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# Current Strategies to Produce 3D Electrospun-Based Wound Dressings for Skin Regeneration

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**ABSTRACT**

Tissue engineering-based wound dressings are a promising treatment approach that mimics the native skin microenvironment to promote effective healing and tissue regeneration. In fact, those advanced wound dressings can be made by electrospinning which has revolutionized the field of wound healing by developing biostructures that closely mimic the skin's extracellular matrix (ECM). To date, most electrospinning research has focused on composition and materials rather than exploring advanced deposition strategies. Conventional electrospinning using random, aligned fibers, co-electrospinning, core-shell approaches, or redesigned collectors has achieved significant results in wound healing, which are discussed in this article. However, these approaches lack the development of realistic 3D structures. To achieve that, advancing approaches through the combination of electrospinning with different techniques such as gas foaming, short nanofiber assembly, or 3D printing significantly enhance skin regeneration of deep wounds (full-thickness and chronic wounds) compared to 2D structures. This review highlights the latest developments, design principles, and recent breakthroughs in electrospinning structures for skin regeneration and provides a fresh perspective for upcoming research in the field of 3D electrospun-based structures.

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Electrospinning; 3D structures; wound healing; Full-Thickness wound; chronic wound

## 1. Introduction

Wound management poses a significant challenge for healthcare systems globally.[1] Wounds can vary from acute superficial injuries such as surgical wounds, to challenging-to-treat wounds, like chronic wounds, burns, and full-thickness wounds which require different medical treatments.[1]

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These wounds exhibit distinct characteristics in terms of etiology, depth, and healing processes. Acute wounds undergo orchestrated biological events leading to the restoration of skin integrity, while chronic wounds persist without progressing through normal healing stages within a reasonable timeframe, typically exceeding 3–4 weeks.[2]

However, full-thickness wounds are injuries that extend through all layers of the skin, including the epidermis, dermis, and subcutaneous tissue and can be acute or chronic depending on the healing duration.[3] The damage to overall skin layers makes the healing process more complex, which encompasses not only skin layers but also blood vessels, nerves, and connective tissue.[1–3] The rising prevalence of bacterial skin diseases underscores the urgency of effective wound management, combined by factors such as bacterial resistance to antibiotics and the increasing incidence of predisposing conditions like aging, obesity, diabetes, and immunodeficiency.[4,5]

The management of full-thickness wounds often requires hospitalization, additional surgeries, nursing care, systemic antibiotics, and the use of complex secondary dressings, resulting in a considerable socioeconomic burden.[5–7] Prioritizing dressings that minimize the need for frequent cleansing and debridement is paramount for clinical efficacy.[7] Despite advancements in skin grafting therapies and cell suspensions, these approaches remain costly, reliant on donor availability, and require specialized expertise.[5,6]

Therefore, the use of wound dressings and local medicaments remains the standard clinical procedure of wound care due to their accessibility and effectiveness. However, addressing the diverse needs of complex wounds requires advanced properties in dressings, such as mimicking the extracellular matrix (ECM) of native skin, anti-inflammatory capabilities, angiogenic potential, and the capacity for biomolecule incorporation to facilitate wound healing and minimize scar formation.[8,9] The demand for innovative advanced wound dressings is corroborated by the advanced wound care market projections that will reach USD 24.8 billion by 2024.[10]

Among biofabrication techniques, electrospinning emerges as a promising approach to produce biostructures that mimic the ECM characteristics, particularly through the fabrication of randomly-oriented nanofiber with diameters ranging from 10 to 300 nm.[11] Electrospun meshes are characterized by high surface area-to-volume ratio, high porosity (60–90%), and interconnectivity, crucial for supporting new tissue growth, promoting exudate absorption, and maintaining a moist wound bed.[12] Furthermore, electrospinning is a versatile technique to produce fibers from nano to micro scale, use different polymers (from natural to synthetic), and tailor the structures' properties ranging the parameters (solution, ambient, and processing). However, the widespread adoption of electrospun wound dressings is limited due to the common 2D structure that limits their application to the superficial wounds. To address this limitation, hybrid approaches combining electrospinning with various techniques, such as different electrospinning variants(e.g., melt electrospinning, wet electrospinning, adding sacrificial substances, cryogenic electrospinning, or using diverse-shape collectors), additive manufacturing(e.g., extrusion), or conventional techniques (e.g., freeze drying), are required to achieve three dimensional (3D) electrospun-based structures.[13] This review aims to provide a comprehensive summary of available electrospun-based strategies for producing 3D advanced wound dressings capable of

promoting wound healing. It covers both conventional electrospinning approaches and their limitations in treating full-thickness, deep, and chronic wounds. Additionally, this document explores the innovative techniques to obtain hybrid structures with 3D architectures by combining electrospinning with freeze-drying, expansion gases or 3D printing among others. Furthermore, the studies discussed about 3D electrospun-based structures highlighted the potential to act as one-single-use advanced wound dressing to promote *in situ* regeneration as mimic the 3D environment of natural tissue. The demonstrated approaches enhanced the wound healing process by improving cell infiltration, hemostatic capacity, nutrient diffusion, and overall tissue regeneration. The future direction of electrospinning is emphasized, focusing on scalability, regulatory approval, and the path to clinical application and commercialization of electrospun wound dressings for skin regeneration. These advancements pave the way for more effective and widely applicable treatments in wound care.

## 2. Skin pathophysiology and wound healing

The skin is the body's largest organ, covering an area of approximately 2 $m^2$ composed of epidermis, dermis, and hypodermis layers.[14] Skin comprises important functions, including: (i) protecting the internal tissues against chemical and physical hazards, (ii) regulating body temperature, (iii) providing sensory, and (iv) synthesizing vitamin D.[15,16] However, several injuries such as trauma, accidental lacerations, surgeries and burns can compromise skin integrity, leading to wounds that often require medical intervention.[7,17–19] While many wounds heal naturally through a cascade of events - haemostasis, inflammation, proliferation, remodeling – others, such as full-thickness wounds (second and third-degree burns) and diabetic ulcers or vascular ulcers, frequently disrupt this process due to chronic inflammation, infection, and oxidative stress, creating significant challenges to treatment[20–23] (Figure 1). Additionally, in full-thickness wounds, the re-epithelialization during the proliferation stage relies on secondary contracture. The lack of residual cells for regeneration, results that tissue repair begins with the formation of granulation tissue, followed by epithelialization over this new tissue.[24] Current interventions, such as autologous skin grafts or cell-based skin substitutes address only part of the challenge. However these approaches have limitations: (i) donor site create additional wounds, (ii) insufficient donor sites for large-area wounds (e.g burn wounds), (iii) patients may experience prolonged hospital stays, (iv) costly.[25,26] Furthermore, skin grafting surgery is normally associated with a higher risk of surgical site infections, and in the case of split-thickness skin grafts (contains the epidermis and a portion of the dermis) the re-epithelization is more prone to secondary contracture.[27,28] Secondary healing leads to scar tissue formation (hypertrophic scars and keloids), without fully restoring the skin's anatomical and structural functions.[14,29]

Therefore, there is a strong need for an efficient multifunctional therapeutic that enables wound repair with functional tissue restoration and decreases scar formation while avoiding infection, and brings relief and quality of life to the patient. Based on this, ongoing research pursues possible therapeutic paths for wound healing.

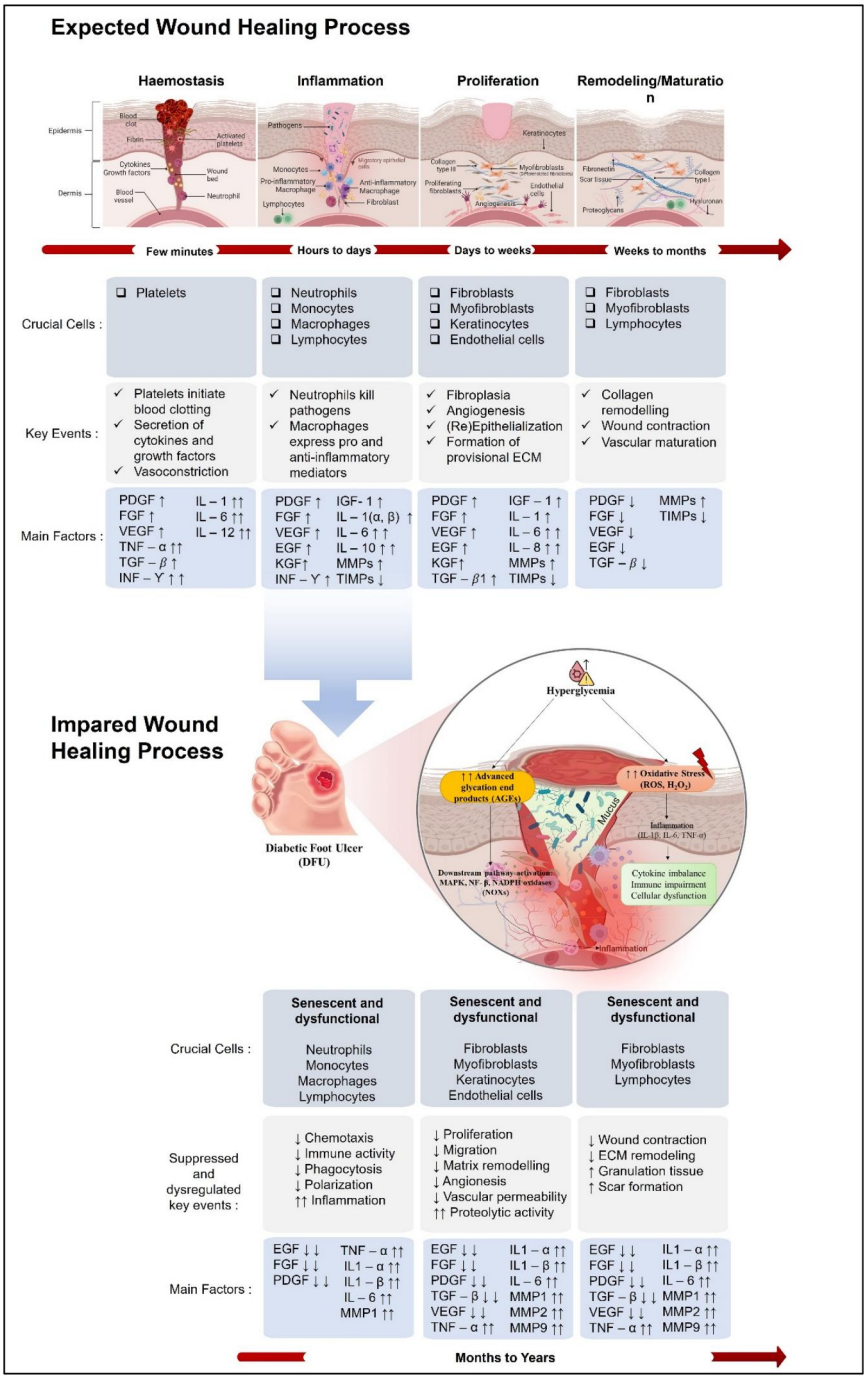


**Figure 1.** Phases of the wound healing process with the involved key events and cells indicated, and the differences in chronic wounds/full-thickness wounds. PDGF: Platelet-derived growth factor; FGF: Fibroblast Growth Factor; VEGF: Vascular endothelial growth factor; TGF: Transforming Growth Factor; INF: Interferon gamma; IL: Interleukin; EGF: Epidermal growth factor; KGF: Keratinocyte growth factor; MMPs: Matrix metalloproteinases; TIMPs: Tissue inhibitors of metalloproteinases. ↑ Increase, ↑ ↑ Highly Increase, ↓ Decrease. Images created in BioRender.

### 2.1. Full-thickness wound management: from traditional to innovative solutions

Wound management has improved significantly over the past years, yet an ideal solution combining bioactivity and structural support remains indefinite.[5,6,20,25,30–33] Traditional methods like gauze and bandage dressings or sutures, provide basic protection but cannot actively stimulate healing, especially in severe wounds.[34,35] Skin glue and stitches offer precise wound closure but are insufficient for deeper wounds, often requiring primary closure through grafting.[35,36] To surpass conventional skin graft limitations, bioengineered substitutes were developed, combining scaffolds with diverse cell sources (autologous, allogeneic, or xenogeneic) to promote wound closure and replace skin functions.[5,6,27] However, challenges persist, ranging from storage demands and complexity to limited scalability and potential infection risks associated with animal-derived products.[5,6,25] Current, commercially available options, including hydrogel dressings, alginates, hydrocolloids, and foams address specific wound needs through moisture retention, bacterial protection, and autolytic debridement, whereas bioactive dressings with honey, silver ions, or hyaluronic acid enhance healing and combat infections.[20,30,31,34,37–39] While effective, they lack the structural complexity necessary for large-scale tissue repair. Thus, the field remains divided between solutions prioritizing either structural integrity or bioactivity.

Advanced wound dressings aim to bridge this gap by offering biophysical (bulk properties, structure, topography, and degradation) and biochemical (release of bioactive molecules such as proteins, growth factors, drugs, etc.) stimuli to turn the materials attractive to remain native cells.[32,33] Electrospinning has emerged as a powerful technique enabling the production of advanced wound dressings as skin substitutes to repair, maintain or reestablish tissue function. This technique is more affordable and simple than other biofabrication strategies like extrusion, bioprinting or photolithography, which deposit materials and/or cells (bioinks) or use high-resolution light patterns, respectively.[16,40,41] Furthermore, this approach extends shelf lifetime, and accelerates the market entry compared to cell-based solutions, due to fewer regulatory hurdles.[32,33]

In the following section, we will focus on electrospinning, as an innovative and simple strategy for produced advanced wound dressings.

## 3. Electrospun-based wound dressings

Electrospinning wound dressings continue to explore novel approaches to reestablish tissue structure, functionality, and esthetics rather than merely protect the wound. This technique uses a high voltage to stretch a polymeric solution.[42] During the process, the solvent gradually evaporates, and non-woven fibers are deposited on the collector.[42,43] As a result, electrospinning is recognized among biofabrication techniques for accurately mimicking ECM, with fibers ranging from nanometers to micrometers, providing a roadmap to cells adhere and proliferate.[11,44,45] Electrospun dressings possess key features for wound management, such as high porosity and a large surface area, which help regulate moisture, oxygen exchange, and nutrient supply while acting as barriers against microbial invasion.[37] Simultaneously, the intrinsic properties of electrospun fibers made them an efficient drug delivery system (DDS) to be used in

wound healing, since they provide a large contact area improving drug dissolution and delivery.[11,45] However, managing complex wounds like full-thickness and chronic wounds requires solutions that go beyond the capabilities of traditional 2D meshes. To address these challenges, skin tissue engineering is shifting toward the development of 3D electrospun structures that better replicate the structural and functional complexity of native skin.

The following sections explore the role of 2D electrospun meshes in wound healing, their limitations, and how advancements in 3D electrospinning are pushing the boundaries of skin regeneration.

### 3.1. Two-dimensional electrospinning approaches

A vast number of 2D electrospun structures with different morphologies and orientations can be achieved by simply modifying the collector, the solution content, and the spinneret (Figure 2).

