J. Innovative Eng. Nat. Sci., vol. 6, no. 1, pp. 133-145, 2026.
http://doi.org/10.61112/jiens.1756938

Review Paper

Journal of Innovative Engineering and Natural Science
(Yenilikçi Mühendislik ve Doğa Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/jiens

Advanced electrospun biomaterials for skin tissue engineering

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ARTICLE INFO

Article history:
Received August 2, 2025
Received in revised form October 11, 2025
Accepted November 25, 2025

Available online

Keywords:
Biomaterials
Electrospinning
Electrospun nanofibers
Skin tissue engineering
Scaffolds

ABSTRACT

Autografts, allografts or xenografts can be applied for treating damaged skin tissue, but these methods have certain disadvantages such as additional damage to donor regions, increased infection risk, and risk of disease transmission. On the other hand, tissue engineered skin substitutes provide more advantageous properties including large sources and good bioactivity. Tissue engineering is an interdisciplinary field of science that aims to develop tissue-engineered substitutes or tissue scaffolds in order to replace, repair, maintain, and regenerate the tissue functions. For producing functional tissue scaffolds for use in tissue engineering, different fabrication methods have been used by scientists. One such technique is electrospinning, which has been recognized as a promising method for creating microstructures that closely resemble the extracellular matrix of skin tissue. In this review article, the aim was first to provide information about skin tissue-related problems, current treatment methods, electrospinning method and its working principle, and to review the recent literature on the applications of electrospinning for use in skin tissue engineering.

I. INTRODUCTION

Skin is a tissue capable of preventing the body from losing water and electrolytes and shielding organs and tissues from external injuries. Wounds that are caused by burn injuries, diabetes, and other conditions alter the skin's appearance and physiological functions. For conventionally treating wounds, skin grafting techniques, including autografts, allografts, and xenografts are commonly employed. Although autografts do not trigger immunoreactions, these increase the risk of infection, cause further damage the donor site, and have a restricted supply. On the other hand, allografts and xenografts come from different origins, but they might transfer diseases and trigger immune responses [1]. At this point, tissue engineering can ensure alternative and innovative approaches for the treatment of skin damage by developing tissue-engineered skin grafts.

By imitating the physiological milieu, tissue engineering is recognized as a reliable technique to treat tissue damage [2, 3]. Furthermore, new developments in nanotechnology offer an opportunity to enhance the characteristics of tissue-engineered scaffold constructs [2, 4]. In tissue engineering, there are some scaffold production techniques such as self-assembly, phase separation, gas foaming, solvent casting, freeze-drying, and three-dimensional (3D) printing. Between these present methods, electrospinning shows promise for using in applications of tissue engineering. Electrospinning method involves using an electrostatic force between a collector and a polymer solution's droplet to create nanofibers [5].

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Non-woven meshes with fibers that range in diameter from tens of microns to tens of nanometers are created via electrospinning. Natural or synthetic materials may be used to eliminate potential risks associated with immunological responses or disease transmission. Moreover, synthetic polymeric materials generally allow greater control over mechanical features and degradation rates [6].

Within the scope of this review article, it was aimed to summarize the literature associated with the use of electrospinning for the applications of skin tissue engineering. For this reason, fundamental information on skin tissue properties, damage, and engineering was first presented. Then it was also aimed to explain the main principle of electrospinning as a scaffold fabrication technique. Furthermore, the materials used for producing electrospun skin tissue engineering scaffolds were also mentioned and some recent studies from related literature were also examined.

II. SKIN TISSUE PROPERTIES

With a surface area ranging from 16,000 to 18,000 cm^2, the skin is the biggest organ in the body of humans and makes up around 16% (5 kg) of the total body mass [7]. A normal and healthy skin tissue is known to regenerate nearly every 28 days [8].

