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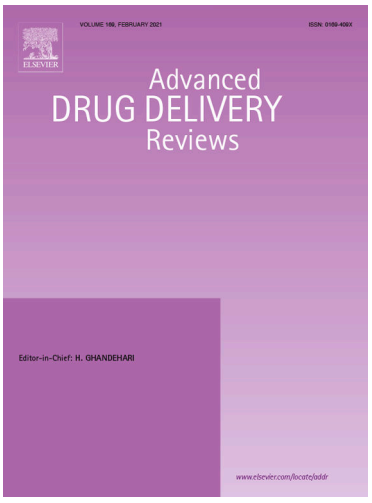

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Electrohydrodynamic Atomisation Driven Design and Engineering of Opportunistic Particulate Systems For Applications in Drug Delivery, Therapeutics and Pharmaceutics

Amna Ali[1], Aliyah Zaman[1], Elshaimaa Sayed[1], David Evans[2], Stuart Morgan[2], Chris Samwell[3], John Hall[3], Muhammad Sohail Arshad[1,4], Neenu Simgh[1], Omar Qutachi[1], Ming-Wei Chang[5], Zeeshan Ahmad[1*].

1. The Leicester School of Pharmacy, De Montfort University, Leicester, UK
2. Genvolt, New Road, Bridgnorth, Shropshire, UK
3. BlueFrog Design, 19 St. Margaret's Street, Leicester, UK
4. Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
5. Nanotechnology and Integrated Bioengineering Centre, University of Ulster, Newtownabbey, Belfast, Northern Ireland, UK

*Corresponding author: zahmad@dmu.ac.uk

Abstract

Electrohydrodynamic atomisation (EHDA) technologies have evolved significantly over the past decade; branching into several established and emerging healthcare remits through timely advances in the engineering sciences and tailored conceptual process designs. More specifically for pharmaceutical and drug delivery spheres, electrospraying (ES) has presented itself as a high value technique enabling a plethora of different particulate structures. However, when coupled with novel formulations (e.g. co-flows) and innovative device aspects (e.g., materials and dimensions), core characteristics of particulates are manipulated and engineered specifically to deliver an application driven need, which is currently lacking, ranging from imaging and targeted delivery to controlled release and sensing. This demonstrates the holistic nature of these emerging technologies; which is often overlooked. Parametric driven control during particle engineering *via* the ES method yields opportunistic properties when compared to conventional methods, albeit at ambient conditions (e.g., temperature and pressure), making this extremely valuable for sensitive biologics and molecules of interest. Furthermore, several processing (e.g., flow rate, applied voltage and working distance) and solution (e.g., polymer concentration, electrical conductivity and surface tension) parameters impact ES modes and greatly influence the production of resulting particles. The formation of a steady cone-jet and subsequent atomisation during ES fabricates particles demonstrating monodispersity (or near monodispersed), narrow particle size distributions and smooth or textured morphologies; all of which are successfully incorporated in a one-step process. By following a controlled ES regime, tailored particles with various intricate structures (hollow microspheres, nanocups, Janus and cell-mimicking nanoparticles) can also be engineered through process head modifications central to the ES technique (single-needle spraying, coaxial, multi-needle and needleless approaches). Thus, intricate formulation design, set-up and combinatorial engineering of the EHDA process delivers particulate structures with a multitude of applications in tissue engineering, theranostics, bioresponsive systems as well as drug dosage forms for specific delivery to diseased or target tissues. This advanced technology has great potential to be implemented commercially, particularly on the industrial scale for several unmet pharmaceutical and medical challenges and needs. This review focuses on key seminal developments, ending with future perspectives addressing obstacles that need to be addressed for future advancement.

Key words: electrohydrodynamic atomisation (EHDA), electrospraying, coaxial, particle engineering, drug delivery systems, core-shell micro/nano particles, dosage design, targeting

Contents

1. Introduction

Particulate systems hosting active pharmaceutical ingredients (API), biologics and functional chemical entities are dynamic and opportunistic platforms to meet key attributes and requirements for specific drug delivery and therapeutic applications (anti-cancer [1], theranostics [2] and tissue engineering [3]). Enhancing fundamental characteristics of particles can be an intricate challenge. However, when overcome, optimum properties are achievable using formulation or emerging engineering technologies; some of which have been adapted to the therapy and drug delivery remits over the last decade. These provide noteworthy improvements (e.g., toxicity and consequential side effects) over conventional drug delivery systems. Engineering methods have been adapted, manipulated and modified to fabricate an array of particulate systems by exploiting the morphology and surface chemistry of resulting structures. Successful engineering of tailored particles enables opportunistic pathways for therapeutic and diagnostic agents to enhance efficacy at specific target tissue and sites of interest. Fine-tuning morphological characteristics of particles during an engineering process or set of processes, yields greater control over particle properties. For example; particle size, size distribution, shape, rigidity and topography are known to influence therapeutic delivery and therefore drug delivery strategies [4]. The particle size for example, determines the surface area of particles which interact with biological milieu and can significantly impact dosing and release characteristics. These, in turn, can affect blood circulation times [5], toxicity, particle stability and drug loading [6] .