In wound healing applications, random orientation of electrospun fibers, achieved by the traditional electrospinning setup, is preferred, since besides mimicking ECM, can trap platelets and growth factors to promote clotting.[46] Alternatively, aligned electrospun fibers (uniaxially or radially), produced by a drum collector, a rotating disk, or through the external manipulation of an electric field, are broadly explored since induce a rapid proliferation of the cells along the fiber axis.[47,48] The fiber alignment extension could be adjusted depending on the collectors' velocity, promoting a better cell ordering which allows for achieving faster wound closure.[49,50] Radially electrospun fibers, achieved through a metal ring with a central point electrode, provide a topographic cue to guide and accelerate cell migration along the radial direction, promoting primary wound closure.[51,52] Patterned collectors also can be used to modulate the fiber geometry, varying from a triangular prism to multiple interconnected tubes, and honeycomb shapes among others.[53–55] Besides fibers orientation, fiber diameter and porosity are also a crucial parameter that influences cell adhesion and proliferation across the structure.[42,52,56] This parameter can be easily tailored by changing high voltage, the distance between the tip and the collector, or optimizing polymers' concentration. Several studies have demonstrated that fibroblasts tend to spread and proliferate fast, and even, induce differentiation to myofibroblasts in nanofibers ranging from $200-450\,\text{nm}$.[57–61] Additionally, besides fibroblasts, the electropsun fiber morphology can also influence macrophage polarization, a critical factor in wound healing.[62] Modulating macrophages from the pro-inflammatory M1 to the pro-regenerative M2 phenotype, as demonstrated in the work of Sadeghi-Soureha *et* al., and Pisani *et* al., where PCL/Gelatin and PLA-PCL meshes, supports anti-inflammatory responses and promotes skin regeneration.[62,63]

Beyond morphology, surface properties like wettability play a vital role in cell attachment.[64] Hydrophilic surfaces, achieved through material selection or surface modifications, can enhance fibroblast proliferation.[65] Natural polymers such as gelatin, collagen, hyaluronic acid, chitosan, and fibrin are biocompatibility and can interact with cells.[66,67] However, natural polymers are prone to hydrolytic degradation, which often requires post-modifications or crosslinking.[68] Combining natural polymers with

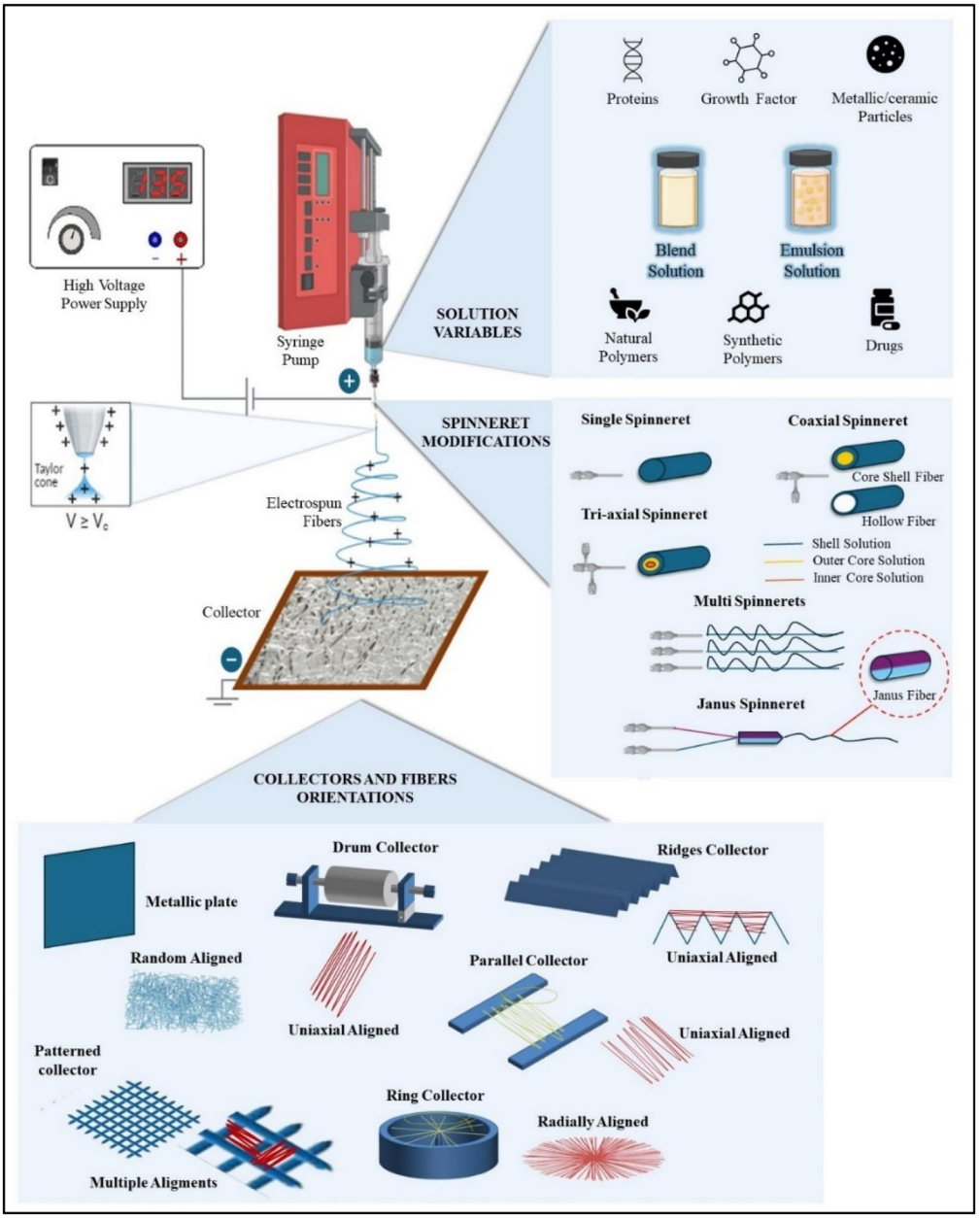


**Figure 2.** Electrospinning apparatus and the possible solution variables, spinneret modifications, and collector adaptions to produce fibers with different orientations. Images created in BioRender.

synthetic polymers, such as Polycaprolactone (PCL), Polylactic acid (PLA) and Poly (lactic-co-glycolic acid) (PLGA) allows them to enhance their performance and reach mechanical properties similar to the native skin (Tensile Strength ∼ 5–30 MPa, Elongation of ∼ 35–155%, and a Young's modulus of ∼ 15–150 MPa).[56,64,69–72]

To stimulate wound healing and prevent infection, electrospinning allows the incorporation of a myriad of compounds, such as drugs, growth factors, metallic/ceramic

particles among others. In this regard, to incorporate drugs or other bioactive molecules into electrospun fibers, methods, such as blend electrospinning, and emulsion electrospinning are used.[73,74] Blend electrospinning blends polymer(s) and solvent or bioactive compounds, while emulsion electrospinning, uses an emulsion containing the aqueous phase (with the bioactive molecule) and the inorganic phase (with the polymer), preserving the structure integrity and bioactivity (e.g., avoiding protein denaturation).[74,75]

Significant progress has been made in developing other spinneret designs besides basic needle spinneret. An alternative approach is coaxial electrospinning, in which the spinneret produces core-shell structured nanofibers through two separated polymer systems.[76] In the wound healing field, this approach is widely used to deliver growth factors, allowing the stabilization and bioactivity of these molecules, while the release is slowly controlled.[77]

Moreover, with the configuration of the spinneret, it is possible to develop multi-material fibers and multifunctional structures, ranging from a hollow core to a triaxial or quad-axial structure.[76,78] Alternatively, co-electrospinning enables the production of multilayer (layer-by-layer, LbL) structures using different syringe pumps simultaneously, combining the desired properties of each polymer. This methodology is widely used to incorporate sacrificial components, as observed in the work of Türker *et al.*,[61] in which hybrid electrospun meshes were fabricated simultaneously with Poly(L-lactide-co-ε-caprolactone) (PLLCL) and Collagen (Col) and sacrificing agent Polyvinylpyrrolidone (PVP). This work aimed to produce a structure that mimics the native structure and microenvironment of skin's ECM, without using toxic solvents, commonly used in the electrospinning of collagen.[61]

2D electrospun-based wound dressings have demonstrated promising results in skin regeneration.[61,79] However, due to the production process and parameters (time and flow-rate), the obtained structures with conventional electrospinning are flat 2D meshes. The fibers are deposited on top of each other, in a limited-size collector by grounded charge. For long production periods, the upper layers start compressing the bottom layers, creating a structure with closely spaced fiber layers, which can limit cell infiltration since the pore sizes can be smaller than the cell size.[13,80] The optimum pore sizes for many cell tissues are in the range 100–500 µm, which are greater than the pores exhibited in density-packed electrospun meshes.[81] As a result, cells can only proliferate on the surface of electrospun meshes, inhibiting cell infiltration into the structure's core. Therefore, the cells will not show filopodia and lamellipodia, which are protrusive extensions that able cells to explore and penetrate electrospun and tissue spaces.[58] Moreover, 2D electrospun meshes offer limited depth for cells to migrate and proliferate in a full-thickness wound. This could hinder the complex formation of multi-tissue structures and cell-to-cell interactions required in the wound healing process.[82] Therefore, to overcome the drawbacks associated with the lack of 3D structure and cellular infiltration, electrospinning techniques have been adapted/combined to produce advanced 3D structures. The production of 3D structures can be achieved by the modification of the collector devices, or by combining different fiber orientations, as well as different processing approaches such as wet electrospinning, adding sacrificial substances, and cryogenic electrospinning and through the combination with other processing techniques.[13,83]

### 3.2. Three-dimensional electrospun-based approaches

3D electrospun-based structures have been proposed as advanced wound dressings in the treatment of full-thickness wounds.[84,85] To achieve the 3D structure several approaches can be used, which can be divided into I) Direct preparation, which involves the adjustment of electrospinning parameters or the introduction of sacrificial agents during the process, usually to increase the porosity, pore size in order to enhance cellular infiltration. While stacking layers and re-designing the collector improves the thickness. II) Post-processing preparation, uses the 2D electrospun meshes combined with other techniques such as salt leaching, gas foaming, freeze-drying and other additive manufacturing processes like 3D printing to create a porous structure with third dimension[52,86,87] (Figure 3).

#### 3.2.1. Sacrificial polymers (porogen)

The use of sacrificial polymers (porogen) increases the structures' porosity through the fast degradation of one of the polymers from the main matrix after exposure to an aqueous medium.[52,86,87] Therefore, commonly used synthetic biodegradable polymers (e.g., Poly(glycolic acid) (PGA), PLGA, and PCL) were electrospun simultaneously with electrospraying of polyethylene oxide (PEO) microparticles to increase the porosity after dissolution of the PEO microparticles.[88] A study performed by Hodge *et al.* [88]

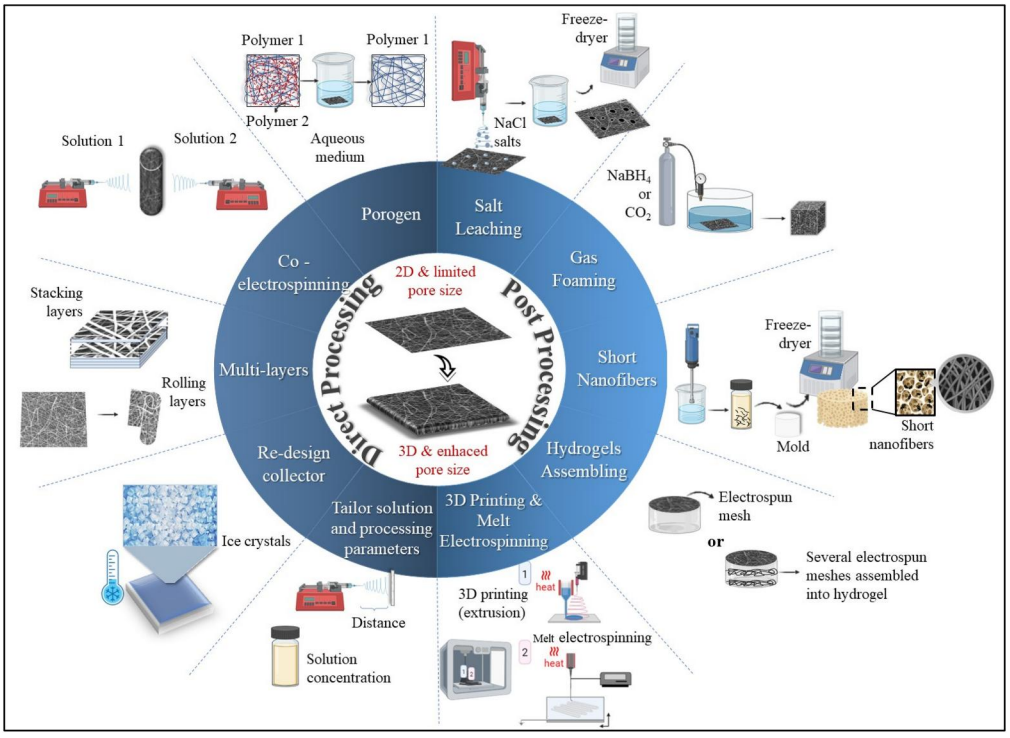


**Figure 3.** Schematic diagram of the current techniques to produce 3D-based electrospun meshes with enhanced pore size. NaCl: sodium chloride; $NaBH_4$: Sodium borohydride; $CO_2$: Carbon dioxide. Image created in BioRender.

demonstrated that after the removal of PEO microparticles cell infiltration around 300–650 µm occurred in PGA, PLGA, and PCL electrospun meshes over 7 days using human dermal fibroblasts.[88] Similarly, besides increasing the porosity, PEO microparticles used as porogen, were shown to enhance electrospun mesh remodeling resulting in greater deposition of collagen and elastin matrix proteins due to higher fibroblast cell infiltration.[89] In the study of Sadeghi-avalshahr *et al.*,[90] PCL and polyvinylpyrrolidone (PVP) electrospun meshes were produced with a final thickness of about 250 µm, followed by the selective removal of PVP. After, collagen/chitosan was grafted through aminolysis on the surface to enhance the cell binding sites besides cell infiltration.[90] The cell proliferation occurred on electrospun PCL meshes from the top to underneath layers of the surface, representing the proper pore size.[90] Another approach of sacrificial agents was used by Gao *et al.*,[91] in which prepared a 3D fiber dressings consisting of a shell layer of PCL/Gelatin (GEL) and an inner layer of iron oxide nanoparticles ($Fe_3O_4$ NP's)/antibiotic (ciprofloxacin hydrochloride) to enhance wound healing. In this work, GEL shell was used as a sacrificial agent during the degradation by collagenase during wound healing, allowing a controllable release of antibiotics in combination with the magnetothermal response.[91] The results showed that 3D fibers structure after GEL degradation promotes stem cell differentiation and accelerates the wound healing by improving re-epithelialization, granulation tissue formation, and collagen deposition. Moreover, *in vivo* healing, in male Kunming mice, revealed that using $Fe_3O_4$ NP's under the control of a magnetic field showed faster healing, which is proportional to the concentration of $Fe_3O_4$ NP's.[91]

### 3.2.2. Multilayering electrospinning

On the other hand, to improve electrospun meshe's thickness, another common approach is through multi-layers also nominated as layer-by-layer (LbL) or sandwich method due to simplicity, the versatility of the layers, and the flexibility of coating layer composition.[92] However, this method requires post-processing steps to stack all layers, which may compromise the process scaling-up. In this sense, Xu *et al.* [93] produced a nanofiber wound dressing prepared through a layered functionality: a hydrophobic PCL top layer with zinc oxide nanoparticles (ZnO NP's) to prevent microbial adhesion, a hydrophilic GEL bottom layer with ciprofloxacin (CIP) for moisture and antibacterial action, and a Janus nanofiber middle layer combining GEL and PCL with CIP and ZnO NP's for infection control. *In vivo* studies on a mouse full-thickness wound model showed enhanced healing rates and promoted angiogenesis, confirming its potential as an effective wound dressing (Figure 4[93]). Similarly, GEL/PVA/chondroitin sulfate (CS) was used to obtain a multilayer 3D nanofibrous (M3DN) structure to be applied in the reconstruction of full-thickness wounds. The layers were attached *via* ethanol solution, followed by a two-stage process with glutaraldehyde to improve the stability of M3DN. Despite the physical shrinkage by ethanol, the mechanical performance was improved compared with the single layers. *In vitro* results show comparable healing properties with commercial dressing, with the advantage that M3DN promotes angiogenesis, considered a potential candidate for full-thickness wounds.[94] Similar to LbL, the electrospun meshes also can be rolled or folded to obtain the desired 3D shape.[48]

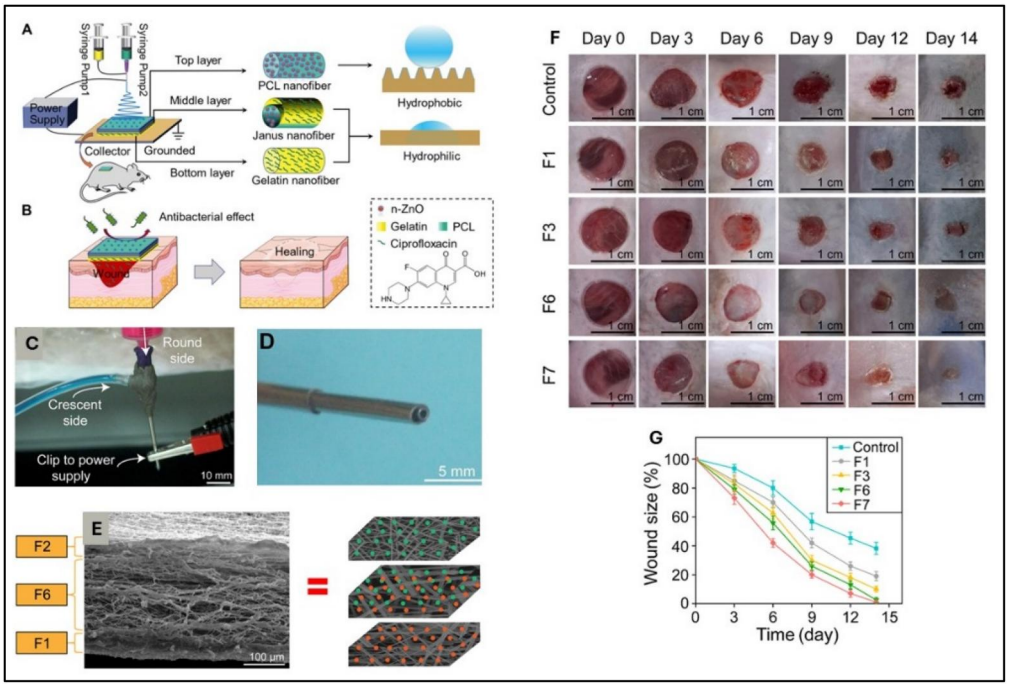


**Figure 4.** Electrospinning process of hierarchical structures to accelerate wound healing. (A) The outer layer is composed of highly hydrophobic polycaprolactone (PCL), the inner layer is composed of highly hydrophilic gelatin, and the middle layer is Janus nanofibers mixed with ciprofloxacin (CIP) and zinc oxide nanoparticles (ZnO NP's). This hierarchical wound dressing can promote full-thickness wound healing in mice. (B) The outer ZnO NP's of the dressing can effectively resist the invasion of *S. aureus* and *E. coli* and cooperate with the function of inner gelatin to absorb wound exudate, which can effectively accelerate wound healing. (C) Modified spinneret. (D) Janus spinneret (E) Scanning electron microscope (SEM) micrograph of the cross-section of the hierarchical mesh structure. *In vivo* evaluation of wound healing in mice. (F) Photographs of wounds treated with gauze (control), F1, F3, F6, and F7 nanofibers on different days. (G) Wound size at different time points. Adapted in part from.[93]