Skin is recognized as an essential organ in the body's "first line of defense" system [9]. Skin acts as an effective barrier towards external harmful substances like ultraviolet rays, mechanical stressors, and microbiological pathogens [10]. The skin's roles include regulating body temperature, secreting and expelling substances having antibacterial and bactericidal qualities, and serving as an exterior barrier against external environment, toxins and infections [11, 12]. Every layer of skin has a distinct purpose, like acting as a barrier structure to prevent injuries, enhancing sensory awareness, synthesizing vitamin D, preventing dehydration, and also supporting the immune system [13]. Skin also helps to maintain bodily fluid balance and takes an important part in thermoregulation and immune defence. To ensure its proper functioning, many cell types—such as keratinocytes, melanocytes, fibroblasts, and endothelial cells—that make up the epidermis, dermis, and subcutis are required [10].

Skin tissue is composed of three different layers which are the epidermis, dermis, and hypodermis [14]. Since the epidermis lacks a blood supply of itself, it depends on the dermis' main papillary layer, which is made up of loosely connecting tissue, to take nutrients and oxygen [15]. In addition to delivering neuronal sensory receptors like Merkel cells that perceive and react to external stimuli, the dermis also controls water content and offers structural and mechanical assistance [16]. Lastly, the hypodermis situated underlying the dermis anchors the skin to underlying bone, muscle, and tendon tissue [17].

III. SKIN TISSUE DAMAGES

Among the leading causes of morbidity and mortality worldwide, skin abnormalities have significant socioeconomic impacts [18]. A wound is the result of a deterioration in the skin’s structural entirety. This may result from tissue rupture because of burns, traumas, congenital abnormalities, or illnesses that cause physical or mental suffering or even long-term damage [19]. Wounds could be categorized as acute or chronic wounds. Usually, acute wounds are defined as those that go through the regular healing phases and typically heal within four weeks. Chronic wounds, on the other hand, are defined as those that do not progress through normal healing

phases and do not start to heal after four weeks [20]. Acute wounds might arise from trauma, burns, or surgeries, whereas chronic wounds might be caused by pressure ulcers, venous ulcers, or diabetic foot ulcers [21].

After a damage occurs on skin tissue, it is necessary to reconstruct the structural integrity of skin for ensuring homeostasis, inflammation, cells migration, reproduction, and also maturation [14]. According to the information from the World Health Organization (WHO), it is reported that each year approximately 180,000 people die from burns (WHO, 2019). Even though the skin's ability to heal or regenerate itself is responsible for the majority of non-fatal wounds and burns, large lesions and chronic wounds can sometimes be non-healing and are thought to be a major cause of morbidity affecting lots of people (WHO, 2019) [22].

Skin tissue behaves as a protective barrier to chemical damage, microbial invasion, or mechanical trauma. Generally, defects or damages of skin tissue are results of serious tissue traumas or burns [23]. Scarring and limited availability of donor sites are the two primary challenges associated with skin transplantation for wound treatment [21]. Full-thickness skin damages or burns have traditionally been treated with autografts, allografts, and xenografts (Figure 1). These methods are restricted in supply, and they run the risk of triggering an immune response or spreading illnesses like primary graft constriction and failure [24]. Autografts are harvested from the same individual, whereas allografts are obtained from a donor of the same species [25]. Autologous skin grafts were the gold standard for wound closing for a long time. Nevertheless, patients with extensive wounds often lack sufficient donor tissue for autologous grafting, necessitating alternative approaches [26]. Moreover, the increasing prevalence of diabetes in the population has contributed to a rise in chronic wound cases [27]. Consequently, the treatment and management of wound-associated skin injuries represent a significant challenge for dermatologists and plastic surgeons [14].