It is therefore crucial to consider both particle surface chemistry and the nature of the intended biological environment (at site of action). The interplay between these governs the degree of interactions, surface charges, adhesive forces, chemical composition and functionality. For instance, extensive studies have shown that zeta potential is dependent on the surface charge of particles [7], facilitates nanoparticle stability and impacts initial adsorption, biodistribution and blood clearance levels [8]. These variable chemistries are therefore essential to determine biocompatibility and toxicity of particle interactions with cellular tissues [9]. Physiochemical properties of particulate drug delivery systems provides an approach to address evolving therapeutic needs where such properties can be pivotal. Applications where cellular uptake poses a challenge, several parameters (i.e. particle size, charge and morphology) can influence how active molecules enter tissues effectively. If the drug delivery system undergoes surface modification, it is imperative that biocompatibility for targeted delivery is not compromised

[10]. Where drugs are encapsulated in matrix type devices, the particle matrix material plays a key role in drug delivery. A controlled release mechanism is often accomplished with the preferred material of choice (e.g., polymer type and length) and offers a known degradation profile with minimal toxicity to surrounding cells [11]. The degradation behaviour is also known to impact cellular uptake for site-specific delivery [12].

Particle engineering includes a host of fabrication techniques and these can deploy either top-down or bottom-up methodologies. Top-down approaches involve size reduction of larger particles and bulk material to the nanometre or micrometer scale [13]. This can be achieved using techniques such as milling, homogenisation and atomisation [14]. In contrast, bottom-up approaches generate nano-scaled architectures through assembly of individual molecules. This is usually accomplished by imparting knowledge of supramolecular chemistry, where interactions such as Van der Waals, hydrogen bonding and hydrophobic forces lead to molecular self-assembly progressing to larger structures [15]. Examples of engineering processes deploying such approaches include microfluidics, electrohydrodynamic atomisation (EHDA) [16], hot-melt extrusion and co-precipitation methods [17].

A plethora of technologies have been used to engineer particles. One of these well-established techniques is spray drying where liquid droplets are atomised by controlled evaporation, eventually forming microspheres. A dry powder is formed consisting of API and other excipients [18]. Spray dried particles can exhibit different diameters (10 - 100μm) [19] and morphologies (crystalline, skin-forming and agglomerate) [20]. Similarly, freeze drying is another solvent-evaporation approach where frozen material containing drug components undergoes sublimation to produce dried powder formulations. Microparticles can also be generated from other engineering methods including nanoprecipitation, microfluidics [21] and supercritical fluid processing, or self-emulsifying techniques such as hot-melt extrusion [22].

The aforementioned advanced particle engineering methods have been utilised for decades, however, are accompanied with several drawbacks and bottlenecks. Engineering processes comprising multiple steps need to be managed effectively and are known to cause reduced production rates, loss of material and are therefore time-consuming. In this regard, EHDA is an efficient technique which can be used to prepare several particulate drug delivery systems with various sizes, compositions and morphologies at ambient conditions in a single-step [23]. This technique has been extensively studied due to its applicability for the preparation of diverse drug delivery systems for different pharmaceutical and biomedical engineering

applications [24]. EHDA generates atomised liquid droplets *via* the deployment of a strong electric field to produce structures at the micrometre and nanometer scales [25]. In addition to one-step engineering, it also offers structure versatility, efficiency in producing particles with desired morphology, narrow size distribution, high drug encapsulation and structure driven control on drug release kinetics [26]. Therefore, an alternative route of engineering is emerging which circumvents limitations associated with established methods for dosage development (i.e. low encapsulation efficiencies, poor drug release kinetics, disruptive engineering conditions and prolonged manufacturing time).

