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Electrospinning technologies for the delivery of Biopharmaceuticals: Current status and future trends

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ABSTRACT

This review provides an in-depth exploration of electrospinning techniques employed to produce micro- or nanofibres of biopharmaceuticals using polymeric solutions or melts with high-voltage electricity. Distinct from prior reviews, the current work narrows its focus on the recent developments and advanced applications in biopharmaceutical formulations. It begins with an overview of electrospinning principles, covering both solution and melt modes. Various methods for incorporating biopharmaceuticals into electrospun fibres, such as surface adsorption, blending, emulsion, co-axial, and high-throughput electrospinning, are elaborated. The review also surveys a wide array of biopharmaceuticals formulated through electrospinning, thereby identifying both opportunities and challenges in this emerging field. Moreover, it outlines the analytical techniques for characterizing electrospun fibres and discusses the legal and regulatory requirements for their production. This work aims to offer valuable insights into the evolving realm of electrospun biopharmaceutical delivery systems.

1. Introduction

Biopharmaceutical is any product manufactured in, extracted from, or semi-synthesised from biological sources. Since the US Food and Drug Administration (FDA) approved 85 recombinant insulin for human patients as the first biopharmaceuticals in 1982, there has been a phenomenal and accelerating development over the years (Clark, AdrianJ.L.; Knight, G.; Wiles, P.; Keen, H.; Ward, J.; Cauldwell, J.; Adeniyi-Jones, R.; Leiper, J.; Jones, R.; Maccuish, A.; et al. BIOSYNTHETIC HUMAN INSULIN IN THE TREATMENT OF DIABETES. Lancet, et al., 1982; Goeddel et al., 1979; Leader et al., 2008). Not only peptides and proteins, which are obvious biopharmaceutical products; however, but also sugars, nucleic acids (NA), living cells, or tissues are categorised in this pharmaceutical group (de la Torre et al., 2021; Walsh and Walsh, 2022). To date, biopharmaceuticals have been developed and used in a wide range of applications. Vaccines, hormones, whole blood (or blood components), immunosera, antigens, cytokines, enzymes, allergens, cell therapies, gene therapies, tissues, monoclonal antibodies, and products derived from recombinant deoxyribonucleic acid (DNA) have all become significant scenarios for the development of biopharmaceuticals

Abbreviations: 3D, Three-Dimensional; AFM, Atomic Force Microscopy; AMD, Age-Related Macular Degeneration; ASO, Antisense Oligonucleotides; ATMPS, Advanced Therapy Medicinal Products; BBB, Blood-Brain Barrier; BMP-2, Bone Morphogenetic Protein 2; BSA, Bovine Serum Albumin; CD, Circular Dichroism; COL1A1, Type I Collagen; COL3A1, Type III Collagen; DMA, Dynamic Mechanical Analysis; DNA, Deoxyribonucleic Acid; DDSs, Drug Delivery Systems; DMF, Dimethylformamide; DSC, Differential Scanning Calorimetry; EDA, Ethylenediamine; ECN, Escherichia Coli Strain Nissle 1917; EDX, Energy Dispersive X-Ray Analysis; ELISA, Enzyme-Linked Immunosorbent Assay; EMA, European Medicines Agency; FDA, US Food and Drug Administration; FTIR, Fourier-Transform Infrared Spectroscopy; GI, Gastrointestinal; GMP, Good Manufacturing Practice; HLB, Hydrophilic-Lipophilic Balance; HP-β-CD, Hydroxypropyl-beta-cyclodextrin; HPLC, High-Performance Liquid Chromatography; HSES, High-speed Electrospinning; IGF, Insulin Growth Factor; ITC, Isothermal Titration Calorimetry; MEMS, Micro-Electromechanical Systems; MHRA, Medicines And Healthcare Products Regulatory Agency; MIRNA, MicroRNA; MRNA, Messenger RNA; MW, Molecular Weight; NA, Nucleic Acids; NPs, Nanoparticles; ODTs, Orally Disintegrating Tablets; O/W, Oil-In-Water; PAN, Polyacrylonitrile; PBS, Phosphate Buffer Solution; PCL, Poly(E-Caprolactone); PEG, Poly(Ethylene Glycol); PEO, Poly(Ethylene Oxide); PLA, Poly(Lactic Acid); PLLA, Poly(L-Lactide); PLLAGL, Poly(L-Lactide-Co-Caprolactone); PLGA, Poly (D, L-lactide-co-glycolide); PU, Polyurethane; PVA, Polyvinyl Alcohol; PVP, Polyvinylpyrrolidone; QC, Quality Control; RNA, Ribonucleic Acid; SEM, Scanning Electron Microscopy; SDF, Stromal Cell-Derived Factor; SIRNA, Small Interfering RNA; SPR, Surface Plasmon Resonance; TGF, Transforming Growth Factor; TGA, Thermogravimetric Analysis; TOF-SIMS, Time-Of-Flight Secondary Ion Mass Spectrometry; VEGF, Vascular Endothelial Growth Factor; W/O, Water-In-Oil; W/O/W, Water-In-Oil-In-Water; XPS, X-Ray Photoelectron Spectroscopy; α-Chy, Alpha-Chymotrypsin; α-SMA, α-Smooth Muscle Actin.

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(Mustafa et al., 2022).

In the case of biopharmaceuticals, 90 drugs were approved by the FDA from 2015 to 2022. The number of approvals is still dramatically growing as many biopharmaceuticals are in preclinical or clinical audit stages (Martins et al., 2022). As of today, there are still more than 2,600 clinical trials on biopharmaceuticals in the active stage and a total of more than 10,000 clinical trials in progress or preparation. The specific numbers of clinical trials in both different types of biopharmaceuticals and various targeted diseases are shown in Fig. 1. High pharmacological activity and low toxicity are the most important features of biopharmaceuticals that make them popular (Leader et al., 2008). More specifically, biopharmaceuticals are primarily large molecules with multiple binding sites, which unlike small molecules, can bind specifically to their targets, potentially avoiding adverse effects (Craik et al., 2012). At the same time, owing to their more complex structure, these large molecules often have physiological functions that other molecules cannot fully replace (Leader et al., 2008; Craik et al., 2012; Moreira et al., 2021). For example, growth hormone is a peptide hormone that promotes growth and development in humans and animals. Its mechanism of action is linked to various biological responses, including promoting bone growth and protein synthesis, accelerating fat metabolism and promoting the growth of muscle tissue (Ranke and Wit, 2018), which cannot be achieved easily by small molecules. Therefore, biopharmaceutical-based therapies are predicted to show great potential for future therapies.

However, the clinical use of biopharmaceuticals has been repeatedly delayed due to several impediments to delivery methods and/or formulation characteristics. Generally, most biopharmaceuticals are delivered by a restricted number of methods (mainly via injection), leading to difficulty in self-administration and, concomitantly, poor patient compliance. Furthermore, once in the bloodstream, the drug is more likely to be cleared by immune cells or eliminated by the liver or kidneys due to its unique physical and chemical structure, thus resulting in a short half-life (Jain et al., 2013; Patel et al., 2014). Although oral administration is a reasonable solution to deal with it, the easy degradation of large molecules, especially proteins, in the gastrointestinal (GI) tract can significantly reduce their bioavailability. On the other hand, the large molecular weight and ionisation properties can potentially prevent the drug from crossing biological barriers such as the intestinal epithelium and blood–brain barrier (BBB), significantly reducing its effectiveness (Aungst et al., 1996; Pardridge, 2020). In addition to the restricted routes of delivery, biopharmaceuticals face another problematic issue, i.e., instability. Some biopharmaceuticals, such as insulin, nucleic acid or adenoviral vector vaccines, including a majority of the Covid vaccines developed in Europe, must be stored at low temperatures (typically 2–8 ℃) for long periods; otherwise will risk inactivation (Emond, 2008). The stringent production and storage conditions will impose a substantial economic and logistical burden, further limiting its dissemination and application (Wolfson, 2008; Zhang et al., 2012).

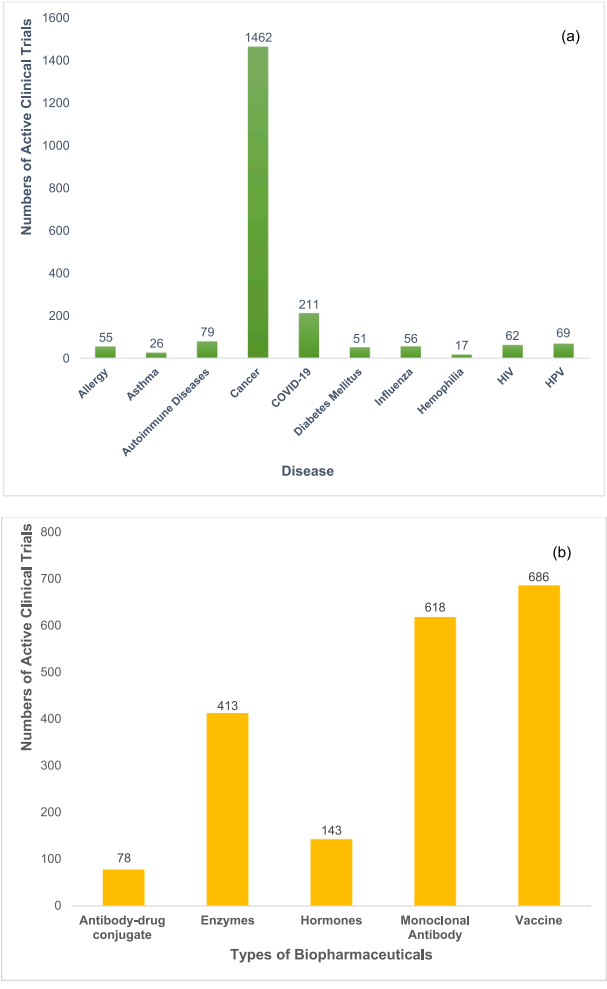

Fig. 1. Distribution of active clinical trials by target diseases (a) and different types of biopharmaceuticals (b) retrieved in Oct 2023.

Under such circumstances, developing delivery systems suitable for biopharmaceuticals is one of the research directions in demand. Several critical factors should be taken into consideration when developing a promising formulation. Firstly, the selected delivery system is expected to protect the biopharmaceuticals from degradation or denaturation, thus increasing their stability and activity in the biological environment (Li et al., 2019). Secondly, specificity is another essential characteristic. Targeted drug delivery can be achieved through specific formulations. Thirdly, achieving slow and controlled release is a crucial aspect for enhancing the therapeutic effects of drug dosage forms, which allows for dose reduction and improved patient compliance. Fourthly, biosafety is also a key factor. As some delivery systems are cytotoxic, biohazardous or even destructive to normal cells, preliminary experiments are necessary to prove their safety. Finally, economics is also a critical factor that determines the potential of formulations under development. The development of delivery systems can be expensive, time-consuming and risky (Fenton et al., 2018; Lau and Suh, 2017; , Chen and Yeh, xxxx; Xiao et al., 2018; Das et al., 2014). However, new formulations can undoubtedly be of more excellent value if they are economically viable and ready for large-scale applications. For example, a novel formulation of proteins stored at room temperature can significantly reduce transport and storage costs and can be the “ideal” candidate (Wolfson, 2008). It is possible, though challenging, to design formulations for achieving desired biopharmaceutical release via different routes of administration by combining biological, pharmacological, and physicochemical principles (Li et al., 2019).

Admittedly, the requirements of formulations, as mentioned above, have also led to the continuous development of drug formulation technology. With the continuous research into drug delivery systems (DDSs), their variety has been gradually enriched. In particular, micro- and nano-formulations, as well as fibrous scaffolds, are all promising dosage forms. Meanwhile, the excipients used in the formulations are becoming more diverse. Nowadays, inorganic materials, lipids, and polymeric materials can be applied to prepare different formulations. Due to their unique advantages, nano-formulations play an essential role in pharmaceutical research (Pant et al., 2019). Drugs encapsulated or delivered by nanocarriers can achieve passive or active targeted delivery. Thus, such a delivery system is particularly suitable for drugs with low solubility, fast clearance, toxicity and instability, which are always found in biopharmaceuticals (Dogra et al., 2019; Sykes et al., 2014). Under such circumstances, novel drug formulations prepared using nanotechnologies such as nanoparticles (NPs), nanofibers, nanogels, micelles, and microspheres are also emerging in increasing numbers (Luraghi et al.,

2021; Lanao et al., 2020). Among these products, nanofibers prepared from biocompatible polymers have garnered significant attention within the field due to their versatility, efficacy, and unique physiochemical characteristics, such as large surface area, small diameter, and high aspect ratio. (Wang et al., 2019; Feng et al., 2019). Additionally, some nanofibers can be targeted to release, thereby reducing the need for drug doses, further reducing their side effects (Narayanaswamy and Torchilin, 2019). For example, nanofiber scaffolds can achieve targeted controlled release of different antitumour drugs (e.g., paclitaxel, cisplatin) to tumour cells, thereby reducing the dose of the drug and increasing the inhibition of tumour cell proliferation (Niu, 2021).

In the last few decades, electrospinning technologies, a novel technique for the preparation of micron- or nano-fibres, have generated interest. Electrospinning technology is a type of electrohydrodynamic technique that uses an electric field to solidify polymer solutions and thus prepare fibres with a large surface area, which is relatively like electrospray technology (Williams et al., 2018). The main difference between them is that electrospinning techniques mainly use higher viscosity solutions to prepare micro- and nano-fibres, whereas electrospray techniques mainly use lower viscosity solutions to prepare micro- or nano- particles (Moreira et al., 2021; Nguyen et al., 2016; Jaworek et al., 2009). Electrospun nanofibres are flexible pharmaceutical intermediates with excellent properties that can be arbitrarily prepared into tablets, capsules, wound dressings, scaffolds, and other final dosage forms that can be prepared by conventional formulation methods such as granulation, tableting, or attaching to other dosage forms to cooperate.

For the materials, the choice of polymers for the preparation of electrostatic spinning is varied, including biocompatible and biodegradable polymers such as gelatine, hyaluronan, chitosan, poly(ε-caprolactone) (PCL), poly (lactic acid) (PLA), poly (ethylene glycol) (PEG), poly (D, L-lactide-co-glycolide) (PLGA), and polyurethane (PU) among many others (Nguyen et al., 2016). However, different polymers, parameters and ambience in preparation will significantly affect the properties of the final product fibres, such as size, morphology, physical structure and even the rate of drug release, which provide more possibilities of using electrospinning technology for the delivery of biopharmaceuticals (Ramakrishna et al., 2013). Fibres with different properties by varying the parameters mentioned above can be produced, thus tailoring them to the needs of different drugs. In addition, as a one-step methodology, electrospinning has the advantages of significant encapsulation efficiency, high drug incorporating capacity, and particle or fibre uniformity, which further facilitate the design of a novel delivery system (Williams et al., 2018; Ramakrishna et al., 2013; Wright, 2015; Pelipenko et al., 2015; Williams et al., 2012; Chou et al., 2015). Moreover, electrospinning is undoubtedly a sustainable production method due to its cost-effective and reproducible characteristics, which aligns with the current desire of research for environmental friendliness (Shi et al., 2015; Krogstad et al., 2013).

This review, which is focused on manuscripts published after 2018, will initially present the principles and background of electrospinning technologies and provide a comparison between melt and solution electrospinning for the delivery of biopharmaceuticals. Moreover, drug incorporation methods, such as blends, emulsions, coaxial and multiaxial (e.g., triaxial) electrospinning, are further compared to evaluate the incorporation efficiency of biopharmaceuticals. On the other hand, the effects of parameters and environmental conditions on the properties of electrospun fibres will be analysed. On this basis, the current applications of these techniques for biopharmaceutical delivery, such as wound dressings, ocular delivery, transdermal delivery, vaginal delivery, and oral targeting of drugs, are further discussed and evaluated. Finally, the potential route of industrial scale-up of these delivery systems and their shortcomings in order to identify further development direction in future research will also be discussed.

2. Electrospinning technology

2.1. Overview of electrospinning

Electrospinning is a versatile technology used for creating nano- and micro-scale fibres, with considerable implications in the biopharmaceutical field. Utilizing high voltage to induce electrical charges on polymeric liquids, electrospinning enables the formation of fibres with applications in drug delivery, wound healing, and more. This technique owes its theoretical foundations to studies dating back to 1882 by Rayleigh, who investigated fluid stability in electric fields (Rayleigh, 1882). Since then, numerous studies have broadened its applicability, particularly in biopharmaceuticals, a field where over 689 articles were published between 2018 and 2023 with the keyword “electrospinning” on PubMed, which is still increasing each year. The basic electrospinning apparatus comprises a spinneret section, a high-voltage power supply in the kV range, and a ground collector, as shown in Fig. 2. The spinneret section houses a reservoir for storing the polymer solution and a syringe pump for controlling the injection rate (Zhang and Yu, 2016; Xue et al., 2019). Upon application of an electric field, the polymeric droplet is subjected to a range of forces, including Coulomb repulsion and surface tension, and forms a Taylor cone (Liao et al., 2018). A stable jet ensues when a critical voltage is exceeded, leading to fibre formation (Luraghi et al., 2021).

During electrospinning, the initially straight jet undergoes bending instabilities due to solvent evaporation, leading to solidified nanofibers (Ding et al., 2018; Sandri et al., 2020). Grounded metal collectors capture these fibres (Barhoum et al., 2019). For specialized applications like drug delivery, where an ordered fibre alignment is crucial, various methods such as rotating mandrels and auxiliary electrodes are employed to improve fibre alignment (Xue et al., 2019; Carnell et al., 2008; Ding et al., 2019). Over one hundred types of polymers have been successfully electrospun into fibres, each with unique properties and potential biopharmaceutical applications (Xue et al., 2019; Wang et al., 2013). Techniques like altering nozzle shapes and electric field parameters enable the production of fibres with specific characteristics (Fig. 2C), expanding the utility of electrospinning in formulating biopharmaceuticals (Wang et al., 2013).

One of the crucial areas for the application of electrospinning is the research of drug delivery with desired properties. By applying such a novel preparation method, could some inherent properties of drug molecules such as solubility be altered and targeted release be achieved (Yang and Pierstorff, 2012; Ahmed et al., 2001). There are many advantages when it is used to prepare DDSs (Bhardwaj and Kundu, 2010; Chakraborty et al., 2009). Firstly, due to the large surface area of the fibres, drugs can be readily adsorbed into the fibres (Buzgo et al., 2015). In addition, by adjusting the morphology and chemical composition of the fibres, the release rate of the drug can be controlled. Furthermore, as fibres often have a highly porous mesh structure, the drug can diffuse freely through the mesh and consequently be used for topical administration or as an implantable device (Hu et al., 2014; Morie et al., 2016; Son et al., 2014; Amler et al., 2014; Amler et al., 2013). Their unique structure and drug release properties, which resemble those of biological surfaces, make them suitable for applications such as dermal drug delivery, oral drug delivery, *in vivo* implant devices, stents and even wound dressings (Choi et al., 2015; Plencner et al., 2014; Liu et al., 2017).