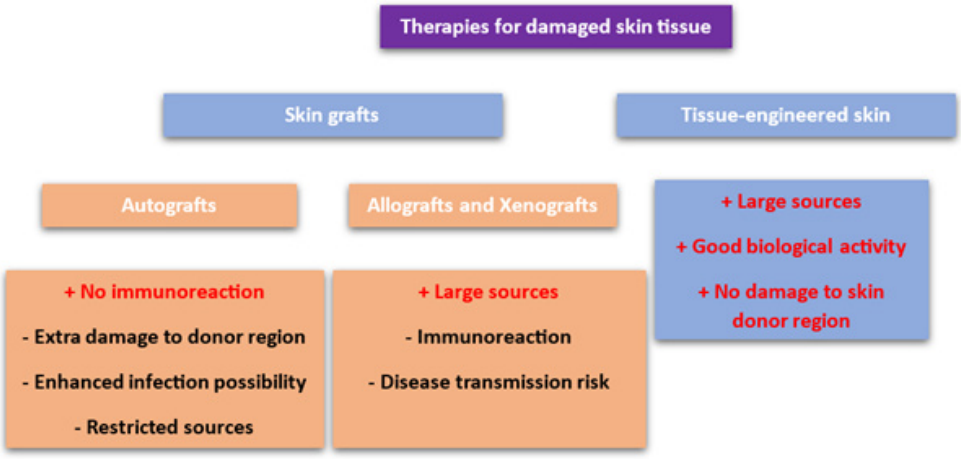

Figure 1. Different treatment techniques for damaged skin tissue, advantages and drawbacks of them [1]

IV. SKIN TISSUE ENGINEERING

Regenerative tissue engineering has become the gold standard field for developing artificial tissues and organ regeneration in order to address significant health challenges in people in the last few years [28]. The main goal of tissue engineering is to either create new, biocompatible alternatives for existing tissues or reconstruct the tissues in order to restore and enhance their function [28, 29].

The basic strategy of skin tissue engineering to solve the problem of identifying the most proper scaffold is to spotlight the shortcomings of autografting and allografting. Despite the fact that those techniques have been quite successful in treating lots of patients having skin disorders, these treatment ways still carry an important quantity of drawbacks [30]. Even though scientists are working to make autografting and allografting better, their attention is currently being directed toward the skin tissue engineering [30].

Skin tissue engineering is a field of science that purposes repairing, regenerating, and reconstructing the skin's structural and functional constituents, decreasing scar creation, and also enhancing the wound healing quality [21]. The main objectives of skin tissue engineering are the efficient healing and full simulation of physiological skin, being close to natural mechanical properties, as well as absence of host toxicity or immunological rejection issue [31].

Three distinct components are involved in tissue engineering of skin: the cells, the matrices like supporting structures, and the diverse growth factors commanding the growth of bioengineered tissue (Figure 2). Manipulating the cells, matrix materials, or the combination of them under in vitro conditions could be used for the manufacture of successfull skin substitutes [32].

Scaffolds are utilized in order to imitate the functions of native tissue but, to date, the functional and anatomical characteristics of the skin could not be precisely matched by a single material [21]. Scaffolds for skin tissue have two purposes: they behave like a wound dressing physical barrier towards external infection and ensure assistance to dermal fibroblasts and keratinocytes for regenerating tissue. In addition, a good scaffold should stop scarring and wound contraction [33].

