Bioactive applications for electrospun fibers

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Abstract

Electrospinning is a versatile technique providing highly tunable nanofibrous nonwovens. Many biomedical applications have been developed for nanofibres, among which the production of antimicrobial mats stands out. The production of scaffolds for tissue engineering, fibres for controlled drug release or active wound dressings are active fields of research exploiting the possibilities offered by electrospun materials. The fabrication of materials for active food packaging or membranes for environmental applications is also reviewed. We attempted to give an overview of the most recent literature related with applications in which nanofibres get in contact with living cells and develop a nano-bio interface.

Keywords: Electrospinning, Biomedical applications, Tissue engineering, Wound dressing, Antimicrobial nanofibres, Membranes, Functional nanofibres

1. Introduction

Electrospinning is the only general technique available for the production of polymeric fibers below the micron scale. Being quite an old technique, it gained renewed interest in recent years with the increasing demand for nanotechnology and its focus on high surface-to-volume ratio and functionalized materials. The rediscovery of electrospinning comes in parallel with a boost in the number of works reporting composite nanofibers from a rich variety of materials. Nanofibres aligned and arrayed, smooth and porous, flat and randomly oriented, raw and highly functionalized nanofibers as well as forming more complex core/sheath nanostructures have been fabricated in order to cover a continuously growing spectrum of applications.

The materials used for electrospinning can be either synthetic or natural and may or not incorporate fillers inside the polymeric matrix in order to provide additional functionalities. The most common filler materials are inorganic nanoparticles (NP), which also exhibit high surface-to-volume ratio and provide synergistic properties to the composite that cannot be easily obtained from the individual components. Due to the very small dimension of nanoparticles, the co-electrospinning of nanofibers represents a natural method for producing blended fibers, but post-functionalization of polymeric scaffolds or producing core/sheath structures are other ways of incorporating nanoparticles to produce novel materials with new functionalities.

Recently, a number of medical uses of nanofibers have been gaining attention in applications such as drug delivery systems, scaffolds for tissue engineering, vascular grafts and wound healing tissues among other. Again, the reason for such an array of uses are the high surface area resulting from the nano-to-micro sized fibers, their high and tunable porosity and their chemical tunability. Additionally, certain nanofibers display a noteworthy capacity for incorporating substances such as metals or small molecules with biological activity as well as complex biological molecules. Electrospun nanofibers and their corresponding nanowebs have also displayed potential for food technology due to the fact that additives such as antimicrobials, antioxidants, essential oils, or even probiotics can be effectively encapsulated into electrospun nanofibrous matrixes. The incorporation of particles into electrospun polymer nanofibers has also been explored by researchers working in membrane technology and for the controlled delivery of chemicals in different air or water treatment technologies.

Relatively low cost equipment, simple basic operation and the promising possibility of large-scale nanofiber production, resulted in a rapid development of electrospinning technique with many papers describing laboratory scale applications. During the last years different modes of production of nanofibers have been extensively explored. Alternative geometrical modifications of the basic set-up equipment and a number of post-processing treatments have been proposed for achieving improved electric-field uniformity and enhanced control over inter-fibre positional ordering and intra-fibre molecular alignment. A large variety of materials and solvents have been combined in order to tailor specific properties and functionalities of electrospun products, even if not all

these achievements are easily transferable to industrial production.

The aim of this review is to summarize and the more recent research contributions to the electrospinning technique and the production of electrospun materials in cases in which a close interaction between living cells and nanofibres is pursued. It reports different applications emphasizing novel implementations in fields in which the bio-nano interface plays a significant role. During the following sections, the main innovations in biomedical, antimicrobial, food packing and environmental fields are outlined. New perspectives for electrospun materials are commented together with references to the introduction of "green electrospinning" products and processes and the alternative methods of scaling-up the production of nanofibers.

2. Electrospinning

2.1 Equipment

In a typical electrospinning set-up, a syringe pump dispenses a polymer melt or solution through a spinneret into a high voltage electric field formed between the spinneret and a grounded plate or collector. The electric field generates a charge build-up within the polymer phase, which causes the solution to adopt a cone-like shape, called Taylor Cone, pointing towards the collector. The fibers form when electrostatic repulsions overcome surface tension and accelerate the polymer liquid towards the grounded collector plate drawing a thin jet of fluid that whips into a fast moving spiral. During its way to the collector, the jet elongates by electrostatic repulsion and the solvent evaporates leaving a solid fibre. This phenomenon results in an intricate mesh of polymeric fibers on the collection plate referred to as mat [1, 2]. Depending on the goal pursued, a number of collector configurations can be used ranging from the simple stationary plate that produces randomly oriented fibre mats to a variety of rotating devices such as rotating drums, disks and mandrels, which allow creating a variety of aligned nanofibers. Rotating collectors are more complex to use because the rotation introduces a mechanical force that plays an important role in determining the degree of fibre anisotropy [3].

So far, more than fifty different polymers (and mixtures) have been successfully electrospun into ultra-fine fibres with diameters ranging from a few nanometres to hundreds of microns. Most of the polymers are dissolved in pure or mixed solvents, which determine the viscosity of the electrospun mixture and results in a complex hydrodynamic behaviour [4]. The polymer fluid is electrospun in a process essentially conducted at room temperature and atmospheric conditions, although generally within chambers having a certain kind of ventilation system in order to prevent the emission of solvent vapours. A DC voltage in the range of kV to tens of kV is applied to generate the necessary charge for the polymer jet to develop. It is remarkable that the same

polymer can be dissolved in different solvents giving rise to different kind of nanofibers [5].

Several alternatives to conventional needle-based electrospinning has been proposed in order to increase the low throughput of conventional single jets. Bubble electrospinning, blown-film methods and multiple pendant drop jets from porous tubes have been reported to be scalable and able to reach productions as high as 10 g/h [6, 7].

In bubble electrospinning, the bubbles create the curved surface analogous to the pendant drop of conventional electrospinning leading to a particularly suitable process for large scale production of nanofibers. Bubble technique involves the formation of multiple electrostatically driven jets of polymer from every charged bubble of polymer solution and, therefore, the system requires higher voltage than that used in conventional needle spinning [8]. Figure 1 shows a laboratory device for bubble electrospinning in which the fibers are collected on a negatively charged metallic collector positioned above the bubble widget [6].
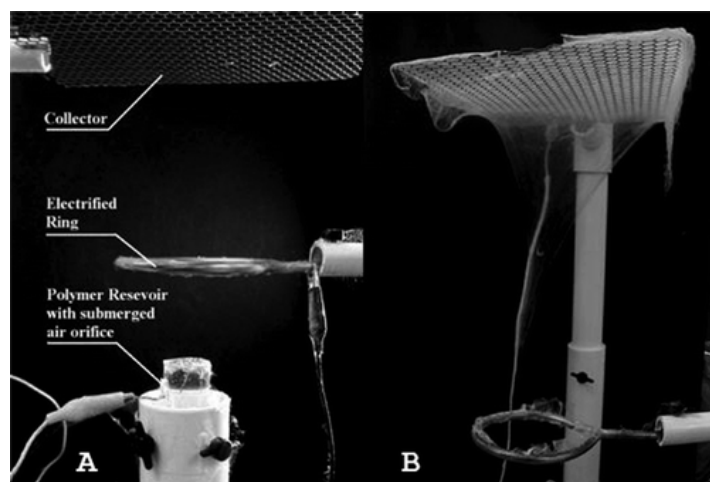

Fig. 1. (A) Experimental setup for bubble electrospinning. Air bubbles rise from the bottom of a polymer solution, and induce polymer jets towards the collector located on top. The electrified ring is used to drive fibers to the collector and air can be blown through it to minimize fibre interception and to twist fibres into a yarn. (B) Fibres are collected on the wire mesh collector [6].

2.2. Processing parameters

It is a well-known fact that electrospinning depends on a number of factors categorized into solution parameters, process parameters, and ambient parameters. All of them are directly or indirectly related to fibre morphology or processability and their correct manipulation allow producing a wide range of nanofibers with the desired morphology and diameter [2]. Solution parameters are viscosity, polymer concentration, polymer molecular weight, conductivity and surface tension. Generally speaking, fibre diameter increases with increasing viscosity and polymer concentration and with decreasing conductivity, while fibre beading can be avoided rising viscosity or using polymers with higher molecular

weight. High surface tension is undesirable as it is associated with jet instability. The main processing parameters are voltage, distance between tip and collector and feed rate. Fibre diameter decreases using high voltage and low feed rate. A too low feed rate, however, results in fibre beading, which is also avoided using a sufficient distance between tip and collector. Conversely, there is a minimum distance required for uniform fibers. Ambient conditions strongly influence the properties of electrified jets and the resulting electrospun materials and even small environmental perturbations can cause significant variations on fibre properties. The two generally controllable ambient parameters are humidity and temperature. Excess humidity results in fibre pores (if desired, pores can be introduced on purpose using combinations of solvents with different volatility) and too high temperature leads to decreased fibre diameter. The optimization of these parameters is complex because of the difficulty of individually manipulating them in larger scale devices and because their influence is different from one polymeric system to another. Even if the accurate control of ambient conditions is difficult, a minimum fine tuning is a prerequisite for obtaining electrospun fibers with the required characteristics of shape and size. Several firms market laboratory-scale

systems ensuring $\pm$ 0.5ºC accuracy in temperature control and $\pm$ 1% in the relative humidity control, but keeping close conditions in industrial equipments is still a challenge.

2.3. Scale-up

The high potential of nanofibrous media in different application fields generates a growing interest in industrial electrospinning. Full scale electrospinning requires processing up to litres of polymer solution or melt per hour. However, most experiments performed in academic laboratories, are performed by spinning volumes of millilitres in the span of a few hours. The scale-up of electrospinning is difficult to optimize because of the poor viscoelastic behaviour of the polymer mixtures, a lack of sufficient molecular entanglements, problems associated to limited solubility, and due to the fact that only a few processing parameters can be effectively chosen directly [9]. Table 1 summarizes the key factors affecting electrospinning scale-up stressing some common drawbacks. It refers to the changes required in the equipment for large-scale production, the problems related to process control and the safety concerns arousing from the processing of large amounts of solvents.

Table 1. Factors influencing the scale-up in electrospinning: alternatives and drawbacks

Factors determining electrospinning scale-up	Alternatives	Drawbacks
Large scale production	(i) Injection system: multi-spinneret components [10, 11] (ii) Collecting devices: cylindrical collector, mandrels, disks with different geometries, conveyors (iii) Modification of electrospinning set-up, e.g. centrifugal electrospinning or free surface systems [7, 13]	(i) Polymer clogging at the spinneret nozzle, alteration of the electric field profile induced by the presence of the electrospinning jets (ii) Limited thickness (iii) Significant variation in fiber diameter and limited configurability of the fabricated fiber assemble (e.g. general lack of fiber alignment, impracticability of core-shell structures)
Accuracy and reproducibility	Climate-controlled setups to ensure temperature and humidity control within certain ranges	High price of the equipment
Environmental safety	Novel formulations of electrospinning: use of aqueous solutions, green processing [20]	Reduced stability and inferior mechanical properties

The scale-up of electrospinning requires changes to the basic laboratory setup described before in order to ensure the requirements of the application within a limited range of variability, particularly in cases with more stringent requirements, such as most biomedical uses, and in larger scale productions. Some of these modifications refer to the injection system, usually based on multi-spinneret fittings, which are arranged either in uniaxial configuration or in circular geometry [10, 11]. Multi-axial (coaxial/triaxial) electrospinning consisting of spinneret components which enable the simultaneous spinning of different liquids, deserve particular attention as they are proposed for the fabrication of the more complex functional nanofibers [12]. The use of rotating devices such as drums, mandrels, and disks with different

geometry and morphology (solid or frame cylinders and various edge morphologies) can be used to obtain highly aligned fibres or for improving conformability and scalability. Of particular interest from the industrial point of view is the singular capability of electrospinning to give rise to complex bi-and tri-dimensional architectures in a single run, for which a proper collector design is essential.

Less conventional approaches such as "free surface" or centrifugal spinning can be useful for increasing the overall set-up throughput and the thickness of the resulting mats, also being suitable for large area deposition [7, 13]. "Free surface" electrospinning is based on the formation of a charged jet from the free

surface of a liquid, without using needles or nozzles [14]. One example of centrifugal spinning is *Forcespinning*™, which based on using centrifugal forces and multiple configurations of spinnerets and is applicable to both polymer melts and solutions [15]. Forcespinning™ is said to produce nonwoven mats with fibre diameters as low as 100 nm with high throughput.

The environmental safety of electrospinning is closely related to the use of solvents, which are a cause for concern not only during processing, but also in the final products. Solvents are a major concern because they can remain even after several days of drying due to strong acid–base interaction and/or hydrogen bonding between polymer and the solvent and their control is essential for biomedical and pharmaceutical applications. The best possibility to avoid toxic solvents is the use of water-soluble polymers and to proceed thereafter to a physical or chemical cross-linking in order to render insoluble mats if required, which may involve solvents [16]. Thermal cross-linking via microwave irradiation has been used to produce antimicrobial polyvinyl alcohol (PVA) fibres with the purpose of avoiding organic solvents or other non-eco benign reagents to produce [17]. Microwave irradiation is also a highly efficient method for fibre processing electrospun fibres, which is not only environmentally friendly but faster, simpler and more economical than conventional methods for operations such as chemical reduction during the incorporation of nano-metals [18]. It has been suggested that the use of solvents could also be avoided by means of suspension electrospinning, which refers to the electrospinning of aqueous dispersions of water insoluble polymers. Emulsion electrospinning can also allow increasing the concentration of polymer in the electrospinning mixture another common drawback of conventional electrospinning, which leads to a reduced productivity [19]. However, poor mechanical properties of the resulting fibres and the fact that not many polymers are suitable to prepare electrospinning suspensions reduce the attractive of suspension

electrospinning. Environmentally friendly electrospinning techniques are generally referred to as "green electrospinning" [16]. A green electrospinning technique has been proposed for the fabrication of stable nanofibers using a Layer-by-Layer (LBL) technique in combination with aqueous polymer electrospinning. In it, water-soluble PVA/PAA nanofibres were coated with PEI/PAA polyelectrolyte. The fibres showed an increase in Young's modulus but became more brittle depending on the concentration of PEI/PAA. The LBL-treated membrane showed strong antimicrobial activity against *Escherichia coli* and the inhibition increased with an increase in polyelectrolyte concentration [20].

3. Antimicrobial materials

Nanoparticles find a plethora of diverse applications ranging from biomedical uses to environmental remediation. The formulation of antibiotic materials is one of their most obvious uses, which emerges from their particular reactivity and high surface area. Many nanoscale antimicrobials are based on metal or metal oxide particles pushed by the fact that the antimicrobial properties of silver, copper and other metals have been known and exploited for centuries on the macroscale. The development of nanofibers provide a new framework allowing the development of nano-engineered surfaces to be used as membranes or different kinds of tissues [21]. Nanofibres allow designing new delivery systems not only for metals, but for the controlled release of many other compounds based on the reservoir-based concept in which a polymer structure surrounds a reservoir with a rate of release modulated by the degradation rate of the polymer, the rate of diffusion or the detachment of a surface coating [3]. Table 2 shows a selection of recent papers dealing with the production of electrospun polymers including nanoparticles or encapsulating chemical compounds in all cases with the aim of preparing antimicrobial nanofibres. In certain cases, the encapsulation of nanoparticles in fibers would allow reducing the concern for the dissemination of nanomaterials into the environment.

