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Electrospun polymeric nanofibres as wound dressings: A review

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ABSTRACT

Skin wounds have significant morbidity and mortality rates associated. This is explained by the limited effectiveness of the currently available treatments, which in some cases do not allow the reestablishment of the structure and functions of the damaged skin, leading to wound infection and dehydration. These drawbacks may have an impact on the healing process and ultimately prompt patients' death. For this reason, researchers are currently developing new wound dressings that enhance skin regeneration. Among them, electrospun polymeric nanofibres have been regarded as promising tools for improving skin regeneration due to their structural similarity with the extracellular matrix of normal skin, capacity to promote cell growth and proliferation and bactericidal activity as well as suitability to deliver bioactive molecules to the wound site. In this review, an overview of the recent studies concerning the production and evaluation of electrospun polymeric nanofibrous membranes for skin regenerative purposes is provided. Moreover, the current challenges and future perspectives of electrospun nanofibrous membranes suitable for this biomedical application are highlighted.

Contents

1. Introduction

Electrospun nanofibres have been applied in the biomedical field, as drug delivery systems [1–3] as well as 3D constructs for tissue regeneration of cartilage [4], bone [5], heart valves [6,7], muscle [8,9], neural tissue [10] and skin [11,12] (see Fig. 1 for further details).

When a skin injury occurs, it is extremely important to re-establish, as quickly as possible, the skin's structure and functions

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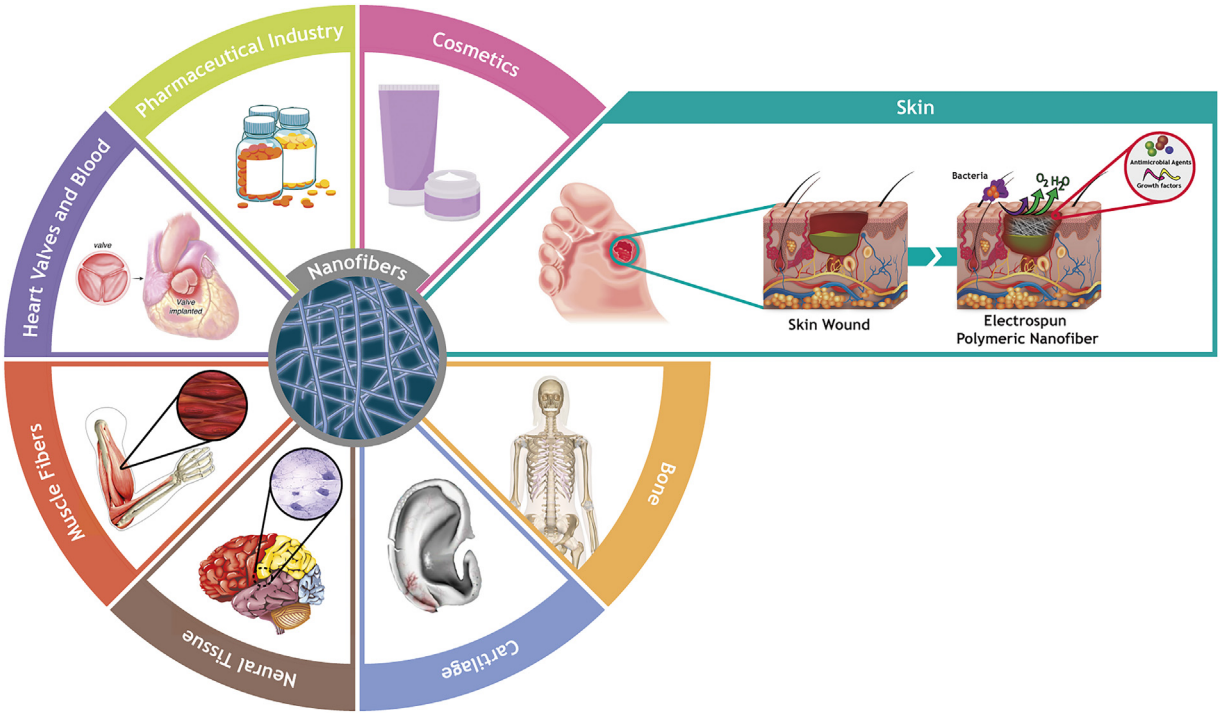

Fig. 1. Illustration of nanofibrous meshes applications in different fields of Biomedicine.

to assure the maintenance of the body's homeostasis. Although the skin exhibits self-regenerative capacity, some types of wounds do not heal as a consequence of extensive lesions and/or chronic wounds [13].

To overcome such drawbacks, new bioactive dressings have been developed or are under optimization to mimic the skin's native structure and are compatible with cell loading (keratinocytes, fibroblasts and stem cells). Depending on their capability to replace epidermis, dermis or both skin layers, they are grouped respectively as epidermal, dermal and epidermal-dermal substitutes [14]. Nonetheless, the associated production costs are high and the bioactive dressings are unable to fully re-establish all native skin features.

Different techniques, including self-assembly, phase separation and electrospinning have been used to produce micro to nano scale meshes aimed to be used as wound dressings [15]. Among them, electrospinning has captured the attention of researchers due to its simplicity, cost-effectiveness and versatility to produce nanofibrous membranes that are capable of mimicking the morphological characteristics of the skin's extracellular matrix (ECM). Moreover, these nanofibrous meshes are also able to support cell adhesion, migration, growth and differentiation as well as angiogenesis, which are vital events for the occurrence of an effective wound healing process [16–20]. In addition, bioactive molecules have also been incorporated into the electrospun nanofibres to improve the biologic performance of these membranes [21].

In the following sections of this review, an overview is provided of the recent studies concerning the production, surface functionalisation and evaluation of electrospun polymeric nanofibrous membranes performance in skin regeneration.

2. Electrospinning set-up used to produce electrospun nanofibrous membranes for the regeneration of skin

An electrospinning apparatus usually is comprised of a syringe pump, a capillary needle (the spinneret), a high-voltage power supply and a metal collector (see Fig. 2 for further details) [14]. During the electrospinning process, a high voltage is generated to produce an electrically charged jet of the polymeric solution that is directed to a collector by the electrostatic forces, resulting in the production of an interconnected fibrous membrane [14,22]. The features of the electrospun membranes are dependent on the properties of the precursor solution (e.g. conductivity, surface tension, viscosity and solvent selection), processing variables (e.g. flow rate, voltage, and the distance between the capillary and the collector) and environmental conditions (e.g. temperature and humidity) [16]. The control of these particular parameters has a direct impact on the mean diameter and arrangement of the produced nanofibres [23]. Moreover, the type of collector used has an impact on the arrangement and packing of the produced fibres, thus determining the morphological and mechanical properties of the electrospun fibres [24]. When the nanofibres are collected in a stationary collector, the membranes produced show a highly porous and randomly-orientated structure that mimics the 3D architecture of collagen fibres found within the ECM of normal skin [25,26]. To the contrary, nanofibrous membranes to be used for muscle and nervous tissue regeneration must display a specific orientation. To obtain meshes with these structural features, researchers are using rotating collectors, where the rotation speed of the collector has a direct effect on the diameter and the alignment of the produced fibres [24,27].

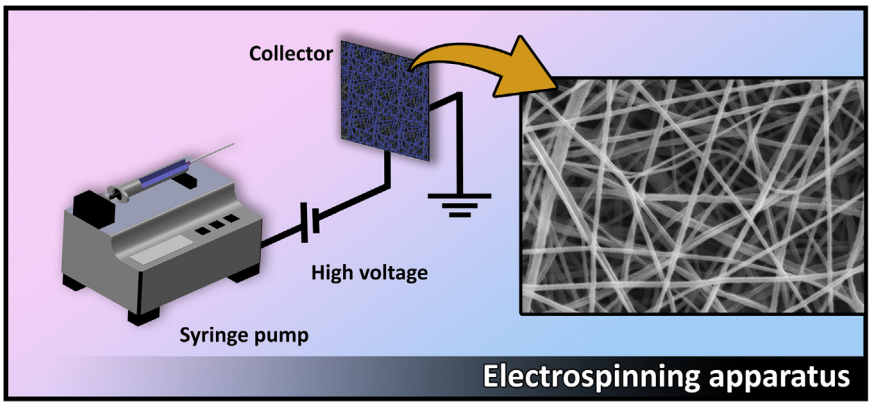

Fig. 2. Representation of the electrospinning setup, that is usually used to produce nanofibrous meshes.

Through the optimisation of the experimental set-up of this technique, researchers have been producing electrospun membranes with particular characteristics that allow them to confer protection to the wound against external contaminants and also display a 3D fibre mesh architecture that mimics skin ECM (as represented in Fig. 3). Furthermore, the high surface-to-volume ratio promotes cell attachment and the microscale interconnected pores are compatible with gas exchange, nutrient supply and control of fluid loss [28–30]. Such properties are vital for assuring the maintenance of a moist environment at the wound site to prevent wound dehydration and enhance angiogenesis and collagen synthesis [31]. Moreover, dressings composed of nanofibres can reduce scar formation, since the biodegradation of fibres provides a suitable roadmap for tissue healing [32,33].

3. Polymers used to produce electrospun nanofibres membranes

3.1. Synthetic polymers

Synthetic polymers have been used to produce electrospun nanofibres membranes. This type of polymers can be tailored to exhibit excellent mechanical properties, thermal stability and an appropriated degradation profile. In 2003, for the first time, Khil et al. used Polyurethane (PU) to produce nanofibrous membranes to be applied as skin substitutes [34]. Their results revealed that the PU membranes were able to control the water vapor transmission rate, displayed an excellent oxygen permeability, and presented fluid drainage ability. The assessment of the biologic performance of the membranes demonstrated their biocompatibility as well as their capacity to avoid exogenous microorganisms penetration into the wound. Moreover, *in vivo* data showed that, after 15 days of treatment, animals treated with PU-electrospun membranes displayed a well-organized dermis and granulation tissue [34]. Kumbar et al. produced Polylactide-polyglycolide (PLGA) nanofibres that were then seeded on the surface with human skin fibroblasts [35]. The results obtained showed that cells were able to spread, adhere and form multiple layers, after 28 days in culture.