### 3.2.3. Re-designed collector

As depicted in Figure 3, another strategy to the attainment of 3D topographical structures in electrospun materials can be reached by the modification of the collector.[95] Strategies conducive to this objective encompass the utilization of a bath collector (wet electrospinning), cold-plate collector (cryogenic electrospinning), or a customized collector.[96–98] In the first case, solvents like water, mixtures of water/ethanol, hexane, or subcritical $CO_2$ fluid serve to create complex nanofiber architectures.[96] The use of those solvents is an important choice in the process since they present low surface tension, otherwise, solvents with high surface tension will result in 2D-packed nanofibers structures.[96] A study developed by Chen *et al.*,[99] demonstrated that 3D PLGA structure produced by wet electrospinning, the fibrous morphology with a pore structure ranging from 5 to 20 μm facilitated Human dermal fibroblasts (HDFs) infiltration at a depth of 1400 μm and promoted cellular proliferation over the structure, improving chronic wound closure within 14 days.[99] In cryogenic electrospinning, the mesh thickness can increase up to 6 mm due to the accumulation of ice crystals.[97] To achieve a

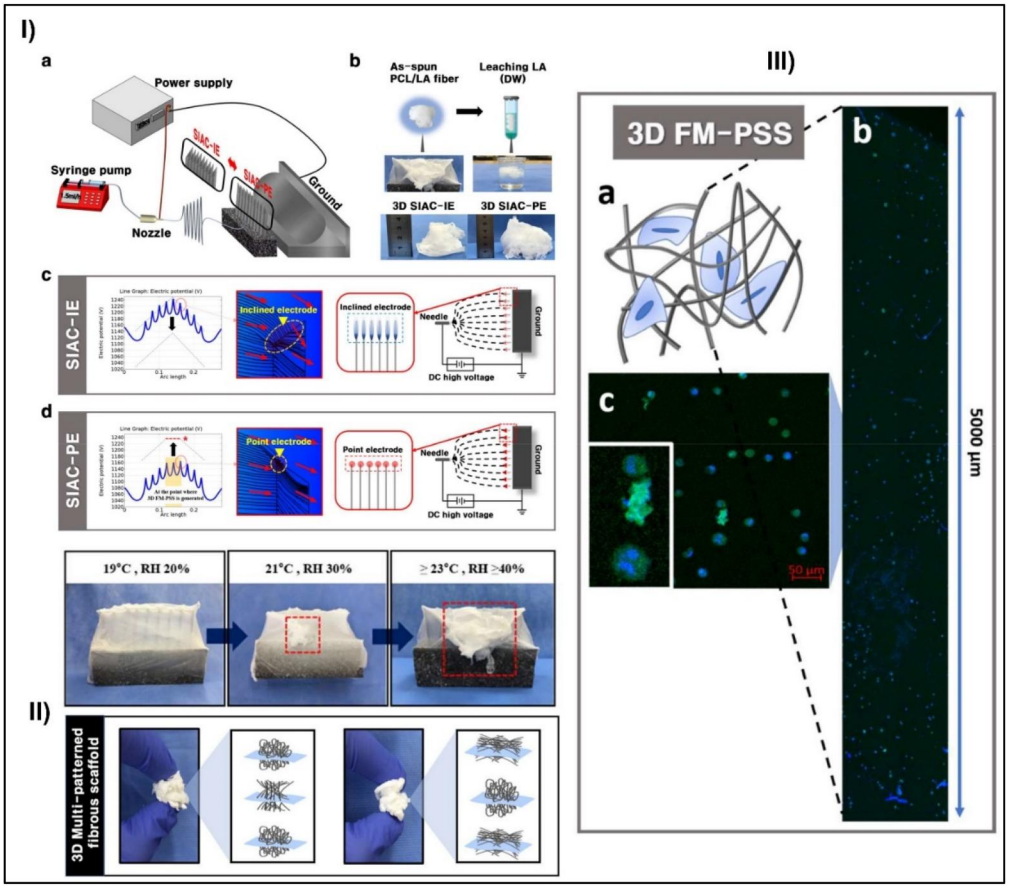


**Figure 5.** (I) Schematic illustration of electrospinning setup. (b) LA leaching process of PCL/LA fibers. Electric field analysis results of (c) sharply inclined array collector with inclined electrode (SIAC-IE) and (d) with pointed electrode (SIAC-PE). (II) Photographs of fibers dependent on RH and temperature (above) and photographs of the 3D cotton-type fibrous structure with customized patterns (bellow). (III) Cells cultured in 3D cotton structure. Z-stack image (b) and confocal microscopy image (c) of the cells on the 3D structure. Images reproduced with permission from.[100]

3D electrospun mesh, researchers developed a new and patterned collector through a directional change of the sharply inclined array collector (Figure 5[100]). Despite the porosities in the study were reduced (17–38%) due to the repulsive forces between the fibers, the porosity increased effectively compared to 2D electrospun meshes and allowed cell infiltration.[100]

A novel collector design was developed by Zhou *et al.* (Figure 6[101]), consisting of two axisymmetric bevels to form a divergence electric field, to produce uniaxially aligned nanofibers of PCL in a LbL approach. In the optimized 3D structure, with 25-mm length collector, the fluorescent cell culture images show aligned nanofibers in which the fibroblasts were stretched along the fiber orientation, as well as are distributed in a 3D space compared to the 2D electrospun meshes. However, fibroblasts were accumulated on the peripheral areas of the 3D scaffold as a result of the higher fiber density in this zone, demonstrating a possible correlation. Nonetheless, the study

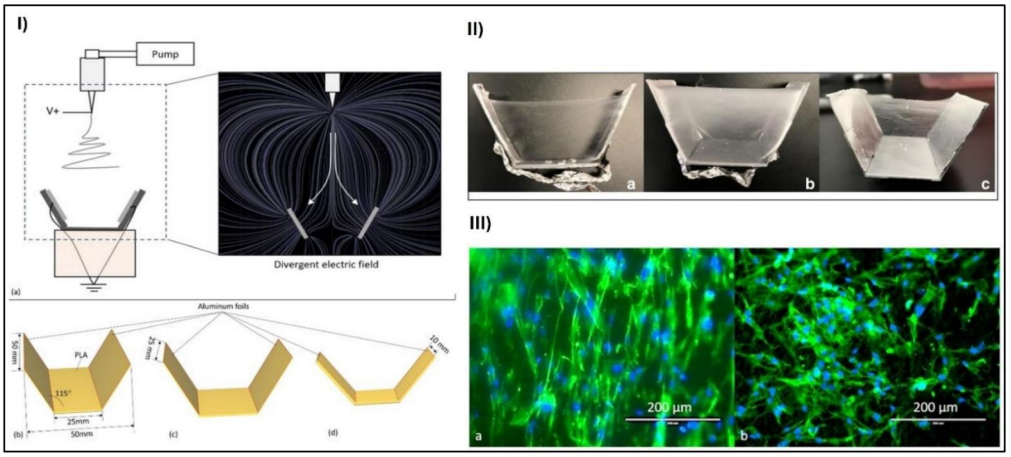


**Figure 6.** (I) Electrospinning set up (a); Illustration of collectors with (b) 50-mm length, (c) 25-mm length, and d 10-mm length. (II) Electrospun nanofiber scaffolds obtained by a 10-mm collector, b 25-mm collector, and c 50-mm collector. (III) Fluorescent images for cells in a 3D scaffold and b a 2D mat. The nuclei were stained blue and the filamentous actins were stained green. Images reproduced with permission from.[101]

highlights an innovative approach to mimic native tissue microstructures and alignment of fibers using electrospinning.[101]

### 3.2.4. Tailor solution and processing parameters

Another way of producing 3D electrospun-based structures was developed by Ghosh *et al.*,[102] by varying PCL and collagen concentration and electrospinning parameters. The authors produced in one-step process a functionally graded structure capable of mimicking the three skin layers to promote full-thickness wounds regeneration. This approach enables the researchers to reach a structure's thickness of $3.0 \pm 0.7$ mm matching the thickness of average human skin ($\sim 3.5 \pm 1.5$ mm).[102]

The shift from conventional techniques to direct processing methods marks a crucial advancement in 3D electrospun mesh fabrication. The application of direct processing techniques has shown significant enhancements in fostering cellular infiltration within electrospun meshes. Although, as derivations of the set-up modifications, direct processing techniques mostly lack control of a real 3D structure and homogeneous distribution of the pores, and thus lacking in biophysical and biomechanical cues.[88,93,99,100,102]

### 3.2.5. Salt-leaching

To achieve significant porosity in the nanofibers, aiming to enhance cells infiltration into the structures, the salt-leaching technique has been employed.[103–105] Normally, sodium chloride salt (NaCl) particulates are used as salt-leaching agents, similar to porogen. Either the salt particles are mixed into the solution to be spun, or they are deposited on the nanofibers during the spinning process through electrospray.[105] However, this procedure often requires the use of post-processing techniques such as freeze-drying to retain the structure. In this context, Ju *et al.*[106] developed 3D

electrospun-based silk fibroin (SF)/polyethylene oxide (PEO) nanofiber using salt-leaching with NaCl crystals for burn applications. Adding NaCl increased the thickness of SF nanomatrix to 2 mm in thickness as well as the porosity (from 4.7 ± 0.7 µm to 11.6 ± 1.8 µm) and pore size (from 53.1 ± 12.7 µm to a size of 164.0 ± 114.9 µm). Biological performance was evaluated in rats as burn model wounds, in which results showed that SF nanomatrix reduces the wound healing period and scar formation, accelerates the re-epithelialization and express anti-inflammatory cytokines, suggesting a promising candidate for burn wound healing.[106] In another work, Juhasz *et al.*[104] used a strategy involving inorganic salts such as calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$) and lithium chloride (LiCl) to synthesize nano and microfibrous cottony 3D structures with electrospinning. The results showed that $CaCl_2$ at 1–3 wt % concentrations yielded the best results, creating a cottony, sponge-like structure, with the highest 3D expansion at 3 w/w %, compared with the other salts. The authors also highlighted that the cation's charge and size significantly influence its interaction with electrospinning solvent to form the 3D structure, but no correlation was found between fiber diameter and salt concentration.[104] However, this study does not present *in vitro* assays to validate the structure performance.

### 3.2.6. Gas foaming

Gas foaming is another technique used to obtain 3D structures from electrospun meshes.[107–109] This technique commonly uses sodium borohydride ($NaBH_4$) to expand 2D electrospun meshes into 3D structures due to the rapid hydrolysis of $NaBH_4$ in methanol solution when in the presence of large amounts $H_2$.[87] Carbon dioxide and subcritical $CO_2$ are other gases that can be applied in this process instead of $NaBH_4$ to surpass the drawbacks of using $NaBH_4$ with materials with weak mechanical properties, that can be destroyed.[110] Hence, 3D polycaprolactone/ε-polylysine modified chitosan (PCL/PCS) nanofibrous structures were obtained by electrospinning and gas foaming process. Then, gelatin, heparin, and vascular endothelial growth factors (VEGFs) were further assembled onto the surface of PCL/PCS fibers by electrostatic adsorption to enhance hydrophilicity. The produced 3D structures were shown to provide an oxygen-rich moisture environment for cell growth (Figure 7[107]). Additionally, 3D structures demonstrated to be more suitable than 2D micro/nanofiber meshes to promote the repair of full-thickness skin defects including wound healing, inhibiting bacteria growth, and inflammation, while promoting hair follicle regeneration, angiogenesis, and collagen deposition.[107]

Similarly, Chen *et al.*,[108] used $NaBH_4$ to expand 2D PCL/gelatin electrospun meshes functionalized with silver-metal organic framework (Ag-MOF) and Curcumin (CUR), whereas Zhang *et al.*,[109] lengthened CS/PVA electrospun meshes into a 3D nanofibrous sponge by gas foaming with 0.1 M $NaBH_4$ for 30 min (Figure 8[109]). In the Chen *et al.*,[108] study, the 3D nanofibrous sponge accelerates the wound coagulation and haemostasis compared to 2D electrospun meshes. Moreover, due to the high surface area alongside, the homogeneous porosity of the 3D sponges hemostatic ability demonstrated higher concentration to the reference value (set to the hemoglobin concentration in blood (50 g/L)), ranging from 267.48 g/L, 270.81 g/L, and 346.36 g/L in 3D, 3D-AgMOF and 3D-AgMOF-CUR samples, respectively. Also, SEM imaging showed that

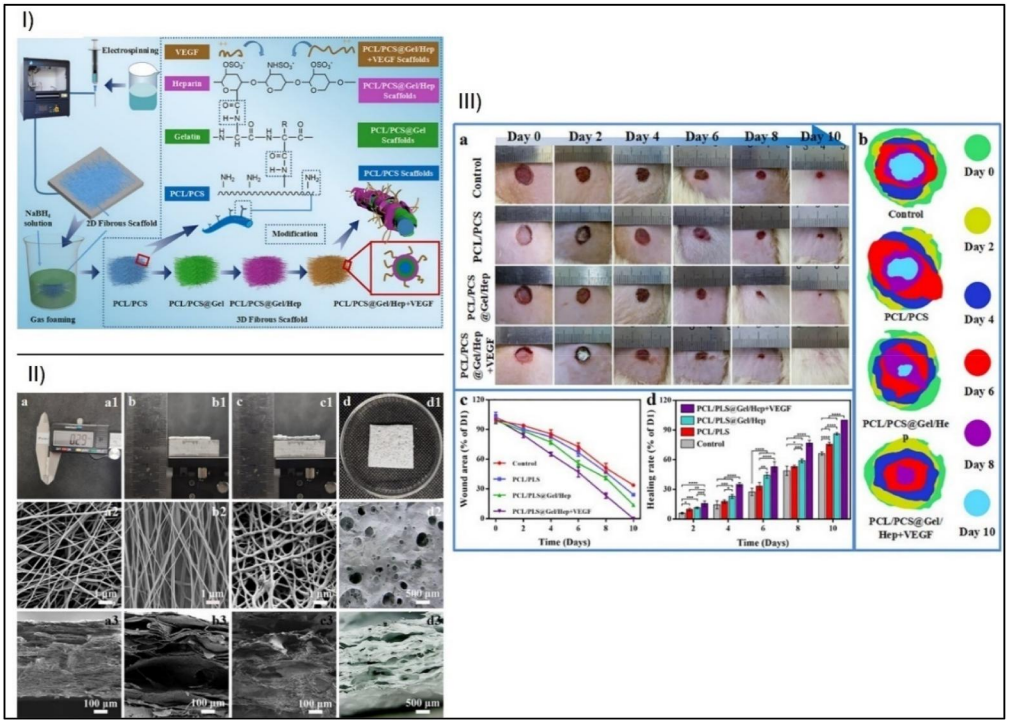


**Figure 7.** (I) Schematic illustration of the preparation of 3D polycaprolactone/ε-polylysine (PCL/PCS) modified chitosan fibrous scaffolds incorporation of bioactive factors for accelerating wound healing. (II) Morphological images of fabricated electrospun PCL/PCS (a1), 3D PCL/PCS (b1), 3D PCL/PCS@Gel/Hep (c1 and d1) nanofibrous scaffolds. SEM pictures of the surface and cross-sectional morphologies of PCL/PCS (a2 and a3), 3D PCL/PCS (b2 and b3), 3D PCL/PCS@Gel/Hep (c2 and c3). VDM images of the surface and cross-sectional morphology of 3D PCL/PCS@Gel/Hep (d2 and d3). (III) Wound images after treated by PCL/PCS, PCL/PCS@Gel/Hep, PCL/PCS@Gel/Hep + VEGF nanofibrous scaffolds at days 0, 2, 4, 6, 8, and 10 (a), diagram of wound size during healing of different fiber scaffolds (b), wound area (c) and healing rate (d) treated with different nanofibrous scaffolds at days 0, 2, 4, 6, 8 and 10. Images adapted from.[107]

blood cells remain entrapped in the 3D network, while in 2D fibrous meshes, the blood cells mostly remain attached to the surface. Whereas, the study developed by Zhang *et al.*,[109] demonstrated the same profile as the previous study regarding the hemostatic ability. Furthermore, the biocompatibility assays showed higher cellular viability in 3D meshes than in 2D meshes. In fact, the nanofiber expanded with $NaBH_4$ presented layers with a gap distance in the range of 140–220 μm, that shown to improve cell infiltration. Also, the expanded structure promotes the regeneration of the functional dermis and the restoration of differentiated adipocytes in the early repair stage. Contrary to the 2D-packed structures, these structures reduced the collagen III deposition during the later stage of repair, which is responsible for scar formation.[16] Gas foaming, particularly with radially or vertically aligned nanofibers, emerges as a strategy to significantly enhance the treatment of deep wounds, namely diabetic wounds. This approach precisely matches wound shape in depth and size, creating an ideal niche for stem cells in a 3D context.[111] The studies present layered 3D electrospun structures produced by

