in bead-less fibers and high surface tension causes instability of jets and generation of sprayed droplets. The low molecular weight of the solution leads to formation of beads while a higher molecular weight solution produces fibers with larger diameters.

3.2.2 Processing parameters

Processing parameters include needle/spinneret to collector distance, flow rate and the applied voltage. The formation of beads is observed when the needle to collector distances is whether too near or too far. Optimum distance gives the fibers sufficient time to dry before settle on the collector surface. When the distance is decreased, there is less time for the jet to solidify before reaching the collector drum; thus, forming defective and large-diameter nanofibers. While increasing the distance results to a smaller diameter of fiber [53], however, there are possibilities of beads formation [54] . High polymer flow rates also result in beaded fibers, which may be due to improper drying time before reaching the collector surface. Increasing the flow rate beyond the critical value also leads to an increase in the pore size and fiber diameter [53]. As the flow rate reduces, a bead-free nanofiber can be obtained. As for voltage supply, higher voltage favours smaller fiber diameter. However, an increase in the applied voltage beyond the critical value also contributes to the probability in the formation of beaded fibers and larger diameter [53]. Thus,

optimum voltage must be investigated, which will produce smooth and smaller nanofibers diameter.

3.2.3 Ambient parameters

Ambient parameters such as temperature and humidity also influence the electrospinning process. Increasing the temperature has two distinct effects on the polymeric solution: increases the evaporation rate of the solvent and reduces the viscosity of the polymer [55]. As the result, the higher temperature of the produce thinner diameter [54] and more homogeneous distribution [55]. In case of humidity, high humidity gives thick nanofibers [54] and beaded fiber is possibly be formed and almost no electrospinning can happen when the humidity is increased. However, increased humidity will also increase the amount of porosity, which may be useful to develop more porous nanofibers [55]. A high humid condition gives an effect on fiber morphology, as the water might condense on the fiber surfaces. Low humidity can increase the solvent evaporation rate [54].

3.3. Electrospun nanofiber as an ideal wound dressing material

In order to successfully employ a nanofibrous scaffold for a specific function, it must possess appropriate physical and biological properties, which match the desired requirements. For wound dressing application, the nanofibrous scaffold should serve a tissue regenerator as well as deliver suitable therapeutic agents [49].

The fiber's structure is especially interesting for wound healing as it is able to promote cell respiration, skin regeneration, moisture retention, removal of exudates, and haemostasis. As compared to typical bandages that do not fulfill the important criteria of wound care, electrospun nanofiber mats meet requirements for an excellent healing environment [56]. The high surface area to volume ratio property is a great advantage for electrospun nanofibers as drug carriers to encapsulate any antimicrobial agent or suitable therapeutics. This feature facilitates the loading of desired amount of drug within the formulated nanofibers [57] and simultaneously accelerates the

solubility of drug in aqueous solution and thus improves the efficiency of the drug [58]. While enhancing the delivery of loaded drugs, the high surface area of nanofiber also aids in fluid absorption [44].

The pores of nanofiber meshes have high-interconnected porosity of 60 to 90%, which enable cells attachment, migration and proliferation for regeneration of the skin. The small pore size of electrospun nanofiber which is less than 1 μm, allows oxygen permeability and prevents bacterial infiltration through aerosol particle capturing mechanisms [46]. The porous nature of electrospun nanofiber prevents the tissue from dehydration [44] as gaseous exchange occurs through the structure, which allows cellular respiration. Concurrently, the interconnected pores are responsible for nutrient supply and exudate control, which maintain moist environment at the wound site thus, enhance angiogenesis and collagen synthesis [59]. In addition, nanofiber mats also provide physical and mechanical protection to the wound [9]. The mentioned features of electrospun polymeric nanofiber fulfill the ideal characteristics of modern multifunctional wound dressings. Therefore, for application on chronic and slow healing wounds, electrospun nanofiber has a potential to be used as an ideal wound dressing considering its properties that contribute towards effective wound healing process.

3.4. Advancement of electrospun nanofiber as wound dressing

The selection of polymer is a crucial step since the type of polymer and interactions between drug-polymer-solvent influence the biocompatibility, mechanical properties, morphology, formation, , and drug release of the drug from the final electrospun nanofiber [60]. Electrospinning allows flexibility in materials and bioactive selection for therapeutic delivery applications. Numbers of polymeric materials; synthetic, semi-synthetic, natural and biological materials have been employed in fabricating nanofibers. Electrospun nanofiber with desired physical and biological properties can be obtained using co-polymerization and polymer mixtures. For instance, the materials selected for wound healing need to have proper swelling

capability for absorption of excess wound exudates and subsequent oxygen permeability for cellular respiration [61]. When there is absorption of the wound exudates by the fiber mats, there is swelling of the dressing materials. Swelling degree, also referred to as fluid uptake or fluid absorption ability of nanofibrous mats, is a significant parameter for the application as wound dressing. Conventional dressing films have limitations for such swelling, which is around 2%. However, due to a higher degree of porosity and incorporation of hydrophilic polymers in the fibrous mats, these can swell by 17-213% of their size [62,63]. The swelling of the mats does not lose its permeability for the gas and moisture from the air and simultaneously keeps the wound area moistened.

As fast healing is concerned, inert wound dressing materials will not be sufficient especially for chronic wounds. Therefore, functionalized biological and biochemical dressing can be developed to accelerate healing [61]. For nanofiber to have a specific function, the desired drug or functional material can be added to a polymeric solution [40] or added after electrospinning process. Numerous studies have been performed with the superiority of electrospun nanofibers in wound dressing. A few of the reported electrospun nanofibers applied in the wound dressing are highlighted in Table 2. Several different kinds of therapeutic agents can be incorporated into or onto the nanofiber for wound healing process, such as antibacterial agents, growth factors or proteins, vitamins, and natural extracts, which has been highlighted in the connecting section.

Figure 3

3.4.1. Electrospun nanofibers of small molecules in wound dressing application

In the case of wound care management, one of major concerns of the delayed wound healing is wound infection due to skin injuries, which are prone to microorganisms contamination. Such infection to the wounds eventually interrupt the healing process and causes severe complications [64]. Therefore, in application of electrospun nanofiber as wound dressing, the