Electrospraying, while similar to electrospinning, is distinguished by its generation of micro- or nano-sized droplets instead of fibres. In biopharmaceuticals, electrospraying is instrumental in creating drug delivery systems for peptides, proteins, and nucleic acids (Moreira et al., 2021). Unlike electrospinning, the electrospraying process yields droplets that undergo rapid solvent evaporation (Jaworek et al., 2009). The comparison between electrospinning and electrospraying in both principle and properties of final products is shown in Fig. 2. Recent innovations in nozzle-ring configurations have allowed for the more stable production of mono-disperse particles, thus broadening its applicability

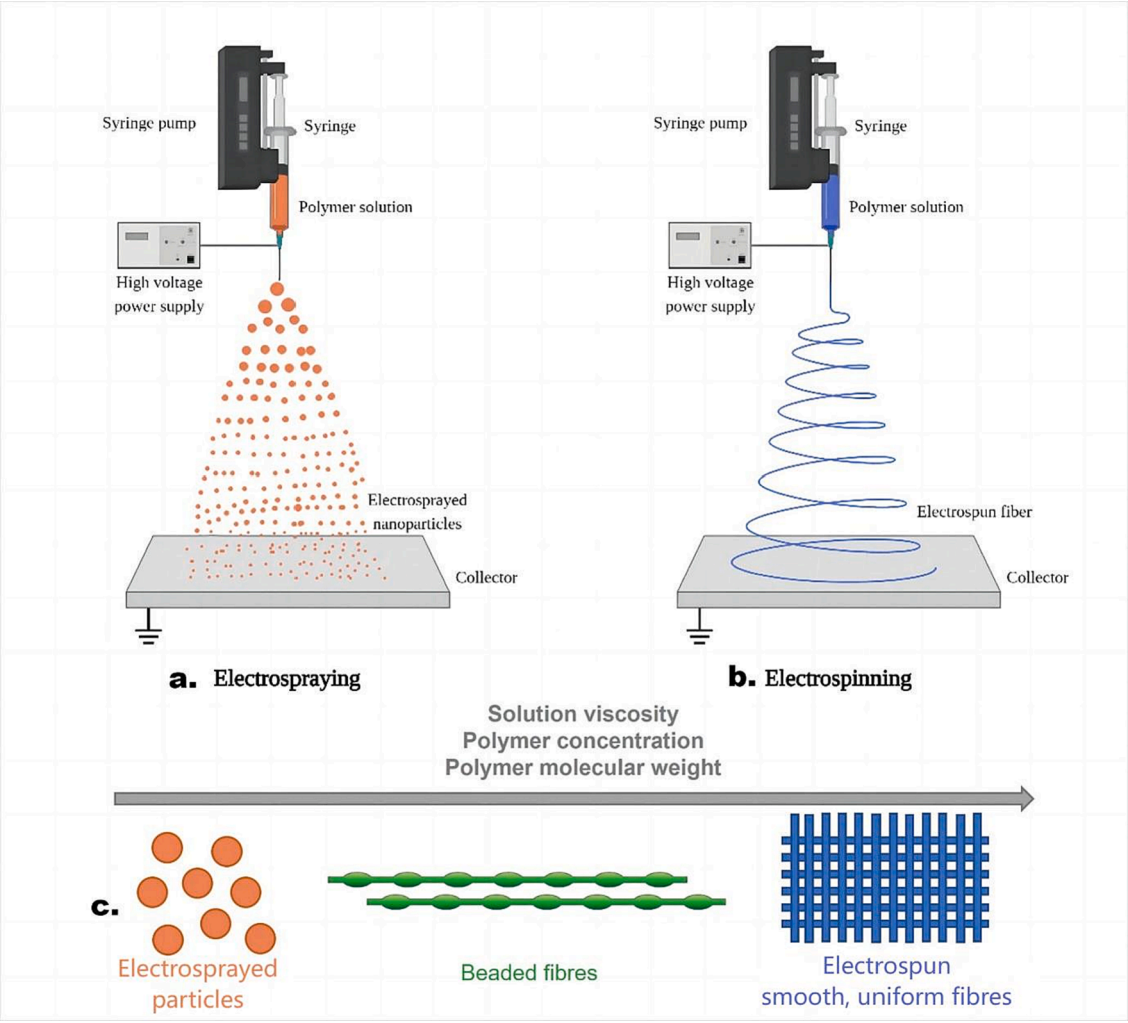

Fig. 2. Schematics of electrospraying (a) and electrospinning (b). Schematic illustration of the influence of formulation parameters on the electrosprayed and electrospun products (c).

in biopharmaceuticals (Xie et al., 2006; Ciach, 2006; Nyström et al., 2015; Nyström et al., 2010; Davies et al., 2005; Xie et al., 2006; Geerse et al., 2001). However, the in-depth discussion on the profound principle and applications of the electrospray process fall outside the scope of this review, and the interested reader is directed elsewhere for a fuller explanation.

2.2. *Different modes of electrospinning*

Electrospinning technology can be divided into solution and melt electrospinning. Admittedly, there have been many reports of synthetic electrospun fibres using natural or synthetic polymers in recent years (Abdelhady et al., 2015; Panthi et al., 2015; Mehrasa et al., 2016; Lee et al., 2016; Li et al., 2016; Zhang et al., 2016). However, it is worth noting that most researchers have focused on the solution but neglected the melt electrospinning technique due to the wide range of applications of solution electrospinning. There are differences in materials, equipment and processes between these two methods. As for the process, specifically, solution electrospinning usually uses a suitable and compatible solvent, either pure water or organic solvents, or an appropriate mixture, to dissolve specific polymers and drugs. Then, the configured solution is ejected under high voltage to form a steady jet, eventually evaporating the solvent and producing solid fibres (Jiang et al., 2015; Muerza-Cascante et al., 2015; Chen and Li, 2015). In contrast, the melt electrospinning process does not need the involvement of a solvent. The drugs and polymers will be heated and melted to the molten state, which is ejected from the needle under high voltage to form a steady melt jet, and eventually cooled during the process of shooting to collectors in order to form fibres. In terms of equipment, as compared to the solution electrospinning equipment, melt electrospinning requires an additional heating device in order to convert the solid state polymeric carrier to the molten state (Detta et al., 2010; Li et al., 2012).

In general, both methods have their own indispensable application scenarios owing to their unique advantages and non-negligible shortcomings. As for the merits of solution electrospinning, wide versatility is its most prominent one. Typically, it can process most polymers and drugs without special restrictions, including biological molecules, such as nucleic acids, proteins and polysaccharides. In addition to its wide range of applications, solution electrospinning also offers the advantage of using relatively simple and easy-to-operate equipment. Nevertheless, the solubility of polymers and drugs in different solvents is one of the most critical factors affecting the feasibility of solution electrospinning (Nagy et al., 2013; Balogh et al., 2014; Chen and Lv, 2015; Ulubayram et al., 2015). In addition, in order to secure solutions with a viscosity above the threshold, larger amounts of polymers are usually required, which restricts the drug-incorporating capacity of solution electrospinning (Khalf and Madihally, 2017). Although water is a promising solvent choice for biopharmaceuticals, there have been a considerable number of electrospun fibres that were produced using organic solvents.

Thus, the release or residual of some solvents is one of the problematic issues that cannot be ignored. On the other hand, melt electrospinning usually involves a direct mixing of drug and polymer in the molten state, avoiding the problem of solvent release or residue. As a result, it is a safer and more environmentally friendly method with a higher biomedical safety profile. Additionally, as the melt-state jet is usually more viscous, the polymer-to-drug ratio is more flexible. Thus, fibres with a higher drug-loading can be prepared. However, as melt electrospinning needs the heating of the feedstock to the molten state at a high temperature (usually over 250 °C), there are high demands on its thermal stability (Mu et al., 2021). Currently, the use of melt electrospinning for biologics, as well as thermosensitive polymers, is still a significant challenge (Dalton et al., 2012; Mazalevska et al., 2013; Patil et al., 2016). Besides, the low electrical conductivity of polymer melts is one of the factors preventing them from electrospinning, which can significantly affect the morphology of the final fibres.

Despite these drawbacks, and there is still relatively little research on melt electrostatic spinning technology, some researchers have demonstrated merits in the properties of electrospun fibres made by melt electrospinning compared to those made by solution methods, such as better drug release rates (Nagy et al., 2013; Balogh et al., 2014). Therefore, melt electrospinning is an up-and-coming biopharmaceutical technology waiting to be explored. In an interesting study, Lian (Lian and Melt, 2017) et al. attempted to produce PCL fibres loaded with curcumin by melt and solution electrospinning, respectively, and tried to compare the differences in their properties as well as in drug release. The authors vary the loading of curcumin in the different fibres and found that its content did not affect the morphology of the melt electrospun fibres. However, in solution electrospun fibres, the aggregation of curcumin affects the stability of the jet and, therefore, significantly enhances the range of diameter distribution of the fibres. At the same time, the loading of curcumin in solution electrospun fibres is limited due to solubility limitations, which are not present in melt electrospun fibres. Furthermore, the higher crystallinity of molten fibres results in a slow and smooth drug release profile, while solution electrospun fibres had a faster release rate and burst release was observed. The comparison of advantages and disadvantages of solution and melt electrospinning are shown in Table 1.

2.3. Drug incorporation approaches for electrospinning

Two main types of drug incorporation methods are commonly used with electrospun fibres, where the drug is either attached to the surface of the fibres or encapsulated within the fibres. Surface adsorption is a relatively simple process, with the amount of drug loaded and the rate of drug release usually dependent on the properties of the fibre surface and the method of adsorption, but the composition ratio and stability are key issues to be addressed. Drug encapsulation, on the other hand, involves the dissolving of the drugs into the electrospinning solution before the electrospinning process, thus directly dispersing them in the fibres. The preparation process and methods determine the structure, incorporation efficiency, and drug release behaviour in such fibres. For example, blend electrospinning (co-electrospinning), emulsion electrospinning and co-axial electrospinning can prepare fibres, especially nanofibers, with utterly different morphology and structure (Buzgo et al., 2018).

2.3.1. Surface adsorption

Of the above drug incorporation methods on nanofibres, surface adsorption is the simplest. The large specific surface area of fibres dictates that a large amount of drug can be adsorbed on their surface. However, since the drug is on the fibre's surface, it is usually released rapidly. Consequently, such a method is generally only suited to deliver the short-term drug. Nitanan et al. (Nitanan et al., 2013) demonstrated that the properties of the electrospun fibre surface, drug properties (pKa, hydrophilicity and molecular weight (MW)), as well as the solvent (concentration), determine the rate of drug adsorption/desorption. The rate of adsorption/desorption is determined by the nature of the solvent (concentration and valence of ions in releasing solution). Under such circumstances, complex optimisation of many parameters is required to achieve a balance between the adsorption/desorption speed of the drug and the expected delivery speed of the molecules on the fibre surface (Buzgo et al., 2018).

As for biopharmaceuticals, many biomolecules can also be loaded onto the surface of electrospun fibres by surface adsorption, such as various growth factors (Nie et al., 2008; Schneider et al., 2009; Dinis et al., 2014). In order to increase the loading capacity of fibres for biomolecules, specific treatments are often applied to their surfaces (Filová et al., 2013). For example, Lam et al. (Lam et al., 2010) increase the loading of epidermal growth factors as well as basic fibroblast growth factors by applying heparin to the surface of the fibres. Moreover, these factors interacted with the heparin molecules in favour of promoting the growth of axons on the scaffold. Nevertheless, it is undeniable that being on the surface of the fibres, many molecules that are more demanding to the external environment are not immune to environmental degradation, which may lead to reduced bioactivity, which limits the development of surface-adsorbed nanofibres for use in biopharmaceuticals (Nie et al., 2008).

2.3.2. Co-electrospinning

Co-electrospinning, also known as blend electrospinning, is the most

Table 1
The advantages and disadvantages of solution vs melt electrospinning.

	Advantages	Disadvantages
Solution Electrospinning	Simple equipment, no heating unit needed, easy to assemble in every lab	Solvent needed, possible solvent residue
		Solubility requirements for polymers and drugs
	Wide range of applications, especially for biomolecules with poor thermal stability	Low loading capacity and inefficiency
	Smaller diameter for solution electrospun fibres (10 nm – 2 μm)	High cost and poor safety of some organic solvents
Melt Electrospinning	No solvents are required, and no solvent residue problems	Complex equipment, heating and control devices needed
	Insoluble drugs and polymers could be used	High viscosity of the melt, the high voltage required, possible air discharge (breakdown)
	Well biomedical safety	
	Higher dose of drug load	High temperature (usually over 250 °C) requirements, not suitable for drugs and polymers with poor thermal stability
	More controlled API release profile	Bigger diameter for molten electrospun fibres (200 nm – 100 μm)
	Low economic cost and environmentally friendly	

basic electrospinning technique a far as equipment and principle are concerned. The API is usually dissolved or dispersed relatively homogeneously in the polymeric solution in co-electrospinning. There is not any spatial structure required. After mixing the drug and polymer solution, a high voltage will be applied, and the solvent will evaporate to obtain the electrospun drug fibres (Fig. 3C&F). In this case, the release of the drug is usually dependent on the properties of the polymer (Moreira et al., 2021). The different desorption/diffusion and dissolution/erosion processes of the polymetric matrix could significantly affect drug release (Maderuelo et al., 2011). For nonbiodegradable polymers, drug diffusion through the polymeric layer is the crucial release factor. However, for biodegradable polymers, the effect of dissolution/erosion of the system cannot be ignored. In addition, other mechanisms might regulate drug releases, such as swelling and osmosis. According to the different mechanisms, the release of the drug in blend electrospinning may correspond to Fickian-type diffusion, polymer swelling diffusion, non-Fickian diffusion or polymer erosion/degradation mechanism (Maderuelo et al., 2011; Gao et al., 1996).

Up to date, most of the applications of co-electrospinning have focused on the delivery of small molecule drugs, including antibiotics and anti-inflammatory drugs (Alhusein et al., 2013; Bhattacharjee et al., 2016; Hong et al., 2008; Maleki et al., 2014; Valarezo et al., 2015; Hu et al., 2016; Um-i-Zahra et al., 2014; Kenawy et al., 2009; Valle et al., 2016) to the point where it has become relatively well-established. As for large molecules, the use of co-electrospinning technology has recently attracted the interest due to its advantages such as one-step formation and ease of processing hence a number of promising applications reported. However, it is undeniable that there are potential problematic issues when it is used for biomolecules. In the case of proteins, which have many ionisation sites and are therefore susceptible to charging upon energisation and tend to migrate towards the surface of the jet via dielectrophoretic motions, co-electrospinning would ultimately result in drug enrichment on the surface of the fibres, leading to an explosive release, which could offer advantages in specific application scenarios, such as sublingual patches and reconstitution injections, but not for applications like wound dressings where a sustained- or controlled-release is required (Sun et al., 2008; Sun et al., 2007; Szentivanyi et al., 2011; Sharma et al., 2013). Furthermore, due to the stability issues in biopharmaceuticals, water is usually the preferred solvent. The poor volatility of water dictates that it will significantly affect the efficiency of electrospinning, while water solubility can also limit polymer selection, compared to organic solvent. As an essential part of biopharmaceuticals formulations, biocompatible polymers (e.g., PCL and PLA) need to be dissolved in the organic solvents. However, prolonged exposure to organic solvents can lead to the denaturation or inactivation of biomolecules, resulting in reduced drug activity and even safety risks. Many attempts have been made to solve the above problems (Mickova et al., 2012). Buzgo et al. (Buzgo et al., 2015) prepared different amounts of PEG-based cross-linkers with a view to changing the release rate and profile of the drug by varying the cross-linking ratio. Additionally, Yang et al. (Yang et al., 2014) employed polar polymers in combination with Bone morphogenetic protein two and silk fibroin to create nanofibres that stimulate osteoblast growth. Nevertheless, it is undeniable that the above studies still have some limitations and hence cannot fully address the release profile and stability issues, which limit the application of blend fibres for biopharmaceuticals. In this context, advanced electrospinning methods, notably emulsion and coaxial electrospinning, offer a more stable approach for fabricating fibres for biopharmaceutical delivery, which can circumvent the direct exposure of sensitive biopharmaceuticals to organic solvents.

2.3.3. *Emulsion electrospinning*

As previously discussed in Section 2.3.2., the challenges with blend electrospinning can be solved by a method called “emulsion electrospinning”. Emulsion electrospinning still requires only electrospinning equipment with a single nozzle (Fig. 3B), but it combines emulsification methods with electrospinning methods to produce fibres with a core–shell structure (Fig. 3F). Without the direct usage of solutions or molten polymers, emulsion electrospinning typically utilises a stable emulsion formed from two or more fluids as the raw material, which consists of two or more phases and can be kept separate throughout the whole process. To disperse and stabilise two immiscible liquid phases, an appropriate surfactant is usually added. Finally, the droplet phase of the emulsion is transformed into the inner core of the fibre, while another phase becomes the shell of the fibre (Moreira et al., 2021).

It is worth noting that emulsion electrospinning technology places higher demands on a broader range of performance parameters. Adequate conductivity of the solution making up the emulsion, the

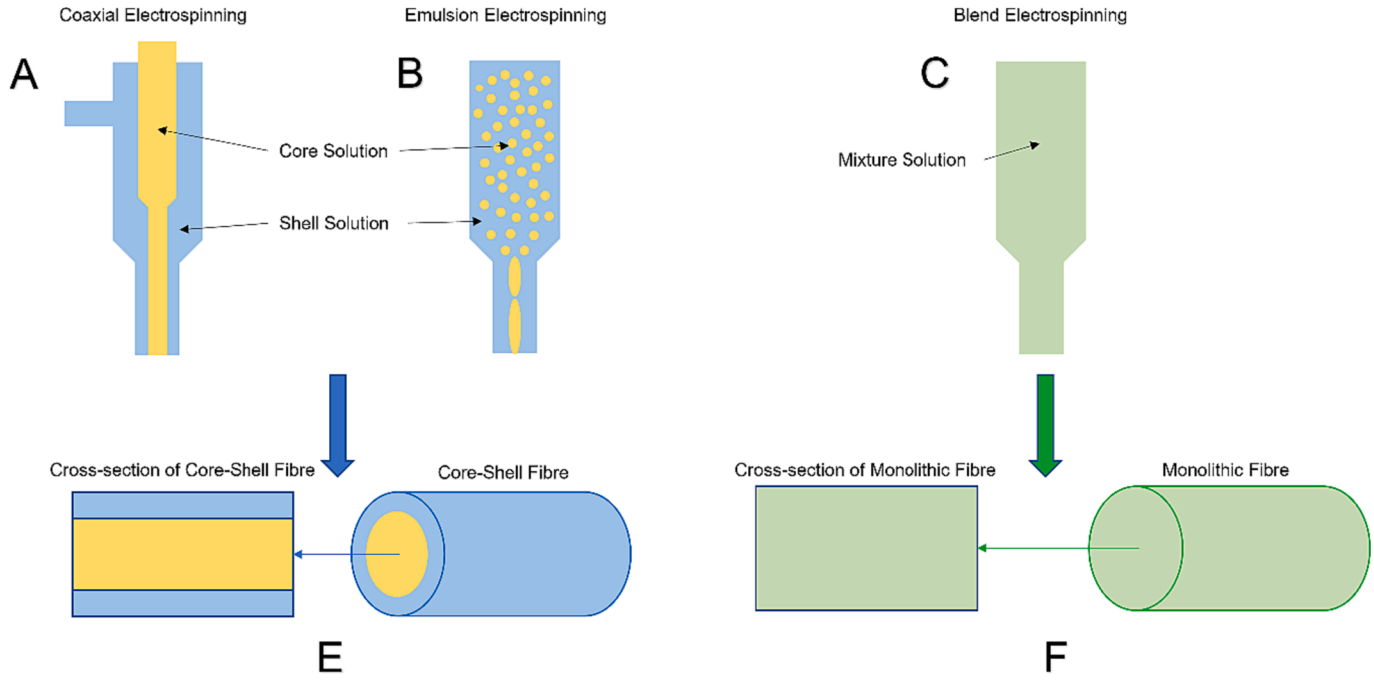

Fig. 3. Schematics of coaxial (A), emulsion (B) and blend (C) electrospinning. Illustration of cross section of core–shell fibres (E) and monolithic fibres (F) produced.

appropriate polymer concentration, molecular weight and surface tension are all key factors affecting the success of electrospinning (Williams et al., 2018). Emulsions commonly used in electrospinning are usually divided into two categories: water-in-oil (W/O) emulsions and oil-in-water(O/W) emulsions (Zhao and Wang, 2016; Li et al., 2010). Undeniably, more complex multiple emulsions exist but are also created based on these two emulsions. W/O emulsions consist of an oleophilic continuous phase as well as a hydrophilic dispersed phase and are typically used to encapsulate more polar or hydrophilic molecules, including most biomolecules. Therefore, it is used more frequently for biopharmaceuticals (Li et al., 2010). The shell polymers are usually oil-soluble polymers, such as PCL, PLA, and PLGA, while the dispersed phase chooses water-soluble ones (i.e., Polyvinyl alcohol (PVA), Polyethylene glycol (PEO), polyvinylpyrrolidone (PVP), cellulose derivatives, chitosan, and alginates). On this basis, high hydrophilic-lipophilic balance (HLB) values emulsifiers, like Span 80, Span 60, as well as Pluronic, are usually chosen to maintain emulsion stability (Tadros, 2004). On the contrary, in O/W emulsions, the hydrophilic solution forms the continuous phase, while the lipophilic solution builds up into the droplet phase (Camerlo, 2014). Furthermore, although the polymers required are similar, low HLB emulsifiers (i.e., Tween 20 and Tween 80) are an essential part of maintaining the stability of the system (Tadros, 2006). On this basis, if the aqueous phase of an O/W emulsion is emulsified, a water-in-oil-in-water (w/o/w) multiple emulsification system can be obtained. However, similar multiple emulsification systems often have higher requirements for emulsifiers and emulsification protocol optimisation, which could refer to the quality-by-design strategies that Badawi et al. demonstrated (Badawi and El-Khordagui, 2014).