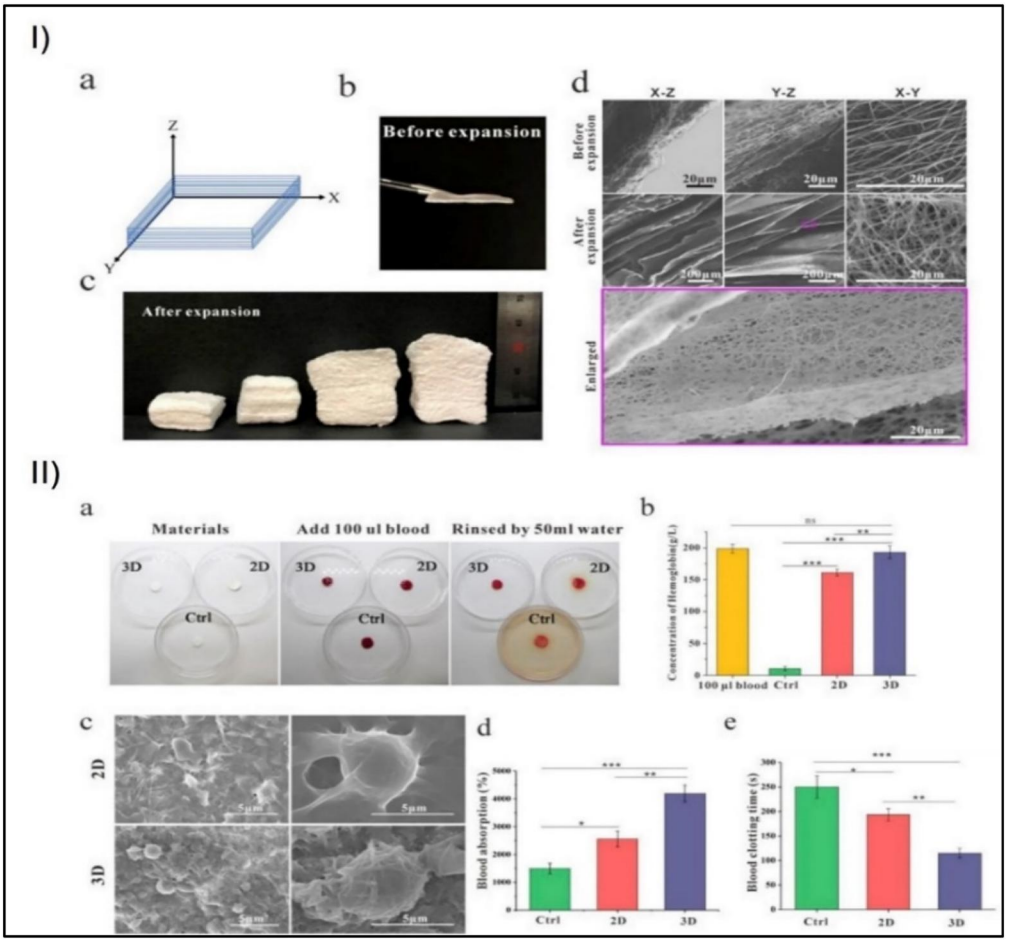


**Figure 8.** (I) The hemostatic activity of the 3D sponge PCL/Gel (a,b) and (c) show the morphological changes of the CS/PVA nanofiber membrane before and after expansion. (d) SEM micrographs of a CS/PVA nanofiber cross-section before and after expansion. The images of the blood-clotting; (II) *In vitro* blood coagulation test. (a) The photographs of the blood-clotting process. (b) The concentration of hemoglobin in the blood clots on the materials. (c) SEM micrographs of the interfacial interaction between the nanofibers with different structures and blood cells; the right side is an enlarged picture. (d) Blood absorption capacity of the medical gauze (control), 2D CS/PVA nanofiber membrane, and 3D CS/PVA nanofiber sponge. (e) Blood clotting time in the case of the medical gauze (control), 2D CS/PVA nanofiber membrane, and 3D CS/PVA nanofiber sponge. Adapted from.[109]

gas foaming that hold promise for clinical wound healing. This technique provides a 3D microenvironment regulating cellular behavior, enhancing hemostatic ability and accelerating healing. Additionally, it is possible to expand not only random 2D electrospun meshes as well as other types of fiber alignment, without compromising the fiber configuration, which could function as a strategy to promote the wound closure of wounds with different etiologies. The discussed studies presented a method to overcome the techniques' drawbacks associated with the degradation of bioactive compounds, incorporating them post-expansion.[107–109,111] The structure functionalization may boost antibacterial activity in full-thickness wounds.

### 3.2.7. Short nanofibers, nanofiber-based sponges, and hydrogel assembling

Apart from these, other 3D structures can be developed using short nanofibers (sNF's) through methods like molding, freeze-drying, and cross-linking to produce ultra-light and superabsorbent porous structures with superior mechanical properties (highly compressive-reversible) compared to traditional 2D nanofibers.[109,110,112] These structures were primarily exploited in bone regeneration, with limited exploration in the context of wound dressing.[110] However, Fu *et al.*,[113] John *et al.*,[114] among others [115–119] have been applying this methodology for wound healing applications. The short nanofibers are usually transformed into a 3D structure by cutting, followed by dispersion in *tert*-butanol, water, or mixtures of ethanol/water due to the accessible freezing temperatures, under high-speed homogenizer or ultrasonication.[120] After, the short nanofibers solution is placed onto molds and then placed at −20 or −80 °C overnight. The freezing stage is the most important phase for the final structure configuration since it influences the ice crystal formation, which influences the pore geometry.[42] As reported, slower rates form larger ice crystals, resulting in poor integrity. In comparison, faster rates promote smaller, more uniform crystals, enhancing pore geometry and structural stability.[120] The medium to disperse the electrospun fibers also is a key parameter in tailoring ice crystal formation. Usually, water is the most used due to the formation of relatively large and non-uniform ice crystals at a slow freezing rate ($0.5-3\,^{\circ}C\ min^{-1}$), causing cracks and poor structural integrity. Otherwise, adding ethanol can effectively improve the size and uniformity of ice crystals, while *tert*-butanol can be used to control the volume and shape of the frozen samples.[120] This labor-intensive, multi-step process increases the risk of failure, being pointed as the main disadvantage.[110] Also, high-speed homogenization and sonication to cut the nanofibers, may partially to totally damage them, compromising their functionality [110,120] Furthermore, after the freeze-drying step, the structures need to be crosslinked, which usually occurs by thermal cross-linking at high temperatures (∼ 180 °C) which restring the use of natural polymers, by chemical cross-linking with glutaraldehyde (GTA) and/or EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)-NHS(N-hydroxysuccinimide) which may induce toxicity.[121] Despite the drawbacks associated, the findings of Fu *et al.*[113] reveal that this methodology increases the fiber diameter and porosity compared with 2D electrospun meshes. After the modification of 3D short nanofibers with polydopamine (PDA) and Nanofat (NF) the ability to promote angiogenesis and cell proliferation as well as collagen deposition in a chronic wound model increased. In John *et al.*[114] work PGLA/GEL and poly-p-dioxanone (PDO)/gelatin short electrospun fiber were prepared by freeze-drying, presenting micro-/macrochannels due to sacrificial templating with 3D printed meshes. Then, was added a synthesized antimicrobial peptide, W379, into the sacrificed macrochannels to enhance the migration and proliferation of keratinocytes and dermal fibroblasts. The results indicated that the macrochannels with diameters of ≈300–500 μm, enable the cells migration and greatly enhance vascularized granulation tissue formation, while the addition of W379 as expected, enhanced wound healing probably by the activation of the p38 MAPK signaling pathway, which has a dysregulated function in diabetic wound healing.[114] In another study, to treat Diabetic foot ulcers (DFUs), were developed 3D PCL/collagen (3D-PC) nanofibrous dressing by short-fiber methodology.[117] The results show an

effective cell infiltration into the 3D-PC after three days with the 3D view analysis demonstrating an increase in depth from 180 to 223 µm on day 3, follow 241 µm on the seventh day, contrarily to the 2D meshes. Moreover, the obtained 3D structure was capable of healing diabetic wounds, without any dressing changes and with blood vessels that appeared on day 7 deprived of significant signs of inflammation. Additionally, the addition of the inhibitor doxycycline hyclate (DCH) and the antibacterial agent cephalexin (CEX) into PC nanofibers demonstrated a good inactivation effect on *E. coli* and *S. aureus*. Therefore, 3D-PC electrospun can be used as a one-time dressing that effectively promotes the wound healing on DFUs (Figure 9[117]). Another study, produced 3D structures using short nanofibers of cellulose acetate (CA) and PCL using thermally-induced nanofiber self-agglomeration (TISA) method to avoid toxic crosslinkers or dispersant polymer solutions.[122] TISA involves processing temperatures higher than the melting point of PCL, into a water bath, followed by freeze drying, and thermal stabilization. This process results in the formation of a porous, interconnected 3D structure similar to ECM. However, TISA nanofibers for skin regeneration purposes is still scarce possibly due to the limitation of using polymers with low melting temperatures that soften when inserted into the organism.[122–124]

In fact, the combination of electrospinning and freeze-drying are a strategy to overcome the limitations of 2D electrospun nanofibers, which had previously been a major challenge in tissue regeneration for applications that required a third dimension.[110] According to several publications cited in this review, 3D nanofiber sponges have demonstrated a superior performance in wound healing due to their high surface area, more interconnected pores, which improve interactions with cells and biological components. The higher porosity, compared with de 2D nanofibers, allows efficient gas and nutrient exchange, as well as cell infiltration which is critical for maintaining cell activity in the healing processes.[113,114,117] A 3D electrospun-based structure could be accomplished through the assembling of electrospun meshes into hydrogels. For instance, Tavakoli *et al.* [118] developed a three-layer composite structure with antibacterial properties against *S. aureus* and *E. coli*. Incorporating insulin-like growth factor 1 (IGF-1) in the polyacrylamide sponge (middle layer), along with stearic acid coating (upper layer) and electrospun meshes of *Aloe vera* and carbon nanoparticles (bottom layer), not only significantly increased cell viability but also accelerated *in vivo* wound healing within 10 days, surpassing other studied groups.[118] Furthermore, other study developed, a 3D hydrogel structure with methacrylate gelatin (MeGel)/PLLA radially aligned nanofiber on top shown to promote diabetic wound healing by significantly enhancing granulation tissue formation, angiogenesis, and ECM deposition, while the radially aligned nanofibers aided cells migrated faster from the center to the borders.[48] The alignment of the 3D structure seems to play a key role in cell elongation, and fostering cell migration which is translated into more angiogenic proteins.[125]

### 3.2.8. 3D printing and melt electrowriting

Until now, the techniques aforementioned do not allow homogenous control of porosity distribution. To overcome such limitations electrospinning has been combined with 3D printing.[126–129] 3D printing offers a significant advantage as it can print almost any geometry that can be created through a computer-aided design (CAD) model.[130] In

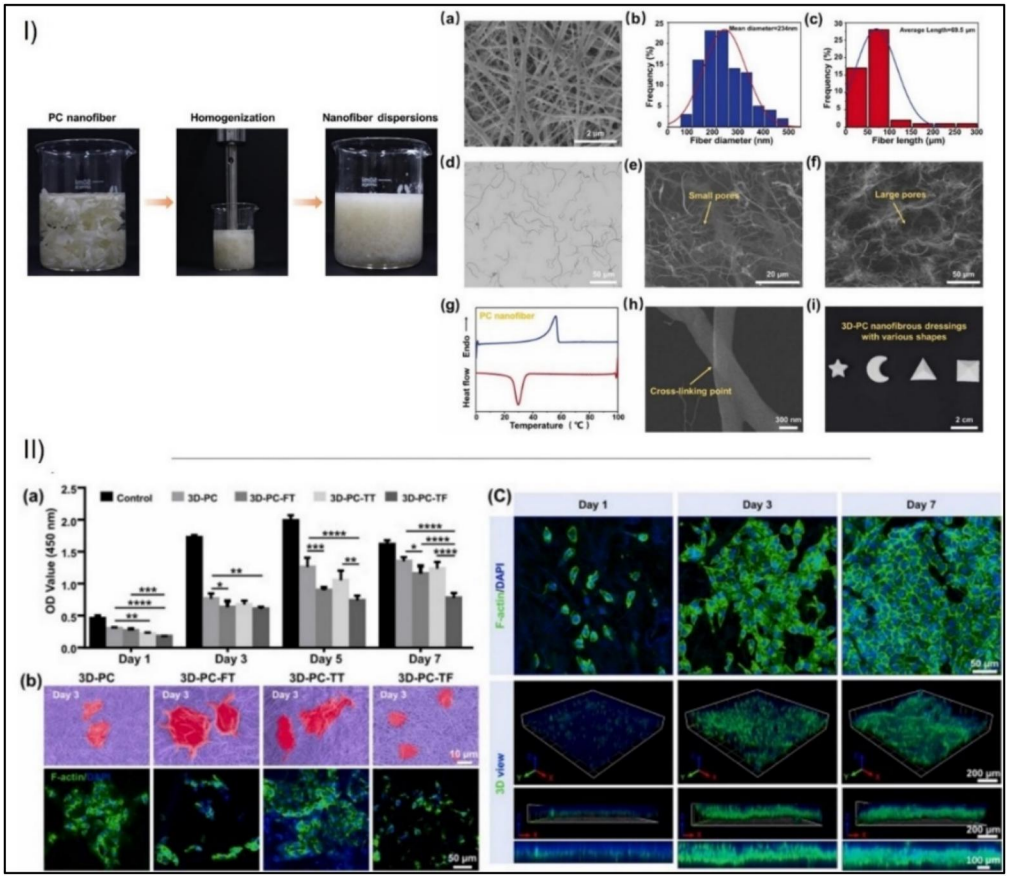


**Figure 9.** (I) Construction of 3D-PC from PC nanofiber. (a) SEM image of PC nanofibers. (b) Diameter distribution of PC nanofibers. (c) Distribution of short fiber length after homogenization of PC nanofibers. (d) Optical microscope image of short fibers after homogenization of PC nanofibers. (e–f) SEM images of microstructure of 3D- PC at various magnifications. (g) Differential scanning calorimetry (DSC) melting (blue line) and crystallization (red line) curves of PC nanofiber. (h) SEM image of the thermal cross-linking point formed between fibers of the 3D-PC after heat treatment. (i) Optical photograph of 3D-PC nanofibrous dressings with diverse shapes. (II) Culture of L929 cells in 3D-short nanofiber structure (a) CCK-8 assay test on each condition dressings after 1–7 days culture. (b) SEM images (cells are highlighted in red) and cell morphology fluorescent images (nucleus [blue, DAPI staining], cytoskeleton [green, F-actin staining]) of L929 cells on 3D short nanofiber dressings after 3 days of culture. (c) The top and 3D views of fluorescent images of L929 cells on 3D-PC by staining with F-actin (green) and DPAI (blue) at culture days 1, 3, and 7. (mean ± SD; $n = 6$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Images reproduced with permission from.[117]

this regard, a multi-layered scaffold capable of mimicking the hierarchical structure of the skin was developed by Mirhaj *et al.*,[126] combining 3D printing and electrospinning.[126] A polyurethane (PU) nanofibrous coating was created using electrospinning, while a 3D printed middle layer was made of Pluronic F127-quaternized chitosan-silver nitrate nanoparticles (F127-QCS-$AgNO_3$) for antibacterial properties, and a bottom layer of core-shell nanofibrous structure of F127-mupirocin/pectin-keratin

(F127-Mup/Pec-Kr) for tissue regeneration.[126] *In vivo* wound closure with a thick epidermis and keratinized layers formation were achieved within 12 days. Moreover, while PU layer allowed moisture retention and prevented the penetration of microorganisms, the 3D printed layer played a significant role in absorbing exudate and possessing antibacterial activity, due to the presence of QCs and $AgNO_3$, and the bottom layer due to the presence of Pec-Kr, caused excellent cell adhesion and angiogenic activity which accelerated the healing process.[126]

In another study, developed by Shahriari-Khalaji *et al.*,[127] a bilayer skin substitute was obtained by a 3D printed layer comprising carboxymethyl chitosan (CMCs) and oxidized alginate grafted catechol (O-AlgCat) on a PCL/gallium electrospun layer.[127] According to the *in vivo* infected burn rat models, the bilayer exhibited a shorter healing time and a more complete skin structure with thinner epithelium and more hair follicles, as well as lower expression of proinflammatory cytokines, higher expression of VEGF, better angiogenesis and collagen deposition than commercial products.[127] Similarly, the conjugation of 3D printing and electrospinning, with aligned nanofibers enables the elongation, guidance, and alignment of cells while facilitating the colonization more efficiently of the scaffold by providing bridging between printed microfibers.[131] Hence, the combination of 3D printing and electrospinning is a suitable strategy to mimic skin structure and could play a key role in cell alignment and migration to improve the regeneration of deep wounds. Although, despite 3D printing enables precise structure design, the resulting large fiber size often hinders cells from recognizing the scaffold as a true 3D environment.[132] Alternatively, melt electrowriting (MEW) is another approach to producing 3D specific geometries. MEW differs from 3D printing for its ability to produce porous structures at micron-scale fiber diameters using an electric field to stretch out the polymer.[132,133] To explore MEW as a powerful tool in skin tissue engineering, Afghah *et al.*[133] produced PCL and PCL bioactive glass fiber meshes combined photocrosslinkable gelatin hydrogel matrices loaded with basic fibroblast growth factor (bFGF) and VEGF individually. The histological evaluation of secondary wound healing, showed that the obtained structures do not promote hypertrophic scar tissue, which is a major concern in the wound healing of full-thickness wounds.[133] Using MEW-based matrices was possible to evaluate the expression and secretion of pro- and anti-macrophages. The produced structure with a rhombus architecture enhanced the secretion of anti-inflammatory factors, suggesting its potential in fostering a wound healing environment, showcasing macrophage responsiveness to 3D environments.[132]

The combination of advanced manufacturing techniques such as electrospinning and 3D printing, alongside MEW, allows the production of tailored and intricate designs, promoting better mimicry of skin layers. Additionally, these techniques overcome the limitations of the more derived conventional methods used in the direct process, such as reproducibility, scalability, and overall efficiency.[126,127,132,133]

In fact, the approach chosen to produce 3D-electrospun based structures for skin regeneration relies upon a comprehensive consideration of the advantages and disadvantages inherent in each technique and take into account the wound requirements. The most relevant aspects to consider in the 3D-electrospun based structures fabrication by the above-mentioned techniques are summarized in Table 1.