However, in other studies, the hydrophobic character of the synthetic polymers used (e.g. Polycaprolactone (PCL) and Poly(glycolic acid) (PGA)), and the absence of peptide sequences on the materials' surface impaired cell adhesion and/or proliferation [36].

3.2. Natural polymers

To circumvent some of the limitations presented by synthetic materials, natural polymers became a viable option because of the availability of peptide sequences at their surfaces that can be recognized by cell surface receptors and, subsequently, trigger cell adhesion and proliferation [21,37].

In 2006, Rho et al. produced a Collagen nanofibrous matrix to be used as wound dressing, for the first time. Their results showed that this matrix exhibited a good tensile strength (≈7 MPa), high porosity and high surface area-to-volume ratio as well as features required for cell adhesion, growth and proliferation. Moreover, *in vivo* assays also demonstrated that collagen nanofibrous membranes were able to improve the healing process [38].

Hyung et al. used Silk Fibroin (SF) to produce nanofibrous membranes and evaluated their biologic performance in *in vivo* assays. The acquired data revealed that the produced SF nanomatrices were able to induce a higher rate of epithelialization and collagen production than commercially available dressings (e.g. Mediofoam® and medical gauze). Furthermore, the SF membranes produced were also able to modulate the concentration of inflammatory cytokines involved in wound healing (IL-10 and TGF-β1). This emphasizes the suitability of SF nanomatrices for the treatment of injured skin, i.e. they were able to decrease injury inflammation and reduce the wound healing period as well as scar formation [39].

Lin et al. produced a biocompatible nanofibrous membrane using Zein (ZN) and Collagen and then evaluated its capacity to be used in the treatment of full-thickness skin wounds induced in mice [40]. Nevertheless, the biodegradation rate and mechanical properties displayed by these natural materials was found to be restrictive, so their application may be avoided in the biomedical field [41].

3.3. Synthetic/Natural blend polymers

The blending of synthetic and natural polymers was also seen as a promising strategy to overcome the limitations of both synthetic and natural polymers. This approach combines the strength and durability of a synthetic polymer with the biocompatibility and bioactivity of natural polymers [42–44].

In 2004, Venugopal et al. evaluated the performance of electrospun matrices prepared with PCL and Collagen blends. These membranes were able to promote cell adhesion and proliferation where their PCL nanofibres counterparts could not. This result can be explained by the excellent intrinsic biological properties displayed by collagen [45]. Nonetheless, the collagen used in tissue engineering applications is usually obtained from animal sources, which is associated to the risk of disease transmission. Therefore, alternative materials have been considered, like gelatin which, despite being a cheaper collagen derivative (obtained through collagen denaturation), exhibits all the required biological properties. Duan et al. investigated the suitability of PCL/Gelatin electrospun membranes to be used as engineered epidermal skin grafts.

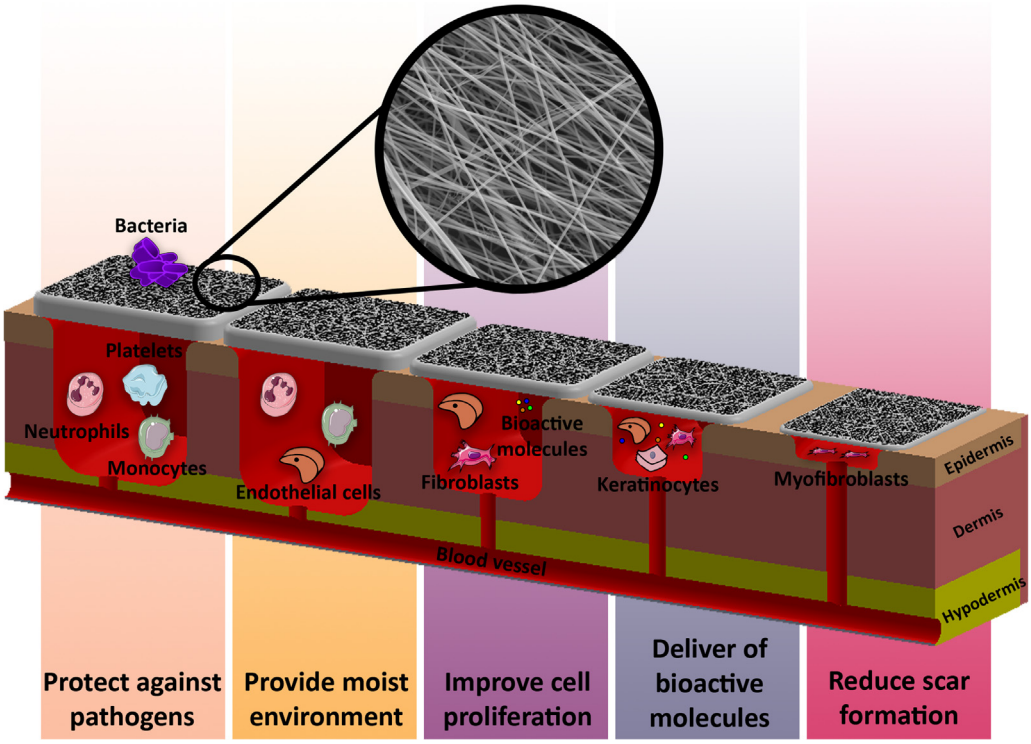

Fig. 3. Representation of the properties that electrospun membranes must display to be used as wound dressings.

Their results showed that HaCaT cells (a human keratinocyte cell line) were able to adhere and spread on the membranes' surface for at least 7 days. Furthermore, the *in vivo* assays demonstrated that the groups treated with PCL/Gelatin membranes exhibited an improved healing process, since the wound closure occurred in a shorter period of time [46]. In order to overcome the drawbacks associated with PCL (e.g. its hydrophobic character as wells as its low degradation profile), other researchers tested alternative materials to produce nanofibrous meshes, like Polyethylene oxide (PEO), Polyvinyl alcohol (PVA), Polylactic acid (PLA) and PLGA.

Zhou et al. produced a water-soluble carboxyethyl Chitosan (CS)/PVA blend to improve chitosan electro-spinnability. Subsequently, mouse fibroblast cells were seeded at the surface of the membranes produced and, after 48 h in culture cells, these were able to adhere and proliferate [47].

Gu et al. used a mixture of PLA and gelatin to produce electrospun meshes. These membranes were able to reduce water loss in wounds, which is essential for avoiding dehydration. Moreover, dermal fibroblasts remained viable, for at least 4 days, after seeded on the top of those membranes [48].

In another study, PLGA was combined with collagen to produce nanofibrous wound dressings that reproduce the native structure and biological function of the ECM of skin. Both *in vitro* and *in vivo* assays showed that PLGA/collagen membranes improve the healing process [49].

Suganya et al. combined poly(L-lactic acid)-co −poly(ϵ −caprolactone) (PLACL) with Aloe Vera (AV) and SF to produce electrospun meshes that promote dermal regeneration. Their results showed that the SF provides a favorable environment for cell adhesion and migration, while the AV components, mannose 6-phosphate and acemannan are known to promote epithelialization and the synthesis of collagen, processes that are essential for an effective healing process [50].

Besides the data available in the above published articles, there are also some patents focused on the application of electrospun membranes as wound dressings. Kataphinan et al. patented their production of a skin mask through the direct deposit of electrospun fibres onto the skin surface [51]. Smith et al. patented their production of electrospun fibres containing a pH-adjusting compound that prevents wound contamination and promotes the healing process [52]. Yarin et al. patented the use of the electrospinning technique for the production of a biodegradable plant-based wound dressing (composed of a biopolymers extracted from plants and synthetic polymers) [53].

Table 1 presents further examples of different electrospun membranes produced with natural, synthetic and blends of natural and synthetic polymers to be applied as skin substitutes.

4. Modification of the surface of electrospun polymeric nanofibres

The modification of a material's surface can be done by changing the topography or the functional groups available [63]. In order to improve the performance of electrospun polymeric nanofibres performance in skin regeneration, the surfaces have been chemically and physically modified with bioactive molecules and cell-recognizable ligands [64]. In the following sections, the most common surface modification techniques used for this purpose are presented.

4.1. Techniques used to functionalise a material's surface

As previously described, the use of synthetic polymers presents some limitations, due to their hydrophobic character and inability to encourage cell adhesion and proliferation [36]. As a possible solution, surface functionalisation techniques, which are represented in Fig. 4, like the wet chemical method, plasma treatment and graft polymerisation are usually applied [64].

The wet chemical method is based on the reaction under acidic or basic conditions, between the mesh and liquid reagents in order to add new chemical groups to the polymeric backbone [65]. Khorsand-Ghayeni et al. produced electrospun PLGA nanofibrous matrices and modified them by adding carboxyl and hydroxyl groups on their surface through alkaline hydrolysis, followed by a

Table 1
Nanofibrous meshes produced through electrospinning that are aimed to be used as wound dressings.