nanofiber is formulated with antimicrobial agents to fight the infection or prevent bacterial invasion and growth. Antibiotics such as triclosan, chlorhexidine, iodine, cetylpyridinium chloride, ciprofloxacin hydrochloride, tetracycline hydrochloride, gentamicin, silver nanoparticles, and metal oxide nanoparticles (e.g. zinc oxide, titanium oxide) have been successfully loaded in fabricating antibacterial electrospun nanofiber wound dressings. Silver nanoparticles (AgNPs) have been widely investigated for its antibacterial ability. For instance, Kohsari and team formulated and reported electrospun chitosan/polyethylene oxide nanofibrous mats containing 0.25% and 0.50% (*w/w*) AgNPs intended for wound dressing application. The results of their studies revealed that both percentages (0.25% and 0.50% (w/w)) of AgNPs had 100% antibacterial activities against both *S. aureus* and *E. coli* bacteria [65]. Ciprofloxacin hydrochloride and tetracycline hydrochloride are widely used antibiotics for the treatment of various infections. These compounds have shown low minimal inhibitory concentration (MIC) for both Gram-positive *Staphylococcus* and Gram-negative *Pseudomonas* bacteria that commonly found on wound, causing wound infection. Alavarse and team investigated the release profile of tetracycline hydrochloride incorporated in poly(vinyl alcohol) (PVA)/chitosan electrospun scaffolds, for wound dressing applications [66]. Results of their experiment showed the inhibition zone formed for PVA/chitosan/ tetracycline hydrochloride scaffolds had a mean diameter of about 8.8 ± 0.4 mm for *E. coli*, 15.6 ± 0.3 mm for *S. epidermidis* and 19.6 ± 0.2 mm for *S. aureus*. In addition, the interconnected and sub-micron sized pores also prevent the penetration of any exogenous bacteria to the wound site. Thus, they concluded that the proposed nanofiber mat could be used for good potential as an antibacterial wound dressing system [66]. Similarly, Kataria and team had investigated electrospun PVA/sodium alginate composites nanofibers transdermal patch loaded with ciprofloxacin [61]. The authors evaluated wound healing performance through *in vivo* studies, where the results showed that the wound healing rate was maximum for drug-loaded PVA/sodium alginate composite nanofibers [61]. Alternatively,

Jannesari et al also had loaded ciprofloxacin HCl into the blend of PVA/polyvinyl acetate (PVAc) to achieve controlled release of the entrapped therapeutics for wound healing administration [67]. In another study, Sirc and team had loaded gentamicin in the middle PVA layer of multilayer electrospun nanofiber mats, for controlled release of the entrapped antibiotic for the effective management of wound condition [68]. The drug release and antimicrobial efficacy were evaluated by the authors where the antibacterial activity was tested on Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*. Their results proved that gentamicin inhibited bacterial growth and showed prolonged retention of gentamicin in the nanofibers with the increasing thickness of the covering layers [68]. Similarly, chitosan/PVA electrospun nanofiber containing gentamicin showed superior antibacterial, cell attachment and proliferation compared to the nanofibers containing lower gentamicin, which was further confirmed by the faster skin regeneration of experimental model [69]. Metal oxides (like zinc oxide) have been shown to possess a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria. In this context, Ahmed and team had fabricated chitosan/PVA/zinc oxide nanocomposite wound dressings and evaluated its' antibacterial activity and wound healing performance in diabetes-induced rabbit model [70]. The wound closure rate was enhanced by the incorporation of zinc oxide nanoparticles in chitosan/PVA mats. The *in vivo* wound healing studies suggested chitosan/PVA/zinc oxide nanofibrous mats accelerated the wound healing in the experimental rabbits when compared to chitosan/PVA nanofibers. Furthermore, chitosan/PVA/zinc oxide showed to possess higher antibacterial potential against *E. coli, P. aeruginosa, B. subtilis* and *S. aureus* when compared to chitosan/PVA nanofibrous membranes. Thus, chitosan/PVA/ZnO nanofibrous membranes were shown to be useful antimicrobial dressings for faster healing of wound [70]. On the other hand, silver sufadiazine was successfully incorporated into the nanofiber composed of PVA and hydrotalcite. The *in vitro* activities reported significant antibacterial efficacy against *E. coli* and

S. aureus with no cytotoxicity [71].Overall, it could be inferred that the nanofiber platform provides the potential for the development of an ideal dressing material for clinical application of non-healing wounds.

3.4.2. Electrospun nanofibers of natural components in wound dressing

Certain essential oils from plant origin have been shown for their intrinsic antibacterial, antifungal and insecticidal properties. Due to their low toxicity and wide availability, such essential oils are preferred over other synthetic antimicrobial agents. In addition, they can be efficiently incorporated in polymeric matrices to create composite materials with excellent antimicrobial activity [72–74]. Liakos and team proposed the use of essential oils (cinnamon, lemongrass and peppermint) as natural antimicrobial agents for electrospun cellulose-based nanofibrous dressing. The resulting dressing was found to be able to inhibit the growth of *E. coli*, even at small percentages of the oils were used. The dressing also reported to possess no cytotoxic effect, and thus are biocompatible and safe to use [72]. Another herbal compound, thymol, which is an important component found in the thyme oil, is used in the healing of wounds. This component has been explored for its antioxidant, antibacterial, antifungal and antiparasite activities. Karami et al investigated the properties and performance of electrospun poly(caprolactone) (PCL), poly(lactic acid) (PLA), and their 50/50 hybrid nanofibrous mats containing this herbal component, thymol (1.2% *v/v*), as wound dressings. The wound healing performance of thymol-loaded 50/50 hybrid of PCL and PLA nanofibrous mats, the commercial wound dressing *Comfeel Plus*, and gauze bandages (control) were evaluated *in vivo* for 14 days. The results after the treatment period revealed that the electrospun thymol-loaded 50/50 PCL/PLA nanofibers mat had a significant wound closure (92.5%) after 14 days, when compared to the control group [75].

Moreover, other natural extracts that possess necessary bioactivities include curcumin, aloe vera, honey, *Centella asiatica* (CA), and bixin, which could also be the alternative natural component for wound healing. Merell and team investigated the feasibility and potential of PCL nanofiber as a delivery vehicle for curcumin, intended for wound healing application [76]. The curcumin-loaded nanofiber was evaluated in diabetic-induced mice having impaired wound healing, where the researchers showed that the animals treated with curcumin-loaded PCL nanofibers showed almost 80% wound closure by day 10. While only approximately 60% wound closure was observed for mice treated with curcumin-free PCL nanofibers [76]. Incorporation of curcumin was also performed in a hybrid electrospun fiber consisting of PCL and gelatin, where the chitosan nanoparticles containing curcumin were reported to facilitate tissue engineering construct [77]. In another study, PCL/montmorillonite nanocomposite was developed to deliver curcumin, which possessed a greater antibacterial potential with low cytotoxicity to confirm the importance of the composite in wound healing [78].