**Table 1.** Fabrication techniques available to produce 3D-electrospun based structures for skin regeneration.

| 3D electrospun-based approach | | Mechanism | Advantages | Disadvantages | Examples of materials used | Main findings | References |
|---|---|---|---|---|---|---|---|
| Direct processing | Direct/ Template-assisted electrospinning | Collecting the electrospun mesh directly from a collector | Possibility to use several materials, Tunable properties (porosity, fiber organization, and geometry). | Control of environmental conditions. | PCL, PLA, PLGA, PVA, chitosan, gelatin, collagen | ↑ porosity<br>↑ cell infiltration<br>↑ cellular proliferation | [96–98,100] |
| | Multilayering electrospinning | Stacking several electrospun meshes layers | Controllable layer number;<br>Increased structure thickness. | Weak interlayer bonding<br>Lack of continuous structure<br>Compact structure<br>Post-processing treatment | PCL, GEL, PVA, chondroitin sulfate | ↑ angiogenesis<br>↑ water absorption<br>↑ mechanical strength | [93,94] |
| | Co-electrospinning | Simultaneous electrospinning of two or more polymeric solutions to create composite meshes | Composite meshes offer tailored properties and functionalities;<br>Enhanced functionalities;<br>Versatility and customization. | Complex setup;<br>Processing challenges<br>Material compatibility;<br>Potential for clogging;<br>Scalability | PLLCL, collagen, PVP | ↑ cell viability<br>↑ cell proliferation<br>↑ collagen deposition | [61,93,94] |
| | Sacrificial agent electrospinning (porogen) | Co-electrospinning of a fast-dissolving polymer with a slow-degrading polymer | Increases the porosity and pore size; Increased cell infiltration; Uniform porous structure. | Difficult to reach a thicker structure | PGA, PLGA, PCL, PEO, PVP, gelatin, | ↑ collagen deposition<br>↑ elastin matrix proteins<br>↑ cell differentiation | [52,86,87,89] |
| | Wet and cryogenic electrospinning | Using a liquid-bath collector or customized cold-plate collector | Simple set-up;<br>Increased structure thickness;<br>Increases the porosity and pore size;<br>Increased cell infiltration. | Uncontrolled shape<br>Limited scalability | PLGA | ↑ cell infiltration<br>↑ cell proliferation | [97,99]. |
| Post-processing | Salt-leaching | Sodium chloride is incorporated into the polymer solution. After the electrospinning process, the salt is leached out | Porous structure;<br>Controlled pore size by adjusting salt particle size;<br>Easy and cost-effective. | Non-homogenous pore distribution;<br>Time-consuming and remaining salt residues;<br>Limited to water-soluble polymers: | HA, collagen | ↑ cell adhesion<br>↑ cell proliferation | [103,104] |
| | Gas foaming | Electrospun mesh expansion by $NaBH_4$ hydrolysis or $CO_2$ depressurization | Highly ordered architecture;<br>Ultra-light structure;<br>Increase pore size and thickness. | $NaBH_4$ only works for hydrophobic polymers<br>Loss of bioactivities<br>Affect the mechanical stability;<br>Limited scalability for industrial production. | GEL, PVA, chondroitin sulfate, ε-polylysine modified chitosan | ↑ hemostatic ability<br>↑ collagen deposition<br>↑ oxygen-rich environment | [107–109,111] |

(*continued*)

**Table 1.** Continued.

| 3D electrospun-based approach | Mechanism | Advantages | Disadvantages | Examples of materials used | Main findings | References |
|---|---|---|---|---|---|---|
| Short nanofibers/ nanofiber-based sponge | Cutting the electrospun meshes into small pieces followed uniform dispersion, molding and freeze-drying | Ultra-light structure; Highly porous structure; Macro and micro-interconnected pores; Increased thickness; Controlled shape and size; Superabsorbent and compressible behavior. | Long multistep process; Required post-processing treatments (crosslinking); Fibers lengths are not controlled/homogeneous. | PCL, PLGA, GEL, collagen, PAM, PDO, Hydrophilic poly(acrylic acid) (PAAc), Polyacrylamide | ↑ cellular infiltration ↑ faster blood vessel formation ↑ enhance vascularized granulation tissue formation ↓reduction of inflammation, | [113,114,117–119] |
| 3D printing (extrusion) | Layer by layer from a digital model or computer-aided design (CAD) file | Controlled morphology; Complex geometries; Customization; On-Demand manufacturing. | Complex process; Specific equipment and costs; | Polyurethane (PU), PCL, Carboxymethyl chitosan, Polyacrylamide (PAAm), pectin/polyacrylic acid (Pec/PAA) | ↑ complete skin structure with hair follicles ↑ absorbing exudate ↑ collagen deposition and skin appendages formation | [126–129] |
| Melt electrowriting | It uses an electric field to stretch fine fibers from a previous molten polymer | Controlled morphology with fiber sizes at micron scale; Mechanical reinforcement High resolution. | Limited to thermoplastics; Equipment complexity. | PCL, Bioative glass | ↓ hypertrophic scar tissue ↑ macrophage anti-inflammatory phenotype | [132,133] |

The arrow ↑ *means an increase in the indicated property, and* ↓ *a decrease.*

**Table 2.** Ongoing clinical trials using electrospun wound dressings.

| Study title | Clinical trial ID | Status | Ref |
|---|---|---|---|
| Study on Regeneration of Skin Defects in Diabetic Ulcers Treated with New Electrospun Material Poly (L-lactide-co-caprolactone) and Formulated Porcine Fibrinogen | NCT06014437 | Study ongoing | [134] |
| A Pilot Study to Evaluate a Temporary Skin Substitute (Spincare® Matrix) for Wound Healing in Recessive dystrophic epidermolysis bullosa (RDEB) Patients | NCT05944250 | Study ongoing | [135] |
| EktoTherix™ Regenerative Tissue Scaffold for Repair of Surgical Excision Wounds | NCT02409628 | Completed. No Study Results Posted | [136] |

## 4. Clinical trials and future directions

Advanced electrospun wound dressings hold great potential to revolutionize wound treatment, especially in terms of encouraging skin regeneration in full-thickness wounds.[5,35] However, few human clinical trials are available to evaluate the performance in clinical and surgical conditions. Over the past seven years, only three clinical studies with the keyword "electrospun wound dressing" have been registered on *ClinicalTrials.gov* (Table 2). This lack of robust, randomized, and blinded trials reduces confidence among regulatory agencies and potential investors/sponsors.[34]

The lack of electrospun wound dressings in clinical trials and commercialization could be related to several challenges that encompass technical, regulatory, and economic aspects.[34,137–141] Most of the studies focus on *in vitro*, and only a minority performed *in vivo* tests in rodents (mice and rats), favored for their ease of handling, inexpensive of keeping and anesthetizing, and provide quicker results.[137] Nevertheless, larger animal models such as pigs and sheep, are also available but lack consensus due to their differing anatomical and physiological features.[138] At the lab-scale level, the use of animals is restricted, triggered by the EU to reduce/leave animal testing. Additionally, limited funding across research groups poses a challenge to advancing preclinical validation tests of electrospun wound dressings. Based on this, new preclinical validation tests are in development, however, it is difficult to achieve a 3D skin model with epidermis and dermis layers that correctly mimic skin diseases.[142] Electrospinning manufacturing processes further complicate scalability due to the need for specialized equipment, including high-voltage power supplies and controlled environments, making it difficult to reproduce results industrially.[137,138] Additionally, the use of organic solvents introduces safety and environmental concerns. Scaling from lab to industrial production is resource-intensive, causing electrospinning to often be regarded as having a low Technology Readiness Level (TRL), delaying clinical trials and market entry.[139,140] Nevertheless, since 2004, there has been a significant increase in the availability of lab-scale electrospinning equipment suppliers, such SKE Research Equipment ® (Italy), Inovenso (Turkey), Bioinicia (Spain), Nadetech Innovations (Spain), Elmarco (Czech Republic), Fnm Co. (Iran), Erich Huber GmbH (E-Spintronic, Germany), Nanoflux (Singapore), in an attempt to diminish this gap between research and industry.[143–149] The wide range of polymers and bioactive compounds/drugs that can be used but are not yet regulated by the FDA or EMA, alongside the extensive and limiting characterization by the regulatory agencies might delay the commercialization of electrospun

**Table 3.** Electrospun wound dressings available on the market.

| Commercialized product | Manufacturer | Product description | Ref |
|---|---|---|---|
| Surgiclot® | St. Theresa Medical Inc., USA | Dextran-based wound dressing infused with human-sourced fibrinogen and thrombin, which acts as a natural clot at the injury site and stops bleeding | [151] |
| Phoenix Wound Matrix RenovoDerm® | Nanofiber Solutions™, USA | 3D advanced wound dressings composed of poly(glycolic acid (PGA) and poly (L-lactide-co-caprolactone) (PLCL) synthetic polymers designed for full thickness wounds inspiring a pro-healing activity. | [152] |
| Restrata® Meshed | Acera Surgical, USA | Polyglactin 910 and polydioxanone electrospun fiber matrix engineered to be similar to human ECM that activates granulation tissue in refractory wounds while maintaining tensile strength with flexibility | [153] |
| Restrata® MiniMatrix™ | Acera Surgical, USA | Polyglactin 910 and polydioxanone Micronized electrospun fiber matrix for wound management including surgical and traumatic wounds | [153] |
| ChitoFib Patch | Nano Medical, Czech Republic | Chitosan-based nano- wound dressing to protect wounds from contamination and promote and accelerate healing. | [154] |
| MIRRAGEN® | Engineered Tissue Solutions (ETS), USA | First bioactive borate-based glass fiber wound matrix on the market to treat diabetic wounds, pressure and venous ulcers, surgical wounds among others. | [155] |
| DermaLayr | NanoLayr, New Zealand | Nanosized electrospun patch of marine collagen to deliver collagen and active ingredients into the dermal skin layers | [156] |
| Matripatch© | matrihealth GmbH ©, Germany | Elastin and collagen wound dressing to promote wound healing. | [157] |
| AquaFiber™ | ActivHeal®, UK | Silver Antimicrobial wound dressing consisting of a high M (mannuronic acid) calcium alginate and carboxymethylcellulose (CMC) for the management of infected wounds. | [158] |

wound dressings.[150] In fact, the multifunctionality of many electrospun wound dressings delays their classification into medical devices since the primary mode of action is sometimes unclear.[34,141] Thus, the identification of standardized methods to characterize advanced wound dressings is extremely complex.

Despite the challenges, ongoing research, collaborations between clinicals and experts in materials science, engineering, and biology, allied with the advances in electrospinning technology have been key to overcoming these barriers. Consequently, more electrospun-based wound dressings are appearing on the market (Table 3).

The ultimate approaches to developing 3D structures to overcome the limitations of the lack a 3D environment for cellular infiltration were exploited, enabling the creation of personalized wound dressings. Following the additive manufacturing advances, it is expected that electrospun dressings shift toward 4D electrospun meshes, introducing an additional dimension that adapts to stimuli such as heat, light, pH, or moisture, allowing dynamic shape and function changes over time.[130,159] Consequently, these materials can improve wound healing and minimize side effects by releasing growth factors or antimicrobial agents.[159] Despite most electrospun meshes being designed for sustained, controlled therapeutic release, 4D meshes could integrate sensors to remotely monitor

healing progress, enabling timely interventions and reducing the need for frequent clinical evaluations.[159–161]

Nevertheless, before moving to 4D electrospun meshes some improvements in the 3D electrospun-based structures can be made. sNF's can be explored as inks for 3D printing, reinforcing biomimetic structures with improved surface area and porosity, as in other fields.[162–164] Furthermore, the adoption of environmentally friendly solvents (water or nontoxic solvents) aligns electrospinning with global sustainability goals while fostering biomedical applications. Additionally, with the limitations of using animal testing, other alternatives are being explored as organ-on-chip, namely skin-on-chip, in which electrospun meshes offer a potential way to assess tissue–tissue interface.[165–167] This opens up the possibility of creating high-throughput, automated, and realistic skin models for skin regeneration, cosmetic, and pharmaceutical applications.[167] At the same time provides a realistic model to study wound healing and gives precise information about the relationship of biomaterials and cells for fostering skin regeneration.

The collaborative efforts of several sectors highlight the enormous potential of electrospun structures in biomedical research, providing revolutionary solutions for skin regeneration. The promising future of electrospinning is emerging as we move forward, holding the promise of changing the boundaries of wound healing and tissue engineering in general.

## 5. Conclusions

Over the last years, electrospinning has provided a simple and versatile technique to produce advanced wound dressings that accurately mimic the skin's ECM. Although, despite the remarkable outputs both *in vivo* and *in vitro* achieved by electrospun structures and even at the industrial scale, the obtained structures with the traditional apparatus and collectors can only provide a 2D mesh. The major limitations of 2D meshes are related to diminished cell infiltration in depth, and especially the lack of 3D environment to mimic the injured tissue, especially in full-thickness and chronic wounds where great tissue areas are damaged. On the other hand, the path of tissue engineering is evolving toward an *in situ* regeneration approach in order to replace expensive cell-based skin substitutes and complex skin grafts. In this sense, different approaches have been applied using electrospinning to reach the third dimension, allowing the structures to possess the necessary physical architecture alongside the chemical and biological components. Thus, the structures recreate a natural tissue-like environment to attract cells and other cellular components such as cytokines, that foster the wound healing process.

Therefore, this article covers the innovative strategies to obtain 3D electrospun-based structures based on direct and post-processing approaches. According to the literature, 3D structures from post-processing methods surpass 2D and direct methods by forming accurate 3D porous structures that cells better recognize as native tissue, evidenced by their superior *in vitro/in vivo properties*. Furthermore, electrospinning's versatility allows these structures to work as one-application dressing due to their multifunctionality (anti-inflammatory or antibacterial properties) reducing the discomfort for patients and costs for health care systems.

However, some challenges persist, limiting the widespread clinical adoption and commercialization of these advanced structures. The challenges are mainly related to the

fabrication process, in which combining different techniques increases the complexity of the process, which might be more difficult to standardize and scale to an industrial level. Additionally, most of the 3D structures incorporate a myriad of growth factors or other bioactive compounds from natural sources. Regarding their effectiveness, these increased their complexity, cost, and concerns about bioavailability, which introduced regulatory hurdles, as agencies faced difficulties in evaluating their efficacy and safety, as well as long-term stability, making them comparable to more expensive cell-based products. Despite most studies proving the biocompatibility of the 3D structures *in vivo*, other important features are often less well described, such as the mechanical properties or degradation rates. The success of the 3D structures, in the scope of *in situ* regeneration, comprises the balance between biophysical, biochemical, and biomechanical stimuli to reach the ideal environment for cell growth and tissue repair. Hence, the focus should be on developing advanced wound dressings that remain affordable and easy to use, ensuring accessibility while still providing effective therapeutic outcomes. In this regard, the 3D electrospun-based structure with the most potential for clinical use should include short nanofibers, nanofiber-based sponges, and hydrogel assemblies. These structures are particularly promising, as they align with existing commercially available products, such as electrospun wound dressings (as outlined in Table 3) and hydrogel sponges, and even hydrofiber-based dressings such as Exufber® and Kaltostat®. Combining these approaches (short nanofibers with hydrogel sponges or short nanofibers and hydrogels) represents a practical and efficient strategy to achieve a 3D structure with native skin ECM nanodetails that could expedite market entry while ensuring the cost-effectiveness of the production process.