Table 2. Antibacterial electrospun fibres

Electrospun material	Antibacterial agent	Microorganism	Reference
Poly(vinyl alcohol-co-vinyl acetate)/octadecyl amine-montmorillonite	Ag NP	*Candida albicans, Candida tropicalis, Candida glabrata, Candida keyfr, Candida krusei, Staphylococcus aureus, Escherichia coli*	[24]
Ethylene vinyl alcohol copolymer (EVOH)	Ag NP	*Listeria monocytogenes, Salmonella enterica*	[25]
Polystyrene (PS)	Ag NP	*Staphylococcus xylosus*	[33]
Polyvinyl alcohol (PVA)/ silk fibroin (SF)	Ag NP	*Escherichia coli, Staphylococcus aureus*	[27]
Ascorbyl palmitate/poly(ε-caprolactone) (PCL)	Ag NP	*Staphylococcus aureus*	[28]
Poly(butylenes succinate) (PBS)	Ag NP	*Staphylococcus aureus, Escherichia coli*	[29]
Nylon 6	Ag NP	*Bacillus cereus*	[30]

Polyacrylonitrile	Ag NP	*Staphylococcus aureus, Escherichia coli, Monilia albicans*	[34]
Polycaprolactone	Ag NP	*Staphylococcus aureus, Escherichia coli, Candida albicans*	[31]
Poly (acrylonitrile-co-methyl methacrylate)	Ag NP	*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Acinetobacter sp, Klebsiella pneumoniae, Micrococcus sp, Staphylococcu*s *epidermidis, Candida sp*	[32]
Polyacrylonitrile (PAN)/β-Cyclodextrin (β-CD)	Cu Nanorods	*Escherichia coli*	[35]
Poly(lactide-co-glycolide) (PLGA)	Hydroxyapatite/CuO	*Escherichia coli*	[36]
Polyvinyl alcohol (PVA)	Epigallocatechin-3-gallate-Cu^{II}	*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli , Pseudomonas nitroreducens Saccharomyces cerevisiae, Candida albicans*	[37]
Polyvinyl acetate	CuO/TiO$_2$	*Staphylococcus aureus*	[38]
Poly(lactic acid) (PLA)	TiO$_2$ NP	*Staphylococcus aureus*	[48]
Nylon 6	TiO$_2$ NP	*Escherichia coli*	[49]
Polyester poly(L-lactide) (PLA)	ZnO NP	*Staphylococcus aureus*	[39]
Cellulose acetate	ZnO	*Methicillin-resistant Staphylococcus aureus, Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae*	[40]
Polyvinyl acetate/titania	Zn-doped-titania	*Escherichia coli, Staphylococcus aureus*	[41]
Poly(lactic acid) (PLA)	Co-Metal Organic Framework (MOF)	*Pseudomonas putida, Staphylococcus aureus*	[42]
Poly(lactic acid) (PLA) and poly(butylene succinate) (PBS)	5-Nitro-8-hydroxyquinoline, 5-chloro-8-quinolinol	*Staphylococcus aureus*	[52]
Poly(lactic-co-glycolic acid) (PLGA)	Amoxicillin adsorbed on nano-hydroxyapatite	*Staphylococcus aureus*	[54]
Polyvinyl alcohol/polyurethane	Gentamicin	*Staphylococcus aureus*	[55]
Poly(vinyl alcohol) (PVA)/poly(ethylene oxide) (PEO)	Metronidazole	*Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Penicillium notatum, Aspergillus flavus*	[56]
Nylon 6,6	Polyacrylic acid grafted rose bengal, phloxine B, azure A and toluidine blue	*Aspergillus fumigatus, Aspergillus niger, Trichoderma viride, Penicillium funiculosum, Chaetomium globosum*	[57]
Eudragit L100 (acrylic polymer from Evonik)	Fluconazole	*Candida albicans*	[58]
Styrene/maleic anhydride copolymers	Grafting of poly(propylene glycol) monoamine (Jeffamine M-600) and 5-Amino-8-hydroxyquinoline	*Escherichia coli, Staphylococcus aureus, Candida albicans*	[70]
Chitosan (CS)/polyvinyl alcohol (PVA)	Clotrimazole	*Candida albicans*	[60]
Polyacrylonitrile	Amidoxime	*Saccharomyces cerevisiae*	[61]
Gelatin	Amphotericin B, natamycin, terbinafine, fluconazole, and itraconazole	*Candida albicans, Fursaium solari, Aspergillus brasiliensis, Aspergillus fumigatus*	[62]
Polyvinyl alcohol (PVA)	Eugenol in aqueous micellar surfactant	*Salmonella typhimuriumListeria monocytogenes*	[66]
Poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA)	*Lawsonia inermis* (henna)	*Escherichia coli, Staphylococcus aureus*	[64]
Poly(ethylene-oxide) (PEO)	Antimicrobial peptide	*Escherichia coli*	[65]
Poly(vinyl alcohol) (PVA)	Organic rectorite (layered	*Staphylococcus aureus, Escherichia*	[45]

	silicate)/sodium alginate	*coli*	
Chitosan (CS)/poly(vinyl alcohol)	Organic rectorite	*Staphylococcus aureus*	[46]
Chitosan (CS)	Organic rectorite/sodium alginate	*Escherichia coli*	[47]
Polyurethane	Tourmaline (silicate)	*Escherichia coli, Enterococci*	[44]
Modified polyurethane (quaternary ammonium salts)	Quaternized polymer backbone	*Staphylococcus aureus*	[67]
Chitosan/poly(ethylene oxide) (PEO)/poly(hexamethylene biguanide) hydrochloride (PHMB)	PHMB	*Staphylococcus aureus, Escherichia coli*	[69]
Styrene/maleic anhydride copolymers/quaternized chitosan	Quaternized chitosan	*Escherichia coli, Staphylococcus aureus*	[68]

Current advances in fibre technology showed the feasibility of metal composites to be used as antimicrobial agents either with surface functionalization of included in the polymeric fibre. Within a group, the toxicity of metals to living organisms increases with atomic weight, leading silver as one of the most toxic elements. The electronegativity of metallic ions, the stability of metal chelates and the possibility of forming insoluble salts are other well-known factors affecting the way metals interact with living cells. Different types of metallic salts, compounds and nanoparticles have demonstrated antimicrobial properties and many of them have been introduced or attached to electrospun nanofibres. Apart from silver, the most relevant case studies focussed on copper zinc and cobalt as indicated below. As the size of the metal particles decreases down to the nanoscale region, the antibacterial efficiency increases because of their larger total surface area per unit volume. The uniformity in the dispersion of nanoparticles within the polymer matrix also influences its antibacterial efficiency and stability [22]. Fibre properties and processing parameters strongly affect not only fibre structure, but also nanoparticle size and dispersion in a complex manner difficult to predict. Moreover, the cost of producing composite mats is a key factor when considering the scale-up and potential market applications of antimicrobial solutions that can be dealt with by considering individual applications [23].

Silver has been widely employed in various nanofibrous architectures. The electrospinning (and centrifugal spinning) of poly(vinyl alcohol-co-vinyl acetate)/octadecyl amine-montmorillonite yielded general purpose nanocomposites with silver nanoparticles with antifungal and antimicrobial activity [24]. Silver ions and silver nanoparticles were compared in antimicrobial ethylene vinyl alcohol copolymer fibers. It was found that in thermally annealed fibers, silver ions were partially transformed into nanoparticles homogeneously distributed along the fibers, producing a substantial decrease in metal release rate [25]. The in-situ reduction of silver with UV irradiation was proposed in PVA or polyvinylpyrrolidone (PVP) prepared by electrospinning [26, 27]. Ascorbyl palmitate, a derivative of vitamin C, was employed to reduce silver ions into silver

nanoparticles in nanofibrous mats made of electrospun poly(ϵ-caprolactone) (PCL) [28]. The deposits of elemental silver were clearly observed on the surface of the fibers as aggregates of nanoparticles of an average size of ˜30 nm (Figure 2A). Poly(butylene succinate) mats containing small (< 10 nm) silver nanoparticles were also be prepared using PVP as capping reducing agent [29] and even the electrospinning solvent was proposed to reduce silver with the electrospinning polymer acting as stabilizing agent [30]. The same results were reported for composite PCL nanofibers with silver particles precipitated onto their surface [31]. Finally, the biosynthesis of silver nanoparticles using *Pseudomonas aeruginosa* was undertaken with silver nitrate as precursor to generate electrospun bionanofibers [32].
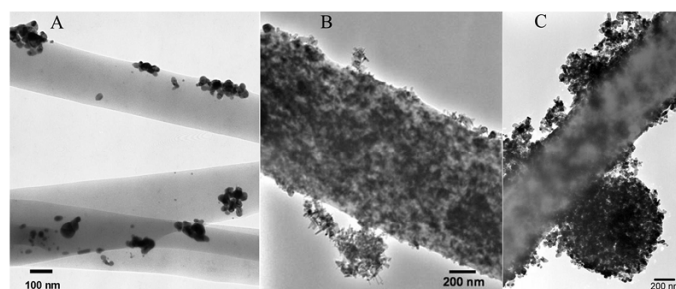

Fig. 2. TEM micrograph of individual Ag-Ascorbyl palmitate/PCL fibers after immersion (6 h) in aqueous solution of $AgNO_3$ (A); ZnO/PLA produced by inclusion within the fibres (B) and by deposition on their surface (C) [28, 39].

A considerable effort has been paid to attach metals on fibre surface, on which they are more accessible even with the drawback of being more loosely attached. Using nanoparticle-decorated fibres was the strategy proposed by several authors to enhance antimicrobial activity. Silver nanoparticles for antimicrobial nonwovens were distributed on the surface of electrospun polystyrene (PS) after reverting the encapsulation of nanoparticles by means of ultraviolet irradiation of oxidative treatments to degrade the layer of polymer that was coating the particles [33]. Electrospun polyacrylonitrile (PAN) nanofibers externally loaded with silver nanoparticles showed excellent results for inhibiting the growth of bacteria and fungi [34]. Li et al. [35], synthesized copper nanorods on the surface of PAN fibers using β-

cyclodextrins (β-CD) to adsorb copper ions from aqueous solution. β-cyclodextrin is a cyclic oligosaccharide consisting 7 glucopyranose units with a central non-polar cavity that can be used to include a wide variety of organic and inorganic compounds. The adsorption of copper ions from $CuNO_3$ on β-CD/PAN fibers followed by annealing in hydrogen atmosphere led to copper metal well-dispersed nanorods on fibre surface [35].

Other metals other than silver have been proposed for antimicrobial composites. Poly lactic-co-glycolic acid (PLGA) electrospun fibers were doped with copper oxide-hydroxyapatite nanocrystals with high antibacterial activity attributed to a synergy between hydroxyapatite and copper oxide [36]. An interesting approach using copper was reported by Sun et al. [37], who reported that copper ions increased the antimicrobial activity of epigallocatechin-3-gallate by limiting its oxidation in electrospun PVA nanofibers. CuO/TiO_2 nanorods were prepared by electrospinning polyvinyl acetate using copper nitrate and titanium isopropoxide as precursors [38]. Electrospun poly(lactic acid) (PLA) was surface-functionalized with nanosized zinc oxide leading to non-woven mats in which ZnO was deposited on the surface or dispersed within the bulk, the former exhibiting higher photocatalytic and antimicrobial activity. Figure 2B shows the "in-the-fiber" ZnO/PLA for which ZnO was mainly inside the fibers even though part of it was also decorating the external surface. The latter was the only location for the "on-the-fiber" type, shown in Figure 2C and characterized by spherical aggregates in the micrometer range loosely attached to the fibers [39]. ZnO nanoparticles embedded in cellulose acetate (CA) electrospun fibres displayed antibacterial behaviour because ZnO decreased surface wettability [40]. Zinc (nitrate) has also been used to functionalize titania nanofibers produced from the electrospinning of titanium isopropoxide in PVA followed by calcination at 600°C. The biological evaluation showed antimicrobial activity for Gram-negative and Gram-positive bacteria due to the effect of zinc ion passing to the culture medium [41].

Besides nanometals, other materials can be used for the controlled release of antimicrobial metals. Quiros et al. [43] prepared composite mats by electrospinning PLA with a suspension of PVP-stabilized Co-SIM-1 (cobalt-based substituted imidazolate). Co-SIM-1 belongs to the family of metal–organic frameworks (MOF), which is a class of hybrid materials in which organic bridging ligands are connected by metal ions to form three dimensional networks [43]. The advantage of MOF is their highly tunable composition, which can be achieved by using different metals or changing the organic linker. The release of metal contained in the structure of MOF gives rise to antimicrobial materials, although it is possible to prepare MOF in which the biological effect lies on the linker. The incorporation to PLA fibres led to a controlled release of cobalt with long-lasting antibacterial activity with the advantage with respect to silver that it is a relatively inexpensive element (Figure

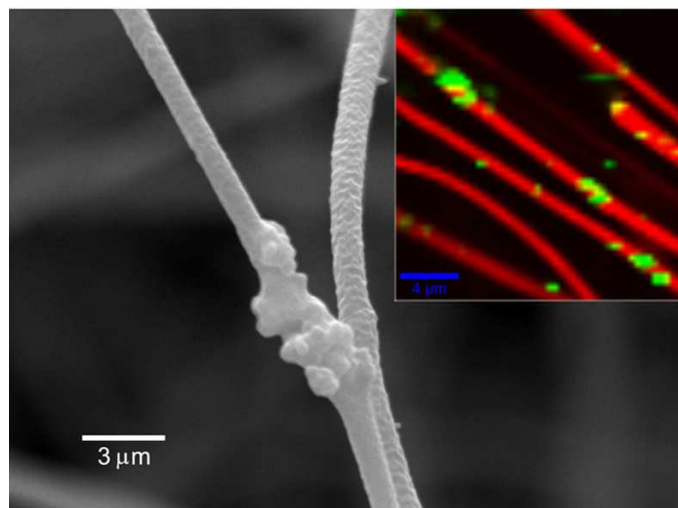3). A load of 6 wt. % Co-SIM-1/PLA decreased 40% the colonization of mats by *P. putida* [42].

Fig. 3. Co-SIM-1 metal organic framework in PLA electrospun mats. Inset: Raman mapping showing the particles included in PLA fibres[42].

Composite antibacterial mats can be prepared by including materials that impart specific surface physical properties to the nanofibers. Tourmaline (a silicate) decorated polyurethane (PU) composite nanofibers prepared as superhydrophilic antibacterial material [44]. The immobilization of a layered silicate (organic rectorite) and sodium alginate into a composite nanofibrous mats of PVA or chitosan has been explored to benefit from the intrinsic bacterial inhibition of both alginate and rectorite [45-47]. PLA/TiO_2 hybrid nanofibres were produced using a hydrothermal processing that led to TiO_2 in anatase form and decorating the fibre surface. The fibres were claimed to possess antimicrobial effect upon ultraviolet irradiation, which rendered biocidal activity during the following hours [48]. However, in another study, nylon-6 nanofibers containing TiO_2 nanoparticles displayed an antibiofouling effect linked to an increase in fibre hydrophilicity [49].

Electrospun polymer fibers have also been extensively used as new devices for the controlled delivery of chemicals thanks to their simple fabrication process and the possibility of efficiently dispensing water insoluble compounds [50]. Different drug incorporation strategies have been proposed for mat loading among which, direct blending is the most thoroughly used. Direct drug blending of the active agent into the electrospun polymeric solutions is a simple one-phase way of loading fibres provided a minimum matching of the hydrophobic-hydrophilic properties of drug and polymer [51, 52].

Surface modification has been frequently proposed for the production of fibres with bioactive molecules on their surface such as cell recognizable ligands for creating biomimetic microenvironments [53].

Drug-loading of hybrid nanofibrous composites has been explored for the delivery of conventional antibiotics. Nano-hydroxyapatite particles with adsorbed amoxicillin were dispersed with into PLGA to form electrospun nanocarriers that were cytocompatible and showed a sustained antibiotic release that inhibited bacterial growth [54]. Gentamicin was incorporated into PVA nanofibers using Nanospider™ needleless technology serving as drug reservoir [55]. The novelty in this case is the method of spinning, which enables effective large-scale production of nanofibrous mats. Nanospider™ was also used for the fabrication of electrospun of metronidazole blended PVA/poly(ethylene oxide) (PEO) [56]. Antifungal nanofiber formulations comprise several examples of blend electrospinning. Kim and Michielsen included antifungal photochemical dyes based on the production of singlet oxygen, which were immobilized by grafting into electrospun nylon [57]. Karthikeyan et al. used acrylic electrospun polymers to prepare a fluconazole topical gel for treating infections produced by *Candida albicans* [58]. Liu et al. used polyhexamethylene biguanide in cellulose acetate and polyester urethane [59]. Several other nanofibres have been developed to include different antimicrobial compounds such as clotrimazole or amidoxime [60-62].