Similarly, aloe vera possesses higher water content (about 99%), which is a fundamental requirement for wound hydration. Additionally, the components in aloe vera are known to responsible for antibacterial, anti-inflammatory, and antioxidant properties. Furthermore, aloe vera can promote fibroblast proliferation, increases collagen synthesis, and therefore enhances the wound healing process [64,79,80]. In a study by Miguel and team, they designed asymmetric electrospun membranes to mimic both the layers of skin for wound dressing purposes. It consists of a top dense PCL layer aimed for mechanical support and a bottom porous layer, composed of chitosan and aloe vera aimed to improve the bactericidal activity of the membrane and ultimately the healing process. From the results, the produced asymmetric membranes displayed a porosity, wettability, mechanical properties similar to those in the native skin. The cell-based studies also showed that the fibroblast cells were able to adhere, spread, and proliferate on the surface of the membranes. The intrinsic structure of the two layers of the

membrane is capable of avoiding the invasion of microorganisms while conferring bioactive properties [64]. Similarly, honey is also known to have an established history in healing and is considered to be the oldest wound dressing. Since ancient times, honey is used as a natural agent for wound healing due to its antibacterial, anti-inflammatory and antioxidant properties. Thus, honey is continued to be used in modern clinical practice for regenerative healing. Due to broad-spectrum antibacterial efficacy, it is effective in preventing infection and clearing infection rapidly. It can provide a moist healing environment and the acidic nature of honey also provides an optimal environment for activity of fibroblast cells [81]. Furthermore, it can provide a non-adherent interface between the dressing and the wound, preventing the dressing from tearing away the newly formed tissues and avoid pain upon dressing removal [82]. Maleki et al fabricated electrospun nanofiber meshes from mixtures of PVA and honey at different weight ratios, loaded with dexamethasone sodium phosphate as the anti-inflammatory agent [82]. The release profile of both PVA and PVA/honey (80/20) nanofibrous mats was similarly establishing that honey does not have a significant effect on the release behaviour of the entrapped drug. The formulations exhibited a large initial burst release, where the release was completed within 1 h. From these findings, the authors concluded that the anti-inflammatory agents could be released at early stages of formulation application, while honey will support as a natural antibiotic to improve the wound dressing efficiency to facilitate healing rate [82]. In another study by Sarkar and team evaluated the ability of low-loaded honey nanofiber for its' antibacterial, anti-inflammatory and antioxidant potential at the at the wound microenvironment. Honey-loaded nanofiber had shown drastical reduction of biofilm formation. From the scratch assay result, the authors depicted that 0.5% honey-loaded nanofiber could be able to maximize reepithelialisation [83]. Alternatively, Sarhan and team developed a nanofibrous wound dressing containing honey/PVA/chitosan (HPCS) with additional natural extracts of *Allium sativum* (AE) and *Cleome droserifolia* (CE) to further enhance healing

process [84]. From the results, it was revealed that HPCS-AE and HPCS-AE/CE nanofiber mats allowed complete inhibition of *S. aureus*, while HPCS-AE/CE nanofiber showed mild antibacterial activity against MRSA. *In vivo* study showed that the developed nanofiber mats could enhance the wound healing process, as proved by the enhanced wound closure rates and histological analysis in mice when compared to untreated groups. Later, when compared to the performance of commercial dressing (Aquacel Ag), HPCS and HPCS-AE/CE had reported similar effects on wound healing process, while HPCS-AE improves wound closure rate [84]. Similarly, CA, another herbal component, is known to possess pharmacological effects on wound healing. The primary active constituents of CA are triterpenoids, which include asiaticoside, madecassoside and madasiatic acid [85]. Asiaticoside possesses strong wound healing properties and is known to reduce scar formation by promoting fibroblast proliferation and ECM synthesis. It has also been reported to increase antioxidant levels and stimulate collagen synthesis [85,86]. The potential effect of madecassoside on wound healing includes antioxidant activity, collagen synthesis and angiogenesis. The last component of CA, asiatic acid, also possesses antioxidant, anti-inflammatory, and neuroprotective properties [85]. Considering the properties of CA extracts, Yao and team fabricated electrospun gelatin nanofiber containing CA extract as topical/transdermal wound dressings. The developed nanofibers had exhibited dermal wound-healing activity on experimental rat model and presented the highest recovery rate of the wound area when compared to those treated with gauze, neat gelatin membranes and commercial wound dressings [87]. A recent report by Dong and team had incorporated the isatis root extract within the electrospun nanofiber formulated using polyvinyl pyrrolidone. Isatis root is a component of traditional Chinese medicine, which is commonly used in the treatment of infected wounds. Here, the researchers approached to deliver the formulation *in situ* using a portable handheld electrospinning device to deposit the fiber bed onto the wound surface. The fibrous structure of the formed nanofiber containing

isatis root possessed desired permeability characteristics and excellent surface wetting. *In vitro* antimicrobial efficacy was further confirmed by the potential wound healing activity in animal model [88]. Similarly, three-layered nanofibrous patches were successfully developed by Shokrollahi et al, where incorporation of PCL and chamomile-loaded carboxyethyl chitosan and PVA was performed to obtain patches with superior mechanical properties. The controlled release pattern of the herbal constituent from the patch was revealed superior antioxidant and antibacterial properties without producing ant cytotoxic property [89]. Liu and team had fabricated sesamol electrospun nanofiber composite to obtain strongest antioxidant efficacy of the herbal constituent projecting towards effective wound healing. In due course, the authors developed the nanofiber using cellulose acetate and zein at a fixed ratio with 5% sesamol to obtain rapid healing of wound via promoting keratinocyte and forming myofibroblasts at the wound area of diabetic animals [90].

Therefore, the findings are positively pointing towards the incorporation of natural constituents in the healing of wounds using this nanofiber platform.

3.4.3. Electrospun nanofibers in wound dressing to deliver biological macromolecules

There are several cytokines, growth factors and chemokines are known to be involved in the cell-signaling network during the process of wound healing [91]. Supplying these macromolecules at the wound site is of prime interest for treating chronic wounds that help remodeling of the normal cycle of wound healing [92]. Thus, different bioactive macromolecules have been incorporated into electrospun fibers to favour the wound microenvironment for rapid healing. However, incorporation of these macromolecules within the nanofibers using electrospin technique is of great challenge, where there is possibility to lose bioactivity. Thus, it is of prime importance to conserve the activity of these macromolecules during the electrospinning process. This can be achieved by the selection of suitable materials and processing conditions [38]. The incorporation of the bioactive molecules was mostly performed by means of blend

electrospinning or coaxial electrospinning. Direct incorporation of GFs is ineffective as GFs rapidly disperse from target sites and also undergo enzymatic degradation in the wound bed, thereby deactivated. Furthermore, the released GFs could be diluted by body fluids such as blood and lymphatic fluids or wound exudate, thus diminish their therapeutic effects at the wound sites [93]. Blend electrospinning often leads to bioactivity reduction as the bioactive molecules are localized on the surface of the fiber. While the core-shell structure produced by coaxial electrospinning could be advantageous in protecting sensitive biomaterial. The shell polymer(s), which surrounds the core, act(s) to protect the core ingredient from direct exposure to the external environment, while also contributes to the sustained release of the bioactive molecule [38]. Furthermore, the encapsulation of biomolecules within drug delivery systems or surface functionalization by an electrospun nanofibers system could be performed to address the problems. The electrospun nanofiber can easily be surface-functionalized or incorporated with GFs [59]. GFs such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) can be incorporated within the nanofiber depending on the demands of the healing process [94]. These components can stimulate the production, differentiation, cell proliferation, and angiogenesis of ECM components, which are important in the healing process of wound. Xie and team created a biomimetic scaffold for wound healing, which was loaded with two different growth factors; VEGF and PDGF-BB to stimulate angiogenesis and cell proliferation [95]. The scaffold performed a dual release pattern of incorporated molecules, by releasing VEGF to help angiogenesis and then slowly release PDGF-BB, which improved epithelium regeneration, collagen deposition and functional tissue remodeling. Macroscopic examination on the improved wounds at different time intervals are represented in Figure 4, where granulation and regenerated epidermis were reported with nanofibers with nanoparticles loaded with VEGF and PDGF-BB. Overall, the nanofibers with dual growth factors had shown to produce faster healing with more cell and hair