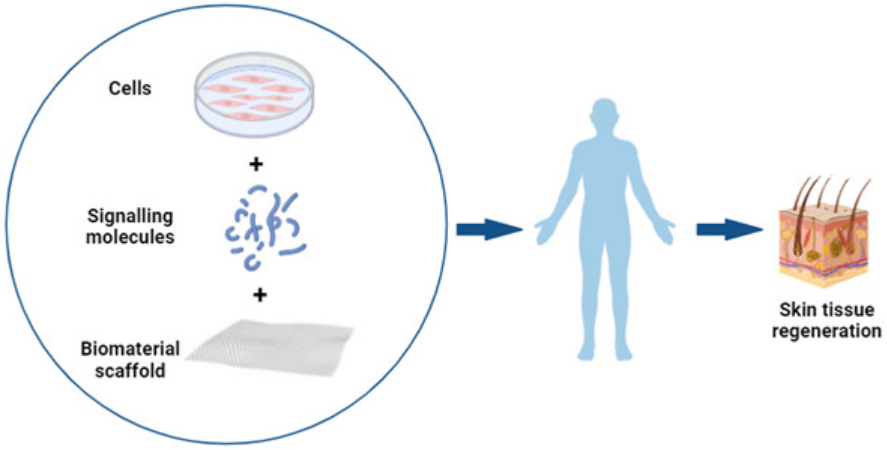

Figure 2. Basic representation of tissue engineering of skin

Tissue-engineered skin requires to: (a) ensure a barrier layer of replenishable keratinocytes, which are the cells that make up human skin's top barrier layer; (b) which is firmly affixed to the dermis underneath it; (c) be highly vascularized; and (d) offer the skin an elastic structural support [34].

Large-scale skin loss wounds must be adequately covered with a dressing to prevent infection [23]. Substitutes for tissue engineering have become a suitable, affordable, and easy way to repair injuries or deformities of the skin [35]. The optimal tissue engineering substitutes should protect the damage from protein loss, enhance the aesthetic

outlook of wound area, prevent invasion of exogenous microorganism, and imitate the roles of native skin [36]. An excellent dermal replacement material in the wound bed has to typically transmit antibacterial qualities, adjust gas/fluid change, alleviate wound pain, minimize scarring, and become biologically compatible and progressively biodegradable [37].

V. ELECTROSPINNING AS A SCAFFOLD FABRICATION METHOD

In tissue engineering, there are several scaffold production methods such as solvent casting and particulate leaching, freeze-drying, 3D printing, etc.. One of the most popular methods for creating high aspect-ratio nanostructures distinguished by great porosity, excellent surface area to volume ratio, and cross-sectional diameters changing from tens to hundreds of nanometers, is electrospinning [38].

Electrospinning can be described as a perfect methodology that uses electrohydrodynamic principles for producing nano or microfibers from a polymer melt or polymer solution [39]. Wide-ranging applications for electrospun nanofibers exist in a number of domains, including tissue templates, bone tissue engineering, filtration creation, drug transport, wound dressings, pharmaceutical components, medical prostheses, artificial organs, and sensing [23].

The fundamental electrospinning apparatus is composed of four main elements which are a high-voltage source that produces an electrical field among a positively-charged syringe needle and a grounded collector, a metallic needle in which the charged solution is enforced in order to stretch with the electrostatic forces, a syringe pump, and a grounded target for depositing the resulting fibers (Figure 3) [40]. The schematic representation of a basic electrospinning set-up is demonstrated in Figure 3. The metallic needle is connected to the high power source by electrical cables, which also keep the distance between the syringe tube and the target quite small [39]. Electrostatic repulsion forces cause the jet to be lengthen as the solvent evaporates during electrospinning process [39]. The next step is the thinning procedure, which causes to the production of a uniform or homogenous fiber structure at the micro- to nanoscale, and this fiber may be gathered in multiple orientations to produce some specialized constructions having distinct mechanical and compositional characteristics [39].

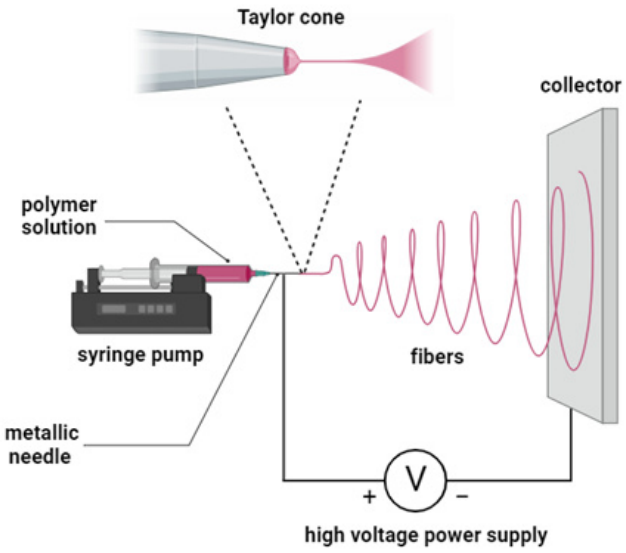

Figure 3. Schematic representation of electrospinning set-up. Figure is created with Biorender.com