When judging the feasibility of a formulation technology for drug delivery, the drug release profile and the influencing factors of products must be essential considerations. In electrospun emulsion fibres, the morphology of the inner core is one of the key influences on the release kinetics, which is influenced by the type of surfactant as well as the nature of the dispersed phase (Wang et al., 2017; Hu et al., 2015). The properties of a polymer can significantly impact the morphology of a core–shell structure formed during the emulsion electrospinning process. Specifically, If the dispersed phase is more viscous, an unbroken core along the fibre axis will be formed. Otherwise, the core phase will break into separated droplets and exhibit non-continuous morphology. The underlying principle behind this phenomenon is related to the increased polymer chain entanglement and cohesion that occurs with higher viscosity solutions (Wang et al., 2017; Hu et al., 2015; Angeles et al., 2008; Xu et al., 2005). On the other hand, diffusion or degradation mechanisms controlled the drug release of emulsion fibres, like blend electrospinning ones (Srikar et al., 2008). The degradation mechanism dominates when fibres use degradable polymers as the shell material. In this case, the polymer matrix is continuously dissolved, and the drug is released from the polymeric structure. Thus, the dissolution of the polymer determines the drug release behaviour (Sill and Recum, 2008; Zeng et al., 2005). If the polymer making up the fibre is non-degradable, the drug release rate is limited by the diffusion rate of the compounds through the polymeric mechanism (Gandhi et al., 2009).

Typically, the rate of drug release from an electrospun emulsion fibre is influenced by a combination of both dissolution of the polymer and the diffusion rate of compounds. Taking a typical W/O emulsion fibre as an example, the outer shell is composed of a water-insoluble polymer such as PLGA. The inner core comprises a hydrophilic polymer such as PVA. Therefore, diffusion of water and drug is a limiting factor for drug release, which depends on the internal structure of the fibre. In instances where the inner core is continuous in nature, the rate of drug release is influenced by the flux of the pharmaceutical agent and the interplay between diffusion gradients and capillary forces within the fibres. Conversely, when the inner core comprises droplets, the release dynamics are mediated by the interconnectedness of said droplets. If the droplets are not interconnected, the initial dissolution of droplets on the surface of the fibre results in a quick release of the encapsulated drug, yet the availability of the drug may be limited. On the other hand, if the droplets are interconnected, sustained drug release is facilitated by the solvent's capacity to permeate deeper into the fibres (Buzgo et al., 2017). Furthermore, it is worth noting that in addition to the factors mentioned above, the stability of the emulsion itself is also a significant influencing factor in the release rate. Core droplets in unstable emulsion spinning fibres tend to agglomerate and accumulate on the surface, resulting in a significant initial release (Xu et al., 2005). Under such circumstances, adding weak electrostatic forces during the process is commonly used to solve this problem. Most biomolecules are electrically charged. Introducing substances of opposite charge into emulsion fibres can increase the interaction with biomolecules and thus slow their release. For example, Zhao et al. successfully produced slow-released PLGA-growth factors fibres by adding cellulose acetate into the formulation (Zhao and Wang, 2016).

Emulsion electrospinning has been utilised for the delivery of different active molecules, especially for some small molecules such as antibiotics (e.g., vancomycin) (Zhang et al., 2016), anti-cancer drugs (e.g., camptothecin, paclitaxel) (Luo et al., 2012; Liu et al., 2012) and anti-inflammatory drugs (e.g., celecoxib and ketoprofen) (Gordon et al., 2015; Basar et al., 2017). In addition, emulsion electrospinning has unique implications for the delivery of biological molecules. W/O emulsions are often chosen for hydrophilic macromolecules, such as proteins, with the active ingredient located in the aqueous phase and the organic solution outside the aqueous phase, after the electrospinning process (Nikmaram et al., 2017). Fibres with a core–shell structure are formed, avoiding direct contact between the biomolecule and the organic solvent, thus ensuring the activity of the biomolecule. At the same time, the active molecules are encapsulated in the centre of the fibres, avoiding the initial burst of release (Xu et al., 2005). For example, the nerve growth factor was delivered by emulsion electrospinning to achieve the constant stimulation of neural cells (Li et al., 2010; Hu et al., 2016). Similarly, an emulsion electrospun with growth factor or peptides was developed to make vascular grafts (Zhang et al., 2013; Tian et al., 2011; Tian et al., 2013; Tian et al., 2013; Wang et al., 2015; Yang et al., 2016). In addition, preparing various stents is another significant application of electrospun emulsion fibres. Emulsion fibres containing Vascular endothelial growth factors and heparin can be used to prepare scaffolds for cardiac tissue engineering to achieve long-term release of Vascular endothelial growth factors and heparin (Chen et al., 2015). Another study with an epidermal growth factor was used for scaffolds for skin tissue engineering (Tian et al., 2011). Similarly, wound dressings containing epidermal growth factors are one of the application scenarios of such technology (Wang et al., 2016; Yang et al., 2011). In the above application, the manufacture of core–shell fibres is achieved by emulsion electrospinning, which avoids direct contact between the drug and the solution, thus enhancing its stability. In addition, the release rate of the drug in the nanofibres is indirectly regulated by modulating the nature of the emulsion. The specific advantages are detailed in Table 2.

However, there are undeniably unresolved technical difficulties with emulsion electrospinning, as shown in Table 2. It is often challenging to produce a homogeneous and stable core–shell structure for the fibres when using low-surface tension solutions as feedstock (Williams et al., 2018). What is more, as mentioned earlier, emulsion instability can significantly affect the release behaviour of the drug and thus reduce efficiency (Moreira et al., 2021). Finally, mechanical mixing or ultrasonication is commonly used to prepare emulsions to homogenise the dispersed phase in a continuous phase. However, such a mixing process may damage the structure and function of the biomolecules, which limits the further development of electrostatic spinning of emulsions for the delivery of biopharmaceuticals (Jiang et al., 2012; Vysloužilová et al., 2017).

Table 2
Advantages and disadvantages of Blend, Emulsion and Coaxial electrospinning for biopharmaceuticals.

Electrospinning Methods	Advantages	Disadvantages	References
Blend	Straightforward user-friendly	Uncontrolled drug release rates, prone to bursts of release	(Buzgo et al., 2018)
	Traditional Method, proven and highly reproducible	Limited stability protection. Biomolecules are easily inactivated when using organic solvent	
Emulsion	Fibres in a core–shell structure, no need to use the complex coaxial spinnerets	Higher requirements for electrospinning parameters	
	Avoids direct contact between the drug and organic solvents, which helps to protect the activity of the biomolecules	Cannot use polymer solutions with low surface tension	(Li et al., 2020; McClellan and Landis, 2016)
	Avoids burst release. The release rate can be indirectly changed by changing emulsion properties	Emulsification, mechanical mixing or ultrasonication may lead to the deactivation of biomolecules	
		Emulsions are more unstable than solutions, which can lead to low efficiency	
Coaxial	Fibres are core–shell structures. The specific distribution of the drug in the fibre can be further controlled	Slow production rates, low efficiency and relatively complex nozzle structures	
	A wider range of applications, allowing the use of materials that are not electrospinnable		(Moreira et al., 2021; Li et al., 2020)
	High drug loading capacity Easily controlled drug release rate	Highly demanding electrospinning parameters, which need to be optimised for the active composition and the different layer structures	
	Avoids contact with organic solvents, while production conditions are milder, thus better protecting the activity of biomolecules	Different properties between layers lead to defects and artifacts	
	Multi-axis electrospinning can produce more versatile fibres		

2.3.4. Coaxial electrospinning

Coaxial electrospinning is another commonly adopted electrospinning technique. Unlike emulsion electrospinning and blend electrospinning, the equipment for coaxial electrospinning has two needles as spinnerets which are placed coaxially (Lu et al., 2016) and are usually filled with different polymeric solutions. Typically, the inner (core) liquid is pumped by the needle in the inner layer, while the outer needle is used to pump the outer shell material. In the presence of an electric field, the two polymeric droplets are ejected along a specific order and then dried to form a fibre with a core–shell structure. The structure of needles and fibres of coaxial electrospinning are shown in Fig. 3A&E.

The success of coaxial electrospinning heavily relies on the formulation design and processing conditions. Firstly, the concentration, entanglement and molecular weight of polymer must be well selected for both the outer and inner layers. More specifically, the polymeric solution forming the shell must be electrospinnable to ensure a stable fibre jet while the inner normally possesses a low concentration of polymer to form a non-spinnable solution. Secondly, the interfacial tension between the inner and outer polymeric solutions needs to be high enough for the drawing of core liquid. Finally, the inner and outer polymeric solutions need to remain stable and especially not interact with each other (Hu et al., 2016; Vyslouzilova et al., 2017).

Coaxial electrospinning is a relatively new and innovative approach in the field of electrospinning, which offers a broader range of applications compared to conventional methods. The technique involves encapsulating a non-electrospinnable core material, such as biomolecular drugs, in an electrospinnable shell to prepare nanofibers. This unique characteristic of coaxial electrospinning is a remarkable advantage over conventional methods, as it enables the creation of nanofibers that are not possible with conventional electrospinning (Buzgo et al., 2018). For instance, protein nanofibers containing ketoprofen were prepared using poly-ethyl pyrrolidone as the shell material due to their favourable electrospinnability. Moreover, coaxial electrospinning also enables the use of a non-electrospinnable solution as the shell material and an electrospinnable solution as the core, as demonstrated in the production of zeatin-ibuprofen fibres, where non-electrospinnable dimethylformamide (DMF) was used as the shell solution and a zeatin-ibuprofen solution as the core. Additionally, coaxial electrospinning has successfully addressed the clogging issue in blend electrospinning, thus efficiently producing drug-loaded nanofibrous scaffolds.

Moreover, coaxial electrospinning offers other advantages, such as being able to prepare electrospun fibres using either miscible or immiscible solutions, the possibility of incorporating biomolecules, sound and controlled drug release rates, and being suitable for formulating unstable compounds due to relatively mild production conditions. Specifically, coaxial electrospinning allows the encapsulation of one or even more APIs in fibres within different layers, minimising the contact of the active substance with solutions (especially organic solutions) that may affect its stability. Therefore, it is favourable for the production and encapsulation of unstable biopharmaceuticals (e.g., proteins, nucleic acids). For example, choosing aqueous protein solutions as core solutions while using organic solvents in the outer layer minimises contact between the two and avoids the harsh environment of the encapsulation process, thus reducing degradation. In addition, the core–shell structure could effectively avoid burst release and potentially extend the drug release time (Moreira et al., 2021). Furthermore, as fibres can be loaded with several different APIs, synergistic therapy is an easily achievable advantage of coaxial electrospun fibres (Lu et al., 2016). In addition, the structure of the extracellular matrix can be mimicked by coaxial electrospun fibres as the thickness of the core and shell can be more freely determined, thus making it easier to prepare biocompatible scaffolds (Li et al., 2020; Li et al., 1803). The outer layer of electrospun fibres commonly used for scaffolds is mostly made of biocompatible polymers, while the inner core is a polymer solution loaded with drugs to achieve therapeutic effects.. What is more, A more controlled drug release rate is

also one of the features of coaxial electrospinning technology. Unlike the emulsion or blend electrospinning, the drug release rate of core–shell structured fibres is influenced by both the degradation rate of the core and shell materials, the type of drug and the diffusion coefficient between the core and shell layers. Thus, drug release profiles can easily be altered and optimised by changing the structure between the core and shell layers. Finally, coaxial electrospinning can also be combined with other functionalisation techniques, thus achieving additional functionality of fibres. Chen et al. covalently bound proteins to the surface of tetracycline nanofibres, thus enabling further functionalisation for tissue repair (Chen et al., 2011). The tetracycline in the inner core of the fibre can be released rapidly to achieve an anti-infective effect, after which the protein loaded on the fibre surface will take effect slowly to promote healing.

From biopharmaceutics a point of view, coaxial electrospinning occupies an important position due to its gentle properties of encapsulating and protecting biomolecules. Any bioactive component, whether DNA, RNA, or protein, which is sensitive to environmental stimuli, has been shown to be rationally encapsulated by coaxial electrospinning. For example, growth factors that can be used for tissue engineering, including vascular endothelial growth factor, nerve growth factor, Bone morphogenetic protein 2, and fibroblast growth factor, have been formulated into different types of core–shell nanofibers (Cao et al., 2011; Jiang et al., 2014; Rubert et al., 2014; Sahoo et al., 2010; Zhang et al., 2016; Zhao and Wang, 2016; Jia et al., 2011; Liu et al., 2011; Lu et al., 2009; Tian et al., 2015). Vascular endothelial growth factor (VEGF) and dexamethasone can be made into nanofibrous membranes with PLGA to achieve slow release and stimulate endothelial cell growth (Jia et al., 2011). Similarly, Su et al. prepared PLCL-shelled nanofibres to deliver BMP-2 and dexamethasone, which significantly delayed the release rate and showed osteoinductive properties (Su et al., 2011). In addition, NGF was encapsulated into nanofibres consisting of a mixture of silk cellulose and PLA for delivery, thereby enabling the promotion of neuronal cell proliferation and differentiation into the desired subtype (Tian et al., 2015). Notably, among lots of research, the BFGF nanofibres prepared by Zhang et al. are interesting. Using the same shell, two fibres with different inner cores can be prepared, that are able to achieve different drug release profiles. The outer shell of fibres is made of PLCL, while the core is made of hydrogel or an emulsion containing heparin and PLGA (Zhang et al., 2016). Under such circumstances, nanofibres with a hydrogel core can achieve rapid release by rapid diffusion of the core solution, while the emulsion core can have an extended-release time. However, both fibres can achieve the proliferation activity of fibroblast. This study is an effective demonstration that drug release rates can be controlled through the coaxial electrospinning process.

However, there are still a few drawbacks of coaxial electrospinning that cannot be ignored. Firstly, the slow rate of production and the complexity of the production process severely limit the diffusion of the technology. Whilst high-speed electrospinning has partly ameliorated this issue, it also places greater demands on polymers, experimental equipment and conditions (Domján et al., 2020). To make matters worse, specific optimisation is required for different active ingredients and outer layer structures to meet compatibility requirements, which may otherwise lead to the formation of artefacts and defects. Besides, different layers of the fibres may have different properties from one another, which might well affect their stability. A comparison of the advantages and disadvantages of coaxial electrospinning with blend electrospinning is detailed in Table 2. Despite a few persistent problems that need further addressing, it is still one of the most suitable electrospinning technologies for incorporating and formulating biopharmaceuticals (Moreira et al., 2021; Buzgo et al., 2018).

Based on the above applications, coaxial electrospinning can be further improved to prepare fibres with more complex morphologies, for example, by introducing three separate needles to prepare nanofibres with multiple structures (Khalf and Madihally, 2017). Recent successful investigations include the preparation of doxycycline and nisin nanofibres (Khalf and Madihally, 2017; Han et al., 2017; Panzavolta, 2016), enabling the delivery of multi-stage active compounds with intricate release patterns (Liu et al., 2014; Han and Steckl, 2013). However, it cannot be ignored that more stringent chemical and physical conditions are needed during the process. In addition, the homogeneous nature of the nanofibres is often difficult to guarantee, and the production efficiency is low. The technology is, therefore, not yet mature and is still in the laboratory stage (Khalf and Madihally, 2017; Khalf and Madihally, 2017; Khalf et al., 2015).

2.3.5. *High-throughput electrospinning technologies*

In addition to the electrospinning techniques described above, high-throughput electrospinning techniques have been developed in recent years. These novel electrospinning methods can effectively increase the output rate and yield of electrospun fibres. As mentioned earlier, the flow rate is a critical parameter for classical electrospinning. In most cases, the optimum flow rate of the polymer solution is usually around 10–100 μl/min, which also limits the yield of electrospun fibres to typically only 1–100 mg/h (Rutledge and Fridrikh, 2007; Theron et al., 2004). To solve these problems, high-throughput electrospinning techniques have been developed. The most straightforward approach is to increase the number of needles and, thus, the fibre yield on top of the classical electrospinning (Xie and Zeng, 2012; Kumar et al., 2010; Angammana and Jayaram, 2011; Zheng et al., 2014; Zheng et al., 2013; Zheng and Zeng, 2014). The arrangement between the needles will affect the distribution and homogeneity of the fibre jet, so needle arrangement systems of different styles and sizes, such as linear, triangular, square and hexagonal, have been developed (Xie and Zeng, 2012). Nevertheless, the disadvantages of this method are mainly the difficulty of finding the optimum needle arrangement, the applied voltage and the distance between the collector and the electrode (Xie and Zeng, 2012; Kumar et al., 2010; Angammana and Jayaram, 2011; Zheng et al., 2014; Zheng et al., 2013; Zheng and Zeng, 2014; Guo et al., 2011; Liu and Guo, 2013). To solve these problems, scientists often use finite element analysis modelling to simulate and optimise the jetting process of multi-needle electrospinning and, ultimately, to determine the appropriate needle arrangement system and parameters (Guo et al., 2011; Liu and Guo, 2013; Tian et al., 2014).

In addition, a novel type of electrospinning methods, needleless electrospinning, has been developed to address the problems of optimising multi-needle methods. Schematics of typical needleless electrospinning equipment are shown in Fig. 4. The underlying concept of needleless electrospinning is the spontaneous organization of fibre jets on the surface of a liquid, which is brought about by the synergistic effect of electrostatic force as well as surface tension. This technique can be implemented utilizing a diverse array of electrodes. The selection of electrodes utilized in needleless electrospinning plays a critical role in determining the outcome of the process, with rod electrodes or the rotating drum, disc, and coil electrodes being regularly utilized in a polymer bath as a means of achieving optimal results, which means part of the fibre generators are in the polymer solution (Lukáš et al., 2009; Shin et al., 2015). The specific structures and methods will not be described here due to space limitations, as details can be found in the review of Buzgo et al. (Buzgo et al., 2018). Methods more closely related to the preparation of biopharmaceuticals are the needleless coaxial electrospinning method and the needleless emulsion electrospinning method. For example, by forming a bimolecular layer of two incompatible solutions on the surface of a rotating wire electrode, a novel coaxial needless electrospinning technique has been developed and is suitable for use in biopharmaceuticals (Forward et al., 2013). In a similar vein, Vvslouzilova et al. developed a new electrospinning technique based on double-slit electrodes (Vyslouzilova et al., 2017). In contrast to nanofibres produced by the classical coaxial electrospinning technique, the version produced by the needleless technique has a higher dose of drug incorporation and better drug release properties.