growth at the site of injury. While compared with marketed antibacterial wound dressing, Hydrofera Blue® had shown slow absorption of the materials and thereby showed slowest healing rate (Figure 4) [95]. In another study, Jin and team incorporated multiple epidermal induction factors (EIF) and performed the electrospinning by two different approaches: blend and core-shell electrospinning. The results showed that the core-shell nanofibers achieved a sustained release profile of the molecules without any burst release. With the sustained release of EIF from the core-shell nanofiber, the percentage of epidermally differentiated adipose-derived stem cells on core-shell nanofibers was significantly higher than that on the blend nanofiber. The authors concluded that the core-shell design for nanofibers serves as an ideal EIF delivery vehicle for wound healing and skin reconstitution [93].

Figure 4

Recent research had proposed the incorporation of hemoderivatives in the treatment of chronic wounds for rapid recovery. Particularly, the lysate of platelets is known to contain the pool of biomacromolecules responsible in the process of tissue regeneration. The biomacromolecules present in the lysate are mainly growth factors, platelet-derived epidermal growth factor, EGF, PDGF, VEGF, transforming growth factor-β (TGF-β), insulin-like growth factor and fibroblast growth factor along with the cytokines, TNF-α and IL-8. The role of these components of platelet lysate is eminent in wound healing, known to accelerate proliferation of fibroblasts and migration of keratinocyte [96–98]. Approached had been made to deliver this platelet lysate using the electrospun nanofibers. A recent report by Cordenonsi et al developed nanofibrous electrospun scaffolds to encapsulate platelet lysate successfully for the purpose of wound healing [98]. The researchers involved pullulan and sodium alginate to develop the scaffold. They developed the electrospun nanofiber and later modified it with the coating of pullulan. Loading of lysate did not alter the nanofiber matrix conformation, however, during crosslinking, it was

found to develop less sharp nanofibers. The authors described this because of the hydrophilicity of the proteins. Alternatively, the coated nanofiber depicted an increase in diameter. Changes in the conformation did not affect adhesion of fibroblasts and its proliferation. It has been concluded that the presence of platelet lysate would facilitate orientation of fibroblasts to myofibroblasts towards healing of wound [98]. In a similar approach to encapsulate platelet lysate within the electrospun nanofiber was reported by Saporito and team, where the authors developed the nanofiber patches using gelatin and chondroitin sulfate, however, their purpose of the application was recovery of the heart following congenital heart defect surgery [99].

The specific property of silk protein has been recently introduced in the faster healing of wounds. Thus, PVA and chitosan were introduced in the development of eletrspun nanofiber to deliver silk protein sericin together with tetracycline. Biocompatibility and antibacterial property of the formulation were found to be contributed by the presence of silk protein, which further showed rapid re-epithelialization, collagen deposition and wound healing when compared to nanofibers without sericin [100]. On the other hand, collagen, the main structural protein of ECM, and chitosan were incorporated into the nanofibrous mat containing silk fibroin and PCL. Excellent biocompatibility with antibacterial potential showed to accelerate healing of the wound with mitigation of scar formation through transforming growth factor-β signaling pathways [101]. Therefore, numerous studies are in progress to develop an ideal wound dressing from which the loaded bioactive molecules will be released at a controlled rate to heal the wound effectively to replace the traditional inert dressings. The versatility of polymers and development techniques of nanofiber scaffolds showed the possibilities of deeper insight into the delivery mechanism and improved performance in the healing of wound.

4. Multilayer electrospun nanofiber

Multilayered electrospun nanofibers are referred to be composed of different layers, where each layer can be separately fabricated with special characteristics as desired to obtain a superior delivery system. Incorporation of functionalized nanocarriers is generally done on the top or mid-layer which provides superiority of controlling wound healing by eliminating contaminants from the wound area, such as bacteria, toxins, viruses, dyes and or metal ions [102,103].

4.1 Layer-by-layer (LbL) assembly technique

Basically, LbL self-assembly is an advanced method of alternating the adsorption of materials onto a surface using complementary interactions, where one layer of material is layered at a time [104]. This method had been extensively accepted by the researchers and thus, applied in the manufacture of multilayer polymer membranes since the study by Decher et al. in 1991 [105]. The researchers reported that a polyelectrolyte consisting of opposite charges could be deposited alternately via electrostatic interaction, thereby producing a multilayered film. This LbL technology had been advanced from two-dimensional to three-dimensional, thereby research and application of this technology is greatly expanded [106]. Multilayer membrane can be bilayer or more than two layers, and the drug release from the membrane can be tailored. For instance, several researchers have studied the fabrication and evaluation of multilayer nanofiber structure consisting of a middle layer that incorporates the active ingredient and covered by different nanofibers layers. They found that the initial burst release is reduced and slower release is achieved with thicker covering layer [107]. This technique is remarkable for its easy synthesis, versatility of substrate, and flexibility of coating layer composition.

In developing artificial tissue scaffolds, the recent efforts are focused on a system that had better mimic the native skin's ECM. Multi-layered scaffold structures exhibit the complex behaviour of ECM thus making it a promising form for tissue regeneration, by including different attracted biomaterials in each layer [108]. This 3D multilayer configuration reduces cell spreading while

enhances the expression of differentiated cell function. The advantage of LbL multilayer electrospinning method is that the fiber diameter, composition, and porosity of each electrospun fiber layer and the total number of layers are tunable [109]. Also, surface modification of the fiber can be performed by using various solution composition, pH, dipping duration and ionic strength or the polyelectrolyte itself (Figure 5) [110]. LbL allows high levels of biocompatibility both *in vitro* and *in vivo* for biomedical applications. It can be used to modify the surface topography and load various biomolecules [111]. LbL approach offers flexibility in material selection and also in structural design [112]. A wide range of materials, including biological substances (e.g. proteins, nucleic acids, saccharides, and virus particles), organic polymers, and inorganic substances can be used in the LbL assembly. Thus, multi-layered thin films can be designed with tailored structures and properties, makes LbL beneficial in biomaterial field. In addition, the ability to incorporate drugs in separate sets of layers of the system is a promising strategy for a sequential controlled delivery system [111]. This can be particularly suitable to the demands on fabrication of drug delivery materials that require complicated design in their components and structures. Moreover, the assembling procedure of LbL can be performed in a mild aqueous medium where the bioactive agent adsorbed using hydrogen bonding, electerostatic, van der Waals or hydrophobic interaction, thus eliminating the use of chemically harsh conditions [113]. The equipment required for the LbL procedure also is relatively simple and inexpensive when compared to the other methods of fabrication, such as self-assembly, nanolithography, gas jet, melt fibrillation, etc., which enables the LbL rapid prototyping and scale-up for commercialization [114].