Nonetheless, it is important to note that as a rule, there is no single fabrication approach that fulfills all the aspects of TE, since each has advantages and disadvantages. Similarly, no single biomaterial is universally accepted for all patients. Thus, it is crucial an extensive evaluation of wound by a professional and take into account the wound requirements. This scenario opens the door to the possibility of personalized medicine, by creating advanced wound dressings specifically designed for individual patients. Looking ahead, the increasing number of industrial electrospinning companies and multidisciplinary collaboration between scientists and healthcare professionals are driving the commercialization of electrospun dressings, bringing us closer to accessible, personalized wound care solutions.

## Authors contributions

Conceptualization, M.F.L.L., A.C.M., J.R.D., and C.A.M.F.; writing—original draft preparation, C.A.M.F.; writing—review and editing, C.A.M.F ., M.F.L.L., A.C.M., and J.R.D.; validation, M.F.L.L., A.C.M., and J.R.D.; resources, M.F.L.L., A.C.M and J.R.D.; supervision, M.F.L.L., A.C.M and J.R.D.; project administration, M.F.L.L., A.C.M and J.R.D.; funding acquisition M.F.L.L., A.C.M and J.R.D. All authors have read and agreed.

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## References

1. Chen, K.; Hu, H.; Zeng, Y.; Pan, H.; Wang, S.; Zhang, Y.; Shi, L.; Tan, G.; Pan, W.; Liu, H. Recent advances in electrospun nanofibers for wound dressing. *Eur. Polym. J.* **2022**, *178*, 111490. DOI: 10.1016/j.eurpolymj.2022.111490.
2. Sen, C. K. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. *Adv. Wound Care* **2021**, *10*, 281.
3. Chen, S.; Liu, B.; Carlson, M. A.; Gombart, A. F.; Reilly, D. A.; Xie, J. Recent Advances in Electrospun Nanofibers for Wound Healing. *Nanomedicine.* **2017**, *12*, 1335–1352. DOI: 10.2217/nnm-2017-0017.
4. Yakupu, A.; Aimaier, R.; Yuan, B.; Chen, B.; Cheng, J.; Zhao, Y.; Peng, Y.; Dong, J.; Lu, S. *Front. Public Heal* **2023**, *11* 1145513.
5. Vig, K.; Chaudhari, A.; Tripathi, S.; Dixit, S.; Sahu, R.; Pillai, S.; Dennis, V. A.; Singh, S. R. Advances in Skin Regeneration Using Tissue Engineering. *Int. J. Mol. Sci.* **2017**, *18*, 789.
6. Clark, R. A. F.; Ghosh, K.; Tonnesen, M. G. Tissue engineering for cutaneous wounds. *J. Invest. Dermatol.* **2007**, *127*, 1018.
7. Las Heras, K.; Igartua, M.; Santos-Vizcaino, E.; Hernandez, R. M. Chronic wounds: Current status, available strategies and emerging therapeutic solutions. *J. Control. Release* **2020**, *328*, 532.
8. Helary, C.; Abed, A.; Mosser, G.; Louedec, L.; Letourneur, D.; Coradin, T.; Giraud-Guille, M. M.; Meddahi-Pellé, A. Evaluation of Dense Collagen Matrices as Medicated Wound Dressing for the Treatment of Cutaneous Chronic Wounds. *Biomater. Sci.* **2015**, *3*, 373–382. DOI: 10.1039/c4bm00370e.
9. Boateng, J.; Catanzano, O. Advanced Therapeutic Dressings for Effective Wound Healing-A Review. *J. Pharm. Sci.* **2015**, *104*, 3653–3680. DOI: 10.1002/jps.24610.
10. Wound Care Market - Forecast to 2024. https://www.marketsandmarkets.com/Market-Reports/wound-care-market., **2019**.
11. Ferreira, C. A. M.; Januário, A. P.; Félix, R.; Alves, N.; Lemos, M. F. L.; Dias, J. R. Multifunctional Gelatin/Chitosan Electrospun Wound Dressing Dopped with Undaria pinnatifida Phlorotannin-Enriched Extract for Skin Regeneration. *Pharmaceutics* **2021**, *13*. DOI: 10.3390/pharmaceutics13122152.
12. Dias, J. R.; Granja, P. L.; Bártolo, P. J. Advances in electrospun skin substitutes. *Prog. Mater. Sci.* **2016**, *84*, 314.
13. Lin, W.; Chen, M.; Qu, T.; Li, J.; Man, Y. Three-Dimensional Electrospun Nanofibrous Scaffolds for Bone Tissue Engineering. *J. Biomed. Mater. Res.* **2020**, *108*, 1311–1321. DOI: 10.1002/jbm.b.34479.

14. Sorg, H.; Tilkorn, D. J.; Hager, S.; Hauser, J.; Mirastschijski, U. Skin Wound Healing: An Update on the Current Knowledge and Concepts. *Eur. Surg. Res.* **2017**, *58*, 81–94. DOI: 10.1159/000454919.
15. Salazar, J.; Carmona, T.; Zacconi, F. C.; Venegas-Yazigi, D.; Cabello-Verrugio, C.; Il Choi, W.; Vilos, C. The Human Dermis as a Target of Nanoparticles for Treating Skin Conditions. *Pharm.* **2022**, *15*, 10.
16. Tottoli, E. M.; Dorati, R.; Genta, I.; Chiesa, E.; Pisani, S.; Conti, B. Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. *Pharmaceutics* **2020**, *12*, 1.
17. Liu, Y.; Liu, Y.; Deng, J.; Li, W.; Nie, X. Fibroblast Growth Factor in Diabetic Foot Ulcer: Progress and Therapeutic Prospects. *Front. Endocrinol.* **2021**, 12, 1348.
18. Wang, J.; Windbergs, M. Functional electrospun fibers for the treatment of human skin wounds. *Eur. J. Pharm. Biopharm* **2017**, *119*, 283.
19. Flynn, K.; Mahmoud, N. N.; Sharifi, S.; Gould, L. J.; Mahmoudi, M. Chronic Wound Healing Models. *ACS Pharmacol. Transl. Sci.* **2023**, *6*, 783–801. DOI: 10.1021/acsptsci.3c00030.
20. Minsart, M.; Van Vlierberghe, S.; Dubruel, P.; Mignon, A. Commercial wound dressings for the treatment of exuding wounds: an in-depth physico-chemical comparative study. *Burn. Trauma* **2022**, *10, tkac024*. DOI: 10.1093/burnst/tkac024.
21. Jin, S.; Newton, M. A. A.; Cheng, H.; Zhang, Q.; Gao, W.; Zheng, Y.; Lu, Z.; Dai, Z.; Zhu, J. Progress of Hydrogel Dressings with Wound Monitoring and Treatment Functions. *Gels* **2023**, *9*, 694. DOI: 10.3390/gels9090694.
22. van Netten, J. J.; Price, P. E.; Lavery, L. A.; Monteiro-Soares, M.; Rasmussen, A.; Jubiz, Y.; Bus, S. A. Prevention of Foot Ulcers in the at-Risk Patient with Diabetes: A Systematic Review. *Diabetes Metab. Res. Rev.* **2016**, *32 Suppl 1*, 84–98. DOI: 10.1002/dmrr.2701.
23. Burgess, J. L.; Wyant, W. A.; Abujamra, B. A.; Kirsner, R. S.; Jozic, I. Diabetic Wound-Healing Science. *Medicina* **2021**, 57(10):1072. DOI: 10.3390/MEDICINA57101072.
24. Ajmal, G.; Bonde, G. V.; Thokala, S.; Mittal, P.; Khan, G.; Singh, J.; Pandey, V. K.; Mishra, B. Ciprofloxacin HCl and quercetin functionalized electrospun nanofiber membrane: fabrication and its evaluation in full thickness wound healing. *Artif. Cells, Nanomedicine Biotechnol.* **2019**, *47*, 228.
25. Tavakoli, S.; Klar, A. S. Bioengineered Skin Substitutes: Advances and Future Trends. *Appl. Sci.* **2021**, *11*, 1493.
26. Jorgensen, A. M.; Varkey, M.; Gorkun, A.; Clouse, C.; Xu, L.; Chou, Z.; Murphy, S. V.; Molnar, J.; Lee, S. J.; Yoo, J. J.; et al. Bioprinted Skin Recapitulates Normal Collagen Remodeling in Full-Thickness Wounds. *Tissue Eng. Part A* **2020**, *26*, 512.
27. Przekora, A. A Concise Review on Tissue Engineered Artificial Skin Grafts for Chronic Wound Treatment: Can We Reconstruct Functional Skin Tissue In Vitro? *Cells* **2020**, *9*, 1622. DOI: 10.3390/cells9071622.
28. Saleh, K.; Strömdahl, A.-C.; Riesbeck, K.; Schmidtchen, A. Inflammation Biomarkers and Correlation to Wound Status After Full-Thickness Skin Grafting. *Front. Med.* **2019**, *6:159*. DOI: 10.3389/fmed.2019.00159.
29. Rivera, A. E.; Spencer, J. M. Clinical Aspects of Full-Thickness Wound Healing. *Clin. Dermatol.* **2007**, *25*, 39–48. DOI: 10.1016/j.clindermatol.2006.10.001.
30. Darwin, E.; Tomic-Canic, M. Healing Chronic Wounds: Current Challenges and Potential Solutions. *Curr. Dermatol. Rep.* **2018**, *7*, 296–302. DOI: 10.1007/s13671-018-0239-4.
31. WoundSource. **n.d.** https://www.woundsource.com/.
32. Gaharwar, A. K.; Singh, I.; Khademhosseini, A. Engineered biomaterials for in situ tissue regeneration. *Nat. Rev. Mater.* **2020**, *5*, 686.
33. Joyce, K.; Fabra, G. T.; Bozkurt, Y.; Pandit, A. Bioactive potential of natural biomaterials: identification, retention and assessment of biological properties. *Signal Transduct. Target. Ther.* **2021**, *6*, 122.
34. Laurano, R.; Boffito, M.; Ciardelli, G.; Chiono, V. Wound dressing products: A translational investigation from the bench to the market. *Eng. Regen.* **2022**, *3*, 182.

35. Augustine, R.; Kalarikkal, N.; Thomas, S. Advancement of wound care from grafts to bio-engineered smart skin substitutes. *Prog Biomater* 2014, 3(2-4):103-113. DOI: 10.1007/s40204-014-0030-y.
36. Kishan, A. P.; Cosgriff-Hernandez, E. M. Recent advancements in electrospinning design for tissue engineering applications: A review. *J. Biomed. Mater. Res. Part A* **2017**, *105*, 2892.
37. Mohamadinooripoor, R.; Kashanian, S.; Arkan, E. An Overview on Wound Dressings and Sutures Fabricated by Electrospinning. *Biotechnol. Bioprocess Eng.* **2023**, *19*, 1.
38. Shi, C.; Wang, C.; Liu, H.; Li, Q.; Li, R.; Zhang, Y.; Liu, Y.; Shao, Y.; Wang, J. Selection of Appropriate Wound Dressing for Various Wounds. *Front. Bioeng. Biotechnol.* **2020**, *8*, 1.
39. Firlar, I.; Altunbek, M.; McCarthy, C.; Ramalingam, M.; Camci-Unal, G. Functional Hydrogels for Treatment of Chronic Wounds. *Gels* **2022**, *8*, 127. DOI: 10.3390/gels8020127.
40. Pereira, R. F.; Barrias, C. C.; Granja, P. L.; Bartolo, P. J. Advanced biofabrication strategies for skin regeneration and repair. *Nanomedicine* **2013**, *8*, 603.
41. Lavielle, N.; Hébraud, A.; Mendoza-Palomares, C.; Ferrand, A.; Benkirane-Jessel, N.; Schlatter, G. Structuring and Molding of Electrospun Nanofibers: Effect of Electrical and Topographical Local Properties of Micro-Patterned Collectors. *Macromol. Mater. Eng.* **2012**, *297*, 958.
42. Dilamian, M.; Joghataei, M.; Ashrafi, Z.; Bohr, C.; Mathur, S.; Maleki, H. From 1D electrospun nanofibers to advanced multifunctional fibrous 3D aerogels. *Appl. Mater. Today* **2021**, *22*, 100964.
43. Vass, P.; Szabó, E.; Domokos, A.; Hirsch, E.; Galata, D.; Farkas, B.; Démuth, B.; Andersen, S. K.; Vigh, T.; Verreck, G.; et al. Scale-up of electrospinning technology: Applications in the pharmaceutical industry. *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnol.* **2020**, 12. DOI: 10.1002/wnan.1611.
44. Dias, J. R.; Baptista-Silva, S.; Sousa, A.; Oliveira, A. L.; Bártolo, P. J.; Granja, P. L. Electrospinning Fabrication Strategies: From Conventional to Advanced Approaches. Electrospun materials and their allied applications, 2020, 1-52." Scrivener Publishing LLC by Wiley-Scrivener in Beverly, Massachusetts, USA.
45. Dias, J. R.; Alves, A. I. F.; Ferreira, C. A. M.; Alves, N. M. Electrospinning Fabrication Strategies: From Conventional to Advanced Approaches. *Electrospun Materials and Their Allied Applications*; John Wiley & Sons, Ltd, **2020**, pp 1–52.
46. Oroujzadeh, M.; Mosaffa, E.; Mehdipour-Ataei, S. Recent Developments on Preparation of Aligned Electrospun Fibers: Prospects for Tissue Engineering and Tissue Replacement. *Surf. Interfaces* **2024**, *49*, 104386. DOI: 10.1016/j.surfin.2024.104386.
47. Xue, J.; Wu, T.; Xia, Y. Perspective: Aligned arrays of electrospun nanofibers for directing cell migration. *APL Mater.* **2018**, *6*, 120902.
48. Wu, S.; Zhao, W.; Sun, M.; He, P.; Lv, H.; Wang, Q.; Zhang, S.; Wu, Q.; Ling, P.; Chen, S.; et al. Novel bi-layered dressing patches constructed with radially-oriented nanofibrous pattern and herbal compound-loaded hydrogel for accelerated diabetic wound healing. *Appl. Mater. Today* **2022**, *28*, 101542.
49. Rezvani Ghomi, E.; Lakshminarayanan, R.; Chellappan, V.; Kumar Verma, N.; Chinnappan, A.; Esmaeely Neisiany, R.; Amuthavalli, K.; Sheng Poh, Z.; Han Siang Wong, B.; Dubey, N.; et al. Electrospun Aligned PCL/Gelatin Scaffolds Mimicking the Skin ECM for Effective Antimicrobial Wound Dressings. *Adv. Fiber Mater.* **n.d.**, *1*, 3.
50. Robinson, A. J.; Pérez-Nava, A.; Ali, S. C.; González-Campos, J. B.; Holloway, J. L.; Cosgriff-Hernandez, E. M. Comparative Analysis of Fiber Alignment Methods in Electrospinning. *Matter* **2021**, *4*, 821–844. DOI: 10.1016/j.matt.2020.12.022.
51. Xue, J.; Wu, T.; Qiu, J.; Xia, Y. Accelerating Cell Migration along Radially Aligned Nanofibers through the Addition of Electrosprayed Nanoparticles in a Radial Density Gradient. *Part. Part. Syst. Charact.* **2022**, *39*, 1.
52. Vong, M.; Radacsi, N. Fabrication of Radially Aligned Electrospun Nanofibers in a Three-Dimensional Conical Shape. *Electrospinning* **2018**, *2*, 1–14. DOI: 10.1515/esp-2018-0001.