The use of a needleless dual-wire electrode in emulsion

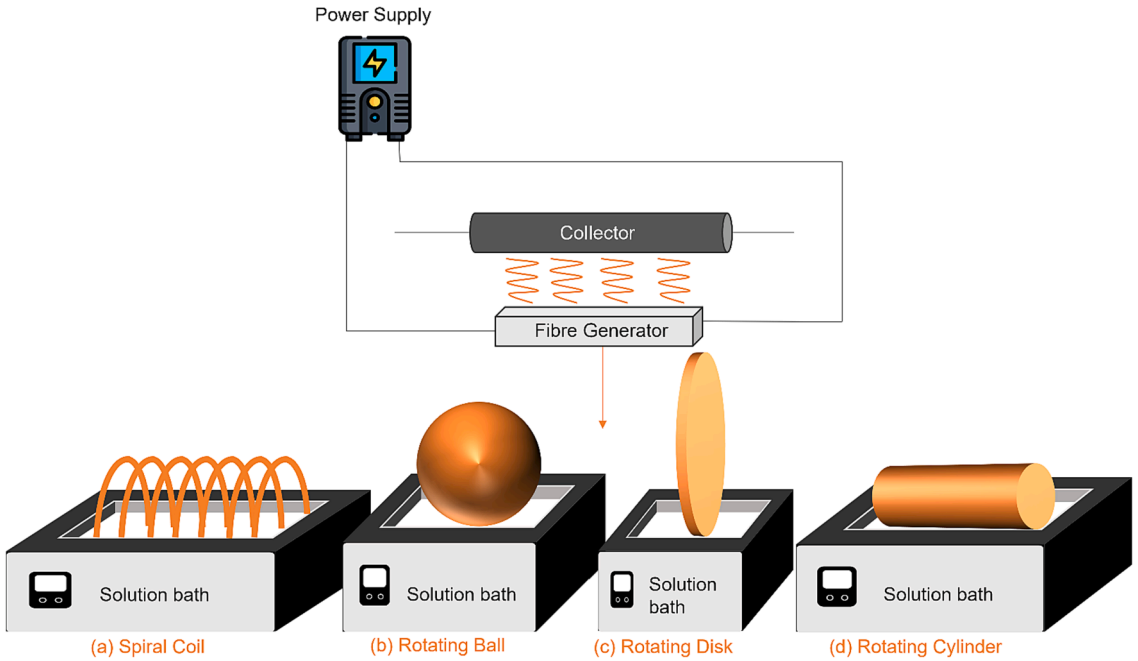

Fig. 4. Schematic of different types of fibre generators: (a) Spiral Coil, (b) Rotating Ball, (c) Rotating Disk, and (d) Rotating Cylinder.

electrospinning has been demonstrated to be a highly effective method for the manufacture of core/shell fibres in a high-throughput approach. This approach, as demonstrated through the producing PCL/Pluronic F68 emulsion electrospun, resulted in the formation of core/shell fibres that exhibited superior enzymatic activity retention and scalable release timings while also allowing for an increased production rate (Buzgo et al., 2018). Additionally, this method has potential applications in the large-scale production of scaffolds loaded with growth factors. Furthermore, core–shell fibres can also be high-throughput manufactured using the novel emulsification centrifugal electrospinning, which utilizes high centrifugal forces to form ultrathin fibres from polymeric solutions (Buzgo et al., 2017). This method has the added advantage of producing fibres with a more 3D shape with expanded holes, which could protect the bioactivity of bioactive molecules, achieve controlled release, and promote osteoblast proliferation.

High-Speed Electrospinning (HSES) aims to elevate nanofibre production rates by accelerating the ejection and solidification of polymeric solutions. This acceleration is achieved by fine-tuning operational parameters such as voltage, flow rate, and spinneret-to-collector distance, often aided by a rotational collector to enhance fibre drawing and alignment (Démuth et al., 2016). The method is particularly promising in biopharmaceutical applications, offering increased production rates that facilitate the shift from lab to industrial scales. It also generates fibres with unique morphologies and enhanced functional properties, vital for encapsulating and controlling the release of biopharmaceuticals (Démuth et al., 2016). Furthermore, HSES accommodates a broad spectrum of bioactive molecules including proteins, nucleic acids, and living cells, thereby expanding opportunities in drug delivery and tissue engineering. The technique's capacity for better fibre formation with aqueous solutions and rapid processing also helps in reducing the degradation risks of sensitive biopharmaceuticals, thus ensuring their post-encapsulation stability and efficacy (Domján et al., 2020). However, challenges exist, primarily in controlling operational parameters to achieve targeted fibre characteristics. The increased processing speed also necessitates a comprehensive understanding and control over polymer solution properties, electric field dynamics, and ambient conditions, and the need for specialised equipment may limit its widespread adoption.

In conclusion, the high throughput production technology of core–shell nanofibres increases the potential for electrospinning technology to deliver biopharmaceuticals. The fibres produced by the methods described above have better properties and could be produced in industrial quantities to address numerous health problems. However, research into these technologies is still at the laboratory stage. Further research is still required on their suitable polymers, excipients as well as drugs in order to achieve the translation from technology in the laboratory to practical applications.

2.4. *Adjustable parameters of electrospinning*

The morphology and properties of electrospun fibres are influenced by several factors, which are known as electrospinning parameters. These parameters are divided into three categories: solution parameters, process parameters and ambient conditions (Bi et al., 2019; Jaiturong et al., 2018; Liu et al., 2017; Pilehvar-Soltanahmadi et al., 2018; Stephansen et al., 2016). Generally, the ideal electrospun products should be smooth, homogeneous micro- or nano-fibres without any bead, which could be achieved by optimising such parameters (Zong et al., 2002). However, parameters interplay with each other and, ultimately, determine the properties of the fibres. Under such circumstances, they need to be constantly adjusted to achieve a balance. The effects of each of these three parameter types on the properties of the fibres are to be described in the following context.

As the name implies, solution parameters are all the properties associated with the electrospinning solution. The viscosity, concentration, conductivity, and surface tension of the solution are all solution parameters. In addition, the properties of drugs, polymers and solvents also belong to the solution parameters. For example, the molecular weight of the drug and polymers and the type of solvent are also critical factors in them. The concentration, the molecular weight of the polymer and drug, and the viscosity are positively correlated. When the molecular weight of the solute is higher and the concentration is higher, then the viscosity of the solution is higher (Litovitz, 1952). Notably, only when the viscosity of the solution is high enough can a stable jet be ejected appropriately through the nozzle to form a spinning stream. In contrast, when the viscosity is not high enough, the jet does not form fibres but instead exists in the form of particles, which is the process referred to above as electrospraying. The critical concentration of the

solution between electrospraying and electrospinning is often referred to as the minimum entanglement concentration. As a rule, the greater the viscosity of the solution, the larger the diameter of the electrospun fibres (Yang et al., 2004). However, too high a viscosity can often lead to complications where fibres made from an excessively viscous solution may not be homogeneous enough, with the presence of beads that can affect the performance of the electrospun fibres and the release of the drug (Shahreen and Chase, 2015). Furthermore, overly viscous solutions may lead to needle blockage, thereby affecting fibre production (Haider et al., 2018). Therefore, the viscosity of electrospinning solutions is usually controlled between 100 and 2000 cp (Pérez-González et al., 2019). Similarly, the conductivity of the solution affects the diameter of the fibres, and solutions with too high or too low conductivity will be unable to be electrospun (Angammana and Jayaram, 2011).

On the other hand, solvent parameters can also significantly affect the properties of the fibres, such as the dielectric constant as well as the volatility. When the dielectric constant of the solvent is large, the bending instability of the jet will increase significantly, resulting in a reduction in the diameter of the resultant fibres (Yang et al., 2004). The volatility of the solvent affects the porosity of the fibres, which is usually positively correlated (Yang et al., 2004). Finally, surface tension is also one of the solution parameters. Surface tension needs to be overcome by electric field forces during the electrospinning process, so it primarily determines the voltage and thus indirectly affects the fibre characteristics.

Voltage is one of the process parameters in addition to the flow rate and tip-to-collector distance. Voltage is necessary for the initiation of the jetting process, where a minimum of 6 kV is usually required to drive the process. However, the voltage needs to be greater than that required so as to overcome the surface tension; otherwise, no jet will be formed. As the voltage increases, the evaporation rate of the solvent also increases, resulting in a reduction in the diameter of the fibres. It is worth noting that too high a voltage can also lead to the formation of beads (Tijing et al., 2018). Therefore, the voltage of the electrospinning process is usually controlled between 15 and 25 kV (Pérez-González et al., 2019). Similarly, the flow rate mainly affects the homogeneity of the fibres. When the flow rate is high, the diameter of the fibres decreases accordingly. However, too high a flow rate can also lead to the creation of beads, affecting the morphology and other properties of the fibres (Zong et al., 2002). Zargham et al. produced the nylon electrospun nanofibers using flow rates of 0.5, 1 and 1.5 mL/hr and observed that when using the flow rate of 0.5 mL/hr, the Taylor cone was most stable and produced the smoothest nanofibers. However, the fibre diameter distribution increase with the flow rate and some beaded fibres occurred when the flow rate was increased by 1 or 1.5 mL/hr (Zargham et al., 2012). Finally, the tip-to-collector distance, which is the distance between the nozzle and the collector, also plays a critical role in electrospinning. Such distance affects the morphology and diameter of the fibres, and too large a distance can also lead to inhomogeneous fibres or even beaded fibres (Robb and Lennox, 2011). Typically, the tip-to-collector distance is between ten and twenty centimetres (Bhattarai et al., 2018).

In addition to the above factors, the influence of ambient conditions on electrospun fibres should also be well recognized. Environmental factors may be seen as simple as the effects of temperature and humidity but can be the most complex influencing factors. Higher temperatures usually accelerate the evaporation rate of the solvent from the jet, resulting in thinner fibres being produced (Yang et al., 2017). Furthermore, temperature also changes the viscosity and surface tension of the solution, thus indirectly changing the properties of the fibres (Lara-Espinoza et al., 2018). It cannot be ignored that continuous fibres may be challenging to form when the temperature is too high, which is related to the termination of the jet due to high temperatures (Cazorla-Luna et al., 2021). On the other hand, humidity can also affect the diameter of the fibres. However, the increase or decrease in fibre diameter with increasing humidity will vary depending on its polymer. The rationale for this may be due to variations in the interaction of moisture and polymers and the different effects of humidity on the evaporation rate of solvents (De Vrieze et al., 2009). However, the humidity of the environment also affects the porosity of the fibres. When the ambient humidity is high, more water will condense on the surface of the fibre, thus increasing its porosity (Casper et al., 2003). A summary of the properties and morphology of the electrospun fibres as affected by the different parameters is presented in Table 3.

It is worth noting that biopharmaceuticals themselves have also been found to alter the properties of nanofibres obtained. Skrlec et al. (Škrlec et al., 2019) reported that Lactobacillus Plantarum could increase the electrical conductivity of polyethylene oxide solutions, thus affecting fibre properties, which was proposed to be related to the proteins and ions added alongside the bacteria. Zupancic et al. (Zupančič et al., 2019) demonstrated that the addition of bacteria could also increase the viscosity of the polymer solution, thereby affecting the fibre diameter. In conclusion, the different electrospinning parameters affect each other and collectively determine the performance of nanofibres. It is impossible to make a superior or inferior order of different parameters, therefore, it is crucial to establish a better balance between the parameters in order to prepare stable and homogeneous electrospun fibres.

3. Applications of electrospinning in biopharmaceutical delivery

Researchers have widely used electrospinning technology in the field of drug delivery for biopharmaceuticals, including proteins, DNA, RNA and even bacteria, living cells and viruses (Carson et al., 2016; Davoodi et al., 2017; D'Amato et al., 2017; McInnes et al., 2018; Achille et al., 2012; Zheng et al., 2020; Salles et al., 2017; Zandi et al., 2020; Nguyen et al., 2015; Guo et al., 2019). The advantages of electrospun fibres include fine and adjustable diameter, large surface area and functionalisation on the surface. Moreover, their structural similarity to the cytoplasmic matrix has contributed to their application and development in the field of drug delivery (Rajput et al., xxxx). In addition, electrospinning technology has the potential to be scaled up and applied to industrial applications thanks to its excellent cost-effectiveness (Ramakrishna et al., 2013). However, electrospinning also has some limitations. Firstly, the polymer chosen should be compatible with the pharmaceutical molecule in question. Secondly, some solvent residues may remain in the fibres after evaporation. Furthermore, aqueous solutions may affect the efficiency and yield of electrospun fibres while the organic solvent may affect the steric structure as well as the stability of the macromolecular drug. Finally, the release of the drug in the fibre can vary widely depending on the nature of the drug molecule as well as the polymer used (Williams et al., 2018).

Electrospun fibres delivery of biopharmaceuticals is a promising technique for various biomedical applications, thus researchers have explored the use of electrospinning to encapsulate and deliver proteins, nucleic acids, bacteria and living cells. Table 4 summarises representative advanced electrospun fibre systems loaded with biologics over a three-year period from 2020 to 2023. Proteins such as enzymes, growth factors, antibodies, vaccines, hormones and chemokines can be stabilised and released by electrospun fibres for different therapeutic purposes. Nucleic acids such as plasmid DNA (pDNA), microRNA (miRNA) and small interfering RNA (siRNA) can also be delivered by electrospinning to modulate gene expression and cellular functions. Moreover, electrospinning can encapsulate bacteria, cells and viruses for applications such as probiotics, tissue engineering and vaccination. This section provides an overview of the recent advances and challenges in each biopharmaceutical application.

3.1. Protein

Proteins are widely used as therapeutic agents for various chronic diseases, such as diabetes, cancer, rheumatoid arthritis and chronic

Table 3
The categories and typical effects of electrospinning parameters.

Types of Parameters	Parameters	Effect	References
Solution Parameters	**Concentration of Solutions**	Fibres will not form at an inadequate concentration	(Litovitz, 1952; Yang et al., 2004)
		Positive correlation with viscosity, indirectly affecting fibre properties	
	Viscosity of Solutions	Low viscosity forms particles instead of fibres	(Yang et al., 2004)
		Positive correlation with fibre diameter Excessive viscosity will result in non-uniform, even beaded fibres Overly viscous solutions may also clog the needle	
	Surface Tension of Solutions	Influences voltage, indirectly affecting fibre properties	
	Molecular Weight of Polymers	Positive correlation with viscosity, indirectly affecting fibre properties	
	Solvent Volatility	Positive correlation with fibre porosity	(Yang et al., 2004)
	Solvent Dielectric Constant	Negative correlation with fibre diameter	(Yang et al., 2004)
Process Parameters	**Voltage**	Fibres cannot be formed when conductivity is too high or too low Negative correlation with fibre diameter Excessive voltage can lead to bead formation	(Tijing et al., 2018)
		The inadequate voltage will not form a jet	
	Flow Rate	Negative correlation with fibre diameter	(Zong et al., 2002)
		Affects uniformity. The excessive flow rate can lead to bead	
	Tip-to-collector Distance	Insufficient distance will affect the formation of the jet	(Robb and Lennox, 2011)
		Affects uniformity. The excessive distance can lead to bead	
Ambient Parameters	**Temperature**	Negative correlation with fibre diameter	(Yang et al., 2017; Lara-Espinoza et al., 2018)
		Indirectly affects fibre properties by influencing viscosity and surface tension	
		The excessively high temperature will result in the inability to form fibres	
	Humidity	Positive correlation to porosity	(Casper et al., 2003; Pelipenko et al., 2013)
		Change fibre diameter	

wounds (Reynolds et al., 2013). However, oral administration of proteins is ineffective due to their degradation in the gastrointestinal tract. Therefore, most proteins are administered by injection, which has drawbacks such as a short *in vivo* half-life and poor patient compliance. Alternative delivery systems that can deliver proteins often suffer from low efficiency and high-frequency dosing due to the low stability and high molecular weight of the drug (Da Silva et al., 2016; Huang and Brazel, 2001). Hence, there is a need for suitable delivery systems that can protect and release proteins effectively. Electrospun nanofibres are a promising formulation approach that can overcome these challenges. This section reviews the recent studies and advanced applications of electrospun fibres for different types of protein delivery.

3.1.1. Bovine serum albumin

Bovine serum albumin (BSA) is a globulin protein derived from bovine serum that consists of 583 amino acid residues, which has advantages over other protein drugs in terms of stability, availability and cost. Thus, it is often used as a model protein to investigate the properties and performance of electrospun fibres for protein encapsulation (Moreira et al., 2021). For example, Homaeigohar et al. developed a biohybrid nanofibrous wound dressing with BSA as the model protein and PCL as the polymeric carrier (Homaeigohar et al., 2021; Homaeigohar and Boccaccini, 2020). The ATR-FTIR studies confirmed that the protein and polymer components were intermiscible with hydrogen bonding interactions, which improved the mechanical properties of the fibres compared to the pure PCL nanofibres. Moreover, SEM images showed that the fibres enhanced cell adhesion and proliferation. Kurpanik et al. also used BSA as a modifier to study the effect of surfactant type and concentration on emulsion electrospinning (Kurpanik et al., 2022). The authors evaluated the stability and release profiles of BSA to assess the potential impact of the emulsifier on the bioactivity of the protein.

Moreover, BSA can serve as a stabiliser for some proteins that are prone to degradation or inactivation at low concentrations. For instance, restriction enzymes or modified enzymes may benefit from the addition of BSA as a “protector” or “carrier” to prevent enzyme breakdown and specific adsorption. Lin et al. developed electrospun nanofibre scaffolds that deliver abaloparatide and aspirin to promote bone repair and regeneration (Lin et al., 2022). In this combined therapy study, nanoparticles of BSA, stabilized by chitosan, were employed as intermediates for the creation of a nanofibrous scaffold through electrospinning. This scaffold serves dual roles as a stabilizer as well as a release modifier for two therapeutic agents, i.e. aspirin and abaloparatide. This innovative approach yielded an optimal drug release profile, with the majority of the aspirin being released within a seven-day period, while the release of abaloparatide was prolonged to over a month. The results underscore the potential of electrospinning as an advanced technique for designing controlled drug delivery systems, particularly in combined therapy applications. However, it is important to acknowledge that proteins have diverse properties, and BSA may not be representative of all protein drugs. Therefore, BSA can be a helpful model in preliminary studies but cannot be used to predict the final performance of other protein drugs of interest. In most cases, experiments using BSA as the primary model only

Table 4
Systems for incorporation of biopharmaceuticals into electrospun nanofibres and their designed use.