Polymers	Solvent	Cell line used in biocompatibility assays	Main findings	Ref
Carboxyethyl chitosan/PVA	Deionized water	Mouse fibroblasts (L929)	Biocompatible nanofibres were prepared using water soluble chitosan. *In vitro assays* showed that fibrous mats promote cell adhesion and proliferation.	[47]
Chitosan/arginine-chitosan	TFA:DCM	Human dermal fibroblasts	The modification of chitosan with L-arginine allowed the production of nanofibrous meshes able to improve the healing process and increased bactericidal activity.	[54]
CS/SF	HFIP:TFA	Mouse fibroblasts (L929)	Blended CS and silk fibroin nanofibrous membranes promoted cell attachment and proliferation.	[55]
CS/PVA	Deionized water for PVA;HOBt, TPP and EDTA for CS	Human foreskin fibroblast	CS/PVA membranes induce a reduction in wound size, during the first week after tissue damage.	[56]
Collagen	HFIP	Normal human oral keratinocytes	The electrospun collagen nanofibrous membranes were produced and characterized, for the first time, aimed to be used as wound dressings.	[38]
Collagen/ZN	AA	L9229 fibroblast cells	The ZN improved the electrospinnability of the blend and fibre tensile strength, while the collagen enhanced cell adhesion.	[40]
Gelatin/PU	HFIP	NIH3T3 fibroblast	The gelatin improved cell adhesion and proliferation, whereas PU allowed the production of elastic nanofibres.	[57]
Gelatin/PLLA	Acetic acid; DCM	WI-38 fibroblast	The electrospun gelatin/PLLA membranes showed controlled water loss, displayed fluid drainage ability, and an excellent biocompatibility.	[48]
Gelatin/PCL	TFE: acetic acid	HaCaT keratinocytes	Membranes exhibited good mechanical properties and did not elicit any toxic effect on cells.	[46]
PCL/SF/HA	HFP; Formic acid:HFP	FEK4 derived from a newborn foreskin explants	The incorporation of hyaluronic acid into nanofibrous scaffolds enhanced cell infiltration both *in vitro* and *in vivo*.	[58]
PCL/SF	THF and DMF	NIH3T3 fibroblasts	PCL/SF nanofibrous matrix provided favourable spatial cues, surface topography and chemistry for cell infiltration.	[59]
PCL/collagen	TFE	Human dermal fibroblast	Core-shell composite nanofibres improved cell-scaffold interactions.	[60]
PCL/ZnO	Acetone	Human dermal fibroblast	PCL/ZnO membranes showed excellent fibroblast cell attachment and good antimicrobial activity.	[61]
PLGA/collagen	HFIP	Human dermal fibroblasts	PLGA/collagen nanofibrous meshes improve the wound-healing process in an early stage.	[49]
PLGA/gelatin	Chloroform: acetone	Postnatal human fibroblasts	Hybrid scaffolds presented the desired bioactivity, hemostasis, and are also capable of encapsulate and perform a controlled release of EGF. Such properties highlight their potential to be applied in skin tissue engineering.	[21]
PCL_HA/CS_ZN	TFE; DMF; AA and EtOH	Human dermal fibroblasts	The produced bilayered electrospun membrane protect the wound as well as enhanced the wound healing process.	[11]
PLACL/SF/AV	DCM: DMF	Human dermal fibroblasts	The synergistic effect of AV and SF resulted in the production of the nanofibrous scaffolds with excellent properties to be used in skin tissue regeneration.	[50]
PCL/AV_CS	TFE; AA and water	Human dermal fibroblasts	The asymmetric membrane containing AV showed enhanced biological properties.	[12]
PLA/MWCNTs/REC	DCM:DMF	L9229 fibroblast cells	The incorporation of inorganic materials improved the thermal stability of the composite nanofibrous membranes. Moreover, these membranes exhibited a biocompatible profile.	[62]
SF	Formic acid	Oral keratinocytes, epidermal keratinocytes	SF nanofibres exhibited a pore size distribution, porosity and surface area-to-volume ratio favourable for cell attachment, growth and proliferation.	[28]

AA: Acetic acid; AV: Aloe Vera; CS: Chitosan; DCM: Dichloromethane; DMF: Dimethylformamide; EDTA: Ethylenediaminetetraacetic acid; EGF: Epidermal growth factor; EtOH: Ethanol; HA: Hyaluronic acid; HFP: Hexafluoro-2-propanol; HOBt: Hydroxybenzotriazole; MWCNTs: multi-walled carbon nanotubes; PCL: Polycaprolactone; PLGA: Poly Lactic-co-Glycolic Acid; PLA: Polylactic acid; PU: Polyurethane; PVA: Polyvinyl alcohol; REC: rectorite; SF: Silk fibroin; TFA: Trifluoroacetic acid; TFE: 2,2,2-Trifluoroethanol; THF: Tetrahydrofuran; TPP: Tripolyphosphate; ZN: Zein; ZnO: Zinc oxide.

collagen coating. Their results demonstrated that the surface functionalisation decreased the PLGA hydrophobicity, which is essential for this material to exhibit a wettability suitable for cell adhesion [66].

The plasma treatment of electrospun polymeric nanofibres has been commonly employed to tailor surface adhesion and optimise wetting properties by changing the surface chemical composition [67]. Depending on the type of plasma used (e.g. oxygen, ammonia, argon), diverse functional groups can be added to the polymer surface in order to improve the biocompatibility of a material [64]. Besides introducing functional groups, plasma treatments can also be used to control surface roughness and induce processes like crosslink formation, graft polymerisation and thin film coating of the polymeric surface [64,68]. Jeong et al. prepared electrospun SF nanofibres and then treated them with oxygen plasma to increase their hydrophilicity. The results obtained revealed a higher cell adhesion and proliferation, for both normal human epidermal keratinocytes (NHEK) and fibroblasts (NHEF), on the surface of functionalised nanofibrous membranes [69].

Graft polymerisation involves the covalent immobilisation of bioactive molecules at the nanofibre's surface to enhance cell adhesion, proliferation and differentiation [70]. In 2013, Gautam et al. fabricated a tri-polymer PCL/gelatin/collagen type I, by grafting collagen type I on electrospun PCL/gelatin mesh to improve fibroblast and keratinocyte cells adhesion and proliferation [71].

Graft polymerisation may require the use of initiators to start the grafting of a monomer on the membrane surface, like when using UV irradiation [72]. Plasma treatment is able, by itself, to

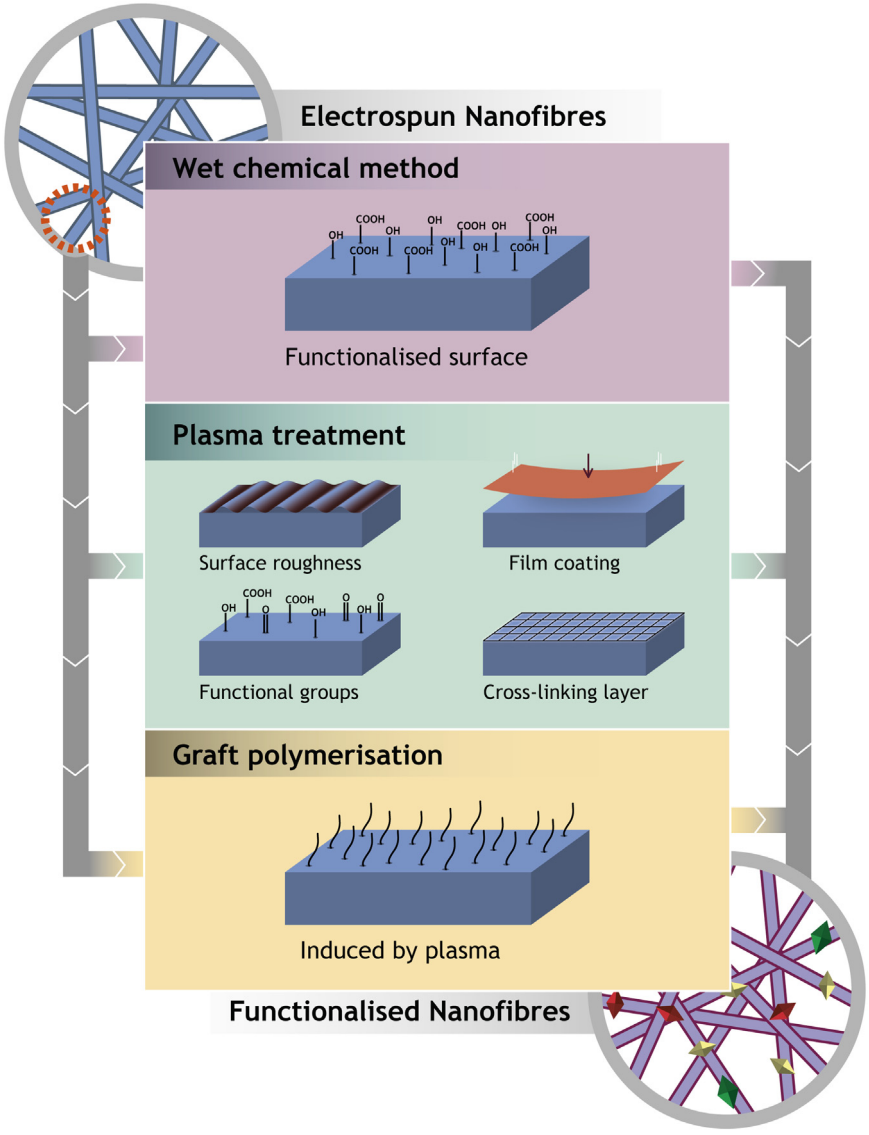

Fig. 4. Representation of the main surface modification techniques used up to now to improve the surface nanofibres properties: wet chemical method, plasma treatment and graft polymerisation.

generate free radicals on the polymeric matrix and therefore initiate the polymerisation reaction. Park et al. produced electrospun biodegradable nanofibrous meshes with PGA, PLLA and PLGA. These nanofibrous surfaces were chemically modified by *in situ* graft polymerisation of acrylic acid using oxygen plasma treatment. The results obtained revealed that the surface-modified membranes showed a significant improvement in cell attachment and proliferation, due to the incorporation of the hydrophilic functional groups [73].

4.2. Surface functionalised nanofibrous meshes to be used as drug delivery systems

A number of nanomaterials provide an excellent platform for local delivery of therapeutic agents due to their functionality and inherent nanoscale morphological characteristics [74,75]. The electrospun nanofibres display a high surface-to-volume ratio that can enhance the solubility of the drug and, consequently, improve its therapeutic effectiveness [76]. Multiple agents (antimicrobial agents, growth factors (GFs), etc.) have been incorporated into nanofibrous meshes by using blend, co-axial and emulsion electrospinning [64,77]. Other, post-electrospinning surface modifications techniques, like physical adsorption, layer-by-layer assembly and chemical immobilisation, have also been used. An overview of the techniques used for incorporating bioactive agents in nanofibrous meshes is provided in Fig. 5 and in the following sections.