The search for eco-friendly natural antimicrobials to replace silver and synthetic chemicals by plant extracts has also been receiving certain attention. Napralert database, the world's largest natural product database currently documents 58850 plant species, from which 6550 plants exhibit significant antimicrobial activity and most of this plants possess extracts that can be used to treat infectious diseases [63]. Avci et al. [64] reported the use of *Lawsonia inermis* (henna) as antimicrobial for the production of PEO and PVA nanofibres with bacteriostatic effect against *Staphylococcus aureus* and *E. coli*. Antimicrobial peptides are a wide family of small proteins with a broad spectrum of antimicrobial activity against bacteria, fungi and viruses. The targeted delivery of antimicrobial peptides using electrospun polymer nanofibers is another possibility for creating antibiotic mats [65]. The solubilisation of lipophilic compounds in surfactant micelles allows generating nanofibers from hydrophilic polymers with high concentrations of lipophilic chemicals, which is the case of many plant derived antimicrobials. Emulsion electrospinning has been used to prepare functional PVA nanofibers loaded with eugenol [66].

Intrinsically (or modified) biocidal polymers have been also explored by modifying the polymer backbone creating, for example, quaternary ammonium moieties [67, 68] or co-electrospinning with polymeric antiseptics [69]. The grafting of styrene/maleic anhydride copolymers with poly(propylene glycol) monoamine (Jeffamine™ M-600) or 5-amino-8-hydroxyquinoline was explored as covalent post-processing of nanofibrous mats for antimicrobial activity [70]. Coaxial electrospinning can be used to prepare fibers from two separate solutions minimizing the interaction with the organic solvents used to dissolve polymers, but it can also be used to process difficult to spin polymers. Biodegradable PLA and chitosan core/sheath (respectively) nanofibers were prepared to overcome the difficulty of electrospinning high molecular weight chitosan due to its high viscosity. The mat benefits from the antibacterial activity of chitosan [71].

4. Biomedical applications

4.1. Scaffolds for tissue engineering

Polymeric electrospun nanofiber matrices of both natural and synthetic origin have been used for a variety of biomedical applications. Among those involving living cells, the development of nanocomposites as cell scaffolds is particularly important22.

A subclass of cell scaffolds in constituted by wound dressing products for burn healing and skin reconstruction, for which the antibacterial behaviour is essential [72]. Tables 3 and 4 summarize the most relevant recent studies published on this topic and show how polymeric nanofiber scaffolds can be controlled in order to achieve a wide range of properties. Tissue scaffolds must exhibit stringent physical and biological properties in order to provide an appropriate surface chemistry and physical structure to facilitate cellular attachment and proliferation. Specifically, the electrospun mat should physically resemble the nanofibrous features of the extracellular matrix (ECM) and mimic its mechanical properties [73].

Chitosan-based nanofibers have been tested as scaffolds for tissue regeneration due to their inherent bioactivity and intrinsic antimicrobial properties. Nevertheless, the electrospinning of pure chitosan solutions remains challenging due to its rigid crystalline structure, limited solubility in common organic solvents and insufficient viscosity to form fibers [74]. The spinnable chitosan concentrations attempted so far are in the 2–8 g/mL range, but higher concentrations are generally required. Mats produced using high molecular weight chitosan are known to improve mechanical stability compared with low molecular weight chitosan and its blends with other synthetic polymers, but the higher the molecular weight, the more difficult to electrospin. Nada et al. [75] reported a modification in the methodology of electrospinning chitosan that allows processing high molecular weight chitosan without additives or blends at much higher concentrations than before. It involves a chitosan derivative, namely 2-nitrobenzyl-chitosan, prepared by reacting chitosan with 2-nitrobenzaldehyde in aqueous solution in order to produce a Schiff base, 2-nitrobenzaldehyde, which protects the amine of chitosan and yields a derivative soluble in trifluoroacetic acid. Neat chitosan is further regenerated using ultraviolet (UV) light by cleaving off iminochitosan. Using this procedure the authors reported spinnable solutions with 10–12 g/mL chitosan with an upper limit established by

the high viscosity of the solution. Chitosan produced in this way and further crosslinked with glutaraldehyde vapour supported cellular proliferation and exhibited excellent biocompatibility with minimal or no death of human skin fibroblasts. The antibacterial activity was also evaluated using an agar disk diffusion assay showing better microbial inhibition than control antibiotics. The rationale is that the presence of amine groups is critical for the antimicrobial activity of chitosan [76]. Fully acetylated chitosan, or chitin, loses its antibacterial activity.

Table 3. Electrospun fibrous materials as scaffolds for tissue engineering

Electrospun material	Antibacterial agent	Cell type/microorganism	Reference
Poly lactic-co-glycolic acid (PLGA)	Ag NP	Human liver carcinoma cell line, Normal human amnion cell line / *Escherichia coli Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes and Salmonella typhimurium*	[86]
Poly(L-lactic acid)-co-poly(ε-caprolactone)	Ag NP	Human dermal fibroblasts / *Staphylococcus aureus, Salmonella enterica*	[87]
Poly(ε-caprolactone) (PCL)	Ag NP	Human Mesenchymal Stem Cells / *Staphylococcus aureus*	[22]
Poly(vinyl alcohol) (PVA)	Au NP (Synthesized from *Couroupita guianensis* leaves extract)	Vero cell lines, and HeLa cancer cell lines / *Candida albicans, Candida krusei, Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Klebsiella pneumoniae, Bacillus subtilis Pseudomonas aeruginosa*	[88]
Poly(D,L-lactide-co-glycolide) (PLGA)	Tetracycline	*Staphylococcus aureus*	[81]
Chitosan/polyethilene oxide	Tetracycline	Human osteoblast-like and human chondrocytes-like cell lines	[72]
2-nitrobenzyl-chitosan (NB)		Human skin fibroblasts / *Bacillus subtilis, Escherichia coli, Aspergillus niger, Candida albicans*	[75]
Mauran from *Halomonas maura* / poly(vinyl alcohol) (PVA)		Mesenchymal stem cell line derived from mouse and mouse connective tissue fibroblast cells	[77]
Poly(aniline-co-3-aminobenzoic acid) (3ABAPANI) copolymer and polylactic acid (PLA)		African Green Monkey fibroblast COS-1 cells / *Staphylococcus aureus*	[78]
Poly(D/L)lactide and diblock copolymers consisting of poly(L/D)lactide and poly(N,N-dimethylamino-2-ethyl methacrylate)		Platelets, erythrocytes, and Thrombocytes / *Staphylococcus aureus*	[79]
Polycaprolactone (PCL)		Astrocytes	[80]

Bacterial electrospun polysaccharides have been exploited for the production of biocompatible nanofibers. Mauran, an extremophilic sulfated exopolysaccharide extracted from *Halomonas maura* was blended with PVA and tested for cell growth showing good migration, proliferation and differentiation of mammalian cells [77]. Other polymers from renewable sources attracted attention for biomedical uses. Poly(aniline-co-3-aminobenzoic acid) (3ABAPANI) and PLA were prepared in three-dimensional networks with enhanced biocompatibility for the proliferation of COS-1 fibroblasts and good antimicrobial capability against *S. aureus* [78]. Polylactide-based stereocomplex diblock copolymers with poly(N,N-dimethylamino-2-ethyl methacrylate) blocks were also explored as electrospun scaffolds. The availability of tertiary amino groups was claimed to impart haemostatic and antibacterial properties to the nanofibrous materials as shown by tests on blood cells and pathogenic microorganisms [79]. PCL is another biocompatible and biodegradable polymer usually employed in tissue engineering applications. PCL was proposed as scaffold for the generation of astrocytes for the regeneration of central nervous system neurons including its co-electrospun with the blue-green microalgae Spirulina, which was shown to enhance cellular metabolism [80]. The capacity of nanofibrous scaffolds for drug loading was also explored for tissue repair applications. PLGA was proposed by Yan et al. for composite fibrous scaffolds proposed for bone defect repair, which deliver tetracycline controlled by the lactidyl/glycolidyl ratio [81].

Different metal nanoparticles have been incorporated to electrospun polymers in order to provide antimicrobial effect. Silver nanoparticles are the most obvious choice due to their strong antibacterial activity, but they have also been found cytotoxic by various researchers [82, 83]. A survey of the existing literature indicated that size, texture, concentration, surface area, surface functionalization, and the presence of toxic residues from previous synthesis steps are the main major factors influencing the bio-kinetics and toxicity of nanostructured scaffolds [84, 85]. Recent studies

Table 4. Wound-dressing electrospun fibrous materials

Electrospun material	Antibacterial agent	Microorganism	Reference
Polylactic acid (PLA)	Ag nanorods	*Escherichia coli*	[99]
Chitosan	Ag NP	*Pseudomonas aeruginosa*	[100]
Chitosan-poly(vinyl alcohol) (PVA)	Ag NP	*Escherichia coli*	[101]
Collagen-Chitosan	Ag NP	*Staphylococcus aureus*	[102]
Polyvinyl alcohol (PVA)	Ag NP	*Escherichia coli*	[104]
Polyvinyl alcohol (PVA)	Ag NP	*Staphylococcus aureus, Escherichia coli*	[17]
Silk Fibroin (SF)	Ag NP	*Staphylococcus aureus, Pseudomonas aeruginosa*	[103]
Polyacrylonitrile (PAN)	Ag NP	*Bacillus cereus, Escherichia coli*	[105]
Polyurethane	Ag NP	*Staphylococcus aureus, Escherichia coli*	[106]
Polyurethane/ hydrolyzed collagen, elastin, hyaluronic acid or chondroitin sulfate	Ag NP	*Escherichia coli, Salmonella typhymurium, Listeria monocytogenes*	[108]
Zwitterionic poly(sulfobetaine methacrylate)	Silver ions	*Pseudomonas aeruginosa, Staphylococcus epidermis*	[107]
Polyurethane (PU)	CuO NP	Methicillin-resistant *Staphylococcus aureus*	[23]
Poly(lactide-co-glycolide) (PLGA)	Bioactive glass	*Streptococci, Lactobacillus paracasei*	[96]
Poly(lactic-co-glycolic-co-hydroxymethyl propionic acid) (PLGH)	Nitric Oxide (NO)	*Acinetobacter baumannii*	[111]
Cellulose acetate (CA) and polyesterurethane (PEU	Polyhexamethylene biguanide (PHMB)	*Escherichia coli*	[59]
Poly(D, L-lactide-co-glycolide) (PLGA)	Vancomycin, gentamicin	*Staphylococcus aureus, Escherichia coli*	[94]
Poly(L-alanine) (PLLA)	Chlorhexidine-gluconate	*Escherichia coli*	[109]
poly(L-lactide-co-D,L-lactide) (coPLA) and coPLA/poly(ethylene glycol)	Ciprofloxacin hydrochloride, levofloxacin hemihydrate, moxifloxacin hydrochloride	*Staphylococcus aureus*	[95]
Poly(ε-caprolactone) (PCL)	Ampicillin	*Staphylococcus aureus, Klebsiella pneumoniae*	[93]
Poly(caprolactone) (PCL)	Rifampicin	*Staphylococcus epidermis, Pseudomona aeruginosa*	[92]
Poly(L-lactide) (PLLA) and PLLA/poly(ethylene glycol) (PEG)	Diclofenac sodium, lidocaine hydrochloride, benzalkonium chloride	*Staphylococcus aureus*	[11]
Poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(ethyleneimine) (PEI)		*Escherichia coli*	[20]
Cyanoethyl chitosan		*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillius subtilis*	[97]
Iminochitosan		*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillius subtilis*	[98]

incorporating nanosilver to different nanofibrous scaffolds for tissue engineering and wound healing stressed the balance between cell proliferation and bacterial inhibition. A relatively low concentration of silver nanoparticles in PCL effectively inhibited the growth of microorganisms without compromising on the cell adhesion, but higher concentrations did [22]. Almajhdi et al. [86] reported cell viability studies revealing that cytotoxicity was highly dependent on silver nanoparticle concentration. They showed that, the anticancer activity against a liver cancer cell line of PLGA increased by increasing the concentration of silver NP up to a certain concentration for which the anticancer and antimicrobial activity of PLGA nanofibers are maximum without cytotoxicity effect on normal cells. The results are difficult to interpret, but the authors concluded that highly porous scaffolds benefit the adherence and proliferation of cells, while silver exerts oxidative stress cytotoxicity preferably on cancer cells. It has also been shown that he proliferation of fibroblasts decreased with an increase in silver concentration in poly(L-lactic acid) co-poly(ε-caprolactone) (PLLCL)

nanofibres. The results of cell proliferation assays suggested that the electrospun PLLCL-Ag (0.25 wt. %) nanofibres are more biocompatible than those bearing higher metal loadings [87]. The antibacterial ability of Ag NP is clear. However, an excess of Ag NP inhibits cell proliferation and migration, so that an optimum concentration of silver seems to exist for every tissue-engineering application. Gold nanoparticles attracted considerable interest due to their remarkable biocompatibility as well as their antimicrobial and anticancer activities [88]. A recent study reported a green synthesis of gold NP employing plant extracts as an alternative to procedures using toxic chemicals. The dispersion was electrospun with PVA the composites displaying high hydrophilicity and biocompatibility. Specifically, the maximum percentage of Vero cell viability was 90% after 72 h incubation. Cell proliferation increased with incubation time indicating that the cell nutrition was not hindered by the organic–inorganic hybrid nanofibers. The presence of Au NP in the electrospun nanofiber showed anti-proliferative effects for MCF-7 and HeLa cell lines, with proliferation percentage of only 8% and 9% respectively after the same period of incubation. The anti-proliferative activity was attributed to Au NP originating a decrease in the membrane potential of mitochondria and increased production of reactive oxygen species. The results also showed significant inhibitory activity of the hybrid nanofibers against a number of microorganisms while PVA nanofibers without Au NP did not show any antimicrobial activity [88].

Electrospun mats with surface antibody functionalization can be used to produce nanofibrous membranes for immuno-enhanced tissue scaffolds. Nano- or microfibrous poly(ε-caprolactone) (PCL) non-wovens with surface covalently immobilised growth factors were used for endothelial cells. Immunohistochemistry provided the biological integrity of immobilised growth factor and at the same time the number of primary endothelial cells or immortalised endothelial cells was significantly enhanced [89]. Growth factors were also immobilized on electrospun PCL nanofibers using several antibodies. The use of autologous biological fluids and cells offers the possibility to implement personalized therapies tailored to specific medical conditions [90]. Poly(ε-caprolactone) coated with self-assembled films of hydrophobin turns hydrophilic and its biocompatibility increased. Using an antibody specific for endothelial cells on the hydrophobin-coated PCL scaffolds, the attachment and retention of endothelial cells resulted significantly promoted [91].

4.2. Wound dressings

Treating wounds or burns is an important health issue because they are common, painful and can result in disfiguring or even threaten life. Infection is a major complication of skin wounds as any open skin lesion is a potential invasion site for microorganisms. Adequate wound management is necessary to prevent infection and requires wound dressing materials that can achieve rapid healing while minimizing infection. Among conventional wound dressing materials cotton gauze is the most used, but it allows fast evaporation of fluids making the wound desiccate. Besides, its porous structure does not provide an efficient barrier against bacterial penetration. Other disadvantages of traditional wound dressings are adherence to wound, ischemia/necrosis, and the need for frequent changes [59]. In general, wound dressings can be classified as passive or active, depending on their roles in wound healing. Passive wound dressings refer to the dressings that only provide cover and physical protection, whereas active wound dressings facilitate wound management and healing. An ideal wound dressing should protect wound from microorganism infection, allow gas exchange, absorb exudate, impart a moist environment to enhance epithelial regrowth, and be painless to remove.

Electrospun nanofiber membranes typically have a high porosity with excellent pore-interconnectivity, which is particularly important for exuding fluid from the wound. The inherent small pores and very high specific surface area allow the nanofiber membranes to inhibit exogenous microorganism invasions and to control fluid drainage. Electrospun membranes made from several biopolymers and polymers incorporating wound healing agents can be applied as wound dressings to improve wound healing performance based on its capacity for serving as scaffold for tissue regeneration and the possibility of delivering bioactive agents during healing [3]. Table 3 is a compilation of recent studies concerning electrospun wound dressings. Most of the works are focused on the use of biocompatible polymers and composites functionalized with antimicrobial NP.