Types of API	Bioactive Molecule	Polymer	Application	References
Protein	BSA (Mw: 66.5 kDa)	PCL	Wound healing N.B. also used as a modifier to other bioactive molecules	(Homaeigohar et al., 2021; Kurpanik et al., 2022)
	Abaloparatide (Mw: 3.96 kDa) (with BSA and Aspirin)	PCL	Induce bone formation	(Lin et al., 2022)
	Alkaline phosphatase (Mw: 86 kDa)	PEO	Comparing study	(Onyekuru et al., 2021)
	β-galactosidase (Mw: 520 kDa)	HP-β-CD	Treatment of lactose intolerance	(Vass et al., 2020)
	α-chymotrypsin (Mw: 25 kDa)	PVA	Dry powder inhalers preparation	(Ito et al., 2022)
	IGF-1 (Mw: 7.65 kDa)	HAMA/PCL	Dural substitutes	(Wang et al., 2022)
	IGF-2 (Mw: 7.5 kDa)	Chitosan/PCL	Wound healing	(Yang et al., 2022)
	VEGF-A (Mw: 45 kDa)	PCL	Neuroprotective Effects	(Wang et al., 2021)
	TGF-β (Mw: 25 kDa)	PCL/PLA	Tissue engineering (cardiac repair)	(Tambrchi et al., 2022)
	IGF-1,VEGF (Mw: 45 kDa)	Silk Fibroin	Bone defect repair and angiogenesis	(Zheng et al., 2022)
	TGFβ3 (Mw: 25.5 kDa)	PCL	Tissue engineering (cartilage)	(Malinauskas et al., 2022)
	VEGF	PCL/Gelatin	Tissue engineering (Patellar Ligament Regeneration)	(Yuan et al., 2022)
	SDF-1α (Mw: 8.0 kDa)	PLGA	Trap the residual GBM cells in the brain	(Molina-Peña et al., 2021)
	SDF-1α/CXCL12 (Mw: 10 kDa)	PLA	Neural Regeneration Therapy	(Castaño et al., 2021)
	TGF-β3 and SDF-1α	Hyaluronic acid	Tissue engineering (articular cartilage repair)	(Martin et al., 2021)
	Thyroid hormone triiodothyronine (Mw: 0,65 kDa) (with ibuprofen)	PLGA/PLLA	Treatment of Acute Injuries of the Central Nervous System	(Dolci et al., 2021)
	Melatonin (Mw: 0.23 kDa)	PCL	Tissue engineering (tendon)	(Yao et al., 2022)
	Melatonin	Chitosan/PCL/PVA	Wound healing	(Mirmajidi et al., 2021)
	Insulin (Mw: 5.81 kDa)	PCL/PEO	Wound healing	(Walther et al., 2022)
	F(ab) (Mw: 50 kDa)	PVP	Inflammatory oral mucosal disease	(Edmans et al., 2022)
	Bevacizumab (Mw: 149 kDa)	PCL	Age-related macular degeneration	(Angkawinitwong et al., 2017)
	Bevacizumab	PCL/Gelatin	Age-related macular degeneration	(de Souza et al., 2018)
	Infliximab (Mw: 144 kDa)	HP-β-CD	Treatment of Crohn's disease	(Domján et al., 2020)
Nucleic acids	plasmid DNA (Mw: around 2000 kDa)	Gelatin-Collagen-PEG	Gene therapy	(Furuno et al., 2022)
	plasmid DNA	Gelatin	Gene therapy	(Tsekoura et al., 2021)
	miR-132/miR-222/miR-431 (Mw: 7.14–8.5 kDa)	Collagen	Promote Axon Regeneration	(Zhang et al., 2021)
	miR-132/miR-31 (Mw: 7.14–8.5 kDa)	PVA	Wound healing	(Bombin et al., 2023)
	miR-181a/b-1 (Mw: 7.14–8.5 kDa)	PEG/PLGA	Tissue engineering (osteogenesis of human mesenchymal stem cells)	(Qi et al., 2021)
	Antisense oligonucleotide	HP-β-CD	Gene suppression	(Hirsch et al., 2023)
Cells	Escherichia coli strain Nissle 1917	PVA/CA	Probiotics delivery (gut)	(Çanga and Dudak, 2021)
	Vaginal lactobacilli (Lactobacillus crispatus ATCC 33820, Lactobacillus gasseri ATCC 33323, and Lactobacillus jensenii ATCC 25258)	PEO	Probiotics delivery (Vaginal)	(Stojanov et al., 2021)
	L. rhamnosus CRL1332	PVA	Probiotics delivery (Vaginal)	(Silva et al., 2021)
	E. coli.	PEO/PVA	Probiotics delivery (gut)	(Diep and Schiffman, 2021)
	Lactobacillus crispatus, Lactobacillus gasseri, and Lactobacillus jensenii	PEO	Probiotics delivery (Vaginal)	(Stojanov et al., 2022)
	Lactobacillus paracasei	PVA-PEO	Probiotics delivery (Oral cavity)	(Hirsch et al., 2021)
	Clostridium butyricum	HP-β-CD	Probiotics delivery (gut)	(Vass et al., 2020)
	Stem cell	Collagen/PLGA	Cardiac patch	(Wee et al., 2022)
	Phage	Gelatin/Silk fibroin	Against multidrug resistant *Pseudomonas aeruginosa*	(Sarhan et al., 2021)
	Phage	Poly(γ-glutamic acid)	Bactericide	(Kasbiyan et al., 2022)

provide a valuable reference for subsequent experiments, which still require the addition of specific active ingredients to investigate their effects on the electrospun fibres.

3.1.2. Enzyme

Apart from BSA, enzymes also serve as another commonly used model drug system for evaluating the structural and activity effects of the electrospinning process on proteins due to the readily availability and stability of enzymes and the well-established activity assay protocols for them. Electrospinning technology has been shown to be a feasible method for enzyme encapsulation in a number of studies (Moreira et al., 2021; Liu et al., 2019; Li et al., 2008; Puhl et al., 2014), especially those involving model enzymes such as alkaline phosphatase, lysozyme and α-Chy.

Alkaline phosphatase and lysozyme are two examples of enzymes that have been frequently used to investigate the feasibility of electrospinning for protein encapsulation in recent years. Alkaline phosphatase is a dimeric metalloenzyme with a molecular weight between 115 and 165 kDa that is widely distributed in the human body (e.g., liver, kidney, bone, intestine). The enzyme has been shown to degrade lipopolysaccharides and thus exert some killing effect on gram-negative bacteria (Moreira et al., 2021). Onyekuru et al. encapsulated alkaline phosphatase into PEO nanofibres by two different methods, blend electrospinning and coaxial electrospinning (Onyekuru et al., 2021). The authors compared the effects of the two preparation methods on enzyme activity by measuring fibre properties and biological activity. In this study, water and ethanol were used as solvents for PEO, while phosphate buffer solution (PBS) was used as a solvent for the enzyme. In the preparation of blend electrospun samples, enzyme and polymer are directly combined before being loaded into the syringe for the spinning process, while ethanol needs to be used to dissolve the polymers. Conversely, during the preparation of coaxial electrospun samples, enzyme and polymer are loaded into separate coaxial nozzles for spinning, while only PBS has been used to dissolve the enzyme, which

prevents direct contact between the enzyme and the organic solvent, thus ensuring a controlled process. Therefore, it was assumed that the core–shell fibres formed by coaxial electrospinning might have preserved better enzymatic activity. However, contrary to the expectations, the results of SDS-Page, MicroBCA and activity assay indicated good ALP integrity and enzymatic activity in both nanofibres produced by blend and co-axial methods without significant differences. Also, their further investigations revealed that ethanol did not significantly affect enzyme activity, but very high voltages resulted in reduced enzyme activity. Under such circumstances, the authors ultimately deduce that proteins retain their activity and structural integrity, even when exposed to organic solvents partially. They further argue that blend electrospinning can, under certain conditions, be as efficacious as coaxial electrospinning for protein encapsulation. Moreover, the blend electrospinning method offers a more streamlined approach, thereby positioning it as a potentially viable alternative.

Another noteworthy study employed β-galactosidase as a model drug to explore the feasibility of High-Speed Electrospinning (HSES) for protein encapsulation. β-galactosidase is an enzyme that catalyses the hydrolysis of β-galactosides into monosaccharides and is widely used in industrial applications. The HSES technique is advantageous for its higher production rates compared to traditional electrospinning methods, making it more suitable for industrial-scale applications. In this context, the authors, P. Vass et al., produces β-galactosidase-cyclodextrin fibres and found that the enzyme activity of β-galactosidase was well-preserved after the HSES process, even after further processing steps like grinding and tableting (Vass et al., 2020). This suggests that HSES could offer advantages in terms of scalability and efficiency, while still maintaining the biological activity of the encapsulated model drug. However, it should be noted that the long-term stability of the enzyme in these fibres was not extensively investigated, which leaves room for further research.

Lysozyme, which is often found in human cell secretions, is another widely used enzyme to evaluate the effects of electrospinning on protein structure and activity. It is an antimicrobial enzyme with a molecular weight of 14.4 kDa. It catalyses the hydrolysis of peptidoglycan in the cell wall of bacteria, thereby destroying their cell wall, mainly for Gram-positive bacteria (Huang and Brazel, 2001). Many researchers have studied the encapsulation of lysozyme in electrospun fibres and have developed various scaffolds, mats or wound dressings composed of nanofibres containing lysozyme. However, the stability and solubility of lysozyme in the electrospinning solvents presented problematic issues to be addressed. Several strategies have been proposed to overcome these challenges. For example, coupling fatty acids with lysozyme can significantly enhance the solubility of the enzyme in dimethyl sulfoxide and prolong its activity (Yoo et al., 2001). Alternatively, electrospinning using lysozyme crystals suspensions rather than solutions is also an effective way to protect the enzyme activity (Puhl et al., 2014). Moreover, the adsorption of proteins onto the surface of nanofibres membranes has also been suggested as an efficient method of encapsulation. Hsin et al. prepared polyacrylonitrile (PAN) nanofibres membranes using electrospinning and modified the surface of the nanofibres with ethylenediamine (EDA) and Stearic Blue F3GA to turn them into dye-affinity membranes (Hsin et al., 2021). The nanofibre membranes are ideal for protein adsorption due to their hydrophilicity, high porosity and large surface areas. The results of the adsorption studies also showed that lysozyme had a good binding affinity to the nanofibres membrane. Further thermodynamic studies demonstrated that the free energy change of the adsorption process was negative, indicating that the process was spontaneous. It is worth noting that the enzyme activity was maximised due to the lack of involvement in the electrostatic spinning process. However, the study did not evaluate lysozyme release, and there was no guarantee that the enzyme could be released stably from this fibrous membrane.

Alpha-chymotrypsin (α-Chy) is another example of an enzyme that has been encapsulated into electrospun fibres. It is a commonly used digestive enzyme with a stable *in vitro* assay for enzyme activity (Hericks et al., 2005). Ito et al. prepared dry powder inhaler dosage forms using electrospun fibre mats containing α-Chy and PVA, as illustrated in Fig. 5(Ito et al., 2022). The authors evaluated the retention of enzymatic activity and the drug release rate from the nanofibres mats. The enzyme activity tests showed that the electrospinning process did not significantly affect the enzymatic activity of α-Chy and that the nanofibres mats had suitable properties for dry powder inhaler dosage forms. The enzymatic activity was found to be maintained for six months after storage compared to freshly prepared nanofibres mats. Therefore, it was concluded that the method offers a novel approach to preparing inhalation formulations. However, drug release experiments were not conducted to assess the release of α-Chy from the nanofibres mats, which was a limitation of their study.

Admittedly, preserving the enzyme activity during electrospinning is challenging. Since the catalytic activity of enzymes depends on their three-dimensional structure and conformation, some factors that may affect their integrity, such as organic solvents and ultrasound, should be avoided, if possible, which further restricts the application and development of enzyme encapsulation using electrospinning technology for large-scale production in industrial settings.

3.1.3. Growth factors and cytokine delivery

Using electrospinning to encapsulate growth factors and chemokines is versatile and has great potential and research value. The use of coaxial and emulsion electrospinning preserves the biological activity of the active factors as much as possible and makes it easier to achieve stable and effective drug delivery. Growth factors are proteins that naturally occur in living organisms and regulate cell proliferation and differentiation. They modulate various cellular activities and functions by acting as intercellular signalling molecules that bind to specific receptors on the surface of target cells, thus inducing cell differentiation and maturation (Wang et al., 2017). However, the functions of growth factors are diverse, depending on their types. For instance, bone-forming growth factors stimulate the differentiation of osteoblasts, whereas vascular endothelial growth factors promote the proliferation of vascular endothelial cells. A related signalling protein is a cytokine which mediates intercellular communication in biological systems. Cytokines are predominantly proteins or glycoproteins participating in various immune responses and embryonic development processes (Morie et al., 2016). Depending on their source and target cells, cytokines have different families, such as chemokines or interleukins. In some cases, the distinction between growth factors and cytokines is not clear-cut, and they tend to be used interchangeably.

Growth factors delivery is a common strategy in tissue engineering that aims to enhance cell migration, growth and differentiation. However, growth factors are unstable and susceptible to rapid inactivation *in vivo*. Moreover, the use of large molecules in excessive doses may cause toxic side effects or even tumour induction (Wang et al., 2017). Therefore, the *in vivo* delivery of growth factors is often inefficient, which is the major change in this application. To overcome this problematic issue, formulations for delivering these proteins need to protect their activity and achieve a sustained release (Ji et al., 2011). Electrospinning is a technique widely used in tissue engineering and for growth factor delivery. Its applications mainly focus on neural tissue engineering, bone and cartilage tissue engineering and skin regeneration.

The main application of electrospinning technology in neural tissue engineering is the fabrication or modification of scaffolds or carriers that can deliver growth factors. For instance, nanofibrous scaffolds composed of PCL or PLGA based nanofibres encapsulating nerve growth factor have been shown to enhance the adhesion and proliferation of PC12 cells, a type of cell that resembles post-differentiated sympathetic ganglion neurons stimulated by nerve growth factor (Hu et al., 2016; Quintiliano et al., 2016). Moreover, nerve growth factor-encapsulated nanofibres prepared by emulsion electrospinning and coaxial electrospinning have been used to fabricate nerve guidance conduits and

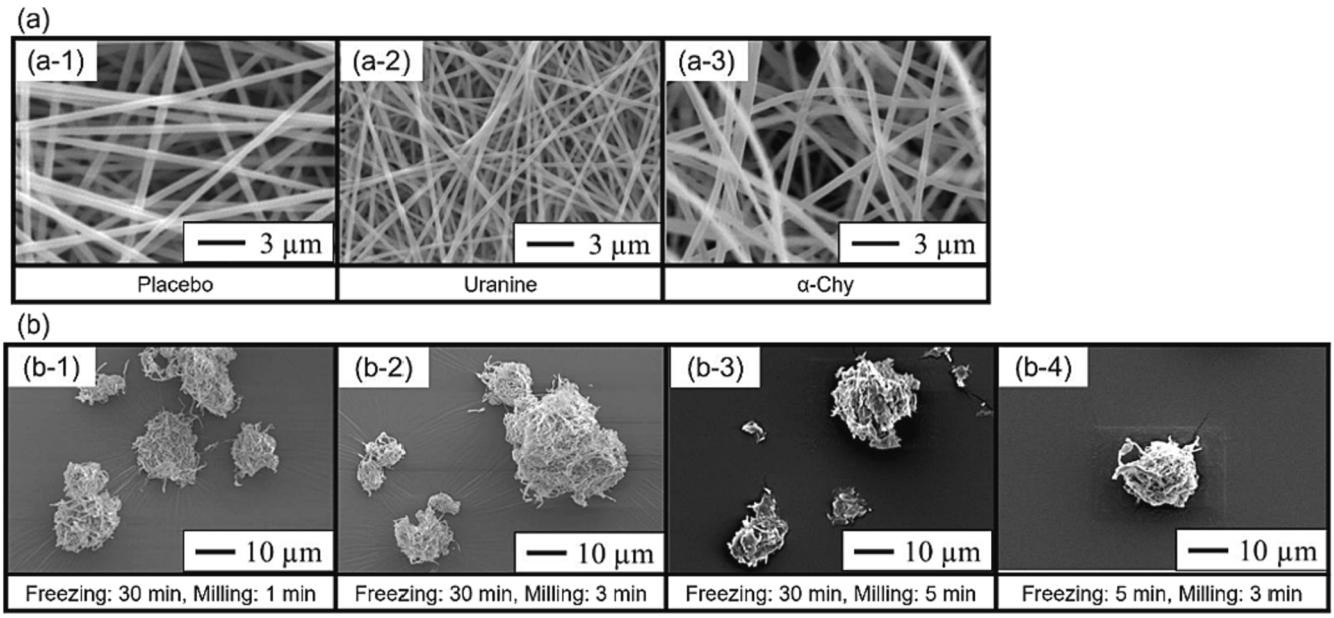

Fig. 5. Scanning electron micrographs of electrospun nanofiber mats and milled nanofiber mats. (a) Nanofiber mats prepared using the electrospinning technique. (a-1) A polyvinyl alcohol (PVA) nanofiber mat without the experimental drugs, (a-2) the PVA nanofiber mat loaded with uranine, and (a-3) the PVA nanofiber mat loaded with α-chymotrypsin (α-Chy). (b) Uranine-loaded PVA nanofiber mats milled by cryo-milling: (b-1) freezing at 30 min and milling at 1 min; (b-2) freezing 30 min and milling 3 min; (b-3) freezing 30 min and milling 5 min; and (b-4.

scaffolds. These delivery systems have been shown to improve neurological recovery by stimulating the synaptic extension of ganglion neurons and preserving the biological activity of nerve growth factors (Kuihua et al., 2014; Whitehead et al., 2018). Furthermore, some studies have attempted to encapsulate multiple growth factors in the electrospun fibres to increase the efficiency of promoting repair. For example, Liu et al. prepared nanofibres that enabled the simultaneous delivery of nerve growth factor and glial cell line-derived neurotrophic factor (Liu et al., 2018; Liu et al., 2018) and further demonstrated that both growth factors participate in neuronal regeneration and differentiation and exhibit synergistic effects on axonal branching and elongation.

Bone morphogenetic protein 2 (BMP-2) is one of the most frequently used growth factors encapsulated into electrospun fibres for bone and cartilage tissue engineering. BMP stimulates DNA synthesis and cell replication, thereby inducing the specific differentiation of mesenchymal cells into osteoblasts. It also plays a major role in the embryonic development and regenerative repair of bone and cartilage by inducing their formation *in vivo*. It is expressed during limb growth, endochondral ossification, early fracture and cartilage repair (Chen et al., 1997; Huang et al., 2010). Due to its nature, BMP-2 is usually encapsulated by coaxial electrospinning. For example, nanofibres constructed from aqueous BMP-2 solutions as the core and hydroxyapatite nanoparticles in a co-blended solution of silk proteins and chitosan as the shell exhibit good osteogenic properties (Shalumon et al., 2015). In addition, electrospun fibres can be used as carriers to deliver BMP-2 along with other drugs, such as dexamethasone, via coaxial electrospinning (Su et al., 2012; Li et al., 2018) to achieve better skeletal osteoinduction. Diverse formulations were utilized in one study. One such formulation involved poly(l-lactide-co-caprolactone) (PLLACL)-collagen as the outer shell, encapsulating dexamethasone and a PBS solution of BMP-2 as the inner core. Another formulation leveraged Zein, a protein derived from maize, in combination with dexamethasone for the outer shell while retaining the original composition for the inner core. Both fibres achieved a sustained release of BMP-2, enhanced mesenchymal stem cell adhesion and growth as compared to pure drug nanofibres, and increased osteogenic marker production effectively.

VEGF is a key growth factor that explicitly stimulates vascular endothelial cell growth and angiogenesis. It also enhances vascular permeability, extracellular matrix degradation, and endothelial cell migration and proliferation (Farokhi et al., 2013). Yuan et al. fabricated a PCL/Gelatin scaffold with a prominin-1-binding peptide sequence for patellar ligament regeneration (Yuan et al., 2022) demonstrating that this scaffold could induce tubulogenesis of human umbilical vein endothelial cells *in vitro* and facilitate collagen deposition in ligaments *in vivo*. Moreover, VEGF nanofibres have been reported to ameliorate ischaemic neuronal injury. In their study, Wang et al. incorporated VEGF into PCL nanofibres membranes. They utilized a layer-by-layer technique, to load the VEGF. The effectiveness of this approach was confirmed through the demonstration of neuroprotective effects. These effects manifested in several ways, including the modulation of anti-inflammatory responses, the enhancement of antioxidant activities, and the preservation of mitochondrial membrane potential (Wang et al., 2021). Furthermore, VEGF can also be co-administrated with insulin growth factor (IGF) to enhance bone defect repair. Zheng et al. prepared a composite scaffold with silk protein and VEGF as the outer layer and IGF-1 as the inner core (Zheng et al., 2022) and it was found that this scaffold could significantly upregulate the expression of osteogenic marker genes and proteins. RNA sequencing results confirmed its superior role in bone defect repair and angiogenesis, which was further verified by *in vivo* experiments in mice. IGF can also be used alone as a wound dressing, and Yang et al. showed that adding IGF-2 to a composite dressing composed of a chitosan sponge and a PCL membrane could stimulate fibroblast migration and proliferation (Yang et al., 2022). Interestingly, a recent study provided novel insights for healing after decompressive craniectomy in patients with traumatic brain injury, who often suffer from postoperative dural defects. More specifically, Wang et al. used coaxial electrospinning to produce nanofibres that could release IGF-1 for a prolonged period, which could act as an adequate substitute for dura mater, increasing the survival and outgrowth of neuronal cells (Wang et al., 2022). Additionally, the nanofibres also prevented cerebrospinal fluid leakage, thus maintaining normal intracranial pressure and better-protecting brain function. This study is a good example of the applications of electrospun fibres in neurosurgery. Moreover, transforming growth factors (TGFβ) are often encapsulated in electrospun fibres to form various scaffolds to promote cartilage regeneration or cardiomyocyte differentiation. In the future,

more electrospun fibres containing growth factors may be used in more medical applications. On the other hand, as with enzymes, there have been studies that have attempted to encapsulate monoclonal antibodies using high-speed electrospinning. Domján et.al have encapsulated Infliximab into the 2-hydroxypropyl-β-cyclodextrin electrospun fibres (Domján et al., 2020). Infliximab is a monoclonal antibody primarily used for the treatment of autoimmune diseases such as rheumatoid arthritis and Crohn's disease. In addition to ensuring structural integrity and bioactivity, the mAb-loaded Hydroxypropyl-beta-cyclodextrin (HP-β-CD) electrospun fibres exhibit controlled release profile that could potentially minimize the frequency of administration, which is particularly significant given that monoclonal antibodies like Infliximab are primarily administered through intravenous injections. Although its long-term stability has not yet been verified, it is still a promising research direction owing to the high production yield, stable bioactivity, and excellent release profile.