4.2.1. Pre-electrospinning surface modification techniques

The blend electrospinning method involves the encapsulation of bioactive molecules that are dissolved or dispersed in the polymeric solution. Then, hybrid fibres are produced when this mixture is electrospun [78]. The encapsulation of the biomolecules within the fibres, assures their sustained release and also avoids the occurrence of an early burst release [79]. Li et al. mixed PVA with sodium alginate and organic rectorite (OREC) (which is recognized as a bacterial inhibitor) and then produced an electrospun nanofibrous mesh. The *in vitro* data showed that the OREC improved the bactericidal activity of the nanofibres [80]. Chouhan et al. developed non-mulberry SF based (NMSF) electrospun mats functionalised with EGF and ciprofloxacin to be used as wound dressings. The results obtained showed that NMS-based mats are biocompatible,

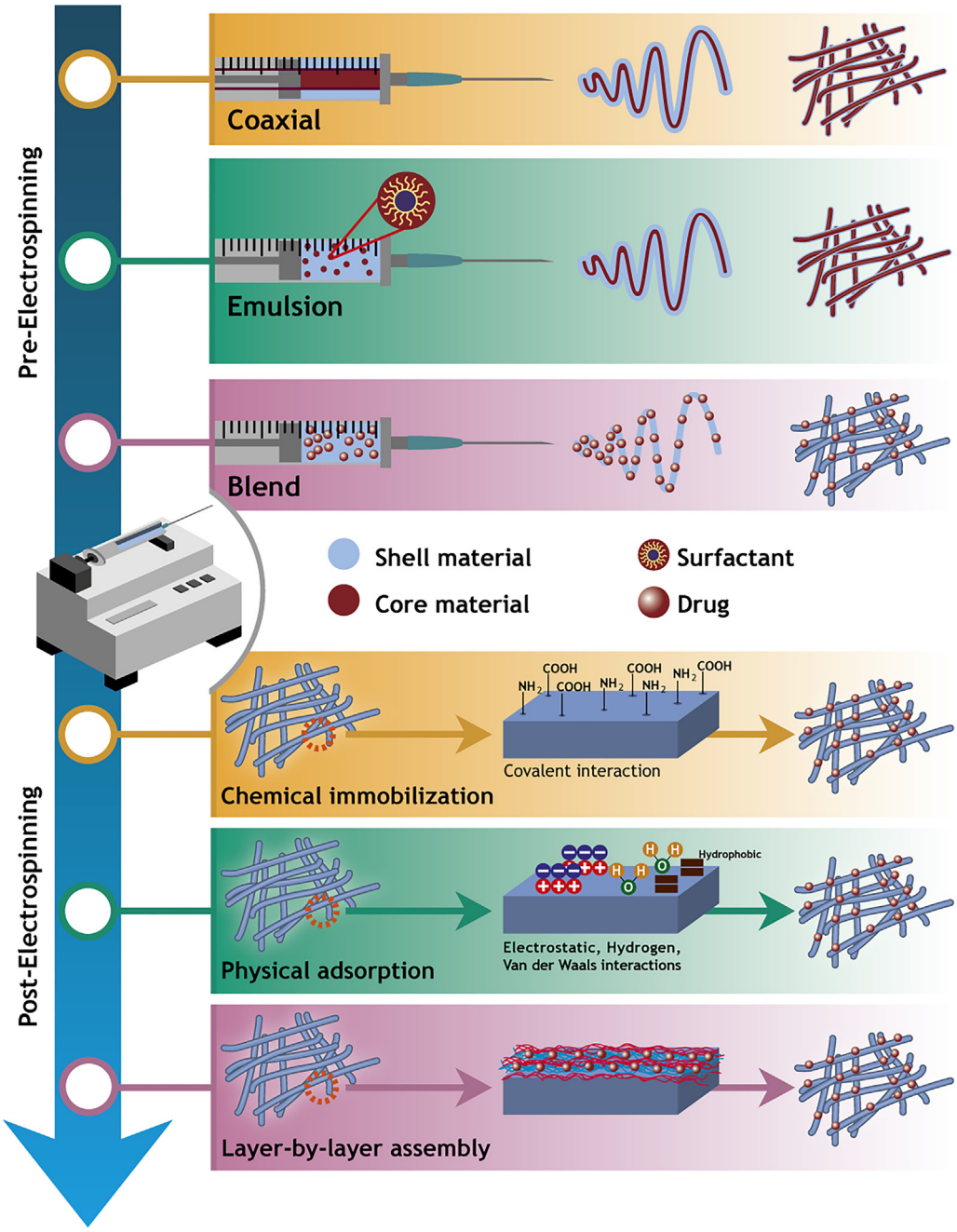

Fig. 5. Illustration of the surface modification techniques used to produce a carrier-based drug delivery nanofibres: pre-electrospinning (blend, co-axial and emulsion electrospinning) and post-electrospinning (physical adsorption, layer-by-layer assembly and chemical immobilization).

display antimicrobial activity and can perform a controlled drug release [81].

Despite the versatility of the blend electrospinning, one major disadvantage of the fibres fabricated with this methodology is the loss of function and activity of the incorporated biomolecules, which compromises the therapeutic effectiveness of the system [79]. To overcome this shortcoming, core-shell fibres have been produced through co-axial and emulsion electrospinning. This type of nanofibres displays an enhanced encapsulation efficiency of drug/bioactive molecules and avoids the direct contact of the biomolecules with the external environment, which is fundamental for unstable biological agents to maintain their biologic activity [82,83].

In co-axial electrospinning, different syringe pumps and coaxial needles are used to produce the core, where the bioactive components are usually loaded, and the shell that provides protection and assures a sustained release of the encapsulated molecules [82]. Maleki et al. compared the capacity of tetracycline hydrochloride-loaded PLGA core-shell nanofibres (produced with a co-axial electrospinning system) with blend fibres (prepared with the same materials) for a sustained drug release [84]. The core shell nanostructures revealed better results.

Emulsion electrospinning can also be used to manufacture core-shell nanofibres without requiring a specific needle setup. This method relies on a chemical separation through the creation of an emulsion within a single solution and the subsequent organisation of the emulsified droplets into two distinct phases, as the solvent evaporates from the electrospun fibres [85]. Wang et al. produced and EGF-loaded PCL/hyaluronan nanofibrous mesh, using an emulsion electrospinning technique. Their findings showed that the

resulting modified hyaluronan-based membranes can encapsulate and release EGF, which is fundamental for skin tissue engineering [86].

4.2.2. Post-electrospinning surface modifications techniques

A number of approaches have been employed to immobilize biomolecules or small cell recognition motifs onto the surfaces of electrospun polymeric nanofibres. Drug immobilization on the nanofibre's surface that is stronger and offers a more stable linkage can be achieved by physical immobilization through simple physical adsorption, layer-by-layer assembly or by chemical immobilization techniques [64].

Physical surface adsorption is the simplest approach for the preparation of surfaces with well-defined properties, and does not rely on chemical processing [87]. Generally, weak nonspecific intermolecular interactions (like those found in electrostatic interactions, hydrogen bonding, hydrophobic interactions and Van der Waals forces) are established between the surface and peptide sequences [88]. One strategy used for a successful physical immobilisation on the surface is based on the use of materials with a high surface area-to-volume ratio, as presented by electrospun polymeric nanofibres. The typical porous structure of these matrices results in a higher drug loading capacity per unit mass than for any other morphologies [64]. Casper et al. produced electrospun PEG nanofibres functionalised with low molecular weight heparin, a highly sulfated glycosaminoglycan that binds GFs, for applications in drug delivery and wound repair. The analysis of their results suggests the conclusion that this functionalisation method enables the binding of the basic fibroblast growth factor (bFGF) on the surface of PEG nanofibres [89].

Over the past few decades, layer-by-layer (LbL) assembly has attracted research attention due to its ability to exert nanometer control over film thickness and provide a simple, useful and versatile methodology for material surface modification [90]. Generally, LbL assembly is a cyclical process embracing the alternated depositing of polymers exhibiting opposite charges at their surface to formulate a coating of polyelectrolyte multilayers (PEMs) or free-standing film. The depositing process can be repeated until a multilayer film of the desired thickness is assembled [64,90,91]. In the assembly process, electrostatic attraction is the main driving force, although, hydrogen bonding, hydrophobic, covalent and biological interactions can also play a vital role [64]. This build-up can precisely control the composition, morphology and structure of the film [91].

In recent studies, LbL assembly has been used in a wide range of applications in the biomedical field, including wound healing. Huang et al. produced cellulose acetate nanofibrous mats that were used as a substrate for LbL films composed of positively charged chitosan derivative, HTCC (lysozyme (antibacterial agent)- N-[(2-hydroxy-3-trimethyl-ammonium) propyl]), and negatively charged sodium alginate. The results obtained showed that the average diameter of fibres increased with the number of coating bilayers applied. Moreover, the produced fibres exhibited antimicrobial activity [92]. Similarly, Xin et al. developed novel cellulose nanofibrous mats coated with SF (negatively charged) and lysozyme (positively charged). The *in vitro* and *in vivo* assays demonstrated that the mats can promote both healing in wounds and avoidance of wound infection [93]. Huang et al. reported the production of biomimetic nanofibrous matrices that were coated using LbL assembly of chitosan (positively charged) and Type I collagen (negatively charged). The LbL structured nanofibrous membranes displayed enhanced *in vitro* cell migration and promoted *in vivo* skin re-epithelialization and vascularisation. These results demonstrate the potential of LbL structured nanofibrous matrices to restore the structural and functional properties of skin [94].

Although the LbL assembly process is simple and mild, it can be affected by many factors such as the concentration and ionic strength of the polyelectrolyte solution as well as the pH, temperature, assembly time and molecular weight of the polymers used [91]. In addition, as the driving force is a result of electrostatic interactions, they are easily leached out, when incubated over an extended period, from the surface of the modified nanofibres. Therefore, chemical immobilisation of bioactive molecules is favoured over physical immobilisation [64].

Table 2
Electrospun membranes loaded with antimicrobial agents to be used as wound dressings.