This review focuses on the EHDA process mainly for particle engineering. The fundamental principles of EHDA technologies are summarised; elaborating on the types of particulate structures which can be formed. Appropriate applications of this technique are discussed with regards to facilitating pharmaceutical and therapeutic strategies with the aim to elucidate the benefits such technologies bring to the fore for addressing challenges during dosage or particle production in the pharmaceutical industry

2. Principles of electrohydrodynamic atomisation technologies

2.1 The electrohydrodynamic atomisation process

Recent advances (from theory to application) in particulate technology have meant techniques such as EHDA have been increasingly utilised to engineer structures with desired properties. According to early studies, the phenomena underpinning EHDA stems from the English physicist Sir William Gilbert, who in the 1600s observed interactions between electrostatic and magnetic forces. Gilbert's pioneering work revealed that a piece of charged amber was drawn up into a conical shape, (what would eventually become known as the Taylor cone/Gilbert-Taylor cone) when exposed to water. The electric field employed by the charged amber resulted in a fine aerosol of charged water droplets from the tip of the cone, thereby distinguishing the behavioural forces between the two materials [27]. In 1882, Lord Rayleigh also observed these interactions and calculated a charge threshold limit which, when reached, can potentially hinder the capability to engineer particles. This is known as the 'Rayleigh criterion limit' where Rayleigh demonstrated that an isolated liquid droplet became unstable and disintegrated into a jet of multiple drops when the critical threshold was exceeded compared to the stabilising effect of the solution surface tension [28]. Further analysis into the realm of EHDA technology was continued by John Zeleny, who worked on the physical behaviour of fluids and their mathematical modelling during the electrohydrodynamic process [29]. Taylors theory emerged

in 1964, which subsequently led to the characterisation of the value at which a cone formed at a liquid surface under the influence of an electrostatic force, and hence confirmed the Rayleigh limit [30]. The formation of a droplet shape now known as the Taylor cone is used as a basis to fabricate highly monodisperse (or near monodisperse) particles under stable processing with structures exhibiting near uniformity and increased likelihood of reproducibility in the medical industry.

The fundamental principal of EHDA is built on the atomisation of formulations by electrical forces when exposed to a high voltage [31]. This results in the formation of fibers or particles at the tip of a nozzle which are deposited on a grounded collection plate [32]. The EHDA set-up entails a simplistic framework comprising of an apparatus which includes a syringe pump connected to a syringe filled with the formulation, a stainless-steel needle (also referred to as nozzle or spinneret), collection plate and a high voltage generator (Figure 1). During the EHDA process, the desired formulation is allowed to flow through a capillary needle at a controlled rate. The ultimate exposure of a strong electrical field to the spheroid droplet results in a build-up of charge on the liquid surface [33]. Increasing the voltage further subsequently leads to the stable cone-jet mode, thereafter, breaking up into finer particulate or fibrous microstructures and nanostructures depending on a number of parameters [30].

2.2 Types of electrohydrodynamic atomisation technologies

EHDA is an umbrella term which encompasses a range of different structure engineering processes. Fiber (electrospinning) and particle (electrospraying (ES)) fabrication techniques make use of identical base technology and the latter is addressed in detail further in this review. The interplay of various parameters merits the atomisation method to be utilised for different therapeutic approaches.

2.2.1 Electrospraying

ES is a versatile jetting technique offering liquid atomisation of formulations consisting of a polymer and API when exposed to a high voltage. Electrostatic forces enable the formation of a Taylor cone and when these cohesive forces of surface tension are finally overcome, it results in uniform sized microparticles or nanoparticles (Figure 2) [14]. ES is a distinctive method in the family of EHDA technologies as particles fabricated by this technique exhibit self-dispersing behaviour due to the Coulomb repulsion. Therefore, the agglomeration and coalescence of particles are reduced, thus inhibiting the potential hazard of nozzle clogging in

the apparatus [34]. Major parameters which influence the morphology, size and encapsulation capacities of the structures formed can be assigned to the flow rate and applied voltage, developing various modes of atomisation [33].

In comparison to traditional systems, ES provides copious benefits for pharmaceutical applications owing to its ability to fabricate uniformly sized particles, high encapsulation efficiencies and the potential to incorporate several bioactive compounds within polymer matrices. As modern technology emphasizes the use of pharmacokinetic and pharmacodynamic profiles, the unique characteristic of particle size distribution is highly acclaimed in the therapeutic industry as it can control the performance of drug delivery systems and in turn minimise variations between batches [35]. Applications of the ES method include the fabrication of particulate delivery systems for anti-cancer therapeutics [36], anti-inflammatory drugs [37], antibiotics [38], proteins including gene and vaccine delivery [39] as well as a wide variety of bioengineering treatments [40], all of which will be discussed in comprehensive detail below.

2.2.2. Electrospinning

Electrospinning is an electrohydrodynamic variant of ES using the same experimental set-up but rather polymeric formulations with a higher viscosity than those employed in ES, resulting in the production of fibers instead of particles [33]. One of the earliest appearances of the electrospinning mechanism in literature is accredited to Morton in his patent submitted in the 1900s, [41] which consequently propagated a number of further studies on the electrospinning technique [42-44]. This emerging technique can create microfibres and nanofibers by the utilisation of electrostatic forces exerted on a solution [45]. The fibrous structures are produced when an electrostatically charged jet containing the polymeric solution elongates at the needle exit by an unstable whipping motion, resulting in a deposition of fibers on the grounded collection plate [46]. Electrospun fibers possess attractive characteristics as they are simple, cost effective and versatile with respect to the range of polymers which can be incorporated within the solution. They have therefore gained curiosity over the past few years in various fields including tissue engineering [47], drug encapsulation [48], wound management devices [49] and gene therapy [50].