53. Mohd Razali, N. A.; Lin, W. C. Accelerating the excisional wound closure by using the patterned microstructural nanofibrous mats/gentamicin-loaded hydrogel composite scaffold. *Mater. Today Bio.* **2022**, *16*, 100347.
54. Daming, Z.; Jiang, C. Electrospinning of Three-Dimensional Nanofibrous Tubes with Controllable Architectures. *Nano Lett 2008,* 8, 3283 - 3287..
55. Nedjari, S.; Awaja, F.; Altankov, G. Three Dimensional Honeycomb Patterned Fibrinogen Based Nanofibers Induce Substantial Osteogenic Response of Mesenchymal Stem Cells. *Sci. Rep.* **2017**, 7, 1.
56. Kasoju, A. P. R. Strategies to Tune Electrospun Scaffold Porosity for Effective Cell Response in Tissue Engineering. *J. Funct. Biomater* **2019**, *10*, 30.
57. Li, Y.; Xiao, Z.; Zhou, Y.; Zheng, S.; An, Y.; Huang, W.; He, H.; Yang, Y.; Li, S.; Chen, Y.; et al. Controlling the Multiscale Network Structure of Fibers to Stimulate Wound Matrix Rebuilding by Fibroblast Differentiation. *ACS Appl. Mater. Interfaces* **2019**, *11*, 28377–28386. DOI: 10.1021/acsami.9b06439.
58. Stachewicz, U.; Szewczyk, P. K.; Kruk, A.; Barber, A. H.; Czyrska-Filemonowicz, A. Pore shape and size dependence on cell growth into electrospun fiber scaffolds for tissue engineering: 2D and 3D analyses using SEM and FIB-SEM tomography. *Mater. Sci. Eng. C* **2019**, *95*, 397.
59. Fan, J.; Zhang, Y.; Liu, Y.; Wang, Y.; Cao, F.; Yang, Q.; Tian, F. Explanation of the cell orientation in a nanofiber membrane by the geometric potential theory. *Results Phys.* **2019**, 15. DOI: 10.1016/J.RINP.2019.102537.
60. Wang, C.; Chu, C.; Zhao, X.; Yang, Y.; Hu, C.; Liu, L.; Li, J.; Qu, Y.; Man, Y. The Diameter Factor of Aligned Membranes Facilitates Wound Healing by Promoting Epithelialization in an Immune Way. *Bioact. Mater.* **2022**, *11*, 206–217. DOI: 10.1016/j.bioactmat.2021.09.022.
61. Türker, E.; Yildiz, Ü. H.; Arslan Yildiz, A. Biomimetic Hybrid Scaffold Consisting of co-Electrospun Collagen and PLLCL for 3D Cell Culture. *Int. J. Biol. Macromol.* **2019**, *139*, 1054–1062. DOI: 10.1016/j.ijbiomac.2019.08.082.
62. Pisani, S.; Dorati, R.; Genta, I.; Benazzo, M.; Conti, B.; Prina Mello, A. A Study Focused on Macrophages Modulation Induced by the Polymeric Electrospun Matrices (EL-Ms) for Application in Tissue Regeneration: In Vitro Proof of Concept. *Int. J. Pharm.* **2021**, *603*, 120712. DOI: 10.1016/j.ijpharm.2021.120712.
63. Sadeghi-Soureh, S.; Jafari, R.; Gholikhani-Darbroud, R.; Pilehvar-Soltanahmadi, Y. Potential of Chrysin-loaded PCL/gelatin nanofibers for modulation of macrophage functional polarity towards anti-inflammatory/pro-regenerative phenotype. *J. Drug Deliv. Sci. Technol.* **2020**, *58*, 101802.
64. Rezvani Ghomi, E.; Khosravi, F.; Neisiany, R. E.; Shakiba, M.; Zare, M.; Lakshminarayanan, R.; Chellappan, V.; Abdouss, M.; Ramakrishna, S. Advances in electrospinning of aligned nanofiber scaffolds used for wound dressings. *Curr. Opin. Biomed. Eng.* **2022**, *22*, 100393.
65. Raja, I. S.; Lee, S. H.; Kang, M. S.; Hyon, S. H.; Selvaraj, A. R.; Prabakar, K.; Han, D. W. The predominant factor influencing cellular behavior on electrospun nanofibrous scaffolds: Wettability or surface morphology. *Mater. Des.* **2022**, *216*, 110580.
66. Ijaola, A. O.; Akamo, D. O.; Damiri, F.; Akisin, C. J.; Bamidele, E. A.; Ajiboye, E. G.; Berrada, M.; Onyenokwe, V. O.; Yang, S. Y.; Asmatulu, E. Polymeric Biomaterials for Wound Healing Applications: A Comprehensive Review. *J. Biomater. Sci. Polym. Ed.* **2022**, *33*, 1998–2050. DOI: 10.1080/09205063.2022.2088528.
67. Deng, X.; Gould, M.; Ali, M. A. A Review of Current Advancements for Wound Healing: Biomaterial Applications and Medical Devices. *J. Biomed. Mater. Res.* **2022**, *110*, 2542–2573. DOI: 10.1002/jbm.b.35086.
68. Wang, F.; Hu, S.; Jia, Q.; Zhang, L. Advances in Electrospinning of Natural Biomaterials for Wound Dressing. **2020**, 1-14. DOI: 10.1155/2020/8719859.
69. Chanda, A.; Adhikari, J.; Ghosh, A.; Chowdhury, S. R.; Thomas, S.; Datta, P.; Saha, P. Electrospun Chitosan/Polycaprolactone-Hyaluronic Acid Bilayered Scaffold for Potential

Wound Healing Applications. *Int. J. Biol. Macromol.* **2018**, *116*, 774–785. DOI: 10.1016/j.ijbiomac.2018.05.099.
70. Shahrousvand, M.; Haddadi-Asl, V.; Shahrousvand, M. Step-by-Step Design of Poly (ε-Caprolactone) /Chitosan/Melilotus Officinalis Extract Electrospun Nanofibers for Wound Dressing Applications. *Int. J. Biol. Macromol.* **2021**, *180*, 36–50. DOI: 10.1016/j.ijbiomac.2021.03.046.
71. Zhou, F.; Cui, C.; Sun, S.; Wu, S.; Chen, S.; Ma, J.; Li, C. M. Electrospun ZnO-Loaded Chitosan/PCL Bilayer Membranes with Spatially Designed Structure for Accelerated Wound Healing. *Carbohydr. Polym.* **2022**, *282*, 119131. DOI: 10.1016/j.carbpol.2022.119131.
72. Chereddy, K. K.; Vandermeulen, G.; Préat, V. PLGA Based Drug Delivery Systems: Promising Carriers for Wound Healing Activity. *Wound Repair Regen.* **2016**, *24*, 223–236. DOI: 10.1111/wrr.12404.
73. Wan, X.; Zhao, Y.; Li, Z.; Li, L. Emerging polymeric electrospun fibers: From structural diversity to application in flexible bioelectronics and tissue engineering. *Exploration* **2022**, *2*, 20210029.
74. Sethuram, L.; Thomas, J.; Mukherjee, A.; Chandrasekaran, N. Eugenol Micro-Emulsion Reinforced with Silver Nanocomposite Electrospun Mats for Wound Dressing Strategies. *Mater. Adv.* **2021**, *2*, 2971–2988. DOI: 10.1039/D1MA00103E.
75. Li, C.; Luo, X.; Li, L.; Cai, Y.; Kang, X.; Li, P. Carboxymethyl Chitosan-Based Electrospun Nanofibers with High Citral-Loading for Potential anti-Infection Wound Dressings. *Int. J. Biol. Macromol.* **2022**, *209*, 344–355. DOI: 10.1016/j.ijbiomac.2022.04.025.
76. Tabakoglu, S.; Kołbuk, D.; Sajkiewicz, P. Multifluid Electrospinning for Multi-Drug Delivery Systems: Pros and Cons, Challenges, and Future Directions. *Biomater. Sci.* **2022**, *11*, 37–61. DOI: 10.1039/d2bm01513g.
77. Chen, J.; Zhang, G.; Zhao, Y.; Zhou, M.; Zhong, A.; Sun, J. Promotion of skin regeneration through co-axial electrospun fibers loaded with basic fibroblast growth factor. *Adv. Compos. Hybrid Mater.* **2022**, *5*, 1111.
78. Zhang, X.; Chi, C.; Chen, J.; Zhang, X.; Gong, M.; Wang, X.; Yan, J.; Shi, R.; Zhang, L.; Xue, J. Electrospun quad-axial nanofibers for controlled and sustained drug delivery. *Mater. Des.* **2021**, *206*, 109732.
79. Tavakoli, M.; Mirhaj, M.; Salehi, S.; Varshosaz, J.; Labbaf, S.; Golshirazi, A.; Kazemi, N.; Haghighi, V. Coaxial Electrospun Angiogenic Nanofiber Wound Dressing Containing Advanced Platelet Rich-Fibrin. *Int. J. Biol. Macromol.* **2022**, *222*, 1605–1618. DOI: 10.1016/j.ijbiomac.2022.09.109.
80. Wu, J.; Hong, Y. Enhancing Cell Infiltration of Electrospun Fibrous Scaffolds in Tissue Regeneration. *Bioact. Mater.* **2016**, *1*, 56–64. DOI: 10.1016/j.bioactmat.2016.07.001.
81. Khorshidi, S.; Solouk, A.; Mirzadeh, H.; Mazinani, S.; Lagaron, J. M.; Sharifi, S.; Ramakrishna, S. A review of key challenges of electrospun scaffolds for tissue-engineering applications. *J. Tissue Eng. Regen. Med.* **2016**, *10*, 715.
82. Norzain, N. A.; Lin, W. C. Fibroblast cell responses to physical cues of the triangular prism micropattern and aligned nanofibrous scaffold for promoting wound closure. *Mater. Des.* **2022**, *220*, 110864.
83. Wang, C.; Wang, J.; Zeng, L.; Qiao, Z.; Liu, X.; Liu, H.; Zhang, J.; Ding, J. Fabrication of Electrospun Polymer Nanofibers with Diverse Morphologies. *Molecules* **2019**, *24*, 834. DOI: 10.3390/molecules24050834.
84. Calzado-Delgado, M.; Olga Guerrero-Pérez, M.; Lun Yeung, K. A new versatile x–y–z electrospinning equipment for nanofiber synthesis in both far and near field. 123AD. DOI: 10.1038/s41598-022-08310-0.
85. Morel, A.; Guex, A. G.; Itel, F.; Domaschke, S.; Ehret, A. E.; Ferguson, S. J.; Fortunato, G.; Rossi, R. M. Tailoring the multiscale architecture of electrospun membranes to promote 3D cellular infiltration. *Mater. Sci. Eng. C* **2021**, 130. DOI: 10.1016/J.MSEC.2021.112427.

86. Chen, Y.; Dong, X.; Shafiq, M.; Myles, G.; Radacsi, N.; Mo, X. Recent Advancements on Three-Dimensional Electrospun Nanofiber Scaffolds for Tissue Engineering. *Adv. Fiber Mater.* **2022**, *4*, 959–986. DOI: 10.1007/s42765-022-00170-7.
87. Jiang, J.; Li, Z.; Wang, H.; Wang, Y.; Carlson, M. A.; Teusink, M. J.; MacEwan, M. R.; Gu, L.; Xie, J. Expanded 3D Nanofiber Scaffolds: Cell Penetration, Neovascularization, and Host Response. *Adv. Healthc. Mater.* **2016**, *5*, 2993–3003. DOI: 10.1002/adhm.201600808.
88. Hodge, J.; Quint, C. The improvement of cell infiltration in an electrospun scaffold with multiple synthetic biodegradable polymers using sacrificial PEO microparticles. *J. Biomed. Mater. Res. Part A* **2019**, *107*, 1954.
89. Hodge, J. G.; Quint, C. Improved Porosity of Electrospun Poly (Lactic-Co-Glycolic) Scaffolds by Sacrificial Microparticles Enhances Cellular Infiltration Compared to Sacrificial Microfiber. *J. Biomater. Appl.* **2022**, *37*, 77–88. DOI: 10.1177/08853282221075890.
90. Sadeghi-Avalshahr, A. R.; Nokhasteh, S.; Molavi, A. M.; Mohammad-Pour, N.; Sadeghi, M. Tailored PCL Scaffolds as Skin Substitutes Using Sacrificial PVP Fibers and Collagen/Chitosan Blends. *Int. J. Mol. Sci.* **2020**, *21*, 2311.
91. Gao, C.; Zhang, L.; Wang, J.; Cheng, Y.; Chen, Z.; Yang, R.; Zhao, G. *Mater. Sci. Eng. C* 2021. DOI: 10.1016/J.MSEC.2021.112542.
92. Jeckson, T. A.; Neo, Y. P.; Sisinthy, S. P.; Gorain, B. Delivery of Therapeutics from Layer-by-Layer Electrospun Nanofiber Matrix for Wound Healing: An Update. *J. Pharm. Sci.* **2021**, *110*, 635–653. DOI: 10.1016/j.xphs.2020.10.003.
93. Xu, H.; Zhang, F.; Wang, M.; Lv, H.; Yu, D. G.; Liu, X.; Shen, H. Electrospun Hierarchical Structural Films for Effective Wound Healing. *Biomater. Adv.* **2022**, *136*, 212795. DOI: 10.1016/j.bioadv.2022.212795.
94. Sadeghi, A.; Fatemi, M. J.; Zandi, M.; Bagheri, T.; Ghadimi, T.; Tamimi, M.; Pezeshki-Modaress, M. Multilayered 3-D Nanofibrous Scaffold with Chondroitin Sulfate Sustained Release as Dermal Substitute. *Int. J. Biol. Macromol.* **2022**, *206*, 718–729. DOI: 10.1016/j.ijbiomac.2022.03.061.
95. Sun, B.; Long, Y. Z.; Zhang, H. D.; Li, M. M.; Duvail, J. L.; Jiang, X. Y.; Yin, H. L. Advances in three-dimensional nanofibrous macrostructures via electrospinning. *Prog. Polym. Sci* **2014**, *39*, 862.
96. Su, Y.; Toftdal, M. S.; Friec, A. L.; Dong, M.; Han, X.; Chen, M. 3D Electrospun Synthetic Extracellular Matrix for Tissue Regeneration. *Small Sci.* **2021**, *1*, 2100003. DOI: 10.1002/smsc.202100003.
97. Lee, J. M.; Chae, T.; Sheikh, F. A.; Ju, H. W.; Moon, B. M.; Park, H. J.; Park, Y. R.; Park, C. H. Three dimensional poly(ε-caprolactone) and silk fibroin nanocomposite fibrous matrix for artificial dermis. *Mater. Sci. Eng. C* **2016**, *68*, 758.
98. Sheikh, F. A.; Ju, H. W.; Lee, J. M.; Moon, B. M.; Park, H. J.; Lee, O. J.; Kim, J. H.; Kim, D. K.; Park, C. H. 3D electrospun silk fibroin nanofibers for fabrication of artificial skin. *Nanomedicine Nanotechnol. Biol. Med.* **2015**, *11*, 681.
99. Chen, H.; Peng, Y.; Wu, S.; Tan, L. P. Electrospun 3D Fibrous Scaffolds for Chronic Wound Repair. *Materials* **2016**, *9*, 272. DOI: 10.3390/ma9040272.
100. Cho, S. H.; Kim, J. I.; Kim, C. S.; Park, C. H.; Kim, I. G. Harnessing the Topography of 3D Spongy-Like Electrospun Bundled Fibrous Scaffold via a Sharply Inclined Array Collector. *Polymers* **2019**, *11*, 1444.
101. Zhou, Y.; Hu, Z.; Du, D.; Tan, G. Z. The Effects of Collector Geometry on the Internal Structure of the 3D Nanofiber Scaffold Fabricated by Divergent Electrospinning. *Int. J. Adv. Manuf. Technol.* **2019**, *100*, 3045–3054. DOI: 10.1007/s00170-018-2899-4.
102. Ghosh, S.; Haldar, S.; Gupta, S.; Chauhan, S.; Mago, V.; Roy, P.; Lahiri, D. Single Unit Functionally Graded Bioresorbable Electrospun Scaffold for Scar-Free Full-Thickness Skin Wound Healing. *Biomater. Adv.* **2022**, *139*, 212980. DOI: 10.1016/j.bioadv.2022.212980.
103. Kim, T. G.; Chung, H. J.; Park, T. G. Macroporous and Nanofibrous Hyaluronic Acid/Collagen Hybrid Scaffold Fabricated by Concurrent Electrospinning and Deposition/