Antimicrobial agents	Polymers	Incorporation technique	Ref.
Ampicillin	PCL; PMMA/nylon;	Blend; Co-axial	[109,110]
Amoxicillin	nano-HA/PLGA; PLGA; PCL	Blend; Nanoparticle incorporation;	[111–114]
Berberine	Collagen/ZN	Blend	[40]
Cefazolin	PLGA; Gel:CS/PEO	Blend; Nanoparticle incorporation	[115–117]
Cefoxitin	PLGA	Blend	[118,119]
Chitosan	PLA; PEO; SF; PCL; AV/PEO; sericin; pectin/organic rectorite	Blend; Layer-by-layer	[12,55,108,120,121]
Cinnamaldehyde	CS/PEO; PLA/β-CD;	Blend; β-cyclodextrin incorporation	[122,123]
Ciprofloxacin	Dextran/PU; PU; coPLA/PEG; PDEGMA/PLACL	Blend	[101,124,125]
Fusidic acid	PLGA	Blend	[126–128]
Gentamycin	PCL; CS	Co-axial; liposomes adsorption	[129,130]
Lysostaphin	Cellulose/CS;	Chemical immobilisation	[131]
Lysozyme	Cellulose acetate	Physical surface adosprtion	[132]
Mefoxin	PLGA; PDLA/PLA	Blend	[119,133]
Mupirocin	PLA	Blend	[134]
Plant extracts	PCL/PVP; PCL; PVA; PLA/HPG	Blend	[135–138]
Rifampicin	PCL; PLGA	Blend	[126,139]
Salicylic Acid	CS/ZN	Blend	[11]
Silver nanoparticles	Gel; CS/PVA; PVA/MTT; PMMA; PLLCL; CS; SF; PCL;PU	Adsorption; Chemical immobilisation; Blend	[98,99,140–142]
Streptomycin	PU/AC/Zein	Blend	[96]
Tetracycline	PEUU/PLGA; PVA/CS	Blend	[100,143,144]
Titania	PVAc; PU; PMMA; Nylon;	Blend; Chemical immobilisation	[145–148]
Zinc	SA/PVA	Blend	[149]

AC: Cellulose acetate; AV: Aloe vera; coPLA: Poly(l-lactide-co-d,l-lactide); CS: Chitosan; Gel: Gelatin; HPG: Hyperbranched polyglycerol; MTT: Montmorillonite; Nano-HA: Nano-hydroxyapatite; PCL: Polycaprolactone; PDEGMA: poly-(2-(2ethoxy)- ethoxy)methoxy methatcrylate; PDLA: Poly-D-lactide; PEG: Poly(ethylene glycol); PEO: Poly (ethylene oxide); PEUU: Poly(ester urethane) urea; PLA: Poly(lactic acid); PLACL: Poly(l-lactic acid-co-ε-caprolactone); PLGA: Poly Lactic-co-Glycolic Acid; PLLCL: Poly(l-lactic acid)-b-poly(ε-caprolactone); PMMA: Poly(methyl methacrylate); PU: Polyurethane; PVA: Polyvinyl alcohol; PVAc: Polyvinyl acetate; PVP: Polyvinylpyrrolidone; SA: Sodium alginate; SF: Silk fibroin; ZN: Zein; β-CD: β- Ciclodextrins.

In order to fabricate biomimetic materials that can withstand long-term survival, a stable covalent binding of functional biomolecules is required to maintain their bioactivity [87]. To accomplish that, chemical immobilisation of primary amine and carboxylate groups have been extensively employed to immobilise bioactive molecules onto the surface of nanofibres for wound healing [64]. In addition, hydrophilic linkers have also been used to bind bioactive molecules, which can be recognized by cells. For this purpose, Choi et al. produced PCL/PEG electrospun nanofibres functionalised with amine groups on their surfaces, using PEG linkers. Then, in order to use these nanofibrous mats in the treatment of diabetic foot ulcers, EGF was chemically bound to the surface of the meshes. The results obtained showed that the EGF functionalised nanofibres exerted a superior therapeutic effect on wound healing in comparison to control groups. In addition, EGF-receptor was highly expressed in keratinocytes, due to the stimulation exerted by the EGF nanofibre group, showing their suitability to be used as skin substitute [95].

5. Bioactive molecules incorporated into electrospun nanofibrous membranes

5.1. Antimicrobial agents

In wound care management, wound infections are a major concern since they delay the healing process, leading to disfigurement or even patient death [96]. To decrease the probability of a wound becoming infected, researchers are currently producing electrospun nanofibres functionalised with antimicrobial agents, such as nanoparticles, antibiotics and plant extracts (see information presented in Table 2). Silver nanoparticles (AgNPs) are the most common antimicrobial agent incorporated into this type of membranes. The antimicrobial activity exhibited by AgNPs against bacteria and fungi is attributed to the release of Ag^+ ions. These ions bind to thiol groups available on enzymes and cell surface proteins, causing bacteria membranes and cellular walls destabilisation or disruption. In 2008, Rujitanaroj et al. produced electrospun gelatin fibres mats loaded with AgNPs that displayed antibacterial activity against *Pseudomonas aeruginosa* (*P.aeruginosa*), *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*), and methicillin-resistant *S. aureus* (MRSA) [97]. Furthermore, Wang et al. functionalised PU/keratin nanofibrous mats surfaces with AgNPs and then evaluated their bactericidal activity using *E.coli* and *S.aureus*, as bacteria model. The results showed antimicrobial activity against both bacteria [98].

In 2012, Uttayarat et al. functionalised SF mats with AgNPs and tested their bactericidal activity against *S.aureus* and *P.aeruginosa* [99]. Their results demonstrated that the SF mats coated with low concentrations of AgNPs ($\leq$4 mM) exhibited similar antibacterial properties to those exhibited by commercially available wound dressings, which contain higher concentrations of ionic silver (Tegarderm™Ag and Aquacel®Ag).

Electrospun membranes may also exhibit antimicrobial activity by incorporating antibiotics within nanofibres structures. Liao et al. incorporated tetracycline hydrochloride within PCL/cellulose/dextran electrospun nanofibrous mats. The bactericidal activity of the membranes produced was evaluated against *S.aureus* and *E.coli*. The results revealed that only the samples loaded with the antibiotic displayed bactericidal activity [100].

Heyu Li et al. prepared poly (di(ethylene glycol) methyl ether methacrylate) (PDEGMA) and PLACL thermoresponsive electrospun fibre mats loaded with ciprofloxacin (commonly used for the treatment of skin infections). The *in vitro* assays demonstrated that the produced mats were able to inhibit *E.coli* and *S.aureus* growth. In turn, the *in vivo* assays highlighted that ciprofloxacin-loaded fibres resulted in better wound healing than commercially available gauzes [101].

Over the past decades, due to the limited number of antibiotics available, researchers have evaluated other materials that can be used as antimicrobial agents. Among them, CS, due to its intrinsic antimicrobial activity, emerged as a viable option [3,102–104]. CS antibacterial activity is attributed to the interactions established between the protonated amino groups of CS and the electronegative residues available on the surface of bacteria (lipopolysaccharides in gram-negative and teichoic acid/peptidoglycan in gram-positive) [105]. These interactions lead to the loss of membrane permeability and cell leakage and, ultimately, to the death of the cell [106]. Ignatova et al. produced CS/PLA electrospun membranes and reported that these membranes inhibit *S.aureus* and *E.coli* growth [107]. Zhao et al. produced an electrospun membrane composed of CS and sericin that did not display any cytotoxic effect for fibroblasts cells, while exhibiting an excellent antibacterial activity against *E.coli* and *Bacillus subtilis* [108].

Table 3
Examples of produced electrospun membranes loaded with growth factors to be used in skin regeneration.

Growth Factors	Polymers	Incorporation technique	Ref.
EGF	PLGA/AV	Emulsion	[160]
	PVA/SF	Blend	[81]
	PLGA	Blend	[161]
	PLGA/Gel	Emulsion	[21]
	PCL/Collagen	Chemical immobilisation	[162]
	PCL/Gel	Chemical immobilisation	[163]
	PVA/carbon nanotubes	Blend	[164]
EGF (BSA, insulin, T3)	PLGA/Collagen	Blend and emulsion	[165]
EGF (insulin, hydrocortisone, retinoic acid)	PLACL	Blend and co-axial	[157]
VEGF (BSA)	PLGA	Emulsion	[166]
VEGF, PDGF, bFGF,EGF	Collagen/HA/gelatin nanoparticles	Blend:EGF and bFGF; Gelatin nanoparticles: PDGF and VEGF	[158]
VEGF and PDGF	CS/PEO and PLGA nanoparticles	Blend: VEGF and PDGF:PLGA nanoparticles	[167]
VEGF and TGF-β3	PLGA	Adsorption	[168]
PDGF	EUP3/gelatin	Blend	[144]
bFGF	Polyplexes of PEI/PELA	Emulsion	[169]
	PELA	Emulsion	[29]
PRP (PDGF, TGF-β, VEGF, IGF, HGF)	CS/PEO	Blend	[159]

AV: Aloe vera; bFGF: Basic fibroblast growth factor; BSA: Bovine serum albumin; CS: Chitosan EGF: Epidermal growth factor; EUP3: Platelet-derived growth factor-BB binding polysaccharide; Gel: Gelatin; HA: Hyaluronic acid; HGF: Hepatocyte growth factor; IGF: Insulin-like growth factor; PCL: Polycaprolactone; PDGF: Platelet-derived growth factor; PEI: Polyethylenimine; PELA: Polyethylene oxide (PEO)/polylactic acid (PLA) block copolymers; PEO: Poly (ethylene oxide); PLA: Poly(lactic acid); PLACL: Poly(l-lactic acid-co-ε-caprolactone); PLGA: Poly Lactic-co-Glycolic Acid; PRP: Platelet-rich plasma; PVA: Polyvinyl alcohol; SF: Silk fibroin; TGF-β3: Transforming Growth Factor-β3; T3: Thyroid hormone triiodothyronine; VEGF: Vascular endothelial growth factor.

In 2015, Antunes et al. used deacetylated/arginine-modified CS to produce electrospun membranes. The membranes produced displayed an enhanced bactericidal activity against *E.coli* and *S.aureus*, due to the surface charge presented by arginine-modified CS. This property allowed these membranes to improve the regeneration of full-thickness wounds in comparison to electrospun membranes manufactured with non-modified CS [54].

5.2. Growth factors

The incorporation of GFs within the structure of electrospun nanofibrous membranes is another strategy that has been used to increase the performance of this type of membranes in the wound healing process (see Table 3). GFs stimulate angiogenesis, cell proliferation, differentiation, and the production of ECM components, events that are fundamental for skin regeneration [150]. Nonetheless, the free administration of GFs is not effective, since they rapidly disperse from the target site and suffer enzymatic degradation or deactivation. Therefore, the encapsulation of GFs within drug delivery systems can provide them with protection against *in vivo* degradation and maintain the required GFs concentration at the target site for extended periods, leading to improved skin tissue regeneration [151,152].