Formulations based on biodegradable polymers have been frequently proposed for wound dressing, PCL [92, 93], PEO [65] and PVA [55, 56] being the most widely studied for local drug delivery in wound dressing applications. These polymers are of particular interest due to their inherent non-toxicity, non-carcinogenicity, high biocompatibility, high degree of swelling in aqueous solutions and the possibility of solid-state crosslinking. Derivatives of PLA such as PLGA or poly(L-lactide-co-D,L-lactide) (coPLA) are also easy electrospun and allows efficient incorporation of low-molecular-weight biologically active substances [94, 95]. A composite was prepared blending nanoparticulate bioactive glass and PLGA to reduce human oral bacteria for dental applications [96]. Bioactive glass is antibacterial itself, but its mechanism of action is still not clear. Nanofibrous 3ABAPANI-PLA was successfully tested for functional wound dressings with high antimicrobial capability [78]. Cyanoethyl chitosan produced nanofibres effective against several Gram negative and Gram positive bacteria with higher antimicrobial effect attributed to the small pore size and higher surface area of thinner fibres [97]. A similar conclusion was obtained for iminochitosan

electrospun in trifluoroacetic acid. In this case, fibres with barbed structures, which displayed higher surface area, gave rise to gauzes particularly effective against a range of microbes [98]. PLA was also used in a number of formulations in which the active compound is silver in different forms. Electrospun fibre films containing silver nanorods were proposed as porous antimicrobial mats for wound dressing based in their remarkable inhibition of the Gram-negative *E. coli* [99]. Chitosan nanofibers containing silver nanoparticles were obtained as composites for wound dressing in which fibre diameter decreased with increasing silver content due to the tuning of surface tension [100]. Other materials incorporating silver nanoparticles and proposed for wound dressing include glutaraldehyde crosslinked mats produced from colloidal dispersions of chitosan-capped Ag-NP in PVA [101], collagen-chitosan [102], silk fibroin [103], PVA [17, 104], PAN [105] and PU [106]. A slightly different approach, based on the immobilization of silver ions was proposed using electrospun fibres of the superhydrophilic zwitterionic poly(sulfobetaine methacrylate) to yield nonadherent, high gas permeation and pathogen-resistant wound dressings, which rendered insoluble mats upon photo-crosslinking [107]. Polyurethanes have a number of biomedical applications besides wound dressings that include blood contact devices or dental implants. The introduction of copper oxide nanoparticles in electrospun PU originate a material made of porous films with antimicrobial activity [23]. Silver-loaded biocomposite polyurethane–extracellular matrix membranes were prepared by electrospinning in order to produce antibacterial fibres, which allowed the sustained release of bactericidal silver ions from silver nanoparticles [108].

The release of antibiotics in wound healing mats produced by electrospinning has also been dealt with a number of authors. Chen et al. developed sandwich-structured nanofibrous membranes to provide sustained-release of the antibiotics vancomycin and gentamicin as well as the anesthetic lidocaine for treating infected wounds [94]. This work used biodegradable PLGA mats using 1,1,1,3,3,3-hexafluoro-2-propanol as solvent. Another biodegradable polymer, poly(L-alanine), has been used to produce blended fibers encapsulating the antibacterial chlorhexidine in an application intended to produce wound healing mats thanks to its excellent thermoplastic and mechanical properties [109]. Blended processing was also used to prepare nanofibres of coPLA and coPLA/poly(ethylene glycol) (PEG) containing several antibiotics. The resulting mat was suitable for applications that require an initial burst release of active substances, which is the particular case of certain wound dressings [95]. Another interesting application was reported by Toncheva et al., who prepared bundles of poly(L-lactide) with and without PEG incorporating diclofenac sodium, lidocaine hydrochloride and benzalkonium chloride as wound dressing materials [11]. The authors concluded that the ionic solutes enhance the conductivity of the spinning solution leading to the

formation of micron-sized bundles of nanofibers during electrospinning. The design of prophylactics against peri-prosthetic infections based on localized antibiotic release is claimed as a way to limit antibiotic resistant strains. PCL nanofibers loaded with rifampicin were studied for drug controlled release against several bacteria in static and in vitro conditions [92]. PCL electrospun fibers containing ampicillin were also tested with similar purpose [93]. In this case, a rapid release of ampicillin took place during the first hour with a maximum period of activity of 96 hours, which could be optimal for certain applications such as surgical sutures to decrease surgical site infections.

Another approach is the incorporation of nitric oxide (NO) in polymeric scaffolds. It has been demonstrated that materials releasing predictable levels of NO are capable of preventing and treating infection causing pathogens [110]. NO induces membrane damage and DNA deamination in bacterial cells while remaining non-cytotoxic to normal human skin cells. NO also plays a significant role in numerous biological functions, including modulating haemostasis, reducing inflammation, and promoting skin healing. Wold et al. described a novel polymer for the storage and controlled delivery of NO in medical applications [111]. The polymer synthesis was based on a carboxyl functionalized polymer backbone poly(lactic-co-glycolic-co-hydroxymethyl propionic acid) prepared by a ring-opening melt polymerization of L-lactide and glycolide with 2,2′-bis(hydroxymethyl)propionic acid. The pendant carboxyl groups were modified to thiol and nitrosated with t-butyl nitrite to yield the corresponding S-nitrosated polymer derivatives. The exposure of these fibers to physiological conditions for 48 h demonstrated that the electrospun matrix maintains its characteristic fibrous form and resulted in reduction in the bacterial counts > 95%.

5. Food Packaging

The dissemination of food-borne pathogens is a major cause for concern. Food safety problems are illustrated by the fact that during 2014 a total of 3157 notifications were transmitted through the EU Rapid Alert System for Food and Feed (RASFF), which supposed a 25% growth with respect to 2013112. The US Center for Disease Control reported that 48 million people (1 in 6) get sick every year due to food eaten in the United States. Bacterial-associated deaths from tainted meats are the most critical issue forcing better food packaging systems. In particular, the inner layer of the meat packaging material, which is in contact with the food surface has to be redesigned to provide better food safety and in a way that it extends the shelf life of food produces inhibiting the growth of pathogens on meat surface [113]. Food borne illnesses causes a large economic burden particularly for the industry of processed and packaged food due to increased controls and a greater awareness of consumers. The need to introduce novel disinfection

systems and to protect surfaces such as membranes and packaging materials also comes from the necessity of avoiding harmful disinfection byproducts produced by conventional disinfection techniques. Moreover, some pathogens are becoming refractory to conventional disinfectants, making it necessary an additional research effort including nanotechnology and materials science.

Active packaging is intended to develop functions such as atmosphere modification in order to prevent or delay food spoilage. The removal of oxygen is a major milestone in active packaging materials for which deoxidizing agents such as iron, oxidative enzymes and ascorbic acid have been proposed among others [114]. Glucose oxidase is the main enzymatic oxygen scavenger and can be integrated into plastic packaging materials. It catalyses the reaction of glucose and oxygen to yield glucuronic acid and is used to remove residual oxygen in foods and beverages and to enhance their shelf life. PVA fibers electrospun with low-molecular-weight chitosan and green tea (tea polyphenols are known to inhibit the growth of food-spoiling microorganisms) and incorporating glucose oxidase yielded membranes with bacteriostatic activity against several bacteria and were proposed for food packaging materials [115]. Some additional examples of nanofibrous webs susceptible to be used as active packaging material can be found. Many antibacterial mats could meet the requirements for antibacterial food storage material, but some were specifically intended for that purpose. Triclosan/(β-γ)-CD complexes were incorporated into PLA electrospun nanofibers. Every molecule of CD was able to encapsulate one molecule of triclosan. The outfit was prepared as a solid inclusion complex later dispersed in the electrospinning solvent apparently with no loss of charge and the nanofibrous webs were proposed for active food packaging in view of their very high surface area and antibacterial behaviour [116]. NiO/TiO$_2$ composite nanofibers with antibacterial activity were prepared by sol-gel electrospinning from a salt of nickel and titanium isopropoxide and electrospun in poly(vinyl acetate). The resulting web was calcined at 600°C in air leaving non-polymeric NiO/TiO$_2$ fibres, which displayed antibacterial behaviour leading to the disruption of cell membranes and the depression of the activity of certain enzymes. The authors proposed its application to inhibit the microbial growth associated with food stuff [117].

An innovative application proposed the use of bacteriophages encapsulated in electrospun fibers [113, 118]. Bacteriophages are viruses that can kill prokaryotes and that have already been used for species-specific control of bacteria during different phases of food production and storage. The possibility of using phages to reduce the concentration of bacterial pathogens gained general attention since the introduction of a *Listeria monocytogenes*-specific phage preparation approved by the Food and Drug Administration in 2006. The electrospraying/electrospinning of phage suspensions results in a quick deactivation of the bacteriophage

attributed to the rapid evaporation of water during single nozzle electrospinning, which leads to a drastic change in the osmotic environment of the electrospinning polymer solution/forming fibers. Coaxial electrospinning was successfully used to encapsulate T4 phage by locating it in the core of the fibers and therefore protected from rapid dehydration by the outer-layer polymer sheath [113]. Once in aqueous buffer medium, a rapid release of T4 phage was observed (100% after 30 min), which was attributed to the high hydrophilicity of PEO that rapidly dissolves in water. An increase in PEO molecular weight resulted in increased fibre diameter and slower release rate. Blending PEO with hydrophobic cellulose diacetate was also proved to avoid the rapid release of the phage via manipulating the ratio of hydrophilic/hydrophobic polymers in the shell [118].

Physical blockage has also been proposed as adhesive biodegradable patch for the protection of pruning locations of plants from fungi attacks. Based on soy protein/polyvinyl alcohol and PCL electrospun nanofibers deposited onto a biodegradable rayon membrane, the mat physically blocks fungi penetration, while leaving sufficient porosity for plant breathing [119].

6. Filters for environmental applications

Nanofibrous mats with antimicrobial functionality have also attracted considerable attention for air and water treatment applications. They offer an efficient way of dealing with the growing concern on the microbiological quality of treated water and filtered air at the same time keeping low operating costs due to their high permeability and reduced loss of pressure energy [120]. A central issue is the microbial growth on filter surfaces referred to as biofouling that increases filter replacement frequency, raises operational costs, increases the use of cleaning products and deteriorates the quality of purified water or air. Fouling is caused by the accumulation of organic and inorganic substances, including particles and biomacromolecules, as well as biofilm formation due to the attachment and growth of microorganisms. Both are interrelated because even if the organic compounds involved in fouling may come from natural organic matter, after the onset of microbial growth they are mostly formed by a class of macromolecular compounds known as extracellular polymeric substances (EPS). EPS are produced by microorganisms as a way to attach to surfaces and mainly consist of polysaccharides and, to a lesser extent, nucleic acids and proteins [121]. Unlike physical or chemical fouling, biofouling is often irreversible and can only be dealt with by raising operating pressures and frequent backwashing or chemical cleanings. Backwashing is usually employed to control biofouling, but the recovery of flow is partial because certain compounds are irreversibly attached to the membrane surface. Biocidal chemicals such as chlorine can lead to oxidative membrane damage and are particularly impractical for reverse osmosis polyamide

membranes. Chemicals also lead to the formation of harmful oxidation by-products such as halogenated hydrocarbons. It is also important to note that biocides are usually unable to remove the whole biofilm, which is in fact a complex community with different species of microorganisms structured in layers when the outer protect the inner [122].

Table 5. Environmental applications of electrospun fibrous materials

Electrospun material	Antibacterial agent	Microorganism	Application	Reference
Polyacrylonitrile (PAN)	Ag NP	*Staphylococcus aureus, Escherichia coli*	Air Filtration	[125]
Polyacrylonitrile (PAN)	Ag NP	*Staphylococcus aureus, Escherichia coli*	Water and air treatment	[127]
SiO$_2$/Polyvinyl alcohol (PVA)	γ -AlO(OH) (Boehmite)	*Staphylococcus aureus*	Water remediation (adsorption)	[128]
Poly(lactic acid)	Sepiolite funtionalized with silver and copper	*Staphylococcus aureus, Escherichia coli*	Water filtration	[124]
Polyamide (PA)	Ag NP, thiocyanatomethylthiobenzothiazole, dibromocyanoacetamide, bronopol, WSPC (proprietary quaternary ammonium salt and chlorhexidine	Culturable microorganisms from a hospital waste water	Water filtration	[120, 123]
Poly(vinyl alcohol) (PVA)	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanone derivative)	*Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhimurium*	Water filtration	[7]
Chitosan-polycaprolactone (PCL)		*Staphylococcus aureus*	Water filtration	[126]
Polyurethane		*Escherichia coli*	Water treatment	[10]
Polyacrylonitrile (PAN)	ZnO	*Escherichia coli*	Photocatalysis and antimicrobial	[136]
Polyvinilpyrrolidone (PVP)/TiO$_2$	Cu NP	*Escherichia coli, Klebsiella pneumoniae*	Photocatalysis and antimicrobial	[132, 133]
Polyvinilpyrrolidone (PVP)/TiO$_2$	Ag NP	*Staphylococcus aureus, Escherichia coli*	Photocatalysis and antimicrobial	[134]
Ag-TiO$_2$/Polyurethane	Ag	*Escherichia coli*	Photocatalysis and antimicrobial	[137]
TiO$_2$/Nylon 6	Ag NP	*Escherichia coli*	Photocatalysis and antimicrobial	[138]
Poly(styrene-co-maleic anhydride) modified with a quaternary ammonium compound		Bovis bacillus Calmette-Guérin (BCG)	Affinity membrane	[143]
Polyethylene oxide/ polycaprolactone and, polyethylene glycol	Atrazine degrading bacterium *Pseudomonas* sp. ADP		Bioremediation	[12]

Recent selected studies on the use of electrospun fibres for water or air cleaning processes is shown in Table 5. The electrospinning of polymers loaded with nanoparticles with biocidal functionality is the most frequent approach. The method allows the encapsulation of active ingredients for controlled release during a prolonged time, but it has to be taken into account that the polymer may impose and excessive diffusion barrier. Also, a high content of particles during the electrospinning can affect the process and deteriorate the mechanical properties of nanofibers. The possibility of introducing antimicrobial particles on the nanofiber surface may result in their loose attachment with the

subsequent reduction of service life and the possibility of spreading nanoparticles into the environment. Nonwoven polyamide nanofibrous membranes were proposed for water filtration with the inclusion of silver nanoparticles using a one-step method including silver nanoparticles in the electrospinning solution [123]. Dasari et al. prepared PLA non-woven mats with silver and copper loaded on a modified sepiolite in order to avoid loose nanometals. In this case, metals were able to diffuse through the encapsulating polymer, which was made porous using a combination of solvents [124]. Antimicrobial air filters based on silver-doped nanofibrous PAN membrane was tested for the filtration of microorganisms and dust

particles and were found to efficiently remove microorganisms and dust from air suitable for hospitals or other places prone to bacterial infections [125].

Functionalized electrospun polyamide fibres including nanosilver, but also biocidals like bronopol or polyquat, were used to produce flat sheet microfiltration membranes for water disinfection. The testing with hospital wastewater showed a particularly high activity for polyquat-loaded mats also showing that the leaching of active material controlled the durability of the biocidal effect [120]. A furanone derivative for antimicrobial action and cell-adhesion inhibition was included in electrospun PVA and tested in upscaled nanofiber production using bubble electrospinning. Apparently, the biocidal chemical did not leach into filtered water but was effective inhibiting surface-attachment of bacteria [7]. The natural antibacterial property of chitosan was also claimed for water filtration in chitosan-PCL mats, which showed effective against *S. aureus* adhesion with high flux, an almost complete removal of 300 nm beads and good membrane integrity [126]

Chemical post-functionalization was used to decorate electrospun PAN with silver, either as Ag^+ or forming silver nanoparticles. A treatment of PAN fibers with hydroxylamine (NH_2OH) created surface amidoxime groups ($-C(NH_2) = N-OH$), which were used to coordinate with silver ions. Silver ions were subsequently photoreduced to metallic silver nanoparticles [127]. SEM images of the decorated nanofibers are shown in Figure 4. Higher amounts of NH_2OH led to more amidoxime groups on fibre surface and higher amount of silver in finished fibres, in all cases evenly distributed on their surface. Another interesting aspect of amidoxime-functionalized fibres is the strong antimicrobial activity of membranes with amidoxime groups, attributed to their capacity to bind with metal ions (such as Mg^{2+} and Ca^{2+}) through coordination. These divalent ions are essential for the stability of the outer layers of bacterial cell membranes and the coordination with amidoxime groups is thought to destabilize them, inhibiting cellular replication and growth. Anyway, during water treatment such metal ions are continuously supplied in the feed stream, so the described sequestering mechanism could not be able to prevent microbial growth.