Figure 5

Alternatively, the incorporation of composite materials is found to be more effective and attractive than a single material. Successful application of composite materials in the preparation of nanofibers is increasing day-by-day, where a combination of two or more materials are

incorporated, that often have very different properties, giving the final material a unique and desired properties [115]. Generally, a composite material is consisting of three different components, the matrix which acts as the continuous phase, reinforcement, which are fibers and particles, acts as discontinuous phase and the fine interphase region [115,116]. However, increasing the volume of the polymer solution and the spinning time, a three-dimensional fibrous structure with mat thickness from tens to hundreds of microns can be achieved [112]. However, a three-dimensional structure is also possible by coating a layer of nanofibers on another electrospun fiber layer. In multilayer electrospinning, initially, a polymer will be electrospun to produce a single layer of the desired polymer. Then, sequentially the other polymer layers will be collected on the same target collector. Thus, a multilayered nanofibrous mesh in an ordered structure consisting of varying polymer layers is obtained [49]. Different layer properties such as dense layer, cellular layer, and porous layer also can be formed by utilizing different solutions and processing parameters. In the LbL assembly process, multilayer films are deposited onto the surface of the substrate via alternate adsorption of the interacting materials. Various deposition materials such as polymer, metal ions, and particles, can be used to fabricate functional LbL structured composite films. The interaction of the materials is via electrostatic interactions, hydrogen bonds, covalent bonds, metal-ligand complexation, hydrophobic interaction and bio-specific interactions. As these properties allow the controlled release of the drug, thus this technique can be considered as suitable for preparing nano-multilayer films loaded with therapeutic agents [117].

4.2 Polymers in LbL nanofibers

The increased popularity of the electrospun nanofibers with the role of delivering drugs on healing wounds effectively provides the platform for ideal dressing with the versatility of polymers and fabrication strategies. Apart from preventing physical damage to the applied wound, these polymers also serve to entrap high loading of the therapeutics (up to 40%) with

controlled release [92]. These polymers are from natural as well as from synthetic sources. The hydrophilic properties of the polymers favours delivery of proteins, peptides and or small molecules for a burst release, whereas hydrophobic polymers provide controlled release. Use of natural polymers possesses benefits of accessibility, biodegradability and biocompatibility [118], however, most of the time these polymers limit electrospinning (e.g., chitosan) [119]. Thus to avoid limitations, pairing techniques could be employed where the electrospinnability could be improved [63,65,66,69,100]. Other examples of popular polymers are hyaluronic acid, collagen, cellulose, gelatin, alginate, keratin, zein, silk fibroin, which were used by various researchers and the outcomes are described in different sections of this article. Alternatively, the synthetic polymers possess characteristics of biodegradability and biocompatibility, where few are hydrophilic and others are hydrophobic. For example, PCL is a hydrophobic polymer and incorporation with poly(ethylene oxide) (PEO) improves surface property [120], similarly, a combination of PCL with PVA has also been explored in the development of nanofibers with superior quality [47]. Alternatively, a combination of PCL and PLGA had been explored for their prolonged half-life [92]. It has been established that the combination of polymers is always proving improved properties of the developed nanofiber to show controlled release of therapeutics. Present researches are incorporating different polymers for different layers of LbL nanofibers. For example, a recent report by Nada and team revealed the development of multifunctional nanoweb [121]. Different therapeutic agents were incorporated into different layers of the LbL naofiber, where diclofenac was incorporated into the adhesive layer. The contact layer was loaded with chitosan iodoacetamide, an antibleeding agent, which was fabricated with PVA. Capsaicin was loaded into the middle layer, which was fabricated with gelatin and crosslinked using glyoxal. The third layer was composed of PVA containing gentamicin, the antimicrobial agent. Overall, this LbL was reported to be cytocompatible and accelerate healing of wound [121]. Therefore, it can be said that there are large numbers of possibilities to fabricate an ideal LbL-

based wound dressing using the polymers towards healing of chronic wounds faster.

4.3 Controlled drug delivery by LbL electrospinning

Local drug delivery is an effective way to accelerate wound healing with minimal side effects [61]. As a better clinical application is needed, a high control of the drug release rate has been demanded in recent years. For instance, biphasic delivery system is a controlled drug delivery system, which contains a fast release that immediately produces the therapeutic effect of the incorporated drug. This is advantageous due to the sustained release of the drug, which correspondingly will avoid the repeated administration. In the case of wound dressing, sustained-release avoids frequent changing of dressings. This biphasic delivery system is possibly achieved by multilayered nanofiber mats, produced by electrospinning of different polymers sequentially [38]. Nanofiber mats capable of controlled release in the wound site, avoiding any high systemic drug level, which may have adverse side effects, as in nanoparticles delivery system [122]. The drug release rate from a nanofiber mat can be adjusted through several ways; selection of suitable polymer, which affects the degradation rate of the nanofiber; through the drug placement (i.e. within or on the surface of fibers); and through adjusting morphological properties, such as the fiber diameter and mesh thickness. In addition, the drug release profile also can be modified by designing the order of the different nanofiber mesh. Multilayer structured nanofibers are able to extend the diffusional path between the drug and the dissolution medium, providing better control in release. Hence, multilayered electrospun nanofiber mesh results in sustained drug release for a prolonged period of time as the drug mobility is controlled. As example, by layering the drug-loaded nanofiber mat between other two-electrospun nanofiber mats, the kinetics of water uptake can be controlled. In short, by employing sequential electrospinning of different polymers that produced multilayered nanofiber mats, a biphasic drug release system can be designed [38] which involve both immediate release and sustained release of the drug.

In LbL assembly, polymers exhibiting opposite charges can be deposited alternately to formulate polyelectrolyte multilayers (PEMs) or free-standing film. Combining the high surface area of electrospun fibers with polyelectrolyte multilayer structures is another innovation of drug incorporation and delivery [45]. PEMs preparation, for instance, is shown to be a suitable form to load nanoparticles [110]. The method involves the deposition of charged substrate; polyanions and polycations alternately which are driven by an electrostatic force, resulting in LbL self-assembled multilayer coating [123]. The depositing process can be repeated until the desired thickness of the multilayer is achieved [59]. The simplicity of synthesis and analysis makes many bioactive agents and chemical drugs have been assembled for topical drug delivery applications mainly on planar substrates such as silicon wafer, quartz slides, and metal oxide [123]. Charged therapeutic components can be easily incorporated into the multilayer assembly. However, it is not the case for insoluble and uncharged drugs incorporation. Thus, approaches such as encapsulation of those drugs into charged particles and prodrug modification can be the alternative. Polymer/silicate composites are good as deposition materials to form LbL structured films to improve the properties of a template, as they possess properties of both organic and inorganic materials [41,53,124]. For the effective drug incorporation and release by the thin films, few considerations should be noted. These are; potential *in vitro* and *in vivo* toxicity of the therapeutic agents and material, stability of the thin film, precise targeting of drugs, and drug release rate and duration [117].