Electrospun nanofibres can be easily functionalised with GFs and, depending on the demands of the healing process, EGF, bFGF, VEGF and PDGF can be incorporated within the nanofibrous meshes [153–155]. Table 3 provides an overview of the GFs that have been added to electrospun nanofibrous membranes. Schneider et al. functionalised SF mats with EGF, which promoted cell proliferation and synthesis of ECM molecules, as well as angiogenesis and granulation tissue formation. The mats produced were able to increase the rate of wound closure by more than 3.5-fold in comparison to non-functionalised silk dressings [156]. In 2015, a core-sheath structure composed of PLGA/gelatin loaded with EGF was produced using emulsion electrospinning. The membranes produced were able to sustain the release of the GF for 9 days, which is essential for improving the healing process. Furthermore, the histological data obtained by the authors confirmed that the collagen synthesis was higher in the group treated with PLGA/gelatin/EGF electrospun membranes in comparison to the group treated with PLGA membranes. These results confirmed that the hybrid membranes displayed the bioactivity and hemostasis required for skin tissue engineering [21].

In another study, Jin et al. incorporated multiple epidermal induction factors (EIF) such as EGF, insulin, hydrocortisone and retinoic acid into a gelatin and PLACL solution. Then, they produced nanofibrous meshes using two different approaches: blend and core-shell spinning. Unlike blend fibres, the core-shell nanofibres produced were able to sustain the release of EIF, which contributed to increase the percentage of differentiated adipose-derived stem cells (ADSCs), which are known to reduce the wound size and enhance the re-epithelialization process [157].

Yang et al. produced nanofibrous membranes loaded with bFGF through emulsion electrospinning that were able to gradually release GF over the course of 4 weeks. A complete re-epithelization was achieved when the bFGF-loaded fibrous mats were applied on the dorsal wounds induced on diabetic rats [29].

Lai et al. manufactured a collagen/HA stacking nanofibrous skin equivalent loaded with multiple angiogenic GFs (VEGF, PDGF, bFGF and EGF), that were either directly embedded in the nanofibres or encapsulated within gelatin nanoparticles. Their results showed that bFGF and EGF (loaded into nanofibres) were released according to the demands of the initial phases of the wound healing process (hemostasis and inflammation phases), whereas VEGF and PDGF (encapsulated within gelatin nanoparticles and posteriorly within nanofibres) were released during the proliferation and remodelling phases of the healing process. This data suggested the authors' proposal for the future application of these membranes in the treatment of wounds [158].

Bertoncelj et al. incorporated Platelet-rich plasma (PRP) into CS/PEO electrospun nanofibres and evaluated its performance in the treatment of chronic wounds. Their *in vitro* results showed that CS/PEO nanofibres exhibited suitable properties to support the release of PRP at a rate that promotes keratinocyte and fibroblast cell growth [159].

6. Recent advances in the production of electrospun membranes for skin regeneration

Despite tremendous advancements, electrospun membranes still present some limitations for wound management, since they are unable to fully reproduce the structural features of native skin. In the next section are described two of the most recent approaches used to improve the wound healing process: production of asymmetric membranes and seeding of stem cells on nanofibrous meshes.

6.1. Electrospun asymmetric membranes

Recently, researchers have begun producing asymmetric membranes to reproduce skin anatomy and to further enhance the healing process. Usually, this type of membranes displays a dense and/or hydrophobic microporous top layer that prevents bacteria penetration as well as a macroporous bottom layer that allows the exudate absorption, gaseous exchange and cell migration/proliferation [170,171]. Wu et al. reported the production of nanofibrous asymmetric membranes, using polymeric self-assembly and electrospinning. Their results showed that the upper and hydrophobic layer (composed of hydrophobic β-glucan butyrate) was waterproof and breathable as well as capable of preventing bacterial penetration and controlling moisture evaporation, while the bottom layer (comprised of hydrophilic β-glucan acetate) exhibited good aqueous stability and swelling ratio and was capable of promoting the wound healing process [172]. Figueira et al. produced a bilayered electrospun membrane with a top layer made of PCL and HA. This layer displayed adequate mechanical properties, porosity and wettability that enabled it acts as a physical barrier against external threats to the wound site. ZN, CS and salicylic acid were also combined to produce the bottom layer. Due to their properties, this layer was able to promote human fibroblast adhesion, spreading and proliferation, while also avoiding the growth of microorganisms, reinforcing its suitability for wound healing [11].

Recently, Miguel et al. produced asymmetric membranes with PCL, CS and AV using an electrospinning apparatus. The top layer of the membrane was produced with PCL (in order to mimic the epidermis) while the bottom layer was produced with CS and AV. The results obtained revealed that the top layer has low porosity and excellent mechanical properties. The porous bottom layer, which reproduces the dermis layer structure, promoted fibroblast cell adhesion and proliferation, which are involved in the production of ECM components and play a pivotal role in the healing process. While the dense top layer avoids bacterial infiltration, the bottom layer, due to its composition, inhibits the growth of *S.aureus* and *E.coli* at the wound site [12].

6.2. Electrospun nanofibres membranes and cell engineering

Due to the crucial role played by cells in the healing process, recent studies report the use of different cell lineages (e.g. fibroblasts, keratinocytes, endothelial and stem cells) for the treatment of cutaneous wounds [173–177]. Among these, mesenchymal stem

cells (MSCs) have been widely used, since they are involved in almost all phases of wound healing and are able to stimulate new blood vessel formation, modulate the inflammatory response, promote the migration of keratinocytes and improving ECM production [178,179]. Furthermore, the use of stem cells derived from adipose tissue (ASCs) for regenerative purposes has also been explored by researchers, who find that these cells, which display a high potential for multilineage differentiation, can be obtained using minimally invasive procedures [180].

When stem cells are directly applied at the wound site, rapid cell death/clearance occur. To overcome this shortcoming, stem cells have been seeded on the surface of electrospun nanofibres surface. The ultrafine fibres of electrospun membranes that mimic the ECM topography promote stem cells survival and proliferation. Moreover, alignment of these fibres can control cellular arrangement and differentiation [181–184].

In 2011, Jin et al. produced electrospun nanofibrous using collagen and then seeded MSCs on the surface of the membranes. The results obtained confirmed that nanofibrous meshes promoted the differentiation of MSCs into epidermal cells [185]. The potential use of biomimetic nanofibre scaffolds, functionalised with bone-marrow-derived mesenchymal stem cells (BM-MSCs) for the treatment of acute full-thickness skin wounds, has also been evaluated by Ma et al. Their results demonstrated that enhanced healing was achieved with local delivery of BM-MSCs since these cells become differentiated into epidermal cells [186].

Additionally, Bayati et al. evaluated the effect of electrospun PCL fibres on ASCs differentiation into keratinocyte and on the healing process. The results obtained revealed an increased cell proliferation and an overexpression of the keratinocyte markers (such as cytokeratin 14, filaggrin and involucrin) [187].

Despite these promising results, cell infiltration into electrospun membranes continues to be limited since cells remain at the surface of the electrospun membranes. Currently, to surpass this limitation researchers are currently investigating the results of cell grafting, cell coaxial electrospinning and using layer-by-layer approaches [176,188].

7. Concluding remarks and future perspectives

In the last decades, tremendous progress has been achieved in the development of therapeutic approaches to be used in the treatment of wounds. Among the types wound dressings developed, electrospun membranes are regarded as one of the most efficient wound dressing materials, since they show morphological similarities with skin ECM, *i.e.* they display a high surface area to volume ratio as well as a porous structure that enhances homeostasis, exudate absorption, gas permeability and cell adhesion, migration and proliferation. Herein, insights concerning the recent advances attained in the production of polymeric electrospun nanofibres meshes to be used as wound dressings were provided. The functionalisation methods used to improve the surface properties of nanofibres or to produce nanofibres for carrier-based drug delivery, such as plasma treatment, LbL and grafting have also been described.

However, despite the recent achievements, further developments of nanofibrous meshes are required to improve the healing process. New asymmetric dressings and electrospun nanofibres loaded with stem cells are currently under development for this purpose. In a near future, other techniques, like 3D printing, may be combined with electrospinning to obtain 3D constructs that reproduce in further detail the structure and properties of the ECM of native skin. The incorporation of sensors into electrospun membranes may also impact the diagnostic and theranostic applications of these membranes. Finally, the combination of electrospun membranes with electrical stimulation, mechanical stress or pulsed magnetic field may also contribute toward improving the healing process.