Mechanical filters were also developed to exploit the physical exclusion of bacteria by nanofibrous nanofibres. PU (non-doped) electrospun 0.25 µm nanofibrous mats were used for water-treatment purposes with bacterial removal efficiency considerably higher than commercial PVDF microfiltration membranes. This was rationalized claiming that bacterial shape, rather than absolute size, is the key factor determining the ability of bacteria to pass through filters [10]. A number of materials with high surface area have been proposed for the adsorption of organic pollutants. They include porous carbons, metal oxides, mesoporous silica, and many other. A hierarchical core/sheath fibre has been produced using a

combination of electrospinning and hydrothermal reaction in which γ-AlO(OH) (boehmite) nanoplatelets were anchored on the surface of electrospun (using PVA as supporting polymer) SiO_2 nanofibers. Boehmite exhibits an octahedral lamellar structure with rich in surface OH groups and behaves as a strong adsorbent for metals and organic compounds. The so-produced self-standing membrane is flexible and easy to handle as a common water treatment membrane [128]. A SEM micrograph of the material is shown in Figure 5. The hierarchical structure consists of one-dimensional nanofibers and two-dimensional nanoplatelets structured in core/sheath fibers. The open structure is said to offer higher contact area, which increases the adsorption efficiency.
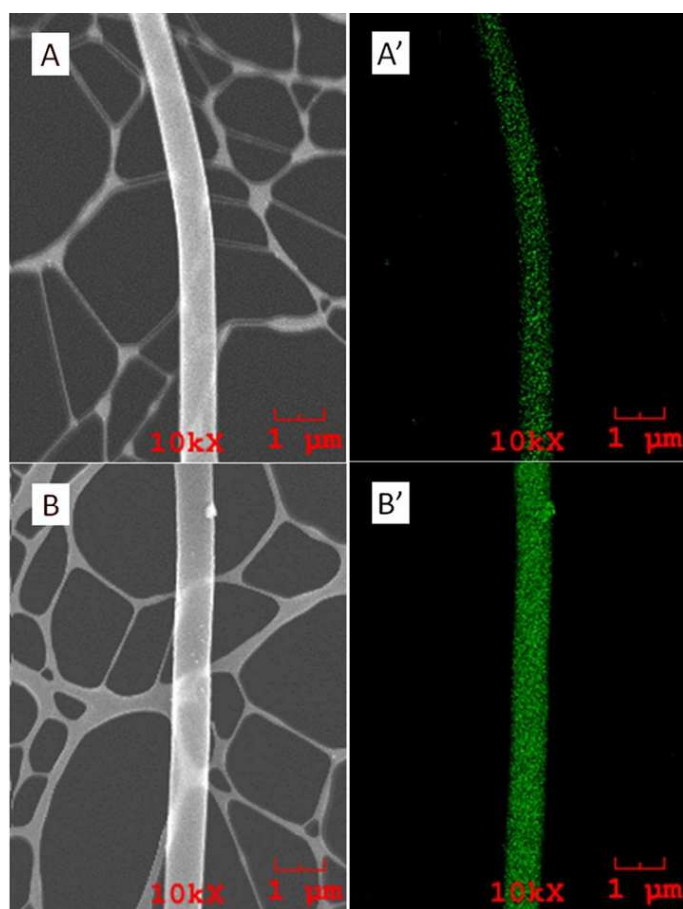

Fig. 4. SEM images (A and B) and elemental mapping images of silver (A' and B') in silver-decorated PAN nanofibers. 1 M NH_2OH 5 min followed by 0.1 M $AgNO_3$ 30 min (A and A'); 1 M NH_2OH 20 min and 0.1 M $AgNO_3$ 16 h (B and B') [127].

Photocatalysis refers to a combination of photochemistry and catalysis for promoting a chemical reaction with the aim of degrading environmental pollutants including pathogens or converting sunlight into hydrogen or other energy carriers. Photocatalytic process can be classified as homogeneous or heterogeneous, the later occurring by means of the generation of electron-hole pairs on the surface of solid semiconductor materials. Titanium dioxide is by far the most extensively used semiconductor photocatalyst either in powdered or supported form [129]. Recently, TiO_2 nanowires or
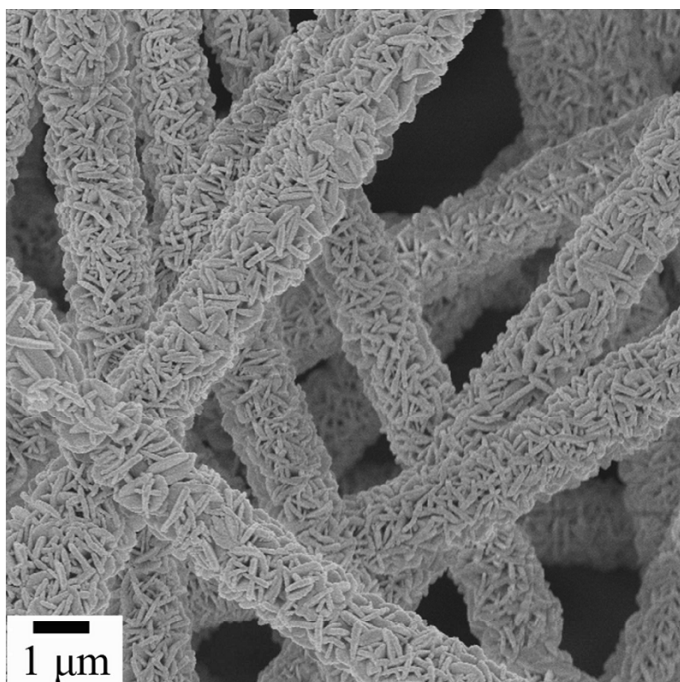
Fig. 5. Boehmite on silica core/sheath fibers [128].

nanotubes have gained attention in search for higher surface areas and more efficient charge separation [130]. Among the variety of ways to prepare TiO₂ 1D nanostructures, the electrospinning of precursors using sol–gel techniques using a polymeric scaffold, further removed by calcination, has received attention due to its ability to provide highly aligned nanofibres with controlled crystallinity and high photocatalytic activity [131]. TiO₂-polyvinylpyrrolidone nanofibers produced using a hydrothermal process with calcination at 700°C and doped with zero-valent copper nanoparticles were tested with the aim of using copper to reduce electron-hole recombination as well as exploiting its antimicrobial properties. The material was a white powder with Cu NP attached to the surface of TiO₂ wires. The antibacterial activity was tested using *E. coli* and *K. pneumoniae* as model organism and were able to degrade dyes in solution besides bacterial inactivation [132, 133]. With the same purpose, nanosilver-decorated titanium TiO₂ nanofibers were prepared with photochemical and antimicrobial activity using a sol-gel-electrospinning process followed by calcination at 400°C with silver particles deposited via photoreduction decorating the outer surface. The authors demonstrated that the inclusion of silver not only introduces antimicrobial activity against *S. aureus* and *E. coli*, but increases photocatalytic efficiency via reducing electron hole recombination rate. The decomposition of NO$_x$ and volatile organic compounds in an air treatment application showed remarkable efficiency [134]. In a similar way, Ag-TiO₂ electrospun composite nanofibers using bulk embedded silver nanoparticles in anatase-rutile, the phase transformation of which decreased leading to a band gap 2.8 eV (3.2 eV without silver). The optimal size of silver nanoparticles was 2–3 nm beyond which, an increased amount of silver decreased

photoactivity, but antibacterial efficiency was not given [135]. The photocatalytic activity of ZnO has been studied using electrospun PAN as platform to create 3D nanocomposites of large (up to 100 m²/g) surface area and antibacterial effect against *E. coli* and Gram-positive *S. aureus* [136]. The same concept has been explored in polymeric non-calcined fibres. Silver-titania in polyurethane nanofibres showed antimicrobial activity against *E. coli* due to the loaded again due to silver antimicrobial activity. The composite mat was intended for biological warfare protection, also showing capacity for the photocatalytic degradation of dimethyl methylphosphonate [137]. Silver-impregnated TiO₂/nylon 6 nanocomposite mats is another example of active fibres with combined antimicrobial-photocatalytic activity [138].

7. Applications with living bacteria

The incorporation of probiotics, which are living organisms, into foodstuff is not an easy task. The viability of probiotics is frequently compromised during food processing or storage operations as well as in the course of gastrointestinal transit [139]. Encapsulation enhances bacterial viability and makes it easier cell preservation and dosage and is usually performed by spray-drying, emulsifying-crosslinking or coacervation [140]. Electrospinning has been proposed as an alternative in view of the possibilities of low-temperature processing and using water-soluble polysaccharides as matrix. The technique is compatible with food grade polymers and biopolymers and results in efficient encapsulation with little denaturation. PVA was used as encapsulating material using a coaxial setup for *Bifidobacterium animalis* subsp. *lactis* Bb12. The cells survived in spite of the osmotic change and the electrostatic field generated and remained viable for 40 days at room temperature and for 130 days at −20°C139. From the same group, Bifidobacterium strains were encapsulated in whey protein concentrate and a pullulan (a carbohydrate) in electrosprayed micro and nanocapsules141. Soluble dietary fibre of different origins was used for the nanoencapsulation of Lactobacillus acidophilus in electrospun PVA nanofibers. *L. acidophilus* was incorporated into the spinning solution to produce nanofiber-encapsulated probiotic with up to 90% bacterial survival and viability retained for several weeks upon storage at 4°C. The nanofibers showed the presence of hemicellulose and their thermal behaviour suggested good behaviour for the protection of probiotics in heat-processed foods [142].

Bioremediation, which uses microbial processes to degrade environmental contaminants, is a complex process usually requiring tailoring to site-specific conditions and an interdisciplinary approach. Keeping organisms alive and active is a difficult task for which electrospun scaffolds have been proposed. The encapsulation of the atrazine degrading bacterium *Pseudomonas* sp. ADP was studied in hollow polymeric

electrospun microfibres by Klein et al. [12]. It is a strain that rapidly mineralizes atrazine under aerobic and denitrifying conditions as well as under non-growth conditions and was included within the core of a hollow fibre. A core solution of PEO dissolved in water was spun with an outer shell made of PCL and PEG. The resultant microtubes are shown in Figure 6. After the encapsulation of bacteria, the degradation rate of atrazine was low due to the stress induced by electrospinning that reduced their metabolic activity. Atrazine degradation rate increased during the following days on stream. However, long-term stability of the scaffold was compromised by the abiotic hydrolysis followed by enzymatic degradation of PCL.
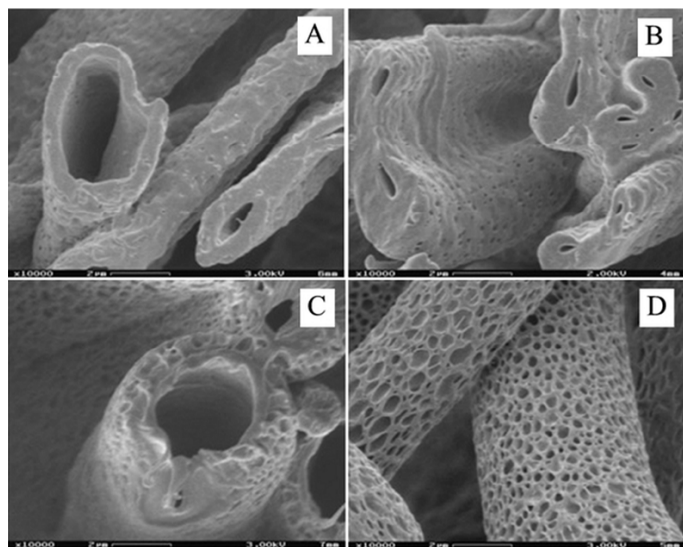

Fig. 6. HRSEM micrographs of microtubes used for encapsulating *Pseudomonas* sp. ADP cells used for atrazine remediation. Shell solutions: PCL-PEG in chloroform-DMF (A and B) and PCL in chloroform-DMSO (C and D) [12].

A closely related application is bacterial capture for analytical purposes, which is intended to increase the sensitivity of pathogen detection. Surface and bulk-modified poly(styrene-co-maleic anhydride) affinity membranes were prepared for capturing microorganisms, specifically the mycobacterium bovis bacillus Calmette-Guérin (usually referred to as BCG). The affinity studies conducted demonstrated that BCG was successfully captured onto the surfaces of the nanofibers functionalized with quaternary ammonium compound irrespective of whether the modification took place after electrospinning (surface-modified) or before electrospinning (bulk-modified) [143].

8. Other applications

The reinforcement of fibres using cellulose fibrillar nanoparticles gained increased attention in order to provide higher mechanical strength to electrospun materials. Nanocellulose is referred to as a number of different names such as nanofibrils, nanowhiskers, nanocrystals and many other that describe a rigid, nanoscale, crystalline material obtained from renewable

souces [144]. The properties of nanocellulose change depending on the source and the way of extraction, but in general it possesses a highly rigid crystalline structure, which seems a natural way overcoming the generally poor mechanical properties of nanofibrous mats [145]. PEG-grafted cellulose nanocrystals were studied for its application as scaffolds in bone tissue engineering [146]. PCL fibers showed improved mechanical properties with maximum tensile strength more than twice that of neat PCL [147, 148]. CA elastic modulus improved from 1.17 GP to 1.68 GPa [149]. The tensile strength of electrospun PS/nanocellulose mats was elevated 170% [150]. PLGA nanofibers modified with cellulose nanocrystals intended for skin tissue engineering improved their mechanical properties and thermal stability also showing better cytocompatibility measured using a fibroblast proliferation test [151]. PVA/nanocellulose fibres also presented excellent mechanical properties [152]. PEO [153, 154], PLA [155, 156], and polyvinylidenefluoride-co-hexafluoropropylene [157] have been recently tested with similar results. Other authors stressed the need for improving the compatibility between the hydrophilic nanocellulose and hydrophobic polymers [158, 159].

Nanomaterials allow fabricating miniaturized biosensors with fast answer and precise and accurate identification. The efficiency of biorecognition is directly linked to the number of biorecognition sites and, therefore, with the surface area of the sensor. The use of electrospun mats take advantage of the high surface or nanofibres to boost the sensitivity of molecular recognition using specific antibodies. In fact, nanobiosensing already constitutes a separate chapter of nanotechnology, which, concerning enzyme-based nanobiosensors, has been thoroughly reviewed recently [160]. Nanobiocatalysis is another emerging field of research in the boundary of nanotechnology and biotechnology in which the immobilization of enzymes at the nanoscale is applied to bioprocessing applications. This exciting subject topic has also been recently reviewed [161].

A particular class of immunoassay applications susceptible to be implemented on nanofibres refer to whole cells. Zha et al. used a polymerizable silane derivative to prepare sol-gel electrospun nanofibrous membranes for immobilizing membrane proteins and antibodies in order to allow targeted cell capture [162]. Electrospun polyhydroxybutyrate fibers were dipcoated by polymethyl methacrylatecomethacrylic acid, and used for the covalent (using COOH groups) immobilization of the dengue virus antibody with substantial signal intensity gained compared with a conventional ELISA assay [163]. Antibody functionalized nanofibrous nitrocellulose was used to prepare a sensor based on a conductometric immunoassay after spray deposition of silver on nanofibres followed by antibody attachment. The detection time of the biosensor was 8 min with a detection limit of 61 CFU/mL for *E coli* [164]. The use of nanofibres with immobilized antibodies as scaffolds for tissue engineering applications was mentioned before.

9. Conclusions and prospective

The electrospinning of polymeric fibres constitutes the first general technique available for producing nanofibres. From laboratory prototypes to commercial applications, the roadmap goes through improvements in large-scale production. For it, more research and development efforts are necessary to upgrade current technology in order to supply large amounts of uniform quality mats with stringent process control and keeping costs as low as possible. The use of large amounts of solvents is a particularly critical issue that forces the design of environmentally clean processes-products, such as those using aqueous solutions.

Many antibacterial nanofibres and composites have been developed during last years, but there still a need to develop of eco-friendly antimicrobial mats using materials from renewable sources. The use of natural antimicrobials and biodegradable polymers would have to prove competitive with more conventional approaches. The testing of antimicrobials has been essentially focused on conventional strains of *E. coli* or *S. aureus*, but further work is required using more pathogenic strains of bacteria and fungi including antibiotic resistant bacteria. The way of inducing cell impairment is simple in certain cases, such as metal or drug releasing mats, but little is known concerning the physicochemical mechanisms of cell attachment, colonization and biofilm formation in nanofibrous supports.