While electrostatic interactions remain widely used in facilitating the formation of the films, other molecular interactions such as covalent bond, hydrogen bond, and host-guest interaction are now well established for LbL assembly, with a variety of material used as film constituents [125]. Different driving forces could work in the formation of LbL assembly in polymers. The forces are well depicted by Zhang and team, where they had mentioned about the formation of (a) electrostatic forces between the compounds with opposite change, (b) hydrogen bond

between the polymers containing hydroxyl groups, (c) charge transfer between electron acceptor and electron donor in non-ionic compounds, and (d) covalent bonds through sharing of electron pairs between atoms [126–128].

This LbL assembly has been utilized in the biomedical field, including application in wound healing. In a study by Pan and team, produced antimicrobial polymer films by employing the negatively charged soy protein isolate (SPI) and positively charged N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) to be alternately deposited on cellulose acetate (CA) based nanofibrous mats via LbL self-assembly technique. The antimicrobial activity results demonstrated that the proposed formulation of HTCC/SPI LbL-film-coated mats had average diameters of inhibition zones against *E. coli* and *S. aureus* of 9.6mm and 11.53mm, respectively, which are the highest antimicrobial activity among samples in their reported study [129]. Deng *et al*. coated the negatively charged cellulose nanofibers by adsorption with the positively charged chitosan-organic rectorite (CS-OREC) intercalated composite and negatively charged sodium alginate which produced by LBL technique. Their results showed that LBL mats with the addition of OREC increase the inhibition percentage on *E. coli*. The addition of OREC to LBL films made the bilayer thicker with enhanced antimicrobial activity [130]. Another study by Huang *et al*., they produced cellulose acetate nanofibrous mats that were used as a substrate for LbL films composed of positively charged chitosan derivative, HTCC (lysozyme (antibacterial agent)-N-[(2-hydroxy-3-trimethyl-ammonium) propyl]), and negatively charged sodium alginate. From the study, the average diameter of the produced fibers increases with the number of coating bilayers applied, and they are also shown to possess antimicrobial activity [131]. Trinca *et al.* also produced electrospun multilayer scaffolds with appropriate mechanical properties for the treatment of wounds. The membranes were designed as double layers; the first layer composed of PCL or PCL/cellulose acetate blend, which acts as mechanical support, and the second layer consisted of chitosan/PEO blend, which performs the role as primary wound dressing. Figure 6

represented the formation of a double layer of the electrospun bed formed using PCL and chitosan/poly(ethylene oxide), and PCL/cellulose acetate and chitosan/poly(ethylene oxide) (Figure 6a,b). Differences in diameters and layers are clearly depicted in Figure 6 c-e, which represented that this double-layered scaffold could be a suitable tool in the treatment of chronic wounds. Additionally, the scaffold's properties, such as mechanical properties, water and moisture uptake, stability in phosphate-buffered saline, moisture permeation, water contact angle met the necessary criteria of dressings for skin lesions. The scaffold also showed low cytotoxicity to L929 fibroblasts and promoted adequate cell proliferation [33]. In another study, Kimna and the team had also prepared a bilayer membrane of gentamicin-loaded zein with controlled antibiotic release characteristics to manage infection during the wound healing process. Zein film served as top layer functions to provide mechanical strength and prevent bacterial infiltration. While the zein layer is placed as the bottom layer, acting as ECM as it is highly porous and capable of oxygen transfer. From the comparison of drug release profiles between monolayer film and bilayer membrane, it was revealed that the cumulative release is about 83% and 94% for monolayer and bilayer membranes after 48 hours respectively [132].

Figure 6

4.3 Advanced bilayer electrospun dressings: asymmetry electrospun membrane

Recently, electrospun asymmetry membranes are newly investigated as a better strategy to reproduce skin anatomy and further enhancing wound healing [133]. To meet all the requirements for rapid wound healing, asymmetric membranes are present as the ideal candidate [44]. Asymmetry membranes feature most of the characteristics of an ideal wound dressing thus, recognized to be promising treatment of skin wound [134]. Asymmetry membranes are two-layered structures that are capable of mimicking the properties of both skin layers; epidermis and dermis. In general, it displays (a) a dense and waterproof top layer that represents the epidermis, which protects the wound site, avoids fast dehydration of the wound surface and avoids bacteria

penetration, and (b) an interconnected porous bottom layer that represents the dermis. It is a sponge-like hydrophilic layer with high absorption capacity that allows adsorption of fluids and drainage of the wounds completely [44,133,134].

In their study, Miguel *et al.* had developed electrospun asymmetric membrane (EAM) composed of two interconnected layers of blend of silk fibroin (SF) and hydrophobic PCL as top layer, which act as epidermis, with waterproof ability and mechanical resistance properties. While the bottom layer consists of the blend of SF and hydrophilic hyaluronic acid (HA) that act as dermis layer, which provides a high hydration capacity, water-sorption and water retention, and simultaneously allows cell attachment, migration, and proliferation. Furthermore, to provide antimicrobial property to the bottom layer, Thymol (THY) was loaded into the nanofiber formulation. The results showed that the produced EAM exhibited the porosity, wettability, and mechanical properties suitable for the healing process. The SEM images (Figure 7) of the normal human dermal fibroblasts cells seeded on the surface of formulated electrospun membranes showed promotion of cell adhesion, where more protrusions of filopodia with enhanced cellular adhesion and proliferation were reported with SF_hyaluronic acid (SF_HA) and SF_HA_THY layers [65]. Earlier, Morgado *et al.* produced PVA/chitosan loaded ibuprofen asymmetrical membranes for skin wound healing, using the supercritical carbon dioxide ($scCO_2$)-assisted phase inversion method. The produced PVA/CS dressings have high water uptake ability and ability to maintain a moist environment needed for wound healing. The dressing is also shown to allow gaseous exchange, prevent microorganisms penetration, and able to remove excess exudates. In vitro studies revealed that the dressings had excellent biocompatibility and biodegradation properties adequate for skin wound healing [134]. Another study was done by Aragon *et al.,* fabricated PCL/PVAc asymmetric membranes loaded carvacrol. This membrane consists of two layers of electrospun PCL and PVAc, which contain the antimicrobial agent and in contact with the skin. Cell assay showed that cells were adhered, homogeneously spread,

migrated on both polymer layers. Moreover, the fluid handling capacity, mechanical properties, antimicrobial efficacies and safety towards human dermal fibroblasts point to the potential of these asymmetric membranes [44].