In the process of electrospinning, between the needle and collector, a high-voltage electrical field is created by utilizing a power supply and electrodes [41]. When an enough amount of high voltage is given to the surface of polymer solution's droplet, this causes it to become charged and stretched [5, 42-45]. Following the slowly forcing out of polymer melt, a hemispherical polymer solution droplet is formed at the needle tip [46]. That droplet of polymer is elongated into a conical form, called as the Taylor cone, and the surface charge on the droplet of polymer enhances with time via enhancing voltage [47]. Electrical field force becomes greater than or overcomes the solution's cohesive force, frequently dominated via surface tension, and then an electrically charged jet of polymer melt or polymer solution is ejected [6]. As the jet approaches to the collector, the jet is stretched or elongated via electrostatic interplays of charges on adjacent parts of the same jet [6]. A polymer jet begins to develop instantly after overcoming the polymer droplet's surface charge, and following the vaporization of the solvent in jet of polymer, the surface charge on jet enhances, which impairs the stabilization of polymer jet [47]. To counteract the instability, the polymer jet is geometrically separated into two jets at first, and thereafter into many jets [48, 49]. Conventional electrospinning techniques produce extremely long fibers with diameters ranging from half to twice the average diameter along their length. These fibers could have a diameter that might be far greater than the nanometer range [6].

There are some parameters that affect the fabrication and properties of electrospun fibers throughout the electrospinning. These parameters involve working distance, voltage, flow rate, spinneret orifice, polymer concentration, viscosity & surface tension, collector geometry, solvent volatility, conductivity, dielectric constant, relative humidity, and temperature. For instance, via lengthening the fiber elongation or flight period throughout the procedure of electrospinning, thinner fibers might be fabricated, which could be performed via enhancing the range among collector and spinneret. For this reason, it is necessary to determine the optimum operating conditions and to optimize the different parameters for producing an effective scaffold for tissue regeneration [14].

It has been possible to effectively electrospun a variety of macromolecules into the ultrafine fibers. Electrospinning is a flexible technology that can generate continuous nanofibers from a large range of materials [39]. This technique permits the fiber diameter to be tuned from nanometers to microns scale [50]. Electrospun matrices with

micro/nanofibers offer a remarkably high specific surface area that may interplay with cells, making them perfect for cell adhesion and proliferation in comparison to other morphologies [23, 51, 52]. Furthermore, the electrospun membranes have a great pore interconnectivity and a highly porous 3D network, depending on how these micro/nanofibers are entangled [53, 54]. Those mats are good nominees for utilization in tissue engineering since they may accurately mimic the varied extracellular matrix in terms of texture and content (depending on the materials used) [23].

Compared with the traditional dressing materials like woven and non-woven gauzes and non-fibrous substances such as hydrogels, electrospun nanofibrous materials offer significant favorable properties like being dermal substitutes because they allow for the creation of structures that nearly similar to the skin's natural extracellular matrix, which allows cells to attach, multiply, infiltrate the scaffold, and induce neodermis regeneration [55, 56].

VI. MATERIALS FOR ELECTROSPUN SKIN TISSUE ENGINEERING SCAFFOLDS

Angiogenesis, gas exchange, moisture maintenance, and tissue mass transport are some important considerations for researchers when building skin scaffolds. Also, skin scaffolds should have the matching values for biodegradability rate and mechanical qualities with those of original skin [2, 18]. Nanofibers provide various benefits as skin substitutes since they imitate the skin's natural extracellular matrix, let oxygen permeation, allow removal of exudates from the wound area, restrict invasion of exogenous microorganisms, shield the wound from fluid loss, and increase the cosmetic look of the healed tissue [57, 58].