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References

[1] N.T.B. Linh, Y.K. Min, et al., J. Biomed. Mater. Res. B Appl. Biomater. 95 (2010) 184–191.
[2] R. Sridhar, R. Lakshminarayanan, et al., Chem. Soc. Rev. 44 (2015) 790–814.
[3] X. Wang, B. Ding, et al., Mater. Today 16 (2013) 229–241.
[4] A. Shafiee, M. Soleimani, et al., J. Biomed. Mater. Res. A 99 (2011) 467–478.
[5] D. Naskar, A.K. Ghosh, et al., Biomaterials 136 (2017) 67–85.
[6] J. Du, T. Zhu, et al., Appl. Surf. Sci. (2018).
[7] P. Ferreira, P. Santos, et al., Eur. Polym. J. 97 (2017) 210–219.
[8] C.E. Cimenci, G. Uzunalli, et al., Acta Biomater. 60 (2017) 190–200.
[9] H. Peng, X. Liu, et al., J. Mater. Chem. B 2 (2014) 6435–6461.
[10] J. Hu, D. Kai, et al., Mater. Sci. Eng.: C 70 (2017) 1089–1094.
[11] D.R. Figueira, S.P. Miguel, et al., Int. J. Biol. Macromol. 93 (2016) 1100–1110.
[12] S.P. Miguel, M.P. Ribeiro, et al., Polymers 9 (2017) 183.
[13] R.F. Pereira, P.J. Bartolo, Adv. Wound Care 5 (2016) 208–229.
[14] R.F. Pereira, C.C. Barrias, et al., Nanomedicine 8 (2013) 603–621.
[15] K. Jayaraman, M. Kotaki, et al., J. Nanosci. Nanotechnol. 4 (2004) 52–65.
[16] D.I. Braghirolli, D. Steffens, et al., Drug Discovery Today 19 (2014) 743–753.
[17] N. Bhardwaj, S.C. Kundu, Biotechnol. Adv. 28 (2010) 325–347.
[18] Z.-M. Huang, Y.-Z. Zhang, et al., Compos. Sci. Technol. 63 (2003) 2223–2253.
[19] S. Zhong, Y. Zhang, et al., Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2 (2010) 510–525.
[20] S. Kumbar, R. James, et al., Biomed. Mater. 3 (2008) 034002.
[21] M. Norouzi, I. Shabani, et al., J. Biomed. Mater. Res. A 103 (2015) 2225–2235.
[22] S.S. Ray, S.-S. Chen, et al., RSC Adv. 6 (2016) 85495–85514.
[23] D. Sundaramurthi, U.M. Krishnan, et al., Polym. Rev. 54 (2014) 348–376.
[24] H. Pan, L. Li, et al., Polymer 47 (2006) 4901–4904.
[25] R. Vasita, D.S. Katti, Int. J. Nanomed. 1 (2006) 15.
[26] Q.P. Pham, U. Sharma, et al., Tissue Eng. 12 (2006) 1197–1211.
[27] T.J. Sill, H.A. von Recum, Biomaterials 29 (2008) 1989–2006.
[28] B.-M. Min, G. Lee, et al., Biomaterials 25 (2004) 1289–1297.
[29] Y. Yang, T. Xia, et al., Biomaterials 32 (2011) 4243–4254.
[30] M. Abrigo, S.L. McArthur, et al., Macromol. Biosci. 14 (2014) 772–792.
[31] P. Zahedi, I. Rezaeian, et al., Polym. Adv. Technol. 21 (2010) 77–95.
[32] Z. Peršin, M. Ravber, et al., Text. Res. J. 87 (2017) 444–459.
[33] A.J. Hassiba, M.E. El Zowalaty, et al., Nanomedicine 11 (2016) 715–737.
[34] M.S. Khil, D.I. Cha, et al., J. Biomed. Mater. Res. B Appl. Biomater. 67 (2003) 675–679.
[35] S.G. Kumbar, S.P. Nukavarapu, et al., Biomaterials 29 (2008) 4100–4107.
[36] M. Tallawi, E. Rosellini, et al., J. R. Soc. Interface 12 (2015) 20150254.
[37] E. Mele, J. Mater. Chem. B 4 (2016) 4801–4812.
[38] K.S. Rho, L. Jeong, et al., Biomaterials 27 (2006) 1452–1461.
[39] H.W. Ju, O.J. Lee, et al., Int. J. Biol. Macromol. 85 (2016) 29–39.
[40] J. Lin, C. Li, et al., ACS Appl. Mater. Interfaces 4 (2012) 1050–1057.
[41] J. Mano, G. Silva, et al., J. R. Soc. Interface 4 (2007) 999–1030.
[42] A. Sionkowska, Eur. Polym. J. 39 (2003) 2135–2140.
[43] E. Marsano, S. Vicini, J. Skopińska, M. Wisniewski, A. Sionkowska, Chitosan and poly (vinyl pyrrolidone): compatibility and miscibility of blends, Macromol. Symp. 218 (2004) 251–260.
[44] M. Piza, C. Constantino, et al., Polymer 44 (2003) 5663–5670.
[45] J.R. Venugopal, Y. Zhang, et al., Artif. Organs 30 (2006) 440–446.
[46] H. Duan, B. Feng, et al., Int. J. Nanomed. 8 (2013) 2077–2084.
[47] Y. Zhou, D. Yang, et al., Biomacromolecules 9 (2007) 349–354.
[48] S.-Y. Gu, Z.-M. Wang, et al., Mater. Sci. Eng.: C 29 (2009) 1822–1828.
[49] S.-J. Liu, Y.-C. Kau, et al., J. Membr. Sci. 355 (2010) 53–59.
[50] S. Suganya, J. Venugopal, et al., Int. J. Biol. Macromol. 68 (2014) 135–143.
[51] D. Smith, D. Reneker, W. Kataphinan, S. Dabney, Electrospun skin masks and uses thereof. Google Patents, The University Of Akron, (2001) WO2001026610 A1.
[52] Daniel J. Smith, Darrell H. Reneker, Albert T. McManus, Heidi L. Schreuder-Gibson, Charlene Mello Michael S. Sennett, Electrospun fibers and an apparatus therefor. Google Patents, The University Of Akron, (2004) US6753454B1.