Figure 7

5. Conclusion

In summary, electrospun nanofibers are found to be advantageous as wound dressing, especially for chronic wounds, owing to its remarkable features that fulfill the modern ideal wound dressing requirements. In order to make useful applications of nanofiber in the biomedical field, various strategies have been developed to incorporate therapeutic agents into the nanofibers as well as to control the drug release from the fiber matrix. LbL technique is one approach in designing a better wound dressing as it resembles more to the skin's ECM and provides a sustained release.

6. Future remarks

Instead of the advantageous role of electrospun nanofibers, its poor mechanical strength limits its widespread use. This poor strength of the nanofibers is resulted due to weak fiber-fiber connections. Additionally, biofouling is another consequence of limiting its application [135]. The electrospun nanofibers with nanowhiskers or nanoparticles, i.e., the multi-layered electrospun nanofibers had been reported to overcome the limitations of electrospun membranes [136]. With the continuous research on electrospinning methods to fabricate different nanofibers urge its improved productivity and scaled up method for commercial production in the industries, which is a great challenge at this moment. Additionally, judicious selection of the polymers for the formation of desired composite or generation of different layers with desired properties is also another challenge as there are numbers of polymers introduced in the field of formulation research and their biocompatibility is of major concern. Further, the optimization parameters for the developed nanofibers need to be standardised so that reproducibility of the batches could be easily determined during commercialization as well as during research stages. Progressing researches in this field could easily overcome the present limitations to bring these electrospun nanofibers as an unprecedented breakthrough in the application of chronic wounds.

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Figure legends:

Figure 1. Contribution of hematopoietic cells to wound healing [10]

Figure 2. Applications of nanotechnology-based deliveries in chronic wound healing [30].

Figure 3: Schematic representation of electrospun nanofibers preparation for application in wound dressing

Figure 4. Wound healing evaluation in rat wound model. A) Representative macroscopic appearance of wound closure at 0, 1, 2, and 4 weeks after treatment of skin wound of control, 2:1 CS/PEO (nanofibers without nanoparticles/growth factors), 2:1 CS/PEO-NPs (nanofibers with nanoparticles loaded with VEGF and PDGF-BB), and Hydrofera Blue® (antibacterial commercial wound dressing); B) quantitative measurement of wound size reduction (* $p<0.01$) [95]

Figure 5: Electrospinning method for drug incorporation.

Figure 6. SEM micrographs of electrospun double layer scaffolds: (a, c, e) PCL + chitosan/poly(ethylene oxide) blend, (b, d, f) PCL/CA + chitosan/poly(ethylene oxide) blend; (a, b) chitosan layer; (c, d) PCL and PCL/CA micrometric fibers under nanometric chitosan fibers; (e, f) lateral vision of the double layer scaffolds, with a 70° tilt angle [33]

Figure 7. After 1, 3, and 7 days of incubation with the membranes. SEM images of normal human dermal fibroblasts cells seeded at the surface of the different electrospun membranes after 1, 3, and 7 days [133]

Table 1: Different types of wound dressing and their limitations [19,26–28]

Types of dressing	Limitations
Gauze dressing	Non-occlusive, pain on removal, needs frequent change, sheds fibers onto wound as contaminant.
Foam	Not suitable for dry and necrotic wound, causes maceration of peri-wound for highly exuding wound.
Film	Accumulates excessive exudate, painful upon removal, causes maceration, have low mechanical protection.
Hydrogel	Not suitable for heavily exuding wound, causes maceration of peri-wound and infection, needs secondary dressing.
Hydrocolloid	Not suitable for heavily exuding wound, most contain gelatin from pig (not acceptable for vegan/certain religion), requires frequent inspection for chronic wound
Alginates	Not suitable for dry and necrotic wound as it causes trauma when remove, needs secondary dressing, needs daily changing.
Silicone	Expensive, cannot be used on patients with silicone allergy, some type need secondary dressing.
Bioengineered skin substitute	Have short half-life, requires strict storage, high cost.
Growth factors	High cost, have poor cell survival and degrades in harsh wound environment.
Iodine	Contraindicated in hypersensitive patients, pregnant or breastfeeding women, in patients with thyroid disease, not applicable for large wound as maximum single application is 50g only, causes stinging or burning on application, requires secondary dressing.
Silver	Expensive, allergic reaction in certain patients, cannot be used with other antimicrobial, can be used for short period of time.
Honey	Pain on application, high cost and bee sting allergy may happen, needs to be sterile, requires secondary dressing, can causes maceration.

Table 2: Summary of electrospun nanofiber for wound dressing application.

Formulation	Drug/bioactive	Objective	Wound healing assessment	Release profile/outcome	Reference
PCL/gelatin o/w nanofiber mat	Ketoprofen	Preparation of single and binary mats by emulsion electrospinning for controlled release of entrapped therapeutic for improved efficacy.	Adhesion study - PCL/gelatin mat showed higher cell attachment performance (90–95%) when compared to, both tissue culture polystyrene and the single PCL mat. Proliferation study – binary mat three times higher cell growth than TCPS and the single PCL mat, due to improve porosity.	Ketoprofen burst release was significantly delayed by the PCL/gelatin binary mat, which was shoed by the sustained release capability of the drug from the formulation over a period up to 4 days.	[70]
PVA/chitosan nanofiber mats	Tetracycline HCl (TCH)	Development of drug free and drug-loaded bio-polymeric electrospun scaffold containing antibacterial for enhanced wound healing property.	Cell viability and scratch assay – TCH loaded nanofiber mat not cytotoxic and good candidate for wound healing.	Drug showed initial burst release profile for first 2 hours, allows effective antibacterial activity against *E. coli*, *S. epidermidis*, and *S. aureus*.	[71]
Silk fibroin (SF)/PCL asymmetric membrane	Thymol (THY)	Preparation of two-layered structure consist of SF/PCL mimicked epidermis and silk fibroin/HA mimicked dermis layer.	SF/HA and SF/HA/THY layer showed higher adhesion and proliferation.	Porosity, wettability, mechanical properties were reported to be suitable in aiding healing and showed antioxidant and antibacterial properties.	[72]
PU/dextran nanofiber mats	Ciprofloxacin HCl	Preparation of drug free and drug-loaded nanofiber scaffold for superior	Adhesion study - PU/dextran and PU/dextran/CipHCl nanofiber showed more fibroblast cell attachment than PU	Cells were interacted favourably with drug-loaded scaffold, good antibacterial activity against gram-	[73]