Polyfunctiontal materials for biomedical applications are still in their infancy in view of the possibilities of nanofibrous mats. From conventional polymers to bacterial polysaccharides, the production of biocompatible fibres offers almost unlimited possibilities. The research performed during last years showed that it is possible to develop electrospun organic–inorganic hybrid nanofibers that are biocompatible and at the same time toxic to cancer cells and pathogenic microorganisms by careful combination of metals or chemicals. The production of wound dressing materials to replace conventional dressings is another expanding filed. However, the interactions of functionalized nanofibres with human cells, including their general toxicological profile, is a very complex issue and is still lacking from generally accepted models. In this regard, stem cell research offers metabolically competent pluripotent stem cells for *in vitro* predictive toxicity and compatibility studies with advantages over established cell lines.

The synthesis of tailored carrier nanoparticles experienced a boosting during last years. More sophisticated materials and increasingly complex chemical functionalization are expected to give rise to drug-loaded fibres for a wide range of applications involving controlled release of active substances. There are clear advantages of nanofibrous carriers compared to conventional dosage forms such as site-specific delivery and multiple drug encapsulation, but additional work has to be performed to tailor the rate of delivery in order to increase therapeutic efficacy by reducing drug toxicity, which is a critical issue for antineoplastic agents, for example.

Electrospun materials can gain a place in food technology as food packaging materials or encapsulating probiotics. Concerning packaging, further research is needed on the inclusion of nanofibres in structured polymeric films before gaining a space in the market. Probiotics are critically affected by stability during processing and storage. Their fate after ingestion is another issue that has to be addressed to. Nanofibres as supports for analytical devices provide high surface area and mass transfer rates, which improve the signal to noise ratio and make them ideal for their use in pathogen detection in many areas. The production of safe food or the control of post-harvest pathogens are areas that may benefit from it.

The environmental applications of electrospun nanofibres are limited by their mechanical properties. The inclusion of reinforcements such as nanocellulose or other organic or inorganic loads increases the mechanical strength of nanofibres allowing their applications as stand-alone membranes or filters. Despite the many advantages of titanium dioxide photocatalysis and extensive lab-research done in this field, there has been little commercial or industrial use of this technology. The major reason for it is the loss of efficiency of supported photocatalysts compared with nanopowdered forms. Nanofibres offer a unique possibility for creating high surface photocatalytic devices for applications such as pollution abatement of artificial photosynthesis.

Abbreviations

3ABAPANI	Poly(aniline-co-3-aminobenzoic acid)
CA	Cellulose acetate
coPLA	Poly(L-lactide-co-D,L-lactide)
ECM	Extracellular matrix
EPS	Extracellular polymeric substances
NP	Nanoparticles
PAN	Polyacrylonitrile
PCL	Poly(ε-caprolactone)
PEG	Polyethylene glycol
PEO	Polyethylene oxide
PLA	Poly(lactic acid) or Polyester polylactide
PLGA	Poly lactic-co-glycolic acid
PLLCL	Poly(L-lactic acid) co-poly(ε-caprolactone)
PS	Polystyrene
PU	Polyurethane
PVA	Poly(vinyl alcohol)
PVP	Polyvinylpyrrolidone
β-CD	β-Cyclodextrin

References

1   Greiner, A.; Wendorff, J. H. "Electrospinning: a fascinating method for the preparation of ultrathin fibers", Ang. Chem. Int. Ed. 2007, 46, 5670-5703.

2   Bhardwaj, N.; Kundu, S.C. "Electrospinning: A fascinating fiber fabrication technique", Biotechnol. Adv. 2010, 28, 325–347.

3   Sill, T.J.; von Recum, H.A. "Electrospinning: applications in drug delivery and tissue engineering", Biomaterials 2008, 29, 1989-2006.

4   Bhattacharjee P.K.; Rutledge G.C. "Electrospinning and Polymer Nanofibers: Process Fundamentals", In Comprehensive Biomaterials; Ducheyne, P.; Healy, K.E.; Hutmacher, D.W.; Grainger, D.W.; Kirkpatrick, C.J., Eds.; Elsevier, 2011, 1, 497-512.

5   Huang, Z.M.; Zhang, Y.Z.; Kotaki, M.; Ramakrishna S. "A review on polymer nanofibers by electrospinning and their applications in nanocomposites", Compos. Sci. Technol. 2003, 63, 2223–2253.

6   Chase, G.G.; Varabhas, J.S.; Reneker, D.H. "New methods to electrospin nanofibers", J. Eng. Fiber Fabrics 2011, 6, 32-38.

7   Gule, N.P.; de Kwaadsteniet, M.; Cloete, T.E.; Klumperman, B. "Furanone-containing poly(vinyl alcohol) nanofibers for cell-adhesion inhibition", Water Res. 2013, 47, 1049-1059.

8   Liu, Y.; Dong, L.; Fan, J.; Wang, R.; Yu, J.Y. "Effect of applied voltage on diameter and morphology of ultrafine fibers in bubble electrospinning", J. Appl. Polym. Sci. 2011, 120, 592-598.

9   Persano, L.; Camposeo, A.; Tekmen, C.; Pisignano, D. "Industrial upscaling of electrospinning and applications of polymer nanofibers: a review", Macromol. Mater. Eng. 2013, 298, 504-520.

10   Lev, J.; Holba, M.; Došek, M.; Kalhotka, L.; Mikula, P.; Kimmer, D. "A novel electrospun polyurethane nanofibre membrane– Production parameters and suitability for waste water (WW) treatment", Water Sci. Technol. 2014, 69, 1496-1501.

11   Toncheva, A.; Spasova, M.; Paneva, D.; Manolova, N.; Rashkov, I. "Drug-loaded electrospun polylactide bundles", J. Bioact. Compat. Polym. 2011, 26, 161-172.

12   Klein, S.; Avrahami, R.; Zussman, E.; Beliavski, M.; Tarre, S.; Green, M. "Encapsulation of Pseudomonas sp. ADP cells in electrospun microtubes for atrazine bioremediation", J. Ind. Microbiol. Biotechnol. 2012, 39, 1605-1613.

13   Chase, G.G.; Varabhas, J.S.; Reneker, D.H. "New methods to electrospin nanofibers", J. Eng. Fiber Fabrics 2011, 6, 32-38.

14   Forward, K.M.; Rutledge, G.C. "Free surface electrospinning from a wire electrode", Chem. Eng. J. 2012, 183, 492-503.

15   Sarkar, K.; Gomez, C.; Zambrano, S.; Ramirez, M.; de Hoyos, E.; Vasquez, H.; Lozano, K. "Electrospinning to Forcespinning™", Mat. Today 2010, 13, 12-14.

16   Agarwal, S.; Greiner, A. "On the way to clean and safe electrospinning—green electrospinning: emulsion and suspension electrospinning", Polym. Adv. Technol. 2011, 22, 372-378.

17   Nguyen, T.H.; Kim, Y.H.; Song, H.Y.; Lee, B.T. "Nano Ag loaded PVA nano-fibrous mats for skin applications", J. Biomed. Mater. Res. B Appl. Biomater. 2011, 96, 225-233.

18   Chen, J.; Li, Z.; Chao, D.; Zhang, W.; Wang, C. "Synthesis of size-tunable metal nanoparticles based on polyacrylonitrile nanofibers enabled by electrospinning and microwave irradiation", Mat. Lett. 2008, 62, 692-694.

19   Qi, H.; Hu, P.; Xu, J.; Wang, A. "Encapsulation of drug reservoirs in fibers by emulsion electrospinning: morphology characterization and preliminary release assessment", Biomacromolecules 2006, 7, 2327-2330.

20   Sridhar, R.; Sundarrajan, S.; Vanangamudi, A.; Singh, G.; Matsuura, T.; Ramakrishna, S. "Green processing mediated novel polyelectrolyte nanofibers and their antimicrobial evaluation", Macromol. Mat. Eng. 2014, 299, 283-289.

21   Knetsch, M.L.W.; Koole, L.H. "New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles", Polymers 2011, 3, 340-366.

22   Sumitha, M.S.; Shalumon, K.T.; Sreeja, V.N.; Jayakumar, R.; Nair, S.V.; Menon, D. "Biocompatible and antibacterial nanofibrous poly(ε-caprolactone)-nanosilver composite scaffolds for tissue engineering applications", J. Macromol. Sci. 2012, 49, 131-138.

23   Ahmad, Z.; Vargas-Reus, M.A.; Bakhshi, R.; Ryan, F.; Ren, G.G.; Oktar, F.; Allaker, R.P. "Antimicrobial properties of electrically formed elastomeric polyurethane-copper oxide nanocomposites for medical and dental applications", Meth. Enzymol. 2012, 509, 87-99.

24   Rzayev, Z.M.O.; Erdönmez, D.; Erkan, K.; Simsek, M.; Bunyatova, U. "Functional copolymer/organo-MMT nanoarchitectures. XXII. Fabrication and characterization of antifungal and antibacterial poly(vinyl alcohol-co-vinyl acetate/ODA-MMT/AgNPs nanofibers and nanocoatings by e-spinning and c-spinning methods", Int. J. Polym. Mater. Polym. Biomater. 2014, 64, 267-278.

25   Martínez-Abad, A.; Sánchez, G.; Lagarón, J.M.; Ocio, M.J. "Influence of speciation in the release profiles and antimicrobial performance of electrospun ethylene vinyl alcohol copolymer (EVOH) fibers containing ionic silver ions and silver nanoparticles", Colloid Polym. Sci. 2013, 291, 1381-1392.

26   Quirós, J.; Borges, J. P.; Boltes, K.; Rodea-Palomares, I.; Rosal, R. "Antimicrobial electrospun silver-, copper- and zinc-doped polyvinylpyrrolidone nanofibers", J. Hazard. Mater. 2015, 299, 298-305.

27   Li, W.; Wang, J.; Chi, H.; Wei, G.; Zhang, J.; Dai, L. "Preparation and antibacterial activity of polyvinyl alcohol/regenerated silk fibroin composite fibers containing Ag nanoparticles", J. Appl. Polym. Sci. 2012, 123, 20-25.

28   Paneva, D.; Manolova, N.; Argirova, M.; Rashkov, I. "Antibacterial electrospun poly(ε-caprolactone)/ascorbyl palmitate nanofibrous materials", Int. J. Pharm. 2011, 416, 346-355.

29   Tian, L.; Wang, P.; Zhao, Z.; Ji, J. "Antimicrobial activity of electrospun poly(butylenes succinate) fiber

mats containing PVP-capped silver nanoparticles",
Appl. Biochem. Biotechnol. 2013, 171, 1890-1899.

30 Shi, Q.; Vitchuli, N.; Nowak, J.; Noar, J.; Caldwell,
J.M.; Breidt, F.; Bourham, M.; McCord, M.; Zhang, X.
"One-step synthesis of silver nanoparticle-filled nylon 6
nanofibers and their antibacterial properties", J. Mater.
Chem. 2011, 21, 10330-10335.

31 Dobrzański, L.A.; Hudecki, A.; Chladek, G.; Król, W.;
Mertas, A. "Surface properties and antimicrobial
activity of composite nanofibers of polycaprolactone
with silver precipitations", Arch. Mat. Sci. Eng. 2014,
70, 53-60.

32 Hafez, E.E.; El-Aassar, M.R.; Khalil, K.A.; Al-Deyab,
S.S.; Taha, T.H. "Poly (acrylonitrile-co-methyl
methacrylate) nanofibers grafted with bio-nanosilver
particles as antimicrobial against multidrug resistant
bacteria", African J. Biotechnol. 2011, 10, 19658-
19669.

33 Song, J.; Wang, C.; Chen, M.; Regina, V.R.; Wang, C.;
Meyer, R.L.; Xie, E.; Dong, M.; Besenbacher, F. "Safe
and effective Ag nanoparticles immobilized
antimicrobial nano-nonwovens", Adv. Eng. Mater.
2012, 14, B240-B246.

34 Shi, Y.; Li, Y.; Zhang, J.; Yu, Z.; Yang, D.
"Electrospun polyacrylonitrile nanofibers loaded with
silver nanoparticles by silver mirror reaction", Mater.
Sci. Eng. C, 2015, 51, 346-355.

35 Li, H.; Li, C.; Zhang, C.; Bai, J.; Xu, T.; Sun, W. "Well-
dispersed copper nanorods grown on the surface-
functionalized PAN fibers and its antibacterial activity",
J. Appl. Polym. Sci. 2014, 131, 41011.

36 Amina, M.; Hassan, M.S.; Al Musayeib, N.M.; Amna,
T.; Khil, M.S. "Improved antibacterial activity of HAP
garlanded PLGA ultrafine fibers incorporated with CuO:
synthesis and characterization", J. Sol Gel Sci. Technol.
2014, 71, 43-49.

37 Sun, L.M.; Zhang, C.L.; Li, P. "Characterization,
antimicrobial activity, and mechanism of a high-
performance (-)-Epigallocatechin-3-gallate (EGCG)-Cu
II/polyvinyl alcohol (PVA) nanofibrous membrane", J.
Agric. Food Chem. 2011, 59, 5087-5092.

38 Hassan, M.S.; Amna, T.; Kim, H.Y.; Khil, M.S.
"Enhanced bactericidal effect of novel CuO/TiO_2
composite nanorods and a mechanism thereof",
Compos. Part B Eng. 2013, 45, 904-910.

39 Virovska, D.; Paneva, D.; Manolova, N.; Rashkov, I.;
Karashanova, D. "Electrospinning/electrospraying vs.
electrospinning: a comparative study on the design of
poly(L-lactide)/zinc oxide non-woven textile", Appl.
Surf. Sci. 2014, 311, 842-850.

40 Anitha, S.; Brabu, B.; Thiruvadigal, D.J.;
Gopalakrishnan, C.; Natarajan, T.S. "Optical,
bactericidal and water repellent properties of
electrospun nano-composite membranes of cellulose
acetate and ZnO", Carbohyd. Polym. 2013, 97, 856-
863.

41 Amna, T.; Hassan, M.S.; Barakat, N.A.M.; Pandeya,
D.R.; Hong, S.T.; Khil, M.S.; Kim, H.Y. "Antibacterial
activity and interaction mechanism of electrospun zinc-
doped titania nanofibers", Appl. Microbiol. Biotechnol.
2012, 93, 743-751.

42 Quirós, J.; Aguado, S.; Boltes, K.; Guzmán, R.;
Vilatela, J.; Rosal, R. "Antimicrobial metal-organic
frameworks incorporated into electrospun fibers",
Chem. Eng. J. 2015, 262, 189-197.

43 Aguado, S.; Quirós, J.; Cavinet, J.; Farrusseng, D.;
Boltes, K.; Rosal, R. "Antimicrobial activity of cobalt
imidazolate metal-organic frameworks", Chemosphere
2014, 113, 188-192.

44 Tijing, L.D.; Ruelo, M.T.G.; Amarjargal, A.; Pant,
H.R.; Park, C.H.; Kim, D.W.; Kim, C.S. "Antibacterial
and superhydrophilic electrospun polyurethane
nanocomposite fibers containing tourmaline
nanoparticles", Chem. Eng. J. 2012, 197, 41-48.

45 Li, W.; Li, X.; Chen, Y.; Li, X.; Deng, H.; Wang, T.;
Huang, R.; Fan, G. "Poly(vinyl alcohol)/sodium
alginate/layered silicate based nanofibrous mats for
bacterial inhibition", Carbohyd. Polym. 2013, 92, 2232-
2238.

46 Deng, H.; Li, X.; Ding, B.; Du, Y.; Li, G.; Yang, J.; Hu,
X. "Fabrication of polymer/layered silicate intercalated
nanofibrous mats and their bacterial inhibition activity",
Carbohyd. Polym. 2011, 83, 973-978.

47 Deng, H.; Wang, X.; Liu, P.; Ding, B.; Du, Y.; Li, G.;
Hu, X.; Yang, J. "Enhanced bacterial inhibition activity
of layer-by-layer structured polysaccharide film-coated
cellulose nanofibrous mats via addition of layered
silicate", Carbohyd. Polym. 2011, 83, 239-245.

48 Gupta, K.K.; Mishra, P.K.; Srivastava, P.; Gangwar,
M.; Nath, G.; Maiti, P. "Hydrothermal in situ
preparation of TiO_2 particles onto poly(lactic acid)
electrospun nanofibers", Appl. Surf. Sci. 2013, 264,
375-382.

49 Pant, H.R.; Bajgai, M.P.; Nam, K.T.; Seo, Y.A.;
Pandeya, D.R.; Hong, S.T.; Kim, H.Y. "Electrospun
nylon-6 spider-net like nanofiber mat containing TiO_2
nanoparticles: A multifunctional nanocomposite textile
material", J. Hazard. Mater. 2011, 185, 124-130.

50 Ignatious, F.; Sun, L.; Lee, C.P.; Baldoni, J.
"Electrospun nanofibers in oral drug delivery", Pharm.
Res. 2010, 27, 576-588.

51 Zamani, M.; Prabhakaran, M.P.; Ramakrishna, S.
"Advances in drug delivery via electrospun and
electrosprayed nanomaterials", Int. J. Nanomed. 2013,
8, 2997-3017.

52 Stoyanova, N.; Paneva, D.; Mincheva, R.; Toncheva,
A.; Manolova, N.; Dubois, P.; Rashkov, I. "Poly(L-
lactide) and poly(butylene succinate) immiscible
blends: from electrospinning to biologically active
materials", Mater. Sci. Eng. C 2014, 41, 119-126.

53 Yoo, H.S.; Kim, T.G.; Park, T.G. "Surface-
functionalized electrospun nanofibers for tissue
engineering and drug delivery", Adv. Drug Deliv. Rev.
2009, 61, 1033-1042.

54 Zheng, F.; Wang, S.; Wen, S.; Shen, M.; Zhu, M.; Shi,
X. "Characterization and antibacterial activity of
amoxicillin-loaded electrospun nano-
hydroxyapatite/poly(lactic-co-glycolic acid) composite
nanofibers", Biomaterials, 2013, 34, 1402-1412.

55 Sirc, J.; Kubinova, S.; Hobzova, R.; Stranska, D.;
Kozlik, P.; Bosakova, Z.; Marekova, D.; Holan, V.;
Sykova, E.; Michalek, J. "Controlled gentamicin release
from multi-layered electrospun nanofibrous structures
of various thicknesses", Int. J. Nanomed. 2012, 7, 5315-
5325.

56 El-Newehy, M.H.; Al-Deyab, S.S.; Kenawy, E.R.;
Abdel-Megeed, A. "Fabrication of electrospun
antimicrobial nanofibers containing metronidazole
using nanospider technology", Fibers Polym. 2012, 13,
709-717.

57   Kim, J.R.; Michielsen, S. "Photodynamic antifungal activities of nanostructured fabrics grafted with rose bengal and phloxine B against *Aspergillus fumigatus*", J. Appl. Polym. Sci. 2015, 132, 42114.

58   Karthikeyan, K.; Sowjanya, R.S.; Yugandhar, A.D.; Gopinath, S.; Korrapati, P.S. "Design and development of a topical dosage form for the convenient delivery of electrospun drug loaded nanofibers", RSC Advances 2015, 5, 52420-52426.

59   Liu, X.; Lin, T.; Gao, Y.; Xu, Z.; Huang, C.; Yao, G.; Jiang, L.; Tang, Y.; Wang, X. "Antimicrobial electrospun nanofibers of cellulose acetate and polyester urethane composite for wound dressing", J. Biomed. Mater B Appl. Biomater. 2012, 100, 1556-1565.

60   Tonglairoum, P.; Ngawhirunpat, T.; Rojanarata, T.; Kaomongkolgit, R.; Opanasopit, P. "Fabrication of a novel scaffold of clotrimazole-microemulsion-containing nanofibers using an electrospinning process for oral candidiasis applications", Colloids Surf. B 2015, 126, 18-25.

61   Sirelkhatim, N.; Lajeunesse, D.; Kelkar, A.D.; Zhang, L. "Antifungal activity of amidoxime surface functionalized electrospun polyacrylonitrile nanofibers", Mater. Lett. 2014, 141, 217-220.

62   Lakshminarayanan, R.; Sridhar, R.; Loh, X.J.; Nandhakumar, M.; Barathi, V.A.; Kalaipriya, M.; Kwan, J.L.; Liu, S.P.; Beuerman, R.W.; Ramakrishna, S. "Interaction of gelatin with polyenes modulates antifungal activity and biocompatibility of electrospun fiber mats", Int. J. Nanomed. 2014, 9, 2439-2458.

63   Mahady, G.B.; Huang, Y.; Doyle, B.J.; Locklear, T. "Natural products as antibacterial agents", Stud. Nat. Prod. Chem. 2008, 35, 423-444.

64   Avci, H.; Monticello, R.; Kotek, R. "Preparation of antibacterial PVA and PEO nanofibers containing *Lawsonia inermis* (henna) leaf extracts", J. Biomater. Sci. Polym. Ed. 2013, 24, 1815-1830.

65   Gatti, J.W.; Smithgall, M.C.; Paranjape, S.M.; Rolfes, R.J.; Paranjape, M. "Using electrospun poly(ethylene-oxide) nanofibers for improved retention and efficacy of bacteriolytic antibiotics", Biom. Microd. 2013, 15, 887-893.

66   Kriegel, C.; Kit, K.M.; McClements, D.J.; Weiss, J. "Nanofibers as carrier systems for antimicrobial microemulsions. II. Release characteristics and antimicrobial activity", J. Appl. Polym. Sci. 2010, 118, 2859-2868.

67   Coneski, P.N.; Fulmer, P.A.; Giles, S.L.; Wynne, J.H. "Lyotropic self-assembly in electrospun biocidal polyurethane nanofibers regulates antimicrobial efficacy", Polymer 2014, 55, 495-504.

68   Ignatova, M.; Petkova, Z.; Manolova, N.; Markova, N.; Rashkov, I. "Non-woven fibrous materials with antibacterial properties prepared by tailored attachment of quaternized chitosan to electrospun mats from maleic anhydride copolymer", Macromol. Biosci. 2012, 12, 104-115.

69   Dilamian, M.; Montazer, M.; Masoumi, J. "Antimicrobial electrospun membranes of chitosan/poly(ethylene oxide) incorporating poly(hexamethylene biguanide) hydrochloride", Carbohyd. Polym. 2013, 94, 364-371.

70   Ignatova, M.; Stoilova, O.; Manolova, N.; Markova, N.; Rashkov, I. "Electrospun mats from styrene/maleic anhydride copolymers: Modification with amines and assessment of antimicrobial activity", Macromol. Biosci. 2010, 10, 944-954.

71   Nguyen, T.T.T.; Chung, O.H.; Park, J.S. "Coaxial electrospun poly(lactic acid)/chitosan (core/shell) composite nanofibers and their antibacterial activity", Carbohyd. Polym. 2011, 86, 1799-1806.

72   Bhattarai, N.; Edmondson, D.; Veiseh, O.; Matsen, F.A.; Zhang, M. "Electrospun chitosan-based nanofibers and their cellular compatibility", Biomaterials 2005, 26, 6176-6184.

73   Liang, D.; Hsiao, B.S.; Chu, B. "Functional electrospun nanofibrous scaffolds for biomedical applications", Adv. Drug Deliv. Rev. 2007, 59, 1392–1412.

74   Martínez-Camacho, A.P.; Cortez-Rocha, M.O.; Castillo-Ortega, M.; Burgos-Hernández, A.; Ezquerra-Brauer, J.M.; Plascencia-Jatomea, M. "Antimicrobial activity of chitosan nanofibers obtained by electrospinning", Polym. Int. 2011, 60, 1663-1669.

75   Nada, A.A.; James, R.; Shelke, N.B.; Harmon, M.D.; Awad, H.M.; Nagarale, R.K.; Kumbar, S.G. "A smart methodology to fabricate electrospun chitosan nanofiber matrices for regenerative engineering applications", Polym. Adv. Technol. 2014, 25, 507-515.

76   Jin, X.; Wang, J.; Bai, J. "Synthesis and antimicrobial activity of the Schiff base from chitosan and citral", Carbohydr. Res. 2009, 344, 825-829.

77   Raveendran, S.; Dhandayuthapani, B.; Nagaoka, Y.; Yoshida, Y.; Maekawa, T.; Kumar, D.S. "Biocompatible nanofibers based on extremophilic bacterial polysaccharide, Mauran from *Halomonas maura*,", Carbohyd. Polym. 2013, 92, 1225-1233.

78   Gizdavic-Nikolaidis, M.; Ray, S.; Bennett, J.R.; Easteal, A.J.; Cooney, R.P. "Electrospun functionalized polyaniline copolymer-based nanofibers with potential application in tissue engineering", Macromol. Biosci. 2010, 10, 1424-1431.

79   Spasova, M.; Manolova, N.; Paneva, D.; Mincheva, R.; Dubois, P.; Rashkov, I.; Maximova, V.; Danchev, D. "Polylactide stereocomplex-based electrospun materials possessing surface with antibacterial and hemostatic properties", Biomacromolecules 2010, 11, 151-159.

80   Kim, S.H.; Shin, C.; Min, S.K.; Jung, S.M.; Shin, H.S. "In vitro evaluation of the effects of electrospun PCL nanofiber mats containing the microalgae *Spirulina* (*Arthrospira*) extract on primary astrocytes", Colloids Surf. B 2012, 90, 113-118.

81   Yan, N.; Zhang, X.; Cai, Q.; Yang, X.; Zhou, X.; Wang, B.; Deng, X. "The effects of lactidyl/glycolidyl ratio and molecular weight of poly(D,L-lactide-co-glycolide) on the tetracycline entrapment and release kinetics of drug-loaded nanofibers", J. Biomater. Sci. Poluym. Ed. 2012, 23, 1005-1019.

82   Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; Kim, Y.K.; Lee, Y.S.; Jeong, D.H.; Cho, M.H. "Antimicrobial effects of silver nanoparticles", Nanomed. Nanotechnol. 2007, 3, 95–101.

83   Asha-Rani, P.V.; Kah-Mun, G.L.; Hande, M.P.; Suresh, V. "Cytotoxicity and genotoxicity of silver nanoparticles in human cells", Nano 2009, 3, 279–290.

84   Singh, S.; Nalwa, H.S. "Nanotechnology and health safety–toxicity and risk assessments of nanostructured materials on human health", J. Nanosci. Nanotechnol. 2007, 7, 3048-3070.

85 Nel, A.; Xia, T.; Madler, L.; Li, N. "Toxic potential of materials at the nanolevel", Science 2006, 311, 622–627.

86 Almajhdi, F.N.; Fouad, H.; Khalil, K.A.; Awad, H.M.; Mohamed, S.H.S.; Elsarnagawy, T.; Albarrag, A.M.; Al-Jassir, F.F.; Abdo, H.S. "In-vitro anticancer and antimicrobial activities of PLGA/silver nanofiber composites prepared by electrospinning", J. Mater. Sci. Mater. Med. 2014, 25, 1045-1053.

87 Jin, G.; Prabhakaran, M.P.; Nadappuram, B.P.; Singh, G.; Kai, D.; Ramakrishna, S. "Electrospun poly(L-lactic acid)-co-poly(ε-caprolactone) nanofibres containing silver nanoparticles for skin-tissue engineering", J. Biomater. Sci. Polym. Ed. 2012, 23, 2337-2352.

88 Manjumeena, R.; Elakkiya, T.; Duraibabu, D.; Feroze-Ahamed, A.; Kalaichelvan, P.T., Venkatesan, R. "'Green' biocompatible organic–inorganic hybrid electrospun nanofibers for potential biomedical applications", J. Biomater. Appl. 2014, 29, 1039-1055.

89 Guex, A.G.; Hegemann, D.; Giraud, M.N.; Tevaearai, H.T.; Popa, A.M.; Rossi, R.M.; Fortunato, G. "Covalent immobilisation of VEGF on plasma-coated electrospun scaffolds for tissue engineering applications", Colloids Surf. B 2014, 123, 724-733.

90 Oliveira, C.; Costa-Pinto, A.R.; Reis, R.L.; Martins, A.; Neves, N.M. "Biofunctional nanofibrous substrate comprising immobilized antibodies and selective binding of autologous growth factors", Biomacromolecules 2014, 15, 2196-2205.

91 Zhang, M.; Wang, Z.; Wang, Z.; Feng, S.; Xu, H.; Zhao, Q.; Wang, S.; Fang, J.; Qiao, M.; Kong, D. "Immobilization of anti-CD31 antibody on electrospun poly(ε-caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells", Colloids Surf. B 2011, 85, 32-39.

92 Ruckh, T.T.; Oldinski, R.A.; Carroll, D.A.; Mikhova, K.; Bryers, J.D.; Popat, K.C. "Antimicrobial effects of nanofiber poly(caprolactone) tissue scaffolds releasing rifampicin", J. Mater. Sci. Mater. Med. 2012, 23, 1411-1420.

93 Liu, H.; Leonas, K.K.; Zhao, Y. "Antimicrobial properties and release profile of ampicillin from electrospun poly(ε-caprolactone) nanofiber yarns", J. Eng. Fiber Fabrics 2010, 5, 10-19.

94 Chen, D.W.C.; Liao, J.Y.; Liu, S.J.; Chan, E.C. "Novel biodegradable sandwich-structured nanofibrous drug-eluting membranes for repair of infected wounds: an in vitro and in vivo study", Int. J. Nanomedicine 2012, 7, 763-771.

95 Toncheva, A.; Paneva, D.; Maximova, V.; Manolova, N.; Rashkov, I. "Antibacterial fluoroquinolone antibiotic-containing fibrous materials from poly(L-lactide-co-D,L-lactide) prepared by electrospinning", Eur. J. Pharm. Sci. 2012, 47, 642-651.

96 Hild, N.; Tawakoli, P.N.; Halter, J.G.; Sauer, B.; Buchalla, W.; Stark, W.J.; Mohn, D. "pH-dependent antibacterial effects on oral microorganisms through pure PLGA implants and composites with nanosized bioactive glass", Acta Biomater. 2013, 9,. 9118-9125.

97 Seyam, A.F.M.; Hudson, S.M.; Ibrahim, H.M.; Waly, A.I.; Abou-Zeid, N.Y. "Healing performance of wound dressing from cyanoethyl chitosan electrospun fibres", Indian J. Fibre Text. Res. 2012, 37, 205-210.

98 Nawalakhe, R.G.; Hudso, S.M.; Seyam, A.M.; Waly, A.I.; Abou-Zeid, N.Y.; Ibrahim, H.M. "Development of electrospun iminochitosan for improved wound healing application", J. Eng. Fibers Fabrics 2012, 7, 47-55.

99 Bai, J.Q.; Li, Y.H.; Duan, Q.; Chang, J.J. "Electrospinning synthesis of PLA fiber film containing antimicrobial silver nanorods", Adv. Mater. Res. 2014, 936, 681-686.

100 Lee, S.J.; Heo, D.N.; Moon, J.H.; Ko, W.K.; Lee, J.B.; Bae, M.S.; Park, S.W.; Kim, J.E.; Lee, D.H.; Kim, E.C.; Lee, C.H.; Kwon, I.K. "Electrospun chitosan nanofibers with controlled levels of silver nanoparticles. Preparation, characterization and antibacterial activity", Carbohyd. Polym. 2014, 111, 530-537.

101 Abdelgawad, A.M.; Hudson, S.M.; Rojas, O.J. "Antimicrobial wound dressing nanofiber mats from multicomponent (chitosan/silver-NPs/polyvinyl alcohol) systems", Carbohyd. Polym. 2014, 100, 166-178.

102 Zhou, T.; Mo, X.; Sun, J. "Development and properties of electrospun collagen-chitosan nanofibrous membranes as skin wound healing materials", J. Fiber Bioengineering and Informatics 2014, 7, 319-325.

103 Uttayarat, P.; Jetawattana, S.; Suwanmala, P.; Eamsiri, J.; Tangthong, T.; Pongpat, S. "Antimicrobial electrospun silk fibroin mats with silver nanoparticles for wound dressing application", Fibers Polym. 2012, 13, 999-1006.

104 Celebioglu, A.; Aytac, Z.; Umu, O.C.O.; Dana, A.; Tekinay, T.; Uyar, T. "One-step synthesis of size-Tunable Ag nanoparticles incorporated in electrospun PVA/cyclodextrin nanofibers", Carbohyd. Polym. 2014, 99, 808-816.

105 Shi, Q.; Vitchuli, N.; Nowak, J.; Caldwell, J.M.; Breidt, F.; Bourham, M.; Zhang, X.; McCord, M. "Durable antibacterial Ag/polyacrylonitrile (Ag/PAN) hybrid nanofibers prepared by atmospheric plasma treatment and electrospinning", Eur. Polym. J. 2011, 47, 1402-1409.

106 Chen, J.P.; Chiang, Y. "Bioactive electrospun silver nanoparticles-containing polyurethane nanofibers as wound dressings", J. Nanosci. Nanotechnol. 2010, 10, 7560-7564.

107 Lalani, R.; Liu, L. "Electrospun zwitterionic poly(sulfobetaine methacrylate) for nonadherent, superabsorbent, and antimicrobial wound dressing applications", Biomacromolecules 2012, 13, 1853-1863.

108 Macocinschi, D.; Filip, D.; Paslaru, E.; Munteanu, B.S.; Dumitriu, R.P.; Pricope, G.M.; Aflori, M.; Dobromir, M.; Nica, V.; Vasile, C. "Polyurethane–extracellular matrix/silver bionanocomposites for urinary catheters", J. Bioact. Compat. Polym. 2014, 30, 99-113.

109 Lin, Y.N.; Chang, K.M.; Jeng, S.C.; Lin, P.Y.; Hsu, R.Q. "Study of release speeds and bacteria inhibiting capabilities of drug delivery membranes fabricated via electrospinning by observing bacteria growth curves", J. Mater Sci. Mater. Medicine 2011, 22, 571-577.

110 De Groote, M.A.; Fang, F.C. "NO inhibitions: antimicrobial properties of nitric oxide", Clin. Infect. Dis., 1995, 21, S162–S165.

111 Wold, K.A.; Damodaran, V.B.; Suazo, L.A.; Bowen, R.A.; Reynolds, M.M. "Fabrication of biodegradable polymeric nanofibers with covalently attached no donors", ACS Appl. Mater. Interfaces 2012, 4, 3022-3030.

112 European Commission "The Rapid Alert System for Food and Feed — 2014 annual report", Brussels, 2015.

113 Korehei, R.; Kadla, J. "Incorporation of T4 bacteriophage in electrospun fibres", J. Appl. Microbiol. 2013, 114, 1425-1434.

114 Dai, R.T.; Sun, C.F.; Wu, G.Q. "Food active packagings and their application", Storage & Process 2001, 1, 4-7.

115 Ge, L.; Zhao, Y.S.; Mo, T.; Li, J.R.; Li, P. "Immobilization of glucose oxidase in electrospun nanofibrous membranes for food preservation", Food Control 2012, 26, 188-193.

116 Kayaci, F.; Umu, O.C.O.; Tekinay, T.; Uyar, T. "Antibacterial electrospun poly(lactic acid) (PLA) nanofibrous webs incorporating triclosan/cyclodextrin inclusion complexes", J. Agric. Food Chem. 2013, 61, 3901-3908.

117 Amna, T.; Hassan, M.S.; Yousef, A.; Mishra, A.; Barakat, N.A.M.; Khil, M.S.; Kim, H.Y. "Inactivation of foodborne pathogens by NiO/TiO$_2$ composite nanofibers: A novel biomaterial system", Food Bioprocess Technol. 2013, 6, 988-996.

118 Korehei, R.; Kadla, J.F. "Encapsulation of T4 bacteriophage in electrospun poly(ethylene oxide)/cellulose diacetate fibers", Carbohyd. Polym. 2014, 100, 150-157.

119 Sett, S.; Lee, M.W.; Weith, M.; Pourdeyhimi, B.; Yarin, A.L. "Biodegradable and biocompatible soy protein/polymer/adhesive sticky nano-textured interfacial membranes for prevention of esca fungi invasion into pruning cuts and wounds of vines", J. Mater. Chem. B 2015, 3, 2147-2162.

120 Daels, N.; De Vrieze, S.; Sampers, I.; Decostere, B.; Westbroek, P.; Dumoulin, A.; Dejans, P.; De Clerck, K.; Van Hulle, S.W.H. "Potential of a functionalised nanofibre microfiltration membrane as an antibacterial water filter", Desalination 2011, 275, 285-290.

121 Hori, K.; Matsumoto, S. "Bacterial adhesion: From mechanism to control", Biochem. Eng. J. 2010, 48, 424-434.

122 Garrett, T.R.; Bhakoo, M.; Zhang, Z. "Bacterial adhesion and biofilms on surfaces", Prog. Nat. Sci. 2008, 18, 1049-1056.

123 De Vrieze, S.; Daels, N.; Lambert, K.; Decostere, B.; Hens, Z.; van Hulle, S.; de Clerck, K. "Filtration performance of electrospun polyamide nanofibres loaded with bactericides", Text. Res. J. 2012, 82, 37-44.

124 Dasari, A.; Quirós, J.; Herrero, B.; Boltes, K.; García-Calvo, E.; Rosal, R. "Antifouling membranes prepared by electrospinning polylactic acid containing biocidal nanoparticles", J. Memb. Sci. 2012, 405-406, 134–140.

125 Chaudhary, A.; Gupta, A.; Mathur, R.B.; Dhakate, S.R. "Effective antimicrobial filter from electrospun polyacrylonitrile-silver composite nanofibers membrane for conducive environment", Adv. Mater. Lett. 2014, 5, 562-568.

126 Cooper, A.; Oldinski, R.; Ma, H.; Bryers, J.D.; Zhang, M. "Chitosan-based nanofibrous membranes for antibacterial filter applications", Carbohyd. Polym. 2013, 92, 254-259.

127 Zhang, L.; Luo, J.; Menkhaus, T.J.; Varadaraju, H.; Sun, Y.; Fong, H. "Antimicrobial nano-fibrous membranes developed from electrospun polyacrylonitrile nanofibers", J. Memb. Sci. 2011, 369, 499-505.

128 Miao, Y.E.; Wang, R.; Chen, D.; Liu, Z.; Liu, T. "Electrospun self-standing membrane of hierarchical SiO$_2$@γ-AlOOH (boehmite) core/sheath fibers for water remediation", ACS Appl. Mater. Interfaces 2012, 4, 5353-5359.

129 Daghrir, R.; Drogui, P.; Robert, D. "Modified TiO$_2$ for environmental photocatalytic applications: a review", Ind. Eng. Chem. Res. 2013, 52, 3581-3599.

130 Paramasivam, I.; Jha, H.; Liu, N.; Schmuki, P. "A review of photocatalysis using self-organized TiO$_2$ nanotubes and other ordered oxide nanostructures", Small 2012, 8, 3073–3103.

131 Chuangchote, S.; Jitputti, J.; Sagawa, T.; Yoshikawa, S. "Photocatalytic activity for hydrogen evolution of electrospun TiO$_2$ nanofibers", ACS Appl. Mater. Interfaces, 2009, 1, 1140-1143.

132 Yousef, A.; El-Halwany, M.M.; Barakat, N.A.M.; Al-Maghrabi, M.N.; Kim, H.Y. "CuO-doped TiO$_2$ nanofibers as potential photocatalyst and antimicrobial agent", J. Ind. Eng. Chem. 2015, 26, 251–258.

133 Yousef, A.; Barakat, N.A.M.; Amna, T.; Al-Deyab, S.S.; Hassan, M.S.; Abdel-Hay, A.; Kim, H.Y. "Inactivation of pathogenic *Klebsiella pneumoniae* by CuO/TiO$_2$ nanofibers: A multifunctional nanomaterial via one-step electrospinning", Ceram. Int. 2012, 38, 4525-4532.

134 Srisitthiratkul, C.; Pongsorrarith, V.; Intasanta, N. "The potential use of nanosilver-decorated titanium dioxide nanofibers for toxin decomposition with antimicrobial and self-cleaning properties", Appl. Surf. Sci. 2011, 257, 8850-8856.

135 Nalbandian, M.J.; Zhang, M.; Sanchez, J.; Kim, S.; Choa, Y.H.; Cwiertny, D.M.; Myung, N.V. "Synthesis and optimization of Ag-TiO$_2$ composite nanofibers for photocatalytic treatment of impaired water sources", J. Hazard. Mater. 2015, 299, 141-148.

136 Lee, H.U.; Park, S.Y.; Lee, S.C.; Seo, J.H.; Son, B.; Kim, H.; Yun, H.J.; Lee, G.W.; Lee, S.M.; Nam, B.; Lee, J.W.; Huh, Y.S.; Jeon, C.; Kim, H.J.; Lee, J. "Highly photocatalytic performance of flexible 3 dimensional (3D) ZnO nanocomposite", Appl. Catal. B Environ. 2013, 144, 83-89.

137 Ryu, S.Y.; Park, M.K.; Kwak, S.Y. "Silver-titania/polyurethane composite nanofibre mat for chemical and biological warfare protection", Int. J. Nanotech. 2013, 10, 771-788.

138 Pant, H.R.; Pandeya, D.R.; Nam, K.T.; Baek, W.I.; Hong, S.T.; Kim, H.Y. "Photocatalytic and antibacterial properties of a TiO$_2$/nylon-6 electrospun nanocomposite mat containing silver nanoparticles", J. Hazard. Mater. 2011, 189, 465-471.

139 López-Rubio, A.; Sánchez, E.; Sanz, Y.; Lagarón, J.M. "Encapsulation of living bifidobacteria in ultrathin PVOH electrospun fibers", Biomacromolecules 2009, 10, 2823–2829.

140 Ghorani, B.; Tucker, N. "Fundamentals of electrospinning as a novel delivery vehicle for bioactive compounds in food nanotechnology", Food Hydrocol. 2015, 51, 227-240.

141 López-Rubio, A.; Sánchez, E.; Wilkanowicz, S.; Sanz, Y.; Lagarón, J.M. "Electrospinning as a useful technique for the encapsulation of living bifidobacteria in food hydrocolloids", Food Hydrocol. 2012, 28, 159-167.

142 Fung, W.Y.; Yuen, K.H.; Liong, M.T. "Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization", J. Agric. Food Chem. 2011, 59, 8140-8147.

143 Cronje, L.; Klumperman, B. "Modified electrospun polymer nanofibers as affinity membranes: The effect of pre-spinning modification versus post-spinning modification", Eur. Polym. J. 2013, 49, 3814-3824.

144 Vazquez, A.; Foresti, M.L.; Moran, J.I.; Cyras, V.P. "Extraction and Production of Cellulose Nanofibers", In Handbook of Polymer Nanocomposites. Processing, Performance and Application; Pandey, J.K.; Tagaki, H.; Nagarito, A.N.; Kim H.J., Eds.; Springer: Berlin 2015, 81-118.

145 Dufresne, A. Nanocellulose: from nature to high performance tailored materials. Walter de Gruyter: Berlin, 2012.

146 Zhang, C.; Salick, M.R.; Cordie, T.M.; Ellingham, T.; Dan, Y.; Turng, L.S. "Incorporation of poly(ethylene glycol) grafted cellulose nanocrystals in poly(lactic acid) electrospun nanocomposite fibers as potential scaffolds for bone tissue engineering", Mater. Sci. Eng. 2015, 49, 463-471.

147 Sutjarittangtham, K.; Tunkasiri, T.; Chantawannakul, P.; Intatha, U.; Eitssayeam, S. "Mechanically improved antibacterial polycaprolactone/propolis electrospun fiber mat by adding bacterial nanocellulose", J. Comput. Theor. Nanosci. 2015, 12, 798-803.

148 Sheng, L.; Jiang, R.; Zhu, Y.; Ji, Y. "Electrospun cellulose nanocrystals/polycaprolactone nanocomposite fiber mats", J. Macromol. Sci. B 2014, 53, 820-828.

149 Sun, C.; Boluk, Y.; Ayranci, C. "Investigation of nanofiber nonwoven meshes produced by electrospinning of cellulose nanocrystal suspensions in cellulose acetate solutions", Cellulose, 2015, 22, 2457-2470.

150 Huan, S.; Bai, L.; Liu, G.; Cheng, W.; Han, G. "Electrospun nanofibrous composites of polystyrene and cellulose nanocrystals: Manufacture and characterization", RSC Advances 2015, 63, 50756-50766.

151 Mo, Y.; Guo, R.; Liu, J.; Lan, Y.; Zhang, Y.; Xue, W.; Zhang, Y. "Preparation and properties of PLGA nanofiber membranes reinforced with cellulose nanocrystals", Colloids and Surf. B 2015, 132, 177-184.

152 Peresin, M.S.; Vesterinen, A.H.; Habibi, Y.; Johansson, L.S.; Pawlak, J.J.; Nevzorov, A.A.; Rojas, O.J. "Crosslinked PVA nanofibers reinforced with cellulose nanocrystals: Water interactions and thermomechanical properties", J. Appl. Polym. Sci. 2014, 131, 40334.

153 Xu, X.; Wang, H.; Jiang, L.; Wang, X.; Payne, S.A.; Zhu, J.Y.; Li, R. "Comparison between cellulose nanocrystal and cellulose nanofibril reinforced poly(ethylene oxide) nanofibers and their novel shish-kebab-like crystalline structures", Macromolecules 2014, 47, 34093416.

154 Naseri, N.; Mathew, A.P.; Girandon, L.; Fröhlich, M.; Oksman, K. "Porous electrospun nanocomposite mats based on chitosan–cellulose nanocrystals for wound dressing: effect of surface characteristics of nanocrystals", Cellulose 2014, 22, 521-534.

155 Cacciotti, I.; Fortunati, E.; Puglia, D.; Kenny, J.M.; Nanni, F. "Effect of silver nanoparticles and cellulose nanocrystals on electrospun poly(lactic) acid mats: Morphology, thermal properties and mechanical behavior", Carbohyd. Polym. 2014, 103, 22-31.

156 Pirani, S.; Abushammala, H.M.N.; Hashaikeh, R. "Preparation and characterization of electrospun PLA/nanocrystalline cellulose-based composites", J. Appl. Polym. Sci. 2013, 130, 3345-3354.

157 Lalia, B.S.; Guillen, E.; Arafat, H.A.; Hashaikeh, R. "Nanocrystalline cellulose reinforced PVDF-HFP membranes for membrane distillation application", Desalination, 2014, 332, 134-141.

158 Martínez-Sanz, M.; López-Rubio, A.; Lagarón, J.M. "Dispersing bacterial cellulose nanowhiskers in polylactides via electrohydrodynamic processing", J. Polym. Environ. 2012, 22, 27-40.

159 Martínez-Sanz, M.; López-Rubio, A.; Villano, M.; Oliveira, C.S.S.; Majone, M.; Reis, M.; Lagarón, J.M. "Production of bacterial nanobiocomposites of polyhydroxyalkanoates derived from waste and bacterial nanocellulose by the electrospinning enabling melt compounding method", J. Appl. Polym . Sci. 2015, doi: 10.1002/app.42486

160 De Cesare, F.; Macagnano, A. "Electrospinning-based nanobiosensor". In: Electrospinning for High Performance Sensors; Macagnano, A.; Zampetti E.; Kny, E., Ed.; Springer International Publishing: Cham (Switzerland), 2015, 225-279.

161 Misson, M.; Zhang, H.; Jin, B. "Nanobiocatalyst advancements and bioprocessing applications", J. Royal Soc. Interface 2015, 12, 20140891.

162 Zha, Z.; Cohn, C.; Dai, Z.; Qiu, W.; Zhang, J.; Wu, X. "Nanofibrous lipid membranes capable of functionally immobilizing antibodies and capturing specific cells", Adv. Mater. 2011, 23, 3435-3440.

163 Hosseini, S.; Azari, P.; Farahmand, E.; Gan, S.N.; Rothan, H.A.; Yusof, R.; Koole, L.H.; Djordjevic, I.; Ibrahim, F. "Polymethacrylate coated electrospun PHB fibers: An exquisite outlook for fabrication of paper-based biosensors", Biosens. Bioelectron. 2015, 69, 257-264.

164 Luo, Y.; Nartker, S.; Miller, H.; Hochhalter, D.; Wiederoder, M.; Wiederoder, S.; Setterington, E.; Drzal, L.T.; Alocilja, E.C. "Surface functionalization of electrospun nanofibers for detecting E. coli O157:H7 and BVDV cells in a direct-charge transfer biosensor", Biosens. Bioelectron. 2010, 26, 1612-1617.