For the purpose of manufacturing a great construct mimicking the structural characteristics of native skin’s extracellular matrix, various types of electrospun matrix materials have been explored [23]. For skin healing, a variety of polymers, both synthetic and natural, have been electrospun into nanofibers [59]. Electrospun wound dressings may be classified into three categories: natural, synthetic or composite polymer types [60].

Particularly, natural polymers, including sodium alginate (SA), gelatin, silk fibroin, chitosan, hyaluronic acid, collagen and some others have received a high degree of interest because of their outstanding biological compatibility and good degradability, cost-effectiveness, and bioactivity [61-63]. Natural polymeric materials possess biological parts which could be specifically recognized via cell integrins derived from plants, inorganic materials, animals, as well as their derivatives. These can also encourage cells for adhesion, migration, reproduction, differentiation, and develop in a way that aids in wound healing [64].

Despite not having cell-binding parts, synthetic polymers often possess acceptable molding and mechanical characteristics [65]. When compared with the natural polymeric materials, synthetic ones show greater mechanical strength, processing resilience, and lesser biological feature [66]. Because of their favorable biodegradability and ease of processing, synthetic polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) have find widespread use in biomedical applications [67, 68].

In addition, to satisfy various purposes, a composite wound dressing can be produced by combining different types of polymers [65]. Many attempts have been made to create composite nanofiber membranes with perfect

bioactivity and customized mechanical characteristics by integrating the benefits of natural and synthetic polymers [69]. Through combination of synthetic polymers (like backbone substitute) and natural polymers (on the surface to increase cell attachment), composite fibrous constructs having optimum mechanical and compatibility features might be created for the usage in biomedical implementations [70, 71].

Several studies related to electrospun scaffolds or wound dressings for skin tissue engineering have been published [72-76]. Since SA, a natural polymer, intrinsically is not spinnable, it can be combined with other spinnable polymers to increase its potential for creating nanofibers. In a study from literature, the primary goal was to increase the spinnability of SA in conjunction with polyvinyl alcohol (PVA) [77]. It was aimed to enhance SA electrification ability utilizing PVA polymer in order to create a new electrospun SA/PVA scaffold that can support skin fibroblasts. According to the findings, the optimal SA:PVA ratio was 1:6.5, which was spinnable at several process parameter values. Under these circumstances, the manufactured scaffold contructs displayed good mechanical, chemical, biological, and physical characteristics. The outcomes showed that at 30 kV, 0.55 µL h^{-1}, and 12.50 cm, consistent and homogeneous nanofibers with a regular size distribution (166 nm) were acquired.

In a study conducted by Lotfi *et al.*, the electrospinning technique was used to create novel PCL/amniotic membrane (AM) scaffolds for skin regeneration [78]. Scanning electron microscope (SEM) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) tests revealed important cellular reproduction and viability on the suggested scaffolds, and these tests demonstrated that scaffolds with a higher amount of AM could achieve higher cell survival and adhesion. Furthermore, the existence of AM in the scaffolds led in keratinogenic differentiation of adipose-derived stem cells (ASCs) even in the absence of epidermal growth factor (EGF). As a result, it was recommended that the PCL-AM scaffold seeded with human ASCs appears to be an encouraging choice for the future skin bioengineering applications to cure skin abnormalities. This work demonstrated that the drawbacks of PCL including high hydrophobicity and poor cellular compatibility, can be addressed by combining AM at varying concentrations with PCL.