[53] Alexander L. Yarin, Soumyadip Sett, Minwook Lee, Suman Sinha-Ray, Biodegradable plant wound dressing composed of electrospun nanofibers. Google Patents, University of Illinois, (2016) US20160219874A1.
[54] B. Antunes, A. Moreira, et al., Carbohydr. Polym. 130 (2015) 104–112.
[55] Z.-X. Cai, X.-M. Mo, et al., Int. J. Mol. Sci. 11 (2010) 3529–3539.
[56] N. Charernsriwilaiwat, T. Rojanarata, et al., Int. Wound J. 11 (2014) 215–222.
[57] S.E. Kim, D.N. Heo, et al., Biomed. Mater. 4 (2009) 044106.
[58] L. Li, Y. Qian, et al., Biomaterials 33 (2012) 3428–3445.
[59] J.M. Lee, T. Chae, et al., Mater. Sci. Eng.: C 68 (2016) 758–767.
[60] Y. Zhang, J. Venugopal, et al., Biomacromolecules 6 (2005) 2583–2589.
[61] R. Augustine, H.N. Malik, et al., J. Polym. Res. 21 (2014) 1–17.
[62] Y. Lu, X. Li, et al., RSC Adv. 4 (2014) 33355–33361.
[63] N. Hilal, M. Khayet, et al., Membrane Modification: Technology and Applications, CRC press, 2012.
[64] H.S. Yoo, T.G. Kim, et al., Adv. Drug Deliv. Rev. 61 (2009) 1033–1042.
[65] T.I. Croll, A.J. O'Connor, et al., Biomacromolecules 5 (2004) 463–473.
[66] M. Khorsand-Ghayeni, A. Sadeghi, S. Nokhasteh, A.M. Molavi, Collagen modified PLGA nanofibers as wound-dressing, The International Conference on Nanostructures (2016).
[67] J.M. Grace, L.J. Gerenser, J. Dispers. Sci. Technol. 24 (2003) 305–341.
[68] S. Yoshida, K. Hagiwara, et al., Surf. Coat. Technol. 233 (2013) 99–107.
[69] L. Jeong, I.-S. Yeo, et al., Int. J. Biol. Macromol. 44 (2009) 222–228.
[70] Z. Xu, L. Wan, et al., Surf. Eng. Polym. Membr. (2009) 80–149.
[71] S. Gautam, C.-F. Chou, et al., Mater. Sci. Eng.: C 34 (2014) 402–409.
[72] O. Burtovyy, V. Klep, et al., ACS Publ. (2009).
[73] H. Park, C. Cannizzaro, et al., Tissue Eng. 13 (2007) 1867–1877.
[74] S. Chen, B. Liu, et al., Nanomedicine 12 (2017) 1335–1352.
[75] F. Song, X. Li, et al., J. Biomed. Nanotechnol. 11 (2015) 40–52.
[76] İmren Esentürk, M. Sedef Erdal, Sevgi Güngör, Electrospinning method to produce drug-loaded nanofibers for topical/transdermal drug delivery applications, İstanbul Ecz. Fak. Derg. / J. Fac. Pharm. Istanbul 46 (2016) 49–69.
[77] L. Weng, J. Xie, Curr. Pharm. Des. 21 (2015) 1944–1959.
[78] C.B.J. Manuel, V.G.L. Jesús, et al., Electrospinning-Material, Techniques, and Biomedical Applications, InTech, 2016.
[79] P.-H. Kim, J.-Y. Cho, BMB Rep. 49 (2016) 26.
[80] W. Li, X. Li, et al., Carbohydr. Polym. 92 (2013) 2232–2238.
[81] D. Chouhan, B. Chakraborty, et al., Acta Biomater. 48 (2017) 157–174.
[82] Y.-N. Jiang, H.-Y. Mo, et al., Int. J. Pharm. 438 (2012) 232–239.
[83] I.C. Liao, S.Y. Chew, K.W. Leong, Aligned core–shell nanofibers delivering bioactive proteins, Nanomedicine 1 (2006) 465–471.
[84] M. Maleki, M. Latifi, et al., Polym. Eng. Sci. 53 (2013) 1770–1779.
[85] P. McClellan, W.J. Landis, BioRes. Open Access 5 (2016) 212–227.
[86] Z. Wang, Y. Qian, et al., J. Biomater. Appl. 30 (2016) 686–698.
[87] T.G. Vladkova, Int. J. Polym. Sci. (2010) 2010.
[88] C. Bellmann, Polymer Surfaces and Interfaces, Springer, 2008, pp. 235–259.
[89] C.L. Casper, N. Yamaguchi, et al., Biomacromolecules 6 (2005) 1998–2007.
[90] J.J. Richardson, M. Björnmalm, et al., Science 348 (2015) aaa2491.
[91] Q. Shi, Z. Qian, et al., Front. Physiol. 8 (2017) 574.
[92] W. Huang, X. Li, et al., Int. J. Biol. Macromol. 53 (2013) 26–31.
[93] S. Xin, X. Li, et al., J. Biomed. Nanotechnol. 10 (2014) 803–810.
[94] R. Huang, W. Li, et al., Biomaterials 53 (2015) 58–75.
[95] J.S. Choi, K.W. Leong, et al., Biomaterials 29 (2008) 587–596.
[96] A.R. Unnithan, G. Gnanasekaran, et al., Carbohydr. Polym. 102 (2014) 884–892.
[97] P.-O. Rujitanaroj, N. Pimpha, et al., Polymer 49 (2008) 4723–4732.
[98] Y. Wang, P. Li, et al., J. Mater. Chem. B 4 (2016) 635–648.
[99] P. Uttayarat, S. Jetawattana, et al., Fibers Polym. 13 (2012) 999–1006.
[100] N. Liao, A.R. Unnithan, et al., Colloids Surf. A: Physicochem. Eng. Asp. 469 (2015) 194–201.
[101] H. Li, G.R. Williams, et al., Int. J. Pharm. 517 (2017) 135–147.
[102] X. Hu, Y. Tang, et al., Carbohydr. Polym. 83 (2011) 1128–1133.
[103] F. Ding, H. Deng, et al., Nanoscale 6 (2014) 9477–9493.
[104] Q. Wang, Y.-M. Du, et al., Wuhan Univ. J. (Nat. Sci. Ed.) 6 (2003) 013.
[105] T.J. Silhavy, D. Kahne, et al., Cold Spring Harb. Perspect. Biol. 2 (2010) a000414.
[106] J.Y. Kim, J.K. Lee, et al., Int. J. Biol. Macromol. 32 (2003) 23–27.
[107] M. Ignatova, N. Manolova, et al., Macromol. Biosci. 9 (2009) 102–111.
[108] R. Zhao, X. Li, et al., Int. J. Biol. Macromol. 68 (2014) 92–97.
[109] H. Liu, K.K. Leonas, et al., J. Eng. Fibers Fabr. 5 (2010) 10–19.
[110] A. Sohrabi, P. Shaibani, et al., Polymer 54 (2013) 2699–2705.
[111] F. Zheng, S. Wang, et al., Biomaterials 34 (2013) 1402–1412.
[112] S. Wang, F. Zheng, et al., ACS Appl. Mater. Interfaces 4 (2012) 6393–6401.
[113] E. Valarezo, M. Stanzione, et al., J. Nanosci. Nanotechnol. 13 (2013) 1717–1726.
[114] P. Sofokleous, E. Stride, et al., Pharm. Res. 30 (2013) 1926–1938.
[115] D.S. Katti, K.W. Robinson, et al., J. Biomed. Mater. Res. B Appl. Biomater. 70 (2004) 286–296.
[116] G. Rath, T. Hussain, et al., Mater. Sci. Eng.: C 58 (2016) 242–253.
[117] Y. Fazli, Z. Shariatinia, Mater. Sci. Eng.: C 71 (2017) 641–652.
[118] X. Zong, S. Li, et al., Ann. Surg. 240 (2004) 910.
[119] K. Kim, Y.K. Luu, et al., J. Controlled Release 98 (2004) 47–56.
[120] G. Doğan, F. Özyıldız, et al., Int. Polym. Proc. 28 (2013) 143–150.
[121] S. Xin, X. Li, et al., Carbohydr. Polym. 92 (2013) 1880–1886.
[122] K.A. Rieger, J.D. Schiffman, Carbohydr. Polym. 113 (2014) 561–568.
[123] P. Wen, D.-H. Zhu, et al., Food Chem. 196 (2016) 996–1004.
[124] A.R. Unnithan, N.A. Barakat, et al., Carbohydr. Polym. 90 (2012) 1786–1793.
[125] Y. Choi, R. Nirmala, et al., Ceram. Int. 39 (2013) 4937–4944.
[126] S.E. Gilchrist, D. Lange, et al., J. Controlled Release 170 (2013) 64–73.
[127] S.S. Said, A.K. Aloufy, et al., Eur. J. Pharm. Biopharm. 79 (2011) 108–118.
[128] S.S. Said, O.M. El-Halfawy, et al., Eur. J. Pharm. Biopharm. 80 (2012) 85–94.
[129] Z.M. Huang, C.L. He, et al., J. Biomed. Mater. Res. A 77 (2006) 169–179.
[130] N. Monteiro, M. Martins, et al., Acta Biomater. 18 (2015) 196–205.
[131] J. Miao, R.C. Pangule, et al., Biomaterials 32 (2011) 9557–9567.
[132] W. Li, X. Li, et al., Carbohydr. Polym. 99 (2014) 218–225.
[133] X. Zong, K. Kim, et al., Polymer 43 (2002) 4403–4412.
[134] R. Thakur, C. Florek, et al., Int. J. Pharm. 364 (2008) 87–93.
[135] S. Suganya, T. Senthil Ram, et al., J. Appl. Polym. Sci. 121 (2011) 2893–2899.
[136] G. Jin, M.P. Prabhakaran, et al., Biomaterials 34 (2013) 724–734.
[137] L.M.M. Costa, G.M. de Olyveira, et al., Ind. Crops Prod. 41 (2013) 198–202.
[138] G. Perumal, S. Pappuru, et al., Mater. Sci. Eng.: C 76 (2017) 1196–1204.
[139] T.T. Ruckh, R.A. Oldinski, et al., J. Mater. Sci. 23 (2012) 1411–1420.
[140] A.M. Abdelgawad, S.M. Hudson, et al., Carbohydr. Polym. 100 (2014) 166–178.
[141] S.J. Lee, D.N. Heo, et al., Carbohydr. Polym. 111 (2014) 530–537.
[142] R. Augustine, N. Kalarikkal, et al., Appl. Nanosci. 6 (2016) 337–344.
[143] Y. Hong, K. Fujimoto, et al., Biomacromolecules 9 (2008) 1200–1207.
[144] A.C. Alavarse, F.W. de Oliveira Silva, et al., Mater. Sci. Eng.: C 77 (2017) 271–281.
[145] T. Amna, M.S. Hassan, et al., Appl. Microbiol. Biotechnol. 93 (2012) 743–751.
[146] S. Hwang, S. Jeong, J. Nanosci. Nanotechnol. 11 (2011) 610–613.
[147] L. Yan, S. Si, et al., Fibers Polym. 12 (2011) 207–213.
[148] H.R. Pant, D.R. Pandeya, et al., J. Hazard. Mater. 189 (2011) 465–471.
[149] K. Shalumon, K. Anulekha, et al., Int. J. Biol. Macromol. 49 (2011) 247–254.
[150] A.A. Ucuzian, A.A. Gassman, et al., J. Burn Care Res. 31 (2010) 158.
[151] J.S. Choi, H.S. Kim, et al., Drug Deliv. Ttransl. Res. 5 (2015) 137–145.
[152] W. Ji, Y. Sun, et al., Pharm. Res. 28 (2011) 1259–1272.
[153] M. Ignatova, I. Rashkov, et al., Expert Opin. Drug Deliv. 10 (2013) 469–483.
[154] P.P. Bonvallet, B.K. Culpepper, et al., Tissue Eng. Part A 20 (2014) 2434–2445.
[155] P. Selcan Gungor-Ozkerim, T. Balkan, et al., J. Biomed. Mater. Res. A 102 (2014) 1897–1908.
[156] A. Schneider, X. Wang, et al., Acta Biomater. 5 (2009) 2570–2578.
[157] G. Jin, M.P. Prabhakaran, et al., Eur. J. Pharm. Biopharm. 85 (2013) 689–698.
[158] H.-J. Lai, C.-H. Kuan, et al., Acta Biomater. 10 (2014) 4156–4166.
[159] V. Bertoncelj, J. Pelipenko, et al., Eur. J. Pharm. Biopharm. 88 (2014) 64–74.
[160] I. Garcia-Orue, G. Gainza, et al., Int. J. Pharm. 523 (2017) 556–566.
[161] E. Yüksel, A. Karakeçili, et al., Int. J. Biol. Macromol. 86 (2016) 162–168.
[162] M. Gümüşderelioğlu, S. Dalkıranoğlu, et al., J. Biomed. Mater. Res. A 98 (2011) 461–472.
[163] R.S. Tığlı, N.M. Kazaroğlu, et al., J. Biomater. Sci. Polym. Ed. 22 (2011) 207–223.
[164] J.L. Liao, S. Zhong, et al., Exp. Therapeut. Med. 14 (2017) 2341–2348.
[165] P. Peh, N.S.J. Lim, et al., Bioconjugate Chem. 26 (2015) 1348–1358.
[166] A. Rosa, D. Steffens, et al., Braz. J. Med. Biol. Res. (2017) 50.
[167] Z. Xie, C.B. Paras, et al., Acta Biomater. 9 (2013) 9351–9359.
[168] M.S. Lee, T. Ahmad, et al., Biomaterials 124 (2017) 65–77.
[169] Y. Yang, T. Xia, et al., Mol. Pharm. 9 (2011) 48–58.
[170] P.I. Morgado, A. Aguiar-Ricardo, et al., J. Membr. Sci. 490 (2015) 139–151.
[171] P.I. Morgado, P.F. Lisboa, et al., J. Membr. Sci. 469 (2014) 262–271.
[172] C. Wu, T. Chen, et al., Biomed. Mater. 11 (2016) 035019.
[173] A.M. Hocking, N.S. Gibran, Exp. Cell Res. 316 (2010) 2213–2219.
[174] M. Benderitter, P. Gourmelon, et al., Health Phys. 98 (2010) 851–857.
[175] K.L. Butler, J. Goverman, et al., J. Burn Care Res. 31 (2010) 874–881.
[176] J.A. Van Aalst, C.R. Reed, et al., Ann. Plast. Surg. 60 (2008) 577–583.
[177] C.H. Lee, H.J. Shin, et al., Biomaterials 26 (2005) 1261–1270.
[178] D. Tartarini, E. Mele, Front. Bioeng. Biotechnol. 3 (2016) 206.
[179] W.M. Jackson, L.J. Nesti, et al., Stem Cells Transl. Med. 1 (2012) 44–50.
[180] L. Frese, P.E. Dijkman, et al., Transf. Med. Hemother. 43 (2016) 268–274.
[181] N.J. Schaub, T. Britton, et al., ACS Appl. Mater. Interfaces 5 (2013) 10173–10184.
[182] A. Nandakumar, R. Truckenmüller, et al., Small 9 (2013) 3405–3409.
[183] H.N. Kim, Y. Hong, et al., Biomaterials 33 (2012) 8782–8792.
[184] X. Ren, Y. Han, et al., Acta Biomater. (2018).
[185] G. Jin, M.P. Prabhakaran, et al., Acta Biomater. 7 (2011) 3113–3122.
[186] K. Ma, S. Liao, et al., Tissue Eng. Part A 17 (2011) 1413–1424.
[187] V. Bayati, M.R. Abbaspour, et al., Anat. Sci. Int. 92 (2017) 509–520.
[188] A. Townsend-Nicholson, S.N. Jayasinghe, Biomacromolecules 7 (2006) 3364–3369.