Leaching of Salt Particles. *Acta Biomater.* **2008**, *4*, 1611–1619. DOI: 10.1016/j.actbio.2008.06.008.
104. Juhasz, A. G.; Molnar, K.; Idrissi, A.; Jedlovszky-Hajdu, A. Salt induced fluffy structured electrospun fibrous matrix. *J. Mol. Liq.* **2020**, *312*, 113478.
105. Rafiq, M.; Khan, R. S.; Wani, T. U.; Rather, A. H.; Amna, T.; Hassan, M. S.; Rather, S.; Sheikh, F. A. Improvisations to electrospinning techniques and ultrasonication process to nanofibers for high porosity: Ideal for cell infiltration and tissue integration. *Mater. Today Commun* **2023**, *35*, 105695.
106. Ju, H. W.; Lee, O. J.; Lee, J. M.; Moon, B. M.; Park, H. J.; Park, Y. R.; Lee, M. C.; Kim, S. H.; Chao, J. R.; Ki, C. S.; et al. Wound Healing Effect of Electrospun Silk Fibroin Nanomatrix in Burn-Model. *Int. J. Biol. Macromol.* **2016**, *85*, 29–39. DOI: 10.1016/j.ijbiomac.2015.12.055.
107. Li, P.; Ruan, L.; Jiang, G.; Sun, Y.; Wang, R.; Gao, X.; Yunusov, K. E.; Aharodnikau, U. E.; Solomevich, S. O. Design of 3D Polycaprolactone/ε-Polylysine-Modified Chitosan Fibrous Scaffolds with Incorporation of Bioactive Factors for Accelerating Wound Healing. *Acta Biomater.* **2022**, *152*, 197–209. DOI: 10.1016/j.actbio.2022.08.075.
108. Chen, J.; Huang, Z.; Zhang, H.; Zhang, Z.; Wang, D.; Xia, D.; Yang, C.; Dong, M. *Chem. Eng. J.* **2022**, *443*, 136234. DOI: 10.1016/j.cej.2022.136234
109. Zhang, K.; Bai, X.; Yuan, Z.; Cao, X.; Jiao, X.; Li, Y.; Qin, Y.; Wen, Y.; Zhang, X. Layered Nanofiber Sponge with an Improved Capacity for Promoting Blood Coagulation and Wound Healing. *Biomaterials* **2019**, *204*, 70–79. DOI: 10.1016/j.biomaterials.2019.03.008.
110. Chen, Y.; Shafiq, M.; Liu, M.; Morsi, Y.; Mo, X. Advanced Fabrication for Electrospun Three-Dimensional Nanofiber Aerogels and Scaffolds. *Bioact. Mater.* **2020**, *5*, 963–979. DOI: 10.1016/j.bioactmat.2020.06.023.
111. Chen, S.; Wang, H.; Su, Y.; John, J. V.; McCarthy, A.; Wong, S. L.; Xie, J. Mesenchymal Stem Cell-Laden, Personalized 3D Scaffolds with Controlled Structure and Fiber Alignment Promote Diabetic Wound Healing. *Acta Biomater.* **2020**, *108*, 153–167. DOI: 10.1016/j.actbio.2020.03.035.
112. Li, Y.; Wang, J.; Qian, D.; Chen, L.; Mo, X.; Wang, L.; Wang, Y.; Cui, W. Electrospun fibrous sponge via short fiber for mimicking 3D ECM. *J. Nanobiotechnol.* **2021**, *19*, 1.
113. Fu, X.; Wang, J.; Qian, D.; Chen, Z.; Chen, L.; Cui, W.; Wang, Y. Living Electrospun Short Fibrous Sponge via Engineered Nanofat for Wound Healing. *Adv. Fiber Mater.* **2022**, 5, 979-993. DOI: 10.1007/s42765-022-00229-5.
114. John, J. V.; Sharma, S.; Tang, G.; Luo, Z.; Su, Y.; Weihs, S.; Shatil Shahriar, S. M.; Wang, G.; Mccarthy, A.; Dyke, J.; et al. Nanofiber Aerogels with Precision Macrochannels and LL-37-Mimic Peptides Synergistically Promote Diabetic Wound Healing. *Adv. Funct. Mater.* **2023**, *33*, 2206936.
115. Mohamed, E.; Wang, Y.; Crispin, P. J.; Fitzgerald, A.; Dahlstrom, J. E.; Fowler, S.; Nisbet, D. R.; Tsuzuki, T.; Coupland, L. A. Superior Hemostatic and Wound-Healing Properties of Gel and Sponge Forms of Nonoxidized Cellulose Nanofibers: In Vitro and In Vivo Studies. *Macromol. Biosci.* **2022**, *22*, 2200222.
116. Xiao, C.; Zhang, G.; Liang, W.; Wang, Z.; Lu, Q.; Shi, W.; Zhou, Y.; Guan, Y.; Lang, M. Preparation of green cellulose diacetate-based antibacterial wound dressings for wound healing. *Front. Mater. Sci.* **2022**, 16. DOI: 10.1007/s11706-022-0599-3.
117. Chang, T.; Yin, H.; Yu, X.; Wang, L.; Fan, L.; Xin, J. H.; Yu, H. 3D PCL/collagen nanofibrous medical dressing for one-time treatment of diabetic foot ulcers. *Colloids Surf. B Biointerfaces* **2022**, 214. DOI: 10.1016/j.colsurfb.2022.112480.
118. Tavakoli, M.; Mirhaj, M.; Varshosaz, J.; Salehi, S.; Mohanna, S. M.; Salehi, S.; Haghighi, V.; Kazemi, N.; Mehrjoo, M.; Shahriari-Khalaji, M. Asymmetric Tri-Layer Sponge-Nanofiber Wound Dressing Containing Insulin-like Growth Factor-1 and Multi-Walled Carbon Nanotubes for Acceleration of Full-Thickness Wound Healing. *Biomater. Adv.* **2023**, *151*, 213468. DOI: 10.1016/j.bioadv.2023.213468.
119. Tavakoli, M.; Mirhaj, M.; Varshosaz, J.; Al-Musawi, M. H.; Almajidi, Y. Q.; Danesh Pajooh, A. M.; Shahriari-Khalaji, M.; Sharifianjazi, F.; Alizadeh, M.; Labbaf, S.; et al.

Keratin- and VEGF-Incorporated Honey-Based Sponge–Nanofiber Dressing: An Ideal Construct for Wound Healing. *ACS Appl. Mater. Interfaces* **2023**, *15*, 55276–55286. DOI: 10.1021/acsami.3c11093.

120. Zhao, G.; Shi, L.; Yang, G.; Zhuang, X.; Cheng, B. 3D Fibrous Aerogels from 1D Polymer Nanofibers for Energy and Environmental Applications. *J. Mater. Chem. A* **2023**, *11*, 512–547. DOI: 10.1039/D2TA05984C.
121. Nair, M.; Best, S. M.; Cameron, R. E. Crosslinking Collagen Constructs: Achieving Cellular Selectivity Through Modifications of Physical and Chemical Properties. *Appl. Sci.* **2020**, *10*, 6911.
122. Xu, T.; Liang, Z.; Ding, B.; Feng, Q.; Fong, H. Polymer Blend Nanofibers Containing Polycaprolactone as Biocompatible and Biodegradable Binding Agent to Fabricate Electrospun Three-Dimensional Scaffolds/Structures. *Polymer* **2018**, *151*, 299–306. DOI: 10.1016/j.polymer.2018.07.074.
123. Pereira, A. L.; Semitela, Â.; Girão, A. F.; Completo, A.; Marques, P. A. A. P.; Guieu, S.; Fernandes, M. H. V. Three-dimensional nanofibrous and porous scaffolds of poly(ε-caprolactone)-chitosan blends for musculoskeletal tissue engineering. *J. Biomed. Mater. Res. Part A* **2023**, *111*, 950.
124. Uyar, M.; Cakmak, S. Three-dimensional macro/micro-porous curcumin releasing polycaprolactone/chitosan nanofiber scaffolds as wound dressing. *Colloids Surf. A Physicochem. Eng. Asp.* **2024**, *688*, 133573.
125. Li, J.; Xiao, L.; Gao, S.; Huang, H.; Lei, Q.; Chen, Y.; Chen, Z. Radial Sponges Facilitate Wound Healing by Promoting Cell Migration and Angiogenesis" , Advanced Healthcare Materials, 2023, 12(11), 2202737. .
126. Mirhaj, M.; Varshosaz, J.; Labbaf, S.; Emadi, R.; Marcus Seifalian, A.; Sharifianjazi, F. An Antibacterial Multi-Layered Scaffold Fabricated by 3D Printing and Electrospinning Methodologies for Skin Tissue Regeneration. *Int. J. Pharm.* **2023**, *645*, 123357. DOI: 10.1016/j.ijpharm.2023.123357.
127. Shahriari-Khalaji, M.; Sattar, M.; Cao, R.; Zhu, M. Angiogenesis, Hemocompatibility and Bactericidal Effect of Bioactive Natural Polymer-Based Bilayer Adhesive Skin Substitute for Infected Burned Wound Healing. *Bioact. Mater.* **2023**, *29*, 177–195. DOI: 10.1016/j.bioactmat.2023.07.008.
128. Pajooh, A. M. D.; Tavakoli, M.; Al-Musawi, M. H.; Karimi, A.; Salehi, E.; Nasiri-Harchegani, S.; Sharifianjazi, F.; Tavamaishvili, K.; Mehrjoo, M.; Najafinezhad, A.; et al. Biomimetic VEGF-loaded bilayer scaffold fabricated by 3D printing and electrospinning techniques for skin regeneration. *Mater. Des* **2024**, *238*, 112714.
129. Tavakoli, M.; Al-Musawi, M. H.; Kalali, A.; Shekarchizadeh, A.; Kaviani, Y.; Mansouri, A.; Nasiri-Harchegani, S.; Kharazi, A. Z.; Sharifianjazi, F.; Sattar, M.; et al. Platelet Rich Fibrin and Simvastatin-Loaded Pectin-Based 3D Printed-Electrospun Bilayer Scaffold for Skin Tissue Regeneration. *Int. J. Biol. Macromol.* **2024**, *265*, 130954. DOI: 10.1016/j.ijbiomac.2024.130954.
130. Radacsi, N.; Nuansing, W. *Fabrication of 3D and 4D Polymer Micro- and Nanostructures Based on Electrospinning*. In: 3D and 4D printing of polymer nanocomposite materials." Elsevier, 2020. p. 191-229. Amsterdam, Netherlands..
131. Vyas, C.; Ates, G.; Aslan, E.; Hart, J.; Huang, B.; Bartolo, P. Three-Dimensional Printing and Electrospinning Dual-Scale Polycaprolactone Scaffolds with Low-Density and Oriented Fibers to Promote Cell Alignment. *3D Print. Addit. Manuf.* **2020**, *7* 105.
132. Mondadori, C.; Chandrakar, A.; Lopa, S.; Wieringa, P.; Talò, G.; Perego, S.; Lombardi, G.; Colombini, A.; Moretti, M.; Moroni, L. Assessing the Response of Human Primary Macrophages to Defined Fibrous Architectures Fabricated by Melt Electrowriting. *Bioact. Mater.* **2023**, *21*, 209–222. DOI: 10.1016/j.bioactmat.2022.07.014.
133. Afghah, F.; Iyison, N. B.; Nadernezhad, A.; Midi, A.; Sen, O.; Saner Okan, B.; Culha, M.; Koc, B. 3D Fiber Reinforced Hydrogel Scaffolds by Melt Electrowriting and Gel Casting as a Hybrid Design for Wound Healing. *Adv. Healthc. Mater.* **2022**, *11(11), 2102068*. DOI: 10.1002/adhm.202102068.

134. Study on Regeneration of Skin Defects in Diabetic Ulcers Treated with New Electrospun Material PLCL/Fg | ClinicalTrials.gov. **n.d.** https://clinicaltrials.gov/study/NCT06014437?intr=Electrospun wound dressing&rank=1.
135. A Pilot Study to Evaluate a Temporary Skin Substitute (Spincare® Matrix) for Wound Healing in RDEB Patients | ClinicalTrials.gov. **n.d.** https://clinicaltrials.gov/study/NCT05944250?limit=100&intr=Electrospun wound dressing&rank=2.
136. EktoTherixTM Regenerative Tissue Scaffold for Repair of Surgical Excision Wounds | ClinicalTrials.gov. **n.d.** https://clinicaltrials.gov/study/NCT02409628?limit=100&intr=Electrospun wound dressing&rank=3.
137. Haier, J.; Schmidt, F. In vivo animal models in tissue engineering. In: Fundamentals of Tissue Engineering and Regenerative Medicine". Berlin, Heidelberg: Springer Berlin Heidelberg, 2009. p. 773-779.. *Tissue Eng. Regen. Med.*
138. Ahmad, N. In Vitro and In Vivo Characterization Methods for Evaluation of Modern Wound Dressings. *Pharmaceutics* **2022**, *15*, 42. DOI: 10.3390/pharmaceutics15010042.
139. Macewan, M.; Jeng, L.; Sallade, E. **2023**.
140. Morris, H. L.; Martins, J. A.; Lach, A. A.; Carr, A. J.; Mouthuy, P. Translational path for electrospun and electrosprayed medical devices from bench to bedside. Elsevier Ltd., Cambridge , UK. *Biomedical Applications of Electrospinning and Electrospraying*; Elsevier, **2021**; pp 423–454.
141. Uhljar, L. É.; Ambrus, R. Electrospinning of Potential Medical Devices (Wound Dressings, Tissue Engineering Scaffolds, Face Masks) and Their Regulatory Approach. *Pharmaceutics* **2023**, *15*, 417.
142. Choudhury, S.; Das, A. Advances in Generation of Three-Dimensional Skin Equivalents: Pre-Clinical Studies to Clinical Therapies. *Cytotherapy* **2021**, *23*, 1–9. DOI: 10.1016/j.jcyt.2020.10.001.
143. NANOFLUX – Electrospinning Equipment and Lab Scale Experimental Equipment. **n.d.** https://www.nanoflux.com.sg/en/.
144. Bioinicia. **n.d.** https://bioinicia.com/.
145. Nadetech Innovations | Nanotechnology Solutions at Your Fingertips. **n.d.** https://nadetech.com/.
146. Nanofibers Are the Future of Your Products | Elmarco. **n.d.** https://www.elmarco.com/.
147. Erich Huber. **n.d.** https://www.ehuber.de/.
148. SKE Research Equipment: Electrospinning/Electrospraing Technology. **n.d.** https://www.ske.it/.
149. Electrospinning Equipment | Nanofiber Machines | Inovenso. **n.d.** https://www.inovenso.com/.
150. Patel, Z.; Gharat, S.; Al-Tabakha, M. M.; Ashames, A.; Boddu, S. H. S.; Momin, M. Recent Advancements in Electrospun Nanofibers for Wound Healing: Polymers, Clinical and Regulatory Perspective. *Crit. Rev. Ther. Drug Carrier Syst* **2022**, *39*, 83.
151. SurgiCLOT® - St Teresa Medical. **n.d.** https://stteresamedical.com/technology/surgiclot.
152. RenovoDerm - Phoenix Wound Matrix - Wound Care Products. **n.d.** https://www.renovoderm.tech/.
153. Restrata: Acera Surgical Inc. **n.d.** https://acera-surgical.com/restratameshed/.
154. ChitoFib Patch - Nano Medical s.r.o. **n.d.** https://nanomedical.cz/en/chitofib-patch-en/.
155. Homepage - Engineered Tissue Solutions. **n.d.** https://engineeredtissue.com/.
156. DermaLayr - NanoLayr. **n.d.** https://www.nanolayr.com/product/dermalayr/.
157. matriheal. **n.d.** https://www.matriheal.com/en.html.
158. ACTIVHEAL®. **n.d.** https://activheal.com/granulating-tissue-wound-assessment/.
159. Lugoloobi, I.; Yuanhao, W.; Marriam, I.; Hu, J.; Tebyetekerwa, M.; Ramakrishna, S. Electrospun biomedical nanofibers and their future as intelligent biomaterials. *Curr. Opin. Biomed. Eng* **2022**, *24*, 100418.
160. Kajdič, S.; Planinšek, O.; Gašperlin, M.; Kocbek, P. Electrospun nanofibers for customized drug-delivery systems. *J. Drug Deliv. Sci. Technol* **2019**, *51*, 672.

161. Dong, Y.; Fu, S.; Yu, J.; Li, X.; Ding, B. Emerging Smart Micro/Nanofiber-Based Materials for Next-Generation Wound Dressings. *Adv. Funct. Mater* **2023**. DOI: 10.1002/adfm.202311199.
162. Liu, Y.; Luo, B.; Liu, H.; He, M.; Wang, R.; Wang, L.; Quan, Z.; Yu, J.; Qin, X. 3D printed electrospun nanofiber-based pyramid-shaped solar vapor generator with hierarchical porous structure for efficient desalination. *Chem. Eng. J.* **2023**, *452*, 139402.
163. Yuan, Z.; Ren, Y.; Shafiq, M.; Chen, Y.; Tang, H.; Li, B.; Newehy, M. E. L.; amshary, H. E. L.; Morsi, Y.; Zheng, H.; et al. Converging 3D printing and electrospinning: effect of poly (L-lactide)/gelatin based short nanofibers aerogels on tracheal regeneration. *Macromol. Biosci.* **2022**, 22. DOI: 10.1002/mabi.202100342.
164. Chen, W.; Xu, Y.; Li, Y.; Jia, L.; Mo, X.; Jiang, G.; Zhou, G. 3D printing electrospinning fiber-reinforced decellularized extracellular matrix for cartilage regeneration. *Chem. Eng. J.* **2020**, *382*, 122986.
165. Lei, F.; Liang, M.; Liu, Y.; Huang, H.; Li, H.; Dong, H. Multi-Compartment Organ-on-a-Chip Based on Electrospun Nanofiber Membrane as In Vitro Jaundice Disease Model. *Adv. Fiber Mater.* **2021**, *3*, 383–393. DOI: 10.1007/s42765-021-00091-x.
166. Chuchuy, J.; Rogal, J.; Ngo, T.; Stadelmann, K.; Antkowiak, L.; Achberger, K.; Liebau, S.; Schenke-Layland, K.; Loskill, P. Integration of Electrospun Membranes into Low-Absorption Thermoplastic Organ-on-Chip. *ACS Biomater. Sci. Eng.* **2021**, *7*, 3006–3017. DOI: 10.1021/acsbiomaterials.0c01062.
167. Sutterby, E.; Thurgood, P.; Baratchi, S.; Khoshmanesh, K.; Pirogova, E. Microfluidic skin-on-a-chip models: Toward biomimetic artificial skin.. *Small* **2020**, 16(39), 2002515. DOI: 10.1002/smll.202002515.