Electrospun nanofibers produced from natural or synthetic polymers or from composites of them, can also be modified by bioactive compounds. Choi *et al.* produced Spirulina extract-alginate PCL nanofibers by utilizing alginate, which contains hydrophilic structures that can hold significant amounts of water, in order to asist the nanofibers's backbone [79]. They analyzed an alginate/PCL nanofibrous wound dressing including Spirulina extract (Spi-Alg/PCL), which improved wound healing via enhancing moisturization of damaged skin, as well as biological safety and efficacy. Alginate increased the effectiveness of Spirulina PCL nanofibers in terms of moisture retention and adhesion capability, which had a significant impact on recovery in the rat skin wound model. It was reported that alginate impregnation enhanced the skin adhesiveness and hydration, accelerating wound healing while avoiding cytotoxicity [79].

In one study, Phulmogare et *al.* examined the impacts of enhancing the concentration of fucoidan (FD) on the properties of nanofibers and their capacity to heal wounds both in vivo and in vitro [80]. They were focused on the creation of PVA/ dextran (DEX) nanofibers through electrospinning, adding FD for wound dressing aims, and fully evaluating their wound healing capacity in vitro and in vivo. Enhancing FD amount (0.25 to 1%) resulted in substantial increases in nanofiber diameter (487.7 ± 125.39 to 627.9 ± 149.78 nm), entrapment efficacy (64.26 ± 2.6 to 94.9 ± 3.1%), and water uptake capabilities (436.5 ± 1.2 to 679.7 ± 11.3%). Nevertheless, the in vitro biodegradation profile reduced as FD concentration increased. Nanofibers containing 1% PVA/DEX/FD showed

slow-release behavior, recommending extended FD availability at the wound area. In vivo investigations in rats with full-thickness wounds showed that employing 1% FD-enriched PVA/DEX nanofibers dramatically increased wound area closure ($p < 0.0001$).

To obtain optimum skin scaffold and to mimic the structure of native skin, electrospinning technique can also be combined with other scaffold fabrication techniques. For instance, Pajooh et *al.* employed a combination of the 3D printing and electrospinning to create a biomimetic bilayer scaffold construct for regeneration of skin [81]. The scaffold's upper layer was built of 3D printed dextran-vascular endothelial growth factor (Dex-VEGF) for triggering angiogenesis and cell migration, while the scaffold's bottom layer was built of electrospun gelatin-keratin (Gel-Kr) nanofibers for inducing cell adhesion. The encouraging findings of that study highlight the developed bilayer scaffold as a promising material for speeding up wound healing [81].

VII. CONCLUSIONS

Treatments for skin injuries mainly include autografts, allografts, and xenografts. However, each of these grafting techniques has its own drawbacks. Therefore, current research has been focused on the development of tissue-engineered skin substitutes in recent years. Electrospinning, one of the commonly used scaffold fabrication methods, is considered a promising approach for producing microenvironments like skin extracellular matrix. This review article aims first to provide information about skin tissue-related problems, current treatment methods, electrospinning method and working principle. Then it aims to review recent literature on the applications of electrospinning in skin tissue engineering.

Natural and synthetic materials can be used to fabricate nanofibrous scaffold structures by electrospinning. Electrospun materials provide high surface area for cells to interact, adhere or proliferate. Therefore, nanofibers produced by electrospinning method can serve as favorable scaffold constructs for skin tissue engineering applications.

Based on the research and studies examined in this review article, it can be concluded that electrospun nanofibrous tissue scaffolds could serve as promising substitutes for skin tissue repair and regeneration. It is widely believed that by utilizing various natural and synthetic polymers in combination with electrospinning as a scaffold fabrication method, promising scaffolds can be developed for skin tissue engineering applications. However, to date, no known skin substitute can fully and completely reconstruct or replace the entire structure of native skin tissue. Future research is expected to enhance the development of tissue-engineered skin constructs generated through the electrospinning method. These advancements in electrospun structures will facilitate the production of more effecient materials for repairing and regenerating damaged skin tissue.

ACKNOWLEDGMENT

This work was also supported by Izmir Bakırçay University Scientific Research Projects Coordination Unit, under grant number BBAP.2024.010.

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