Due to their similar properties, electrospun fibres delivering cytokines have similar applications to those delivering growth factors. For instance, Martin et al. electrospun hyaluronic acid into nanofibres and then incorporated Stromal Cell-Derived Factor-1α (SDF-1α; SDF) and Transforming Growth Factor-β3 (TGF-β3; TGF) to develop a scaffold for cartilage healing (Martin et al., 2021). *In vitro* experiments showed that the scaffold could favourably enhance mesenchymal stem cell recruitment. However, it was found that the release of SDF-1α hindered the local regeneration of new cartilage tissue when attempts were taken to implant the scaffold into a large animal model of cartilage defect repair to test the biological activity of both factors and their effect on cartilage healing. Although the underlying mechanism was not fully clarified, this study illustrates the importance of *in vivo* studies on the efficacy of nanofibres. A different study on chemokines was carried out to functionalise the nanofibres surface using DF-1α/CXCL12 chemokines as chemotactic agents, thereby increasing their migratory properties towards the olfactory sheath cell-derived cell line TEG3 cells and ultimately facilitating the culture of olfactory sheath cells that can be used for transplantation to treat spinal injuries (Castaño et al., 2021). On the other hand, electrospun nanofibres containing chemokines can also be used due to their therapeutic effect. SDF-1α can regulate the migration of cancer cells. Molina-Pe˜na and his colleagues encapsulated SDF in PLGA nanoparticles and electrospun them with chitosan as nanofibrous scaffolds to trap glioblastoma cells (Molina-Peña et al., 2021). They found that the scaffold had good cytocompatibility and stability, as well as a slow degradation profile, allowing for efficient and long-lasting trapping of glioblastoma cells, and therefore has potential for further development as a post-tumour therapy device. However, most of the current research on growth factors and chemokine delivery is still focused on tissue engineering, and encapsulating growth factors and chemokines into nanofibres to achieve different therapeutic effects may be a new promising area for extensive research.

3.1.4. Hormone

Hormone delivery has emerged as a promising research field for the electrospun delivery of biomolecules in recent years. Hormones are bioactive molecules that modulate various physiological functions. Hormones can be either encapsulated within electrospun fibres or conjugated to their surface and subsequently released in a controlled manner by different stimuli, such as pH, temperature, enzymes, or light. A wide range of hormones have been investigated for electrospinning encapsulation in current research, including insulin, melatonin, growth hormone and adrenal glucocorticoids (Stojanov and Berlec, 2020). Insulin is a hormone that regulates blood glucose homeostasis, which is synthesised by the pancreatic beta cells and secreted into the circulation when glucose levels increase after a meal. It is vital for maintaining normal metabolism and preventing diabetes mellitus, a disorder characterised by hyperglycaemia and impaired insulin action or secretion. As a chronic disease that affects hundreds of millions of people globally, insulin is essential as a therapeutic option. However, due to the nature of the hormone, most insulin formulations require frequent subcutaneous injections, resulting in poor patient adherence (Alejandro Juárez et al., 2021). Therefore, many studies have been undertaken to explore the potential of electrospinning technology to encapsulate insulin and thus modify its delivery mode.

Interestingly, unlike most other proteins encapsulated by electrospinning, many electrospun fibres that encapsulate insulin employ blend electrospinning to incorporate insulin into nanofibers for oral delivery. For example, both fish myoplasmic proteins and gelatin nanofibers have demonstrated the ability to encapsulate insulin effectively and protect it from enzymatic degradation by alpha-chymotrypsin (Stephansen et al., 2016; Stephansen et al., 2015). Remarkably, the organic solvents used in the electrospinning process (e.g., hexafluoroisopropanol) do not compromise the biological activity of insulin (Xu et al., 2015). In these studies, insulin preserved its biological activity well after electrospinning. The released insulin from the nanofibers still exhibited the capacity to induce Akt phosphorylation and adipogenic differentiation of preadipocytes. In addition, insulin nanofibers have been developed as oral mucosal adhesive dosage forms. Besides chitosan-based nanofibers, which are commonly used in conventional oral mucosal adhesive films, Voronova et al. developed reduced graphene oxide-modified polyacrylic fibre mats for insulin delivery (Voronova et al., 2022). In addition to having good adsorption properties for insulin, such mats can be reloaded-released, further reducing the cost of use. It shows great potential for application in the delivery of insulin to the oral mucosa and cornea. On the other hand, several insulin-loaded electrospun fibres have been developed as wound dressings for wound healing in diabetic patients. Due to properties such as sustained release required for wound dressings, most of these fibres are fabricated using coaxial electrospinning techniques. For instance, core–shell electrospun fibres with PLGA were used as the outer shell material for an insulin-loaded inner core (Lee et al., 2020). In an *in vivo* model, the insulin-loaded core–shell electrospun fibres successfully exhibited a wound-healing effect that was significantly superior to that of blend electrospun fibres. Additionally, Walther et al. successfully achieved controlled insulin release using nanofibers composed of PCL and PEO with an insulin core (Walther et al., 2022). The nanofibers were also used to produce a wound dressing that was shown to direct cell migration and absorb wound exudate. Furthermore, a significant increase in wound healing biomarkers was detected around human skin wounds treated with the dressing, demonstrating its biocompatibility and efficacy in enhancing wound healing simultaneously. Therefore, the authors concluded that the insulin-loaded electrospun wound dressing has the potential for clinical application.

Another hormone frequently encapsulated in electrospun nanofibers is melatonin, which has been found to have wound-healing properties, and therefore electrospun wound dressings incorporating melatonin have been highly pursued in recent years. Melatonin is a hormone that regulates the circadian rhythm in humans and other animals. It is synthesised by the pineal gland in the brain and secreted into circulation at night when it signals the body to prepare for sleep. Melatonin also has other functions, such as modulating immune responses, antioxidant activity, and influencing seasonal rhythms. Mirmajidi et al. prepared an innovative three-layer electrospun nanofibres wound dressing, as shown in Fig. 6 (Mirmajidi et al., 2021). The outer layer was composed of CS-PCL nanofibers, while the middle layer was composed of PVA and melatonin nanofibers, thus forming a sandwich structure which successfully enables the sustained release of melatonin. The structure also helps the wound dressing to have a high hydrophilic effect and is suitable for cell attachment. Further *in vivo* experiments in rat skin and histopathological evaluation showed that regeneration of the epithelial layer, wound remodelling, collagen synthesis and reduction of inflammatory cells were observed after the application of the wound dressing. At the same time, there was a significant increase in the expression of a range of genes associated with wound healing, including transforming growth factor-β (TGF-β1), α-smooth muscle actin (α-SMA), type I

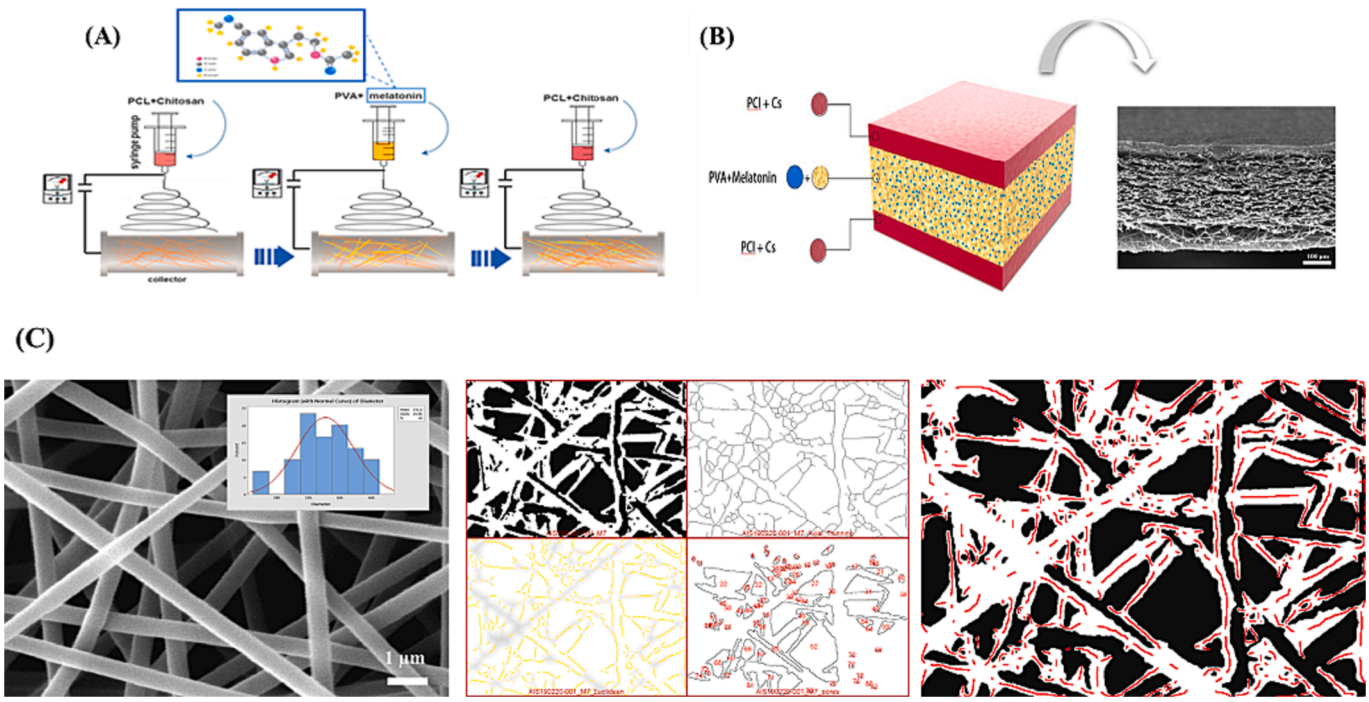

Fig. 6. Fabrication of the three-layer wound dressing. (A) Schematic micrograph of three-layer wound dressing (PCL + Cs/ PVA + MEL/ PCL + Cs), (B) SEM images of the three-layer wound dressing of PCL + Cs/ PVA + MEL/ PCL + Cs cross-section (Scale bar = 100 μm), and (C) Processing SEM image by imageJ software to optimize PVA nanofibers, scale bar 1 μm. Reprinted with permission from Mirmajidi et al. (2021).

collagen (COL1A1) and type III collagen (COL3A1). Based on this, the authors concluded that this dressing could potentially promote wound healing. In a similar vein, another study involved melatonin loading into a PCL and sodium alginate scaffold, which allows for the slow release of melatonin through implantation into the body, thus enabling an accelerated process of tendon injury repair (Yao et al., 2022). Thus, it is demonstrated that electrospun nanofibers also have promising applications in tendon tissue engineering.

Other examples of hormone encapsulation in electrospun fibres are the triiodothyronine delivery system, where Dolci et al. have successfully demonstrated the anti-inflammatory and promyelinating effects of triiodothyronine and ibuprofen by co-encapsulating them in coaxial nanofibers (Dolci et al., 2021). There have also been studies on electrospun fibres to improve the delivery of existing hormone-based drugs. Prednisolone was loaded into the nanofiber network to achieve better disintegration properties and improved release profiles (Celebioglu et al., 2021). Simultaneously, a comparison with commercially available prednisolone formulations revealed distinct advantages associated with orally disintegrating tablets (ODTs). Specifically, these ODTs exhibited a higher drug load capacity and a faster onset of action in comparison to the traditional, commercially available prednisolone tablets. These attributes highlight the potential for ODTs to improve drug delivery efficiency and therapeutic response. In summary, electrospinning technology has a wide range of applications for the delivery of hormonal drugs. The application of nanofibers may further change the current route of delivery of hormonal drugs.

3.1.5. *Antibodies and vaccines*

Electrospinning can also be used to encapsulate antibodies, which are particularly promising for targeted therapy of diseases such as cancer due to their high specificity and low toxicity. To the best of our knowledge, the first attempt to incorporate antibodies into electrospun nanofibres was reported by Gandhi et al. in 2009, who used a blend of PCL and antibodies against the αVβ3 integrin to demonstrate the feasibility and release kinetics of this approach (Gandhi et al., 2009). However, most antibody-based drugs are still delivered by intravenous injection, and topical delivery remains underexplored. One of the few antibodies that have been studied for topical delivery using electrospun fibres is bevacizumab, which inhibits VEGF, a critical factor in angiogenesis and tumour growth. Bevacizumab is approved for treating various cancers, such as colorectal, lung, ovarian, cervical and brain cancers, as well as wet age-related macular degeneration (AMD), an eye disease that causes vision loss (de Souza et al., 2018). Several types of electrospun fibres have been developed for delivering bevacizumab to the eye. Souza et al. used PVA as the core material and a blend of PCL and gelatin as the shell material (de Souza et al., 2018). The final product showed good biocompatibility and sustained release of bevacizumab *in vitro* and in cellular assays, offering a novel therapy for AMD as an alternative to intravitreal injections. Angkawinitwong et al. also fabricated bevacizumab-loaded nanofibres using PCL as both the core and shell material (Angkawinitwong et al., 2017) and found that the pH of the bevacizumab solution influenced the stability and release kinetics of the antibody. Nanofibres prepared at the isoelectric point pH of bevacizumab showed a constant release for more than two months *in vitro*. It was proposed that this approach could reduce the dosing frequency for patients and improve treatment outcomes.

Electrospinning technology also has the potential to be used in the preparation of vaccines. In the context of the COVID-19 pandemic, various protein and nucleic acid vaccines have been developed and tested. However, electrospinning has been underutilised for processing large-molecule vaccines compared to similar electrospray technologies due to scalability issues. One of the few studies in this area reported the encapsulation of virus-like particles into electrospun fibres as a dry formulation for vaccines (Dowlath et al., 2021). The potential of electrospinning for developing oral vaccines is also worth exploring, considering its advantages in providing stability protection and flexible release profiles for biological macromolecules such as proteins.

In conclusion, the case studies covered in this section demonstrate the application of electrospinning technology for formulating various functional proteins with a range of advantages, including enhanced stability and modulated release properties, which enable more efficient delivery of different protein drugs or potentially transform their current

delivery methods to achieve targeted delivery and controlled release. These formulations may improve their efficacy while reducing side effects and increasing patient compliance. However, there are still many research gaps in this area, such as the development of novel electrospinning techniques or devices that can overcome the limitations of conventional methods, such as low throughput, high voltage, and solvent toxicity, and future advances in electrospinning technology may be required to overcome the current limitations of protein delivery that rely on injections.

3.2. Nucleic acids

Nucleic acids are macromolecular compounds with therapeutic potential. Advances in biotechnology have enabled the use of DNA and RNA as preventive or therapeutic agents for various diseases that are refractory to conventional treatments, such as cancer, neurodegenerative disorders, transthyretin amyloidosis, immune diseases and COVID-19. However, nucleic acids face challenges of low stability and susceptibility to degradation and inactivation in biological environments, which is the same as other biopharmaceuticals. Moreover, some nucleic acid drugs may elicit toxicity or immune responses. Therefore, appropriate formulations are critical for nucleic acid drugs (Stojanov and Berlec, 2020). Under such circumstances, electrospun fibres are one of the candidate formulation methods. Nanofibres are currently employed for the delivery of plasmid DNA, miRNAs and siRNAs (Furuno et al., 2022; Tsekoura et al., 2021; Zhang et al., 2021; Bombin et al., 2023).

pDNA is a circular DNA molecule that can replicate autonomously from the host chromosome. pDNA has various applications in pharmacy, such as vaccine production and gene therapy, which involves introducing pDNA into target cells to correct genetic defects or enhance gene expression (Stojanov and Berlec, 2020). Furuno et al. developed gelatin electrospun nanofibers that were cross-linked with phenolic hydroxyl groups on the surface by treating gelatin with horseradish peroxidase and hydrogen peroxide (Furuno et al., 2022). The cross-linked nanofibers could immobilise pDNA on their surface and deliver it to cells for

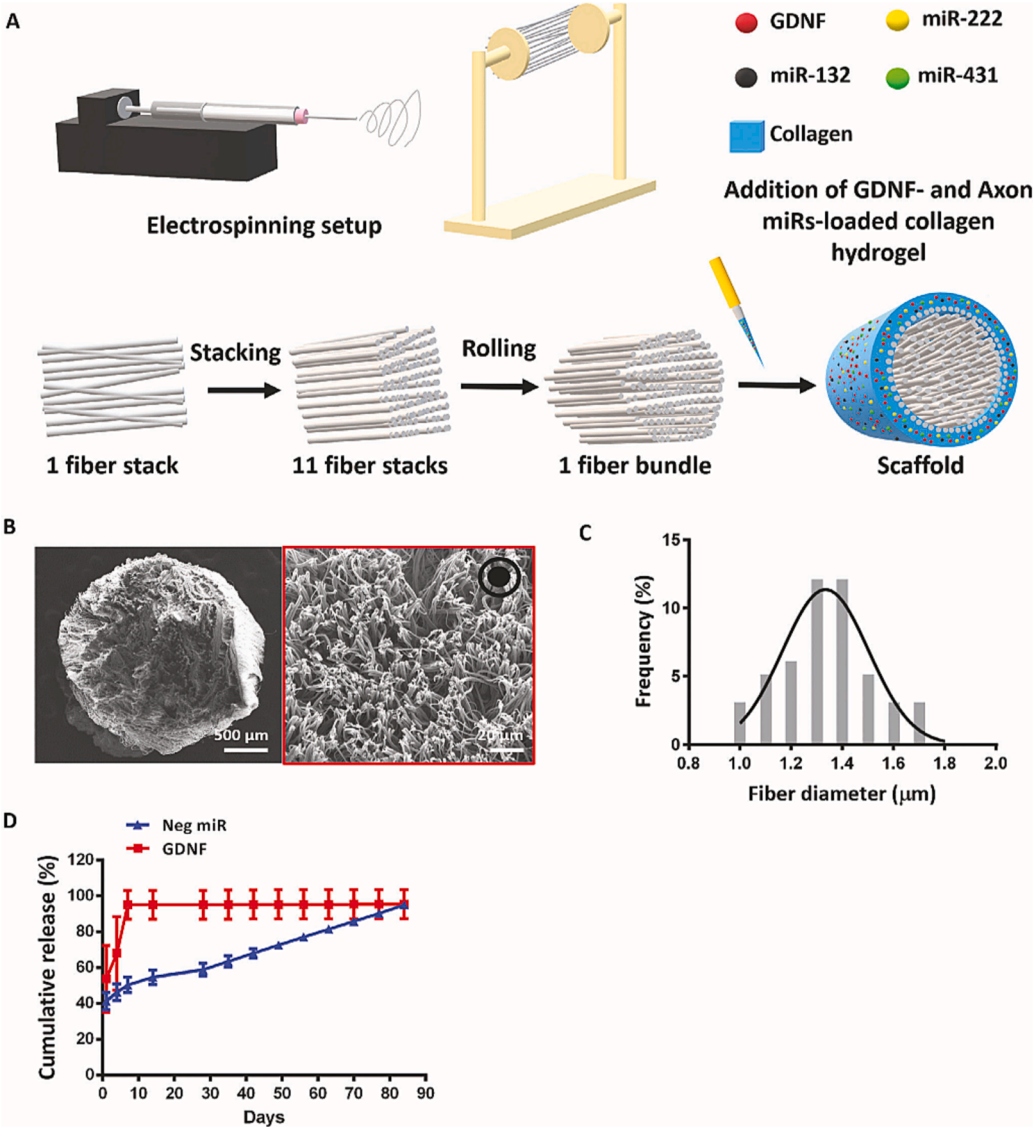

Fig. 7. Fiber-hydrogel scaffold was successfully fabricated. A) Scaffold fabrication schematic diagram. (B) SEM image of the entire scaffold and high magnification of PCL electrospun fibres (red box) where the black arrow pointing out of the paper at the top right indicates the directionality of the fibres. C) A total of 50 fibres were quantified and the average fibre diameter was 1.35 ± 0.19 µm. (D) Cumulative release of Neg miR and GDNF over time. All data are represented as mean ± SD Reprinted with permission from Zhang et al. (2021). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

transfection. The authors demonstrated that cells cultured on the nanofibers could express genome editing molecules such as Cas9 protein and guide RNA, enabling targeted gene knock-in and knock-out. They suggested that the nanofibers have the potential for gene therapy through genome editing. Moreover, they modified the cross-linking method of the gelatin nanofiber system and obtained similar results using glutaraldehyde as a cross-linker (Furuno et al., 2022). In contrast, Tsekoura et al. reported that multilayer electrospun fibre mats were more advantageous for gene delivery (Tsekoura et al., 2021). They fabricated a bilayer mat using collagen nanofibers in the first layer and a mixture of gelatin, collagen and PEG in the second layer. The second layer was electrospun with a non-viral complex containing pDNA as the main active component. The bilayer mats showed higher transfection efficiency than single-layer nanofiber mats made of composite materials.