Formulation	Drug/bioactive	Objective	Wound healing assessment	Release profile/outcome	Reference
		antibacterial potential.	nanofiber. Proliferation study - PU/dextran and PU/dextran/CipHCl nanofiber showed significant increased cell growth after day 6 and 9 culture.	negative and gram-positive organisms.	
PVA/chitosan/starch nanofiber mats	-	Preparation of nanofibrous mat and to evaluate the mats for antibacterial, cytocompatibility and *in vitro* wound healing assay.	Incorporation of starch in the formulation was demonstrated by the enhanced closure efficiency of the gap and capability of healing as evidenced by the *in vitro* scratch assay. Chitosan also played a role in healing, where higher concentration exhibited superior healing properties. Finally the authors reported a combination of PVA+chitosan+starch at a ratio of 9:1:1 could exhibit excellent wound healing properties.	Formulated nanofiber had suitable cytocompatibility and cell viability. Excellent antibacterial property was evidenced against gram-positive and gram-negative organisms.	[74]
Zein/Ag nanocomposite mats	Silver	Preparation of nanofibrous mat containing antibacterial agent for wound healing purpose.	Proliferation study – increased fibroblast cell growth on day 3 and 6 for both pure zein and zein/Ag nanofiber Adhesion study – fibroblast cell observed to attach well on both mats	Good cytocompatibility and cell attachment on composite nanofiber and had high antibacterial activity against *E. coli* and *S. aureus*.	[75]
PVA/PVAc composite nanofibrous mats	Ciprofloxacin HCl	Development of biomedicated electrospun nanofiber mats for controlled release of incorporated therapeutics.	-	The authors reported the effect of incorporated polymers and the drug-loaded amount on different characterization factors. It was reported to affect initial burst release and	[76]

Formulation	Drug/bioactive	Objective	Wound healing assessment	Release profile/outcome	Reference
				subsequent release rate, weight loss, and affected degree of swelling. The authors suggested the PVA/PVAc blend to obtain controlled release rate for longer period of time.	
PVA/SA composite nanofiber transdermal patch	Ciprofloxacin	Preparation of composite nanofiber containing antibiotic and evaluate *in vivo* wound healing performance.	Wound closure – animal treated with PVA and PVA/SA nanofiber showed faster healing rate. PVA/SA nanofiber showed maximum rate of wound healing of 17 days.	Higher healing rate and higher collagen content demonstrated by drug-loaded patch as compared to unloaded patch.	[58]
PCL nanofiber	Curcumin	Preparation of drug free, drug-loaded nanofiber. And to evaluate antioxidant and anti-inflammatory properties.	Wound closure activity in experimental animals with the curcumin loaded PCL nanofiber revealed superior wound (nearly 80%) by day 10 of the experiment when compared to drug free PCL nanofiber (60% only).	Curcumin loaded nanofiber reduced inflammatory induction and has ability to maintain viability of cell under oxidative stress.	[77]
PVA/SA	ZnO	Preparation of composite nanofibrous mat containing nanoparticle antimicrobial with different concentration.	Adhesion study – cell adhere well on both SA/PVA mats with (0.5% and 1.0%) and without ZnO	Formulated nanofibrous mats showed superior antibacterial activity, which might be explained due to the existence of ZnO in the formulation.	[78]
PVA/chitosan	ZnO	Preparation of nanofibrous mat with and without antimicrobial agent.	Wound closure – wound contraction in rabbit treated with chitosan/PVA/ZnO nanofiber is significantly higher on day 4, as compared to chitosan/PVA nanofiber. Chitosan/PVA/ZnO nanofiber	The formulated chitosan/PVA/ZnO nanofirous structure was showed higher antibacterial prospective against *S. aureus*, *P. aeruginosa*, *B. subtilis* and *E. coli*, when compared to the	[79]

Formulation	Drug/bioactive	Objective	Wound healing assessment	Release profile/outcome	Reference
			achieved 90.5% wound closure on day 12.	animal group treated with chitosan/PVA nanofibrous membranes.	
PCL membranes functionalized Maillard reaction products (MRPs)	Fructose arginine (FA) and glucose-arginine (GA)	Development of PCL membranes functionalized with GA and FA using the MRPs. This was supposed to improve their biological properties and provides control over antimicrobial contamination at the site of application.	Proliferation study - cells proliferated and reached a confluent monolayer faster in PCL_FA than PCL and PCL_GA membranes	Functionalized PCL nanofiber through MRPs with FA and GA resulted in membranes production of superior quality. The nanofiber possessed features to control the wound in a superior way due to its porosity, wettability and mechanical property. These features could help in absorption of wound exudate, supply of wound healing nutrients and gas exchange for faster healing of the wound. The membranes found to exhibit inhibitory properties against growth of *S. aureus* and *P. aeruginosa.* Finally, this formulation was also established to be biocompatible.	[80]
CS/PEO/AgNPs, nanofiber mats	Silver nanoparticle, *F. vulgaris*	Preparation of AgNP containing CS/PEO nanofiber mats where herbal extract from *F. vulgaris* was used as a green reducing agent. The herbal extract produced antimicrobial nanofibrous	-	Superior antibacterial efficacy of the CS/PEO mats containing AgNPs was reported. A 100% control on *S. aureus* and *E. coli*. Growth was obtained with 0.25% and 0.50% bioactive *F. vulgaris*-AgNPs. There was a sharp increase in silver ion release was reported from the nanofiber	[60]

Formulation	Drug/bioactive	Objective	Wound healing assessment	Release profile/outcome	Reference
		mats was prepared for the purpose of wound dressing applications.		mats within first 8 hours. This rise was recorded in mats containing 0.25% and 0.50% AgNPs, which were shown to release silver ion slowly.	
CS/PVA asymmetrical membrane	Ibuprofen	Preparation of wound dressing using asymmetrical membrane PVA/CS. This dressing was prepared by a novel non-residue technology; using the supercritical carbon-dioxide ($scCO_2$) assisted phase inversion method.	Important steps in wound healing, cell adhesion and proliferation were reported in the experimental cells when treated with PVA/CS. Additionally, the asymmetric membrane showed biocompatibility.	The top thin layer of nanofiber membranes (around 15 mm) was showed to allow gaseous exchange easily. Further, this layer was reported to provide a barrier for the invading microorganisms. It also provided a sponge bottom layer, which could be able to eliminate excess exudates coming out from the wound.	[81]
CS/PVA	-	Preparation of CS/PVA nanofibres with different proportions the polymers and the electrospinning parameters for wound dressing purpose.	Wound closure – significant reduction in length of epidermis gap and dermis area were observed on 14^{th} day of treatment of the treated group as compared to the normal and diabetic control groups.	PVA/Chitosan nanofiber wound dressings showed high moisture vapour transmission rate and good antimicrobial activity. PCNWD substrate did not show cytotoxicity effects and had excellent odour absorbing capability	[82]

PVA: polyvinyl alcohol, o/w: oil in water, ZnO: Zinc oxide, HCl: hydrochloride, HA: hyaluronic acid, SA: Sodium alginate, PEO: polyethylene oxide, Ag: silver, PVAc: polyvinyl acetate, SF: silk fibroin, PU: polyurethane, PCL: polycaprolactone, CS: chitosan