2.2.3 Microbubbling

Research over the last decade has led to the exponential development of EHDA processing techniques in the pharmaceutical industry. Coaxial EHDA, an extension of EHDA consists of an outer and inner flow from a coaxial needle thereby forming a coaxial electrified jetting system. The coaxial EHDA method has been extensively used for encapsulation systems, from which techniques such as microbubbling have stemmed from. Microbubbling has been established by Farook *et al* in 2007, where the novel preparation of gas filled microparticles (or bubbles) was initially reported. In this, microbubbles were prepared using a glycerol medium which flowed through capillary tubes and formed a stable Taylor cone. Simultaneously, a secondary inner needle incorporated the gaseous phase which ultimately produced monodisperse microbubbles that were less than 10 μm in size [51]. The fabrication of microbubbles is of great interest in medical therapeutics [52] for applications such as diagnostic imaging [53], drug delivery vehicles [54], and biomedical engineering [55].

Li *et al* for example, employed a co-axial electrospray method for the generation of microbubbles to be used as ultrasound contrast agents for medical imaging. In this study, the microbubble shell was comprised of a mixture consisting of polyethylene glycol (PEG) and glycerol. The addition of PEG 400 significantly reduced the viscosity and microbubble size, hence improving microbubble stability. The ability to fine-tune the formulation and operating parameters deems the co-axial jetting system favourable as fabricated microbubbles can exhibit an array of sizes and shell thicknesses. Thus, the microbubbling process as reported in this study was found to be an exceptional technique, finding applications in imaging where microbubbles play an efficient role as drug carriers [56]

2.2.4 Printing

Alternatively, printing has also been developed using EHDA technology which can provide medical pathways to bioengineering [57], making use of 3D EHDA printed structures and more recently near field electrospinning. Printing is a novel approach which involves the use of various biomaterials to tailor topographies or coarse structures using desired quantities of materials, which, when deposited, result in fabricated three-dimensional structural scaffolds [58]. Near field electrospinning is an emerging printing technique which provides exceptional control by allowing customisable adjustment of the voltage potential where the employment of an electric field drives the ink and eventually prints micropatterns and nanopatterns on functionalised topographical structures [59]. Printing techniques can provide applications in tissue regeneration [60], targeted drug delivery [61] and biomedical engineering [62].

3. Principles of electrohydrodynamic atomisation technologies with respect to particle engineering

Significant strides have been made to accomplish engineered particles for different drug delivery platforms. To target and achieve specific therapeutic approaches, the influence of different solution and processing parameters can be adjusted, all of which impact the electric field and variations in resulting jetting modes. The characteristic versatility of the EHDA technique can therefore be employed as a useful tool for the deposition of an array of monodisperse particles.

3.1 Solution parameters

Several fluidic properties affect the characteristics of different atomised structures that are used for various engineering platforms. The electrohydrodynamic model, specifically ES, is reliant on sufficient electrical conductivity which can impact the formation of a Taylor cone and the particle production efficiency [63]. During the atomisation step, droplets exposed to an electric field result in an accumulation of charge on the surface [26]. This involves the interaction of electrostatic forces between charged particles and the grounded collection plate, where low conductivity is preferential for the generation of monodisperse electrosprayed particles [33]. For this reason, the electrical conductivity of the solution should be considered as a key parameter when optimising the ES method and has been previously demonstrated by scaling laws [64]. The dielectric constant or relative permittivity determines how easily the atomised liquid can be polarised due to the application of an electric field. It has been studied that a solution with electrical conductivity as well as relative permittivity culminates into steady jet formation upon the addition of an electric field [65].

Surface tension is another crucial parameter which facilitates the ES method in achieving a hydrostatic equilibrium and subsequently a stable Taylor cone. A key requirement for the EHDA process is the atomisation of the droplet from the needle tip and hence the surface tension of the solution is important [66]. As electrostatic coulomb forces increase, the effect of surface tension decreases. Solutions with high surface tension can result in jet instability whereas low surface tension formulations in conjugation with low viscosity, form optimised particles in a controlled manner [67].

Another formulation variable for the generation of successful ES is the visco-elastic properties of the solution [66]. Viscosity is a critical factor that distinguishes the ES and electrospinning methods. Solutions exhibiting low viscosity have been used ideally for the formation of stable cone jetting which results in particles with uniform size (or near uniform) and smooth surface morphology [68]. Increasing the viscosity further subsequently leads to the generation of continