Chapter

Electrospun Nanofibers: Characteristic Agents and Their Applications

Lingayya Hiremath, O. Sruti, B.M. Aishwarya, N.G. Kala and E. Keshamma

Abstract

This study aimed to introduce antibacterial nanofibers, produced by electrospinning as a novel technique in constructing nanostructured materials. The large size and less bioavailability due to impenetrable (or partial/improper penetration) membrane has resulted in production of nanofibers. These nano sized Fibers were successful in delivering the active ingredients and served the purpose of using plants for its cause. Some of the active ingredients include antimicrobial compounds that are incorporated into various products to prevent unwanted microbial growth. As higher bioavailability is one of the most crucial parameters when it comes to medical solutions, electro spun nanofibers are highly preferred. This method is preferable for organic polymers as they have high flexibility, high specific surface area and surface functionalization. Electrospinning technology has been used for the fabrication and assembly of nanofibers into membranes, which have extended the range of potential applications in the biomedical, environmental protection, nanosensor, electronic/optical, protective clothing fields and various other fields.

Keywords: electrospinning, nanofiber, antibacterial agent, metallic nanoparticle, tissue engineering

1. Introduction

Nanofibers are fabricated thin threads arising from physical processes using synthetic chemicals. Nano, when used as a prefix, restricts the diameter of the thin fibres to nano range i.e., 20–400 nm [1]. Nano being a common term these days has proved it worth in various fields, well known being drug delivery and other biological ones. The high surface to volume ratio serves as the driving factor in most cases for its application. This is also the reason fibres when made in nano-range perform better (form highly porous mesh) and have been universally used [1].

Our purpose here is to highlight the technique, electrospinning, which is used to make nano-fibres with antimicrobial properties. It is a popular technique in tissue engineering that uses polymer solutions and strong electric fields to produce nanofibers as close as the natural extracellular matrix (especially in tissue engineering) [1, 2]. When it comes to industries, scalability is a major issue and electrospinning technique is favoured due to this reason and it has a simple setup [3]. Synthetic fibres are used more often than natural ones. There is no standard size of a nanofiber when it comes to biological usage. This is due to the stability issue and hence the porosity, morphology and shape are all variables and are adjusted to create the best possible product [2]. These products are also affected by the technique used for the production (here electrospinning- electric field, flow rate etc.). Environment and solution used to be the other two affecting parameters. There are a lot of correlations to be taken care of before finalising the nanofiber structure.

As of now, we have an idea of what electrospun nanofibers are, however why these fibres are important is still a mystery to solve. With some basic knowledge of biology in earlier classes, we have concluded that staying away or preventing contact with microorganisms can reduce a lot of biological stress in our body. In short, using antimicrobials is a good option when it is available. Thus, Electro spun nanofibers when incorporate antimicrobial properties, become a great deal of interest even to common population e.g., electro spun nanofibers with essential oils (to prevent the side effect from synthetic compounds) [4], Electro spun nanofibers of zein and PVA have been proposed as carriers and stabilisers of epigallocatechin-gallate (EGCG) [5]. There are few studies on the incorporation and release profile of a drug loaded in biodegradable electro spun nanofibrous membranes, based on the drug-polymer interactions, on top of its ability to hinder bacterial growth. A polymer blend composed of/poly (butylene adipate-co-terephthalate) (PBAT)/ poly (lactic acid) (PLA), loaded with different. Cloxacillin (CLOX) contents were fruitfully produced by using electrospinning technique (as shown in the following **Figure 1**) [6]. The confirmation for the encapsulation of drug was done using characterisation techniques like Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The effect was measured by the pH when the drug (20% of CLOX) was released (antibacterial activity).

Along with antimicrobials, these nanofibers can possess anti-inflammatory and antioxidant properties as well. Such properties tend to degrade in terms of effect when not incorporated properly but with a controlled release, this degradation can be avoided [5]. A detailed description of this technique, usage and the flaws will be discussed in the later part of this chapter.

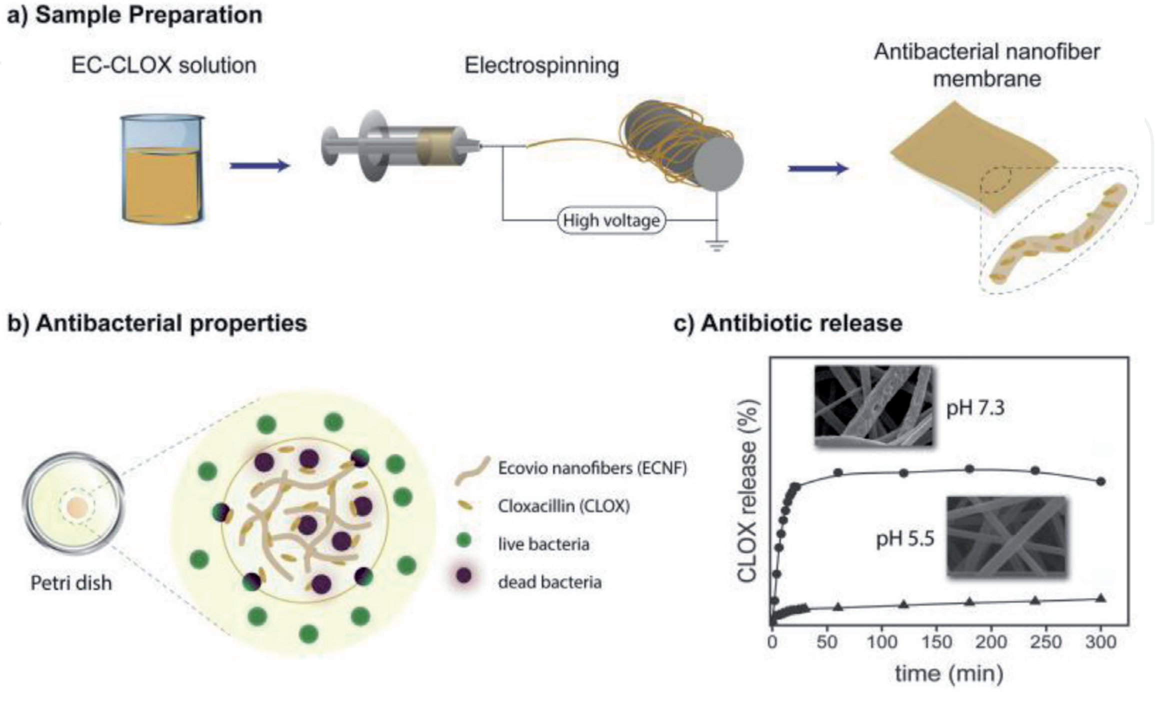

Figure 1.
Antibacterial activity and effect of pH on its release (electrospun nanofibers containing cloxacillin) [6].

2. Nanofiber's production

Fibres can be natural - from animals or plants, or synthetic – man made. When fibres come from plants, cellulose is the polymer made from the sugar glucose that makes those fibres well-built. Natural fibres from plants include cotton, jute, hemp, sisal, and flax. Silk is formed from the cocoons of silkworms. Wool is soft hair that is cut from animals like sheep, goats, alpacas, llamas, and even rabbits. Both silk and wool are protein. Cashmere is an extra-soft fur from goats. Mohair is wool from angora goats. Angora rabbits give us angora fibres [7].

A lot of different polymers can be made into fibres. Fibres are formed when polymer chains are all lined up in the same direction [7]. Metallic fibres, Carbon fibres, fibreglass, mineral fibres, and polymer fibres are all subtypes of synthetic fibres [8].

Diverse types of natural and synthetic types of polymers are used to make nanofibers henceforth they exhibit unlike properties and applications. Instances of manmade polymers include poly lactic acid, polyurethane, polycaprolactone, polyethylene-co-vinyl acetate plus poly 3-hydroxybutyrate-co-3-hydroxyvalerate. Natural polymers comprise cellulose, collagen, gelatine, keratin, silk fibroin, then alginate and chitosan. Polymer chains are linked via covalent bonds [9].

There are many different methods to make nanofibers, including bicomponent extrusion, drawing, electrospinning, thermal-induced phase separation, self-assembly template synthesis and centrifugal spinning [9, 10]. Electrospinning is an extensively used method of producing nanofibers.

2.1 Electrospinning mechanism

When the electrostatic force is applied on solutions or melts, electro-spinning produces fibres with diameters ranging from micrometre to nano-meter scale. A general electrospinning setup consists of three primary components: a syringe with a metallic needle, a high voltage power supply (usually in the kV range), and a grounded collector. When we consider a typical electrospinning course, high power is applied on melts/solutions. Consequently, suspended droplets are formed. A suspended droplet will collapse into a conical droplet as the electrostatic repulsion

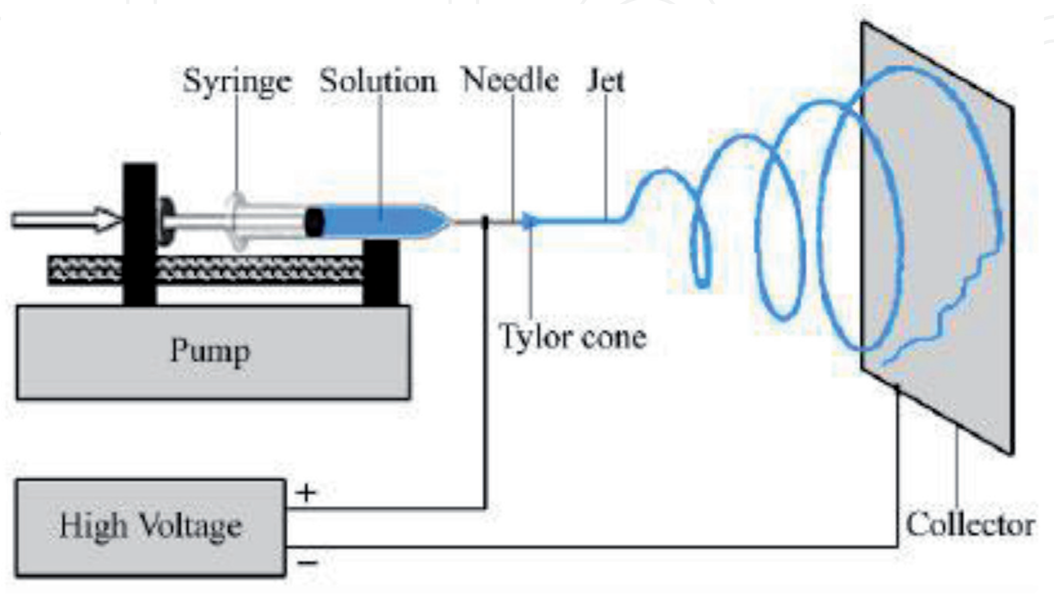

Figure 2.
Electrospinning setup [11].

starts to overcome the surface tension of the fluid. A fine, charged jet of polymer solution is ejected from the tip of the needle as the electrostatic force overcomes the surface tension of the conical droplet. The action between the electric field and surface tension of the fluid outstretches the jet stream and then it encounters a whipping motion and that results in the evaporation of the solvent. This led to the jet stream to be stretched out continuously as a thin and long filament. Subsequently this filament will harden and will be eventually settled onto a grounded collector, and finally results in the development of a uniform fibre (**Figure 2**) [11].

2.2 Electrospun nanofibers with antimicrobial properties

The electro spun nanofibers built-in with antibacterial agents have been fabricated for antimicrobial applications. The electro spun nanofibers exhibit enhanced antimicrobial performance compared to conventional antimicrobial materials. They play significant roles in wound-dressing materials, filtration, tissue scaffolds, protective textiles, and biomedical devices [12].

The electro spun nanofibers with antimicrobial properties fabrication methods are grouped into two categories. Antimicrobial nanofibers can be obtained by one step process or by the following two steps. In one stage process, the suspensions with a mixture of antimicrobial agents and polymer undergo electrospinning. The formulation of this homogeneous mixture is censorious to make up a smooth and continuous nanofiber. The properties of electrospinning solutions are affected by antimicrobial agents. Such vital characteristics that play a significant role in the process and resultant are conductivities and viscosities.

Whereas in the two steps method include, producing an initial electro spun polymeric nanofibers and then post-functionalizing nanofibers with antimicrobial materials. Multiple functionalization approaches have been managed to link the antimicrobial agents onto surfaces of electro spun nanofibers by using various chemical and physical methods [13].

Antimicrobial electro spun nanofibers built-in with different antimicrobial agents: including metallic nanoparticles (silver, zinc, titanium, copper, and cobalt), carbon nanomaterials, antibiotics, and antimicrobial biopolymers.

3. Antimicrobial agents

3.1 Volatile oils

Volatile oils well known as Essential oils are plant derived concentrated hydrophobic and volatile compounds. They are a combination of different compounds such as carvacrol, eugenol, and cymene derived from aromatic plants. The best examples of essential oils are terpenoids and hydrophobic phenolic compounds [14, 15]. The hydrophobic nature of essential oils decides their activity mechanism against microbes. These essential oils break up into the bacterial plasma membrane lipid bilayer and then disrupt its structure. This alters the permeability of membrane to ions and other cellular contents. Consequently, the proton pump collapses and results in cell death [16].

Sadri and his team prepared PEO nanofibers/electro spun chitosan, to which they linked two distinct types of thyme essential oils into this nanofiber. They used broadleaf and narrow leaf thyme essential oils to their study. The nanofibers/chitosan along with the thyme oils were trialled against *P. aeruginosa* and *Staphylococcus aureus*. After 24 hrs, the inhibition of narrow life was reported as 8 and 15 mm were as in case of broad life it was 10 and 19 mm for *P. aeruginosa* and *S. aureus*, respectively.

Accordingly, the broad leaf resulted in more antibacterial activity than narrow leaf spices in the presence of above-mentioned bacteria's [17].

3.2 Herbal bioactive components

There are many studies that prove the potent antibacterial property of plants-derived herbal bioactive components against a wide range of food borne pathogens. The widely researched bioactive components with antimicrobial properties are gingerol, allicin, shikonin, asiaticoside, and curcumin etc. Curcumin (Cur) which is derived from the rhizome of *Curcuma longa* L. is well-known for its valuable properties, including anti-inflammatory, antioxidant, and anticancer features [16, 18–20].

3.3 Silver

Amongst metallic nanoparticles, silver nanoparticles are the most studied and have been demonstrated to be the most effective antimicrobial agents. Ag is a known biocidal agent that is effective against a range of types of fungi, bacteria, and viruses; on the other hand, it is non-hazardous to human cells. The simplest and most frequently used method for combining Ag nanoparticles with electro spun nanofibers is the suspension of Ag nanoparticles directly into the electrospinning polymer solutions [21, 22].

A research team formulated cellulose acetate nanofibers with the use of electrospinning methods. Cellulose acetate nanofibers were transformed into cellulose nanofibers using alkaline hydrolysis. In addition to this, silver nanoparticles were added to the cellulose nanofiber. Developed antibacterial silver cellulose nanofiber activity was examined against *E. coli* and *S. aureus* grown on Lysogeny broth [LB] medium. After 18 hrs of contacting 1% silver nanoparticles, the inhibition zone was spotted with 16- and 14.4-mm diameter against *E. coli* and *S. aureus*, respectively. Besides, it was also proved that antibacterial activities of the Ag nanofibers were directly influenced by the rising concentration of Ag nanoparticle contents [23].

3.4 Zinc and copper

ZnO appears to restrain the growth of strongly resistant bacteria. There are some reports about the significant antibacterial activity of ZnO, which is credited to the production of reactive oxygen species [ROS], causing the production of oxide substances.

Since olden times, Copper has been used for manufacturing utensils as it is a powerful natural biocidal metal. When bacteria encounter copper, there will be cell wall deformation which causes the death of bacteria. To deal with bacteria, many researchers have developed a method where a polymeric matrix was supported with copper by electro-spinning [24]. Ahire and his research team used electrospinning of Poly-D and PEO, L-lactide method to combine Cu nanoparticles into nanofibers. Due to the presence of copper nanofibers, *S. aureus* and *P. aeruginosa* were reduced by 50% and 40%, respectively after two days [25].

3.5 Antibacterial drugs

For wound health, filtration, and active packaging systems antimicrobial nanofibers incorporated with antibacterial drugs have become one of the promising nano-scale materials. A vast range of antibacterial drugs such as peptides and antibiotics have been formulated physically or chemically within electro spun nanofibers or on their surfaces. The polymer degradation, release profile, and release pathway of antibacterial drugs from electro spun nanofibers has a linkage with the release mechanism of antibacterial drugs which may be regulated through the composition of polymer and fibre morphology [26].

Antibacterial drugs encapsulated in electro spun nanofibers have been proved to sustain the antibacterial property over a longer time compared to the un-encapsulated form. A sodium alginate electro spun nanofibers loaded with ciprofloxacin was developed by a team and it was tested for its antimicrobial activity against *Staphylococcus aureus*. The minimum inhibitory concentration [MIC] of ciprofloxacin required is found to be 0.125 μg/mL through this study [27]. Similarly, another team worked on developing nisin nanofibers/cellulose acetate which resulted in approx. 99.9% reduction of *S. aureus* [28].

4. Applications of electro spun nanofibers (ESNF)

Electrospinning offers many advantages like control over morphology, porosity and composition using very simple equipment. Due to its different applications in various fields like filtration products, biomedical applications, and tissue engineering to produce artificial blood vessels, non-woven fabrics, fuel cells, fibre mats etc. [29]. Electrospinning technology has been used for the fabrication and assembly of nanofibers into membranes, which have extended the range of potential applications in the biomedical, environmental protection (**Table 1**), nano sensor, electronic/optical, protective clothing fields and various other fields [30].

4.1 Health applications

The ESNF have shown great capacity in the human healthcare applications, for tissue or organ repair and regeneration, as biocompatible and biodegradable medical implant devices, in medical diagnostics and instrumentation, as protective fabrics against environmental and infectious agents in hospitals and as vectors to deliver therapeutics and drugs [31].

For drug delivery or bio separation, nanofibers with strong paramagnetic properties prepared by the coaxial technology, such as Gd_2O_2S, possibly doped with Eu or Dy, were recommended [32].

For controlled delivery of drugs, molecular medicines, body-care supplements and therapeutics nanofibers are used as a promising tool by cosmetics and pharmaceutical industries. To give an example such as DNA which is attached covalently to a patterned array of carbon fibre and inserted into cells by centrifuging these cells onto the array will not affect cell's viability and the expression of genes encoded by

Materials	Solvent system	Materials	Solvent system
Cellulose acetate	Acetone/DMAc	Chitosan	TFA
Chitin	HFIP/PBS	Hyaluronic acid	DMF/water
Silk fibroin	Formic acid	Fibrinogen	—
Gelatin	TFE/HFIP	Elastin	Water
Collagen	HFIP	Soy protein	HFIP
Wheat protein	HFIP	Whey protein	Acidic aqueous solution

Table 1.
Natural biopolymer electrospun nanofibers [30].

the inserted DNA. This could build a way for the development of a 'smart' polymeric drug delivery system [31]. After alignment, stacking, mechanical properties, diameter, porosity, and biodegradability optimisation nanofiber-based scaffolds have been explored to enhance the repair or regeneration of various types of tissues, including heart, blood vessel, nerves, skin, musculoskeletal system, and tissue interfaces [33].

4.2 Wound dressing

The naturally extracted bioactive agents using electrospinning technique have been majorly promoted for the development of advanced level of dressings which paves way for rapid and efficient wound repair. Electrospun scaffolds consists of several advantages over the traditional dressings for the treatment of chronic as well as acute wounds, high absorption of exudates from the site of wound, efficient exchange of gases and nutrients for cell's proliferation, protection of the injured tissue, and the possibility to release functional molecules [34].

The distinctive features of ESNF scaffolds such as their inter-fibre and intra-fibre pores and high surface area stimulate the fibroblastic cells response by rapidly initiating cell signalling pathways. Additionally, electrospinning technique can be used because of its application in the fabrication of cosmetic masks which are used for skin cleansing and skin healing. The high surface area of an electrospun skin mask facilitates the flow of additives from and to the skin (**Figure 3**) [30].

Many crude extracts of plants have been successfully encapsulated into electrospun fibres, such as *Centella asiatica*, baicalein, green tea, *Garcinia mangostana*, *Tecomella undulata*, *Aloe vera*, *Grewia mollis*, chamomile, grape seed, *Calendula officinalis*, *Indigofera aspalathoides*, *Azadirachta indica*, *Memecylon edule* and *Myristica andamanica* which has been used for wound healing [34].

ESNF has been effectively explored as a wound healing dressing material. By developing nanofibers to provide topographical and biological cues, the migration

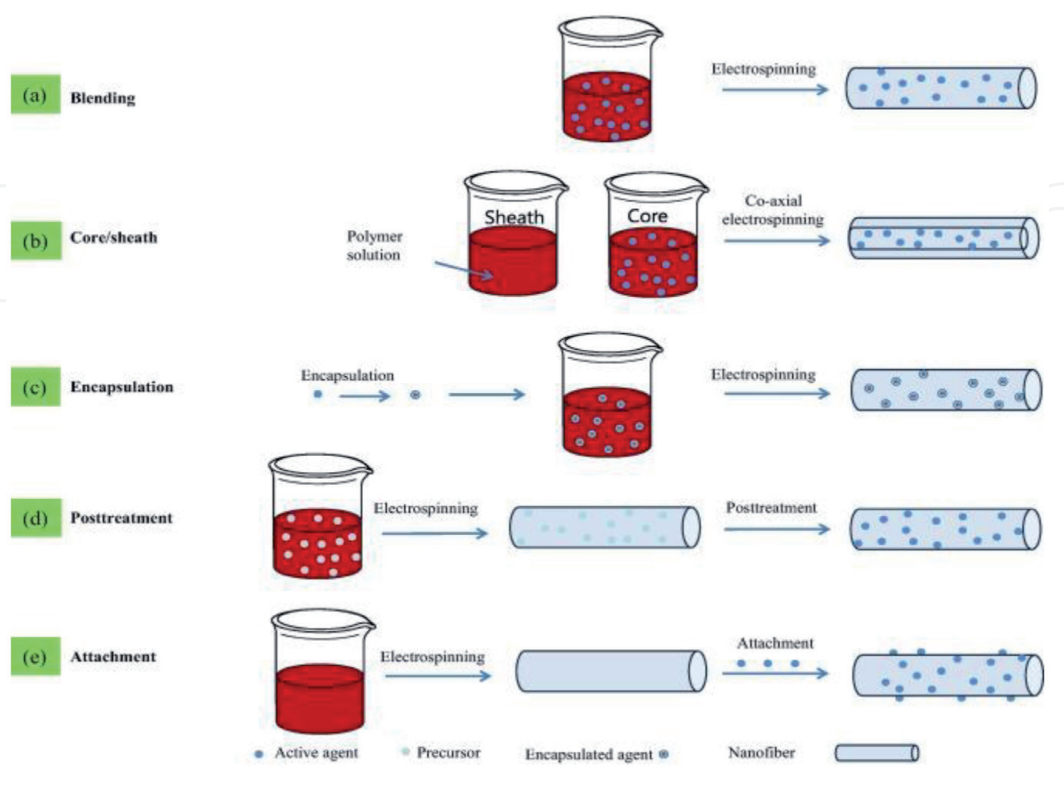

Figure 3.
Various strategies used to prepare suitable wound dressing [31].

and infiltration of repairable cells can be improved. Once the nanofiber-based scaffolds have been optimised accordingly in vitro for the promotion of cell migration and/or delivery of biomolecules they will be subjected for wound healing evaluation in vivo using a mouse, rat, or rabbit model [33].

4.3 Tissue engineering

In tissue regeneration, biocompatible and biodegradable fibrous scaffolds are usually preferred over traditional scaffolds because of their uniqueness and capacity to provide the target cells or tissues with a local environment by imitating the extracellular matrix. Hence, the use of ESNF in tissue engineering is increasing day by day [30].

Osteogenic properties in medicinal plants such as *Cissus quadrangularis (*CQ) and Asian *Panax ginseng* root have been suggested for regeneration of bone. The combined effect of CQ and hydroxyapatite (HA) has been explored by producing PCL-CQ-HA electrospun scaffolds. Proliferation of human foetal osteoblasts (hFOBs) on the composite scaffolds and increased adhesion was observed. Furthermore, increased levels of mineralisation and osteocalcin expression were detected which are fundamental in bone formation [34].

Bio or natural polymers (hyaluronic acid, alginate, collagen, silk protein, fibrinogen, chitosan, starch, and poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV)) have been mainly focused by the researchers until recently for tissue engineering, because these polymers showed excellent biocompatibility and biodegradability. However, in recent years, attempts have been made to utilise a wide range of natural and synthetic polymers for the regeneration of new tissues, specifically cartilage tissue, dermal tissue, and bones.

A synthetic polymer poly (lactic acid-co-glycolic acid) (PLGA) is the ideal material for tissue regeneration because of its tuneable and biodegradable nature, easy spinnability, and the presence of multiple focal adhesion points [30].

4.4 Food industry

ESNF is used for the encapsulation of plant extracts with the aim of preserving the integrity and controlling the release of the active ingredients in food processing and packaging. Electrospinning majorly offers the advantage of being a cost-effective manufacturing procedure that operates at room temperature and it is compatible with most edible polymers and materials approved for food contact in these sectors [34].

A hydrophobic prolamin, Zein which can be extracted from corn consists of marvellous film-forming properties with a high thermal resistance. Earlier, zein films were used as edible coatings on tomatoes to delay the colour changes, weight losses, and on nuts to delay rancidity during storage. However, the zein based electrospun mats may provide additional attributes for food packaging [35].

Functional molecules extracted from plants have been exploited for prolonging food shelf-life and avoiding bacteria colonisation in food packaging applications. In one of the modern studies, electrospun mats of β-cyclodextrin (PVA/CEO/β-CD) and PVA containing cinnamon essential oil (CEO) have been developed and tested against *S. aureus* and *E. coli*. The combination of CEO with β-CD enhanced the antibacterial action of this essential oil [34].

A cost competitive plant protein which is a soy protein is partially purified and concentrated from soybeans in various forms, such as soy protein concentrate, defatted soy flour, and soy protein isolate (SPI). Though there is a great amount of interest in developing soy protein as an electrospinning matrix, pure soy protein cannot be electrospunned easily.

5. When are electrospun nanofibers not a good option?

Electrospinning is an impressive technique however, the size of the fibres being nano, is a disadvantage when it comes to control. The limited control of the pore size (Electrospun scaffold) is a diameter dependent which reflects on the cellular infiltration (decrease due to smaller average pore size) [36].

Another reason being the degradation effect (introduced in the latter half of the introduction). The rapid degradation of nanofibrous constructs can adversely affect the ability of the scaffolds to support tissue growth. The structure of the nanofibers plays an important role, especially when it comes to nanoscale, the high surface area to volume ratio serves as the reason for its selection. However, in case of degradation effect, due to this property, the nanofibers are prone to hydrolytic degradation. Hence long-term processes should not employ such scaffolds as before the entire process (observation, selection or any other research studies) is completed, the culture will have no support to grow [36]. Crystallinity in polymers can treat this problem however the size of the fibres (diameter) are still a variable with a high probability of variation (purpose dependent and needs a lot of testing before it can be finally put into use). The poor infiltration of cells into scaffolds is still an issue to deal with, especially when we want to add various properties into nanofibers.

As mentioned in the introduction, electrospinning is an easy to setup and scalable technique. The cost parameter is in our favour whereas the volume imposes some difficulties in terms of production. It is quite difficult to produce a large volume scaffold and if the critical factors do not meet the threshold level the final structure might not be at its best form. This will drastically affect the application part. This will also affect special properties like antimicrobial/inflammatory/oxidant. The release of the drug will be questionable in such cases (**Table 2**).

The drug loading process when it includes a high amount of drug can result in a burst. When we submerge the fibres in aqueous solution (prone to hydrolytic degradation), the antimicrobial properties (e.g., antibiotics) are released in a short duration (might not last till it's required). This issue can be solved by using different set-ups. We have learned that electrospinning technique is an easy to set-up one, here if we want to use such nanofibers for a longer duration, a different set-up is required which introduces more complication [37].

When environmental factors are taken into consideration, most frequently relative humidity is studied. When this parameter is a settable, considering higher RH leads to thinner fibre diameter, an appropriate high RH level could be selected.

Diameter	Fibre composition	Application
438 ± 156 nm	Electrospun, aligned, and randomly oriented PCL	In vitro culture of meniscal fibrocartilage cells and human MSCs
519 ± 127 nm		
430 ± 170 nm	Electrospun, aligned, and randomly oriented PLLA	In vitro culture of human tendon stem cells
450 ± 110 nm		
657 ± 183 nm	Electrospun, aligned PU	In vitro culture of human ligament fibroblasts
300–900 nm	Electrospun PLGA nanofibers on top of microfibers	In vitro culture of porcine MSCs

Table 2.
Variations in polymer nanofibers size in ligament and tendon tissue engineering [36].

Since high RH levels may lead to beads, too high RH levels cannot be selected. Whereas if an ambient RH cannot be controlled. Then RH is considered a disturbance of the electrospinning process and the required jet diameter, responding to the desired fibre diameter, should change with an ambient RH. The system's velocity should be adjusted along with the flow rate. This is done to obtain a required jet diameter (the application of electric field in the polymer solution will cause the droplets to take a conical shape) and control the fibre's diameter. This is where production will become an issue. Such adjustments are difficult and are highly variable. Hence, relative humidity should be studied to decide what type of parameter it is (settable or disturbance) to decide the production rate. The operating regime should be selected to achieve the desired fibre diameter while maximising production rate [38].

Along with relative humidity, temperature should also be taken into consideration. The average diameter of nanofibers produced by electrospinning changes significantly through variation of temperature and humidity. At a relatively higher temperature the solvent evaporation rate will increase and the viscosity of the precursor solution to be electrospun will decrease, and as a result thinner nanofiber would be obtained [39].

6. Limitations

Though there are many reports on the successful presentation of electrospinning as a useful platform technique for the fabrication of nanofibers from a variety of materials, several issues are yet to be explained. Electrospinning process simulation models need to be optimised by considering all the liquid properties for electrospinning and all the processing parameters for better elucidation of the phenomenology of electrified jets. If it is successful, one should be able to analyse the behaviour of the electrified jet for the deterministic fabrication of electrospun nanofibers with well-controlled size, structure, and morphology [33].

Even though there is increase in the usage of natural biopolymers in the electrospinning technique for food packaging has developed a massive growing interest in the recent years but due to lesser flexibility of these materials ultimately leads to difficulty of processing in traditional equipments, and most of them are hydrophilic materials which means that they lack necessary mechanical properties and good barrier properties to moisture and oxygen [35].

7. Conclusion

As discussed above, electrospinning is one of the most efficient techniques used for the synthesis of nanomaterials [30]. It enables the incorporation of unique properties including large surface area, small size, and high activity, which are expected to develop advanced packaging systems for fulfilling consumers' needs. However, nano or micro sized components may lead to environmental pollution or even health risks due to their migration into food and drinks, whereas our knowledge regarding the potential threats from the used nanomaterials is still relatively lacking [35]. Research and experimentation by various organisations along with academic individuals have stated that the bioactive molecules that were naturally derived were better incorporated into polymeric nanofibers and also improved the membrane and scaffolds manufacturing using such electrospun nanofibers [34].

Although ESN includes antimicrobial and loading other similar agents, the spectrum of this range is limited to some curing agents. The further investigation is to be focused on broadening this spectrum resulting into a diversified product with composite materials [33]. Currently, the control on the deposition deposition, porosity, inter-linkage and intra-linkages made nanofiber accessible in almost all the fields- food industry, wound healing management etc. [30]. Environmental field applications with surface functionalized nanofibers are facing a few challenges that need to be tackled which include capacity reduction and kinetic slowness after surface modifications. The level of research to uplift the current properties for targeted action is not up to the mark, hence needs further investigation by the agricultural and food industries for a real time response. Apart from this, combining the nanofibers with microfluidic systems is still challenging because it requires nanofibers with the well-controlled diameter and orientation, as well as the reproducibility to place them at specific positions and with the right orientation [33].

Author details

Lingayya Hiremath[1*], O. Sruti[1], B.M. Aishwarya[1], N.G. Kala[1] and E. Keshamma[2]

1 Department of Biotechnology, R.V. College of Engineering, Autonomous Institution Affiliated to Visvesvaraya Technological University, Belagavi, Bengaluru, Karnataka, India

2 Department of Biotechnology, Maharani's Science College for Women, Bangalore, Karnataka, India

*Address all correspondence to: lingayah@rvce.edu.in

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