RNA, as well as DNA, is a nucleic acid with therapeutic potential. One type of RNA that can be delivered via electrospun fibres is miRNA, which is a small non-coding RNA that regulates gene expression by binding to complementary sequences on target messenger RNA (mRNA), leading to mRNA degradation or translation inhibition. miRNAs play essential roles in various biological processes such as cell differentiation, proliferation, apoptosis and metabolism. Moreover, miRNAs are also involved in many diseases, especially cancer, where they can act as tumour suppressors or oncogenes depending on their target (Stojanov and Berlec, 2020). For example, a three-dimensional fibrous hydrogel scaffold loaded with a cocktail of miRNA-132/miRNA-222/miRNA-431 (see Fig. 7) has been successfully used to achieve effective therapeutic effects in spinal cord injury (Zhang et al., 2021). The three-dimensional (3D) scaffold consists of electrospun fibres and a collagen matrix, which jointly enables the sustained release of miRNA and guides axonal regeneration (Zhang et al., 2021). Furthermore, this method can be combined with methylprednisolone further to promote functional recovery and even remyelination over several years. Similarly, miRNA electrospun fibre scaffolds can also be effective in the treatment of fractures and osteoporosis. Qi et al. incorporated miR-181a/b-1 into PLGA electrospun fibres to form scaffolds and found that the addition of RNA did not significantly affect the morphology of the fibres but effectively improved their biocompatibility (Qi et al., 2021). Further *in vitro* cellular experiments confirmed the osteoinductive capacity of the nanofibres, and it was also observed that adipose-derived mesenchymal stem cells cultured on the aforementioned nanofibres produced more osteogenic markers and higher levels of expression of bone-related genes, suggesting their ability to enhance osteogenic differentiation potential.

In addition to regulating gene expression, some miRNAs can also promote wound healing by modulating cellular processes such as inflammation and angiogenesis. For example, miRNA-31 and miRNA-132 have been found to be associated with wound healing in various studies. Therefore, a novel electrospun wound dressing was developed to deliver both RNAs in an alginate/polyvinyl alcohol/ciprofloxacin nanofibre wound patch (Bombin et al., 2023). After cross-linking with glutaraldehyde, the dressing was biocompatible and antimicrobial active without cell attachment, further reducing secondary injury. *In vivo* wound healing experiments in mice have also demonstrated that the dressing accelerates the rate of wound closure, epidermal thickness and the number of blood vessels compared to dressings without the drug.

Another type of RNA that can be delivered via electrospun fibres is siRNA, which can also regulate gene expression by silencing specific genes. For instance, Chew et al. have encapsulated Glyceraldehyde 3-phosphate dehydrogenase siRNAs into PCL and PCLEEP nanofibres and transfected them into HEK 293 cells (Rujitanaroj et al., 2011; Cao et al., 2010). The results of these experiments showed acceptable transfection efficiencies. Also, bioactivity assays demonstrated that the electrospinning process does not inactivate RNA, and that the fibres encapsulated with the drug protect the RNA in them to remain active for at least 7 weeks in a physiological state.

Expanding the scope of nucleic acids for electrospun delivery, antisense oligonucleotides (ASOs) have emerged as a promising therapeutic agent. ASOs are single-stranded DNA or RNA molecules that can specifically bind to target mRNA sequences, thereby modulating gene expression. Utilizing high-speed electrospinning technology, a recent study successfully formulated ASO-loaded fibres with up to 9.1 % drug loading. The high-speed electrospinning process enabled the production of these fibres at a rate of approximately 330 g/h, addressing challenges related to scale-up and downstream processing. Importantly, these ASO-loaded fibres demonstrated long-term stability over a one-year period (Hirsch et al., 2023). The significance of this study lies in its innovative use of HSES technology for the formulation of ASOs. This advancement not only allows for higher drug loading but also offers a scalable and efficient method for the continuous production of ASO-containing fibres. It thereby broadens the range of nucleic acids that can be effectively delivered through electrospun fibres and opens up new avenues for the treatment of various diseases.

In summary, although not as abundant as proteins, some therapeutic nucleic acids have been attempted for delivery using electrospun nanofibres with the aim of achieving long-lasting therapeutic effects. Currently, wound dressings and tissue engineering scaffolds are the most important applications for electrospun fibres loaded with nucleic acids.

3.3. Bacteria, cells and viruses

Electrospinning can also be applied to deliver active biological agents such as bacteria, live cells and viruses, owing to the specific properties of electrospun fibres. As discussed in Section 2, electrospun fibres, especially core–shell ones, can stabilise the active agents within them and enable their long-term survival and therapeutic value. One of the applications of this technique is the delivery of probiotics, which are live microorganisms that confer health benefits to the host, especially in the digestive system. Probiotics can be obtained from natural sources or supplements, but their stability is often compromised by the harsh environment of the human GI tract (Çanga and Dudak, 2021). By encapsulating probiotics in biodegradable nanofibres, electrospinning can protect them from degradation and allow their controlled release. Some recent studies on the electrospun delivery of probiotics are listed below.

One of the target strains for electrospun delivery of probiotics is Escherichia coli strain Nissle 1917 (EcN). Çanga et al. used an inclined double-nozzle electrospinning system to prepare nanofibres composed of cellulose acetate and PVA, which successfully encapsulated EcN (Çanga and Dudak, 2021). Cellulose acetate is believed to enhance the stability of the bacteria under gastric conditions, while PVA protects them from inactivation by organic solvents. The authors evaluated the bacterial activity in the nanofibres by simulating digestion *in vitro* and found that more than half of the bacteria survived after 100 min, whereas all free cells died. The study concluded that dual-nozzle electrospinning is a promising probiotic encapsulation system. In contrast, Diep et al. used alginate as the main component and added PEO, polysorbate 80 and E. coli to produce nanofibres for intestinal probiotic delivery (Diep and Schiffman, 2021). By adjusting the component ratio, they obtained smooth fibres with bulges around the bacteria, demonstrating the feasibility of this nanofibres system. Cases of encapsulation of probiotics using high-speed electrospinning technology have also emerged in recent years. In addition to the advantage of mass production, high-speed electrospinning can better ensure the activity of probiotics thanks to the greater use of aqueous solutions. Currently, application areas for electrospun fibres produced by this technology include oral probiotics as well as the delivery of anaerobic probiotics from the gut (Hirsch et al., 2021; Vass et al., 2020).

Another application of nanofibres containing probiotics is the prevention or treatment of genitourinary infections, especially in vaginal formulations. Human vaginal Lactobacillus rhamnosus CRL1332, a probiotic that inhibits genitourinary pathogens, has been encapsulated in electrospun fibres to develop vaginal products. The nanofibres with

Lactobacillus rhamnosus showed a significantly longer activity and shelf life in oxygen-depleted packaging, maintaining their pathogen inhibitory effect for 360 days at 4 °C (Silva et al., 2021). Stojanov et al. encapsulated three more species of Lactobacillus vaginalis (Lactobacillus crispatus ATCC 33820, Lactobacillus gasseri ATCC 33323, and Lactobacillus jensenii ATCC 25258) into PEO nanofibres and introduced different fluorescent proteins among the different Lactobacillus species (Stojanov et al., 2021). As shown in Fig. 8, since these fluorescent proteins have different expression levels in different bacteria, the authors aimed to quantitatively differentiate and track these solid Lactobacillus vaginal delivery systems in the vaginal environment, providing a platform for further research on vaginal Lactobacillus nanofibres delivery. Moreover, they added sucrose as a dressing to the nanofibres in another study (Stojanov et al., 2022). The results indicated that sucrose stimulated the growth of lactic acid bacteria and enhanced the survival of probiotics in the polymer solution when added to the electrospun formulation. These studies demonstrate the potential of composite nanofibres as a solid vaginal probiotic delivery system.

Electrospun fibres can also directly place active cells on nanofibres scaffold fabrication to achieve better repair and regeneration effects in tissue engineering. Zhao et al. encapsulated endothelial cells in hydrogel microspheres and deposited them with VEGF to prepare nanofibrous scaffolds with cell-encapsulated microspheres (Zhao et al., 2021). They selectively disrupted the microspheres to release the cells, resulting in a bioactive nano scaffold with three cell-encapsulated tissue-like layers. The authors confirmed the good bioactivity and stretchability of the scaffold, as well as its excellent pro-vascularisation structural regeneration properties. Another application for electrospun fibre-encapsulated cells is stem cells. A recent study was the encapsulation of stem cells in nano/micron bimodal-compliant collagen and PLGA fibres produced by bimodal electrospinning to construct heart patches (Wee et al., 2022). *In vitro* experiments with bone marrow mesenchymal stem cells showed that the stem cells in this cardiac patch maintained the long-term stem cell capacity to function as cardiomyocytes and secrete restorative factors, improving cardiac function. This stem cell fibre patch can potentially be a powerful platform for stem cell transplantation.

Viruses, which cannot survive independently, can also be encapsulated in electrospun fibres. Although most viruses are pathogenic, rational viral delivery can achieve effective therapeutic effects in some conditions. For example, phages are added to wound dressings to kill multidrug-resistant Pseudomonas aeruginosa. Sarhan et al. encapsulated phages in gelatin/fibroin nanofibres (Sarhan et al., 2021). They proved that phages in this system have good biological activity *in vitro*. The fibre also has good biocompatibility and antibacterial activity against multidrug-resistant Pseudomonas aeruginosa, making it a promising wound dressing. In contrast, Kasbiyan et al. loaded phage into hydrogels obtained by cross-linking γ-polyglutamic acid electrospun nanofibres and prepared hydrogels that retained their bactericidal activity for wound treatment (Kasbiyan et al., 2022). Another therapeutic use of viruses is in viral vector vaccines. Badrinath et al. used PLGA nanofibres to load the vaccinia virus for local delivery (Badrinath et al., 2018). Due to its oncolytic activity, the virus has a potential therapeutic effect on localised colorectal cancer. The nanofibres avoid the immunogenicity caused by the systemic use of the virus and enhance the apoptosis of colon cancer cells, thus having a promising application prospect.

In summary, this section has reviewed the recent advances in electrospinning nanofibres for the encapsulation and delivery of various biological agents, such as bacteria, cells and viruses. Electrospun nanofibres can preserve the biological activity and stability of these agents while enhancing their bioavailability and targeting. Therefore, electrospun nanofibres represent a novel and promising delivery platform that may overcome some of the limitations of conventional delivery methods, such as toxicity and cost-effectiveness.

4. Quality control

Electrospun fibres have been extensively employed in a variety of biopharmaceutical delivery systems due to their exceptional properties. Nonetheless, the majority of studies have mainly concentrated on the design and preparation of formulations, with relatively limited research on fibre quality control. Additionally, unlike standardised analytical methods for conventional dosage forms, significant disparities exist in the analysis and characterisation methods for electrospun fibres across different studies. The lack of standardised analytical methods makes it difficult to compare the properties of electrospun fibres obtained from different research preparations, which in turn leads to a lack of quality control methods in industry, limiting the further development of large-scale production. This section endeavours to systematically classify commonly used characterisation methods for assessing electrospun fibres based on functionality, evaluate their effectiveness, and offer a reference for future industrial-scale quality control.

4.1. Morphology visualisation

Fibre morphology is one of the most notable properties of electrospun fibres. Optical microscopy, scanning electron microscopy (SEM), especially with Energy Dispersive X-Ray Analysis (EDX), Atomic force microscopy (AFM) X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) serve as effective instruments for observing and assessing both the surface and internal structures of fibres. Optical microscopy represents the most straightforward and quickest method, typically employed for the preliminary evaluation of fibre morphology. Although it offers limited magnification, it can ascertain whether continuous fibres have been successfully prepared, thereby indicating overall success. If not, subsequent experiments become unnecessary, and formulation parameters require adjustment (Walther et al., 2022). SEM stands as the predominant method for examining fibre surface and morphology. As an electron microscope, SEM generates an image of a sample by scanning the surface with a focused electron beam. The electrons interact with the atoms in the sample, producing various signals containing information about the sample's surface morphology and composition (Fang and Reneker, 1997). The structure and state of electrospun fibres can be effectively characterised by SEM, encompassing aspects such as surface morphology, diameter size, pore distribution, uniformity, and the presence of beaded structures. If nanofibres encapsulate cells, SEM results can further analyse the interaction between fibres and cells, determining cell proliferation, migration, and differentiation on the fibre scaffold. In general, an EDX device is often equipped as an additional attachment to SEM instruments. EDX is usually used for micro-area compositional analysis and is capable of determining the elemental composition and content within materials (Scimeca et al., 2018). For the characterization of electrospun fibres, EDX can effectively identify the types and quantities of elements in nanofibers, such as carbon, oxygen, nitrogen, or metals. It can also detect impurities or contaminants in nanofibers and quantify the doping or loading levels of nanoparticles or other additives in the nanofibers (Duan et al., 2007). Furthermore, it can provide information on the uniformity and homogeneity of the nanofibers. Therefore, it is typically used in conjunction with SEM to achieve better characterization results. Owing to its relatively straightforward operation and abundant information provided, SEM with EDX remains the primary method for characterising the morphology of electrospun fibres in most studies.

AFM is another valuable technique for characterising the surface topography and mechanical properties of electrospun fibres at the nanoscale (Marrese et al., 2018). AFM operates by scanning a sharp probe across the fibre surface, providing high-resolution images and detailed information on the fibre's morphology, diameter, and surface roughness (Adhikari et al., 2021). Additionally, it can be employed to measure the local mechanical properties of individual electrospun

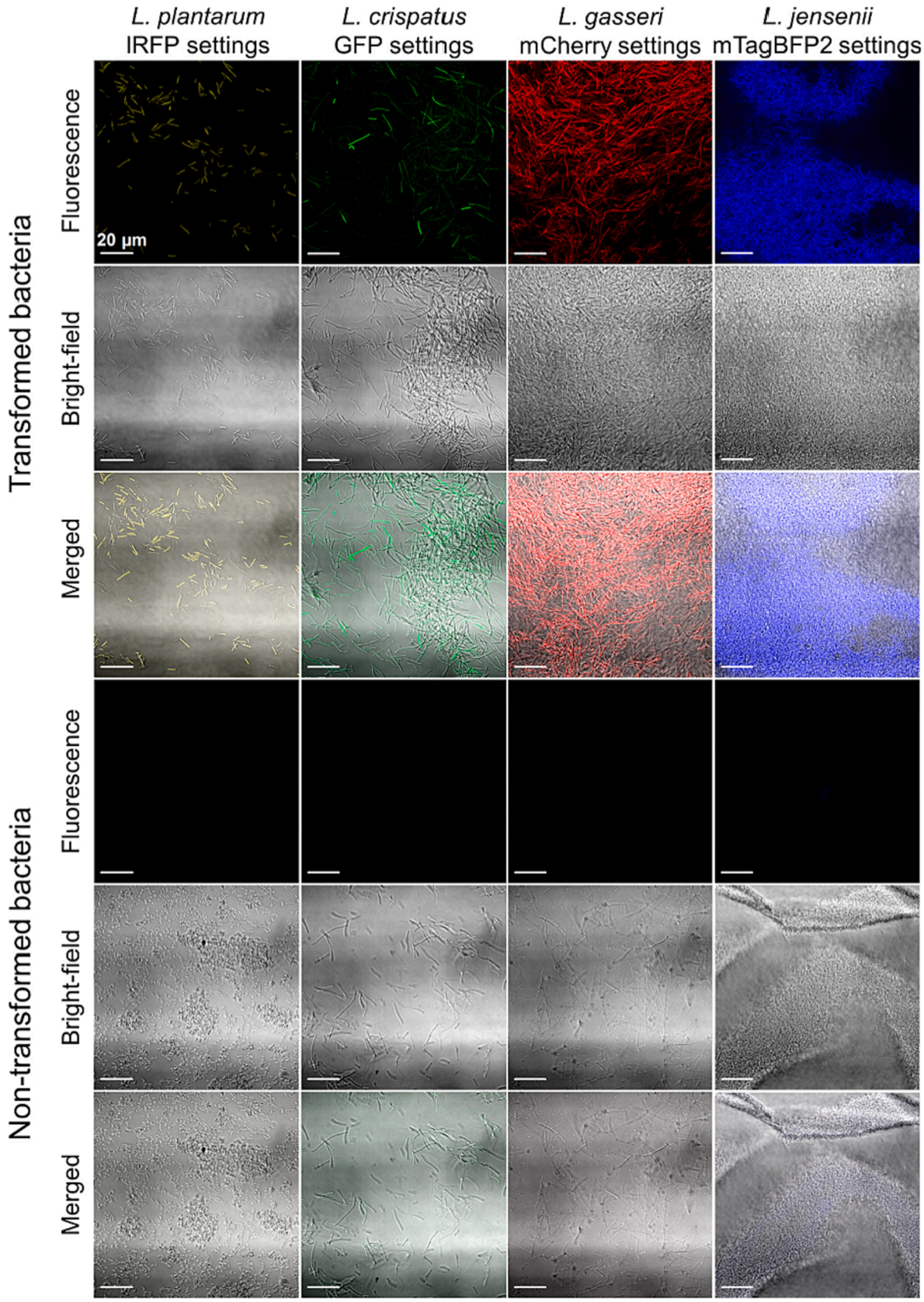

Fig. 8. Representative confocal microscopy images of the lactobacilli expressing the different fluorescent proteins, as L. plantarum expressing IRFP, L. gasseri expressing mCherry, L. crispatus expressing GFP, and L. jensenii expressing mTagBFP2, in comparison to the nontransformed bacteria. Reprinted with permission from Stojanov et al. (2021).

fibres, such as elastic modulus and adhesion forces. This information is crucial for understanding the relationships between electrospinning process parameters, fibre structure, and the resulting mechanical performance (Tung et al., 2019). By leveraging AFM, researchers can optimize electrospinning conditions to fabricate electrospun fibres with desired properties for targeted drug delivery applications. However, AFM has certain limitations that need to be considered. The technique can be time-consuming and requires extensive sample preparation, making it less suitable for rapid, large-scale analysis. Similarly, Raman microscopy is sometimes utilised to examine electrospun fibre properties. Furthermore, XPS and ToF-SIMS are another two valuable techniques for the comprehensive characterisation of electrospun fibres (Seah, 1980). XPS offers insights into the elemental composition and chemical state of the fibre surfaces, providing detailed information on functional groups and bonding, which is particularly useful for evaluating surface modifications, polymer-drug interactions, and assessing the presence of residual solvents (Omastová et al., 2019). ToF-SIMS, on the other hand, allows for the identification of molecular fragments and the distribution of elements or specific chemical species on the fibre surface with high spatial resolution. It aids in understanding the spatial distribution of drugs and other components within the fibres, which can be crucial for controlled release applications and optimising fibre properties (Adala et al., 2021).

However, it is important to note that all AFM, XPS and ToF-SIMS primarily provide surface information, and their depth profiling capabilities are limited. Additionally, these techniques can be time-consuming, require careful sample preparation, and may necessitate the use of expensive, specialised equipment. Consequently, although they can provide more accurate information, it is often beneficial to employ them in conjunction with other complementary techniques to obtain a more comprehensive characterisation of electrospun fibres, ensuring the development of optimal drug delivery systems. Thus, optical microscopy and SEM/EDX continue to be the primary tools for the visualisation of electrospun fibres.

4.2. Chemical structure analysis

The chemical structure of the fibre is also one of the important characteristics of the fibre, which is commonly tested by Fourier-transform infrared spectroscopy (FTIR) and Raman Microscopy. FTIR is a straightforward, rapid, sensitive, and non-destructive analytical method, making it highly suitable for characterising electrospun fibres. It generates characteristic absorption spectra by utilising the interaction between infrared light and the vibrations of molecules within the material. By comparing the absorption peaks at varying wavenumbers, the presence of chemical bonds and functional groups in the fibres, as well as their relative content, can be inferred. For instance, FTIR can detect various functional groups in electrospun fibres, such as carboxyl, hydroxyl, ester, and amino groups, thereby determining the presence of polymers and drugs in the fibres and their interactions. Moreover, FTIR can evaluate the impact of the electrospinning process on the structure of polymers and drugs, as well as the stability and degradation of electrospun fibres under different conditions. By comparing FTIR spectra before and after electrospinning, changes in molecular bonds and crystallinity between polymers and drugs during the electrospinning process can be identified, thus revealing any structural alterations in the polymers and drugs (Xu et al., 2009). Likewise, by comparing FTIR spectra over time, chemical bond changes or breakages in the fibres can be observed, reflecting their stability and degradation. However, FTIR does have limitations, such as the inability to distinguish between different polymer structures or orientations and the inability to detect residual solvents in the fibres. Consequently, it needs to be employed alongside other techniques for a comprehensive characterisation of electrospun fibres. On the other hand, Raman microscopy is another method to test the chemical structure of electrospun fibres. It is an analytical technique that merges traditional optical microscopy with Raman spectroscopy, an effective method for investigating molecular structures, and integrating structural information with spatial information, granting researchers more efficient analysis. For electrospun fibres, Raman microscopy can effectively disclose diameter, morphology, orientation, crystallinity, phase composition, and other information about the fibres. Simultaneously, the distribution and interaction of drugs in the delivery system can be evaluated, and it can even be employed to detect drug release and fibre formation (Walther et al., 2022).Moreover, numerous industrial Raman spectrometers are available for online or real-time analysis of samples. Consequently, it possesses the potential for quality control in the electrospun fibre production in the industry. However, this method necessitates comprehensive training and intricate data processing for the analysis and interpretation of multiple variables. Additionally, fluorescent components may impact the results.

4.3. Thermal analysis

Thermodynamic properties are one of the important physical properties of nanofibres and are related to the thermal stability and bioavailability of the formulation. Commonly used thermal analysis techniques for electrospun fibres encompass differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC is a thermal analysis method that measures the heat flow difference between a sample and a reference material during heating and cooling processes, enabling the analysis of the sample fibre's phase transitions, crystallisation, glass transition temperature, melting point, and other properties. During the electrospinning process, the polymer material is elongated, subjecting it to mechanical stress, which may alter the glass transition temperature. Furthermore, when the fibre raw material comprises two or more polymers, they may interact and form a co-crystal mixture. Such mixtures may display a lower melting point compared to a single polymer, affecting the long-term stability and storage temperature of the formulation. Therefore, DSC is employed to measure relevant properties to ensure stability. Additionally, it can further assess stability by measuring possible polymorphs or metastable structures in a sample over time. Moreover, thermal information of the fibre can be used to determine the fibre's structure and composition, as well as the amorphous/crystalline structure of drugs and polymers, which aids in a better understanding of fibre properties and guides the design and optimisation of electrospun fibres (Shen et al., 2022; Ibrahim and Klingner, 2020). In contrast, TGA measures the mass change of sample fibres under different temperatures and atmospheres, primarily characterising the thermal performance of electrospun fibres. The main application is to measure the moisture content and residual solvents in fibres, which can be calculated by measuring the mass loss of the sample within the temperature ranges of 50–90 °C and 100–200 °C. Furthermore, crystallinity, crosslinking degree, degradation temperature, and even drug encapsulation efficiency and release behaviour can be determined by measuring the mass loss of the sample at different temperatures in various stages (Ibrahim and Klingner, 2020; El-Newehy et al., 2012). Thus, it has extensive application value.

4.4. Mechanical property measurement

Given the need for designing DDSs scaffolds, the structural integrity and mechanical strength of electrospun fibres are important properties that should not be ignored. Currently, the commonly used methods for measuring the mechanical properties of electrospun fibres include single-fibre tensile testing, single-fibre bending testing, and fibrous web tensile testing. Single fibre tensile testing uses equipment such as AFM or micro-electromechanical systems (MEMS), as mentioned in 4.1 before, to apply tensile force to individual electrospun fibres, measuring their stress–strain curves and calculating parameters such as elastic modulus, tensile strength, and elongation at break. It usually provides the most accurate mechanical property data for individual electrospun fibres but is complex, time-consuming, prone to sample loss and affected by

boundary conditions and environmental factors (Rashid et al., 2021; Croisier et al., 2012). Similar to single-fibre tensile testing, single-fibre bending testing uses microfabricated channels or cantilever beams to fix a single electrospun fibre at both ends, applying a load perpendicular to the fibre axis and measuring parameters such as deflection and bending stiffness. Although this method avoids the local damage and stress concentration problems caused by clamping in single fibre tensile testing, it requires precise microfabrication techniques and is limited by the sensitivity and resolution of the testing instruments. Both methods aim to further determine the mechanical properties of single fibres (Croisier et al., 2012).

In contrast to the other methods, the fibre network tensile method uses universal material testing machines or other instruments to perform planar stretching of the entire electrospun fibre network, measuring its stress–strain curve and calculating parameters such as average elastic modulus, average tensile strength, and average elongation at break. The purpose of this method is to directly reflect the potential load-bearing conditions of the entire electrospun material in actual use. However, it cannot distinguish the anisotropic effects in different directions and is influenced by factors such as network structure, porosity, and thickness (Rashid et al., 2021). Measuring the mechanical properties of electrospun fibres helps to elucidate the relationship between fibre diameter, structure, composition, and fibre performance, providing a basis for optimising the electrospinning process and improving the design of delivery systems.

In addition, the mechanical properties of electrospun fibres at different temperatures are one of the properties that should not be neglected, which can be well characterised by Dynamic Mechanical Analysis (DMA). DMA is a paramount technique used in the domain of material science to study and characterise materials (Ago et al., 2013). Particularly beneficial for probing the viscoelastic behaviour of polymers, DMA applies a sinusoidal stress to a material, while the resultant strain is measured, thereby enabling the determination of the material's complex modulus. This elucidation of viscoelastic properties is essential as it furnishes insight into the material's response to mechanical stress under varying temperatures. The characterisation of electrospun fibres, particularly those loaded with biopharmaceuticals, necessitates a comprehensive understanding of their mechanical and thermal properties. Through DMA, researchers can ascertain the mechanical integrity and stability of the fibres under varying thermal conditions, which is imperative for their application in drug delivery systems. For example, the thermo-mechanical behaviour of electrospun thermoplastic polyurethane fibres was investigated using DMA, illuminating the behaviour of these materials under different conditions (Alhazov et al., 2013). Furthermore, the assessment of viscoelastic properties in lignin-based electrospun fibres was conducted using isochronal DMA, elucidating the improved thermomechanical performance when embedded with cellulose nanocrystals (Ago et al., 2013). The viscoelastic properties gleaned through DMA can also shed light on the drug release kinetics from the electrospun fibres, thereby informing the design and optimisation of drug delivery systems.

4.5. *Drug release and stability studies*

In the characterisation of electrospun fibres, drug release tests are crucial for determining the efficacy and applicability of these systems in medical and pharmacological arenas. The properties that these tests measure include the rate and extent of drug release, the effect of drug encapsulation and release on the fibre morphology, and the ability of the system to maintain a sustained therapeutic effect. Currently, there are numerous methods for studying drug release from electrospun fibres. The most commonly employed method is *in vitro* release testing, which involves placing the electrospun fibres in a container with a buffer solution or simulated physiological fluid and measuring the concentration of the drug in the solution at different time points. The concentration is typically determined using high-performance liquid chromatography (HPLC) to ensure the highest level of experimental accuracy. To guarantee the repeatability of the experiment, Walther et al. have developed a method using an automatic sampler for periodic automatic sampling and quantitative analysis, which avoids the loss of medium transfer and improves the stability and repeatability of the results. Additionally, fluorescence microscopy and bioactivity tests are also effective means for studying drug release from fibres. These two methods involve using fluorescently labelled drugs or biopharmaceuticals to observe changes in fluorescence intensity of electrospun fibres under different conditions or using cell culture or animal models to evaluate the effects of drug or biologic release from electrospun fibres on biological systems, both of which are effective characterisation methods (Xue et al., 2019).

For drug release studies of biopharmaceuticals, enzyme-linked immunosorbent assay (ELISA) is a convenient, efficient, highly sensitive, and low-cost method. It uses specific antibodies to detect proteins or peptide biopharmaceuticals in electrospun fibres. By binding the drug to a specific antibody and using enzyme-labelled secondary antibodies to generate a signal, the amount and release of the drug can be calculated. Therefore, it is suitable for the release measurement of proteins or peptide drugs in electrospun fibres (Rashid et al., 2021). Notably, in some specific cases, to ensure the accuracy of the results, two or more methods can be combined to measure the drug release performance of electrospun fibres. All in all, drug release tests can be used to better simulate the release process of nanofibres in an *in vitro* or *in vivo* environment, to better understand their release properties, and to guide the development of nanofibres.

On the other hand, the stability of electrospun fibres is of paramount importance when considering their use in the delivery of biopharmaceuticals. The nature of the APIs embedded within these fibres, their sensitivity to environmental conditions, and their requirement for a sustained release profile necessitate stringent stability tests. Physical stability tests are employed to examine the fibres' response to a range of conditions, such as varied temperatures, light exposure, and humidity levels, which mimic the storage and physiological environments the fibres will encounter. Chemical stability tests are necessary to assess the integrity of the APIs within the fibres under different chemical conditions, ensuring they maintain their therapeutic efficacy and do not undergo degradation or undesirable reactions. Furthermore, bioactivity test is crucial for the characterisation of electrospun fibres. Different activity assays are usually required for different types of biopharmaceuticals, but the following is a list of common assays that can be used to infer their biological activity. HPLC and ELISA, as mentioned in the last paragraph are two commonly used and effective methods, In addition, Surface Plasmon Resonance (SPR), Circular Dichroism (CD) Spectroscopy, and Isothermal Titration Calorimetry (ITC) can also be used to test the bioactivity of biopharmaceuticals. Specifically, SPR can assess the binding affinity between the biopharmaceuticals and their targets, which is a direct indication of their bioactivity, while CD can be used to evaluate the secondary and tertiary structure of the biopharmaceuticals, which are crucial for their bioactivity. As for ITC, besides studying the interactions between the biopharmaceuticals and the polymeric matrix, it can also evaluate the binding interactions between the biopharmaceuticals and their biological targets. However, it is worth noting that none of the above methods can fully determine the activity of a biopharmaceutical. The best test for their specific activity remains validation by *in vitro* and *in vivo* experiments based on their specific types and functions. Moreover, mechanical stability tests ensure the robustness of these fibres to withstand physical stress, preventing premature release or degradation of the drug. These tests are integral to the development of efficient, safe, and reliable electrospun fibres for biopharmaceutical delivery, thus driving their potential to revolutionize drug delivery systems.

In summary, through a variety of analytical methods, researchers can evaluate the morphology, composition, thermal performance, mechanical properties, and other important physicochemical properties of electrospun fibres, as well as the distribution, stability, and

biocompatibility of drugs in the fibres. Based on the analysis results, scholars can choose suitable polymers, solvents, drugs, and process parameters to prepare uniform, defect-free fibres, adjust drug incorporation and release kinetics, and ultimately prepare electrospun fibres with specific structures/morphologies (such as core–shell structures, porous structures, etc.) for intelligent and controllable drug release according to different therapeutic targets. On the other hand, the use of analytical methods and the establishment of standardised analytical toolsets can effectively achieve quality control of electrospun fibres, laying a solid foundation for large-scale production and industrial applications of electrospun fibres.

5. Regulatory and future perspectives

As a DDS, electrospun fibres should be subject to regulation and guidance from relevant government departments. However, possibly due to the absence of mature commercial products on the market, the regulation of electrospun fibres remains a blank slate. Since the diameters of most electrospun fibres are on the nanoscale, they are often classified as nanomaterials and can be regulated under the scope of nanomedicine. In the past, there have been issues in the field of nanomedicine, such as unclear boundaries and a lack of regulatory guidance documents. For example, organisations such as the US National Institute of Health, the European Science Foundation, and the European Technology Platform have not reached a consensus on the definition of nanomaterials. However, it is worth noting that in 2022, the FDA issued a document called "Drug Products, Including Biological Products, that Contain Nanomaterials—Guidance for Industry", which provides a detailed definition of nanomaterials and reviews their current medical applications, particularly offering guidance on their production and use in the industrial sector (Uhljar and Ambrus, 2023). Therefore, most electrospun fibres can refer to this situation for regulation.

The regulation of electrospun fibres for delivering biopharmaceuticals is even more complex. Depending on the type and origin of the biopharmaceuticals, the intended use and indications of the product, and the potential risks and benefits to patients, regulations for electrospun fibre-delivered biopharmaceuticals should vary accordingly. For example, in the UK, electrospun fibres with biopharmaceuticals may be regulated by the Medicines and Healthcare products Regulatory Agency (MHRA) as advanced therapy medicinal products (ATMPs) or medical devices (Gizaw et al., 2018). Additionally, in Europe, they may be regulated by the European Medicines Agency (EMA). However, in the US, electrospun fibres for biopharmaceutical delivery may be regulated by the FDA as biopharmaceuticals or combination products (Gizaw et al., 2018). Therefore, electrospun fibres for biopharmaceutical delivery may need to meet different regulatory requirements and standards in terms of quality, safety, efficacy, and performance in each region (Gizaw et al., 2018).

Based on the current regulatory information for biopharmaceuticals, researchers may need to pay attention to the following aspects when attempting to develop nanofibers as potential biopharmaceutical delivery systems, as these are crucial for subsequent industrial production and regulation. First, the quality and purity of electrospun fibres and biopharmaceuticals are significant, such as their composition, structure, morphology, stability, sterility, and bioburden. Second, the safety and efficacy of electrospun fibres and biopharmaceuticals, such as their biocompatibility, toxicity, and immunogenicity, cannot be ignored. An intuitive suggestion is to use safe, approved polymers and excipients to meet biocompatibility and biodegradability safety requirements whenever possible. In addition, attention should be paid to the possible toxic solvent residues during the electrospinning process, and the content of residual solvents should be analysed. If possible, use non-toxic solvents instead of toxic ones (Uhljar and Ambrus, 2023). Third, the performance and functionality of fibres, such as their mechanical properties, degradation behaviour, drug release kinetics, cell interactions, tissue integration, and gene expression, will also be important regulatory aspects. Precise, reproducible production processes, uniform and stable mechanical properties, and reliable *in vitro* and *in vivo* animal study results are all essential components of successful electrospun fibre products (Li et al., 2022). Fourth, large-scale manufacturing and processing of electrospun biopharmaceutical delivery systems are also important, such as their methods, parameters, equipment, validation, standardisation, and quality control (QC) should also be considered. Finally, after completing the above sections, the clinical trials and marketing authorisation of the final product, such as design, implementation, analysis, reporting, review, approval, labelling, and post-marketing surveillance, will be the last step in the entire regulatory process (Li et al., 2022; Puhl et al., 2022). Only when all of these aspects have comprehensive regulation can the final electrospun fibre product be ensured to be a safe and effective biopharmaceutical delivery system.

Admittedly, the level of detail and stringency of these aspects may vary depending on the specific regulations and guidelines applicable to each product in each region. For nanofiber delivery systems, the current basic research is extensive, but to date, only a few products have been approved for the market, such as PK Papyrus® (Vascular Intervention) (PK, xxxx), ReBOSSIS® (bone-void/defect-filling material) (What, xxxx), and Rivelin® (patch for mucosal delivery) (Bioinicia, xxxx). Although there are clear regulatory approaches for general drug formulations, the regulation of drug delivery systems containing electrospun fibres and all nanofibers is unclear. At present, there is still a need for substantial research to fill the gap in this field.

To be specify, a comprehensive understanding of electrospun fibres regulatory implications warrants future investigation. Firstly, future studies should focus on the standardization of electrospinning processes to ensure consistent and reproducible fibre characteristics, which is crucial for meeting regulatory requirements. Secondly, further in-depth research is required to understand the long-term stability and safety of these fibres in biological systems. This includes an investigation of potential cytotoxic effects and the immunogenic response invoked by the fibres, which are critical for assessing their biocompatibility. Additionally, as biopharmaceuticals often involve complex molecules such as proteins and peptides, the effect of electrospinning on the structural integrity and bioactivity of these molecules should be studied more comprehensively. Lastly, there is a need for stringent guidelines to assess the release kinetics of biopharmaceuticals from the fibres in both *in vitro* and *in vivo* settings. Together, these research directions will help establish a robust regulatory framework for the use of electrospun fibres in biopharmaceutical delivery, facilitating their translation from lab-scale to clinical application.

In conclusion, the regulatory aspects of electrospun fibres and biopharmaceutical delivery systems require researchers to consider several factors, including the quality, safety, efficacy, and performance of the product, as well as the manufacturing process and clinical trials. As the regulation of these fibres is still under development, researchers must stay informed and adapt to new regulations as they emerge. By considering these regulatory aspects and following the appropriate guidelines, researchers can help ensure that their electrospun fibre products are safe, effective, and market-ready.

6. Conclusion

This review highlights the potential of electrospinning technology for encapsulating and delivering a wide range of biopharmaceuticals. Electrospinning is a specialized process that uses an electric field to spin polymer solutions or melts into fibres. There are several types of electrospinning systems, including blend, emulsion, and coaxial electrospinning. These systems can be used to encapsulate proteins, nucleic acids, and living cells within electrospun fibres while maintaining their structural integrity and biological activity. The type and nature of the polymers used in electrospinning, as well as the parameters of the process itself, determine the structure and mechanical properties of the resulting fibres. Coaxial electrospinning is currently favoured for

biopharmaceuticals because it avoids contact between the drug and organic solvents, ensuring maximum bioactivity and controlled drug release rates. Recent advances in high-throughput electrospinning methods have improved productivity and provided a foundation for industrial applications. In summary, electrospinning technology has shown great promise in drug formulation and biopharmaceuticals, with numerous use cases in drug delivery and tissue engineering. Further research should focus on scaling up production for industrial applications and the pharmacology of electrospun fibre formulation prior to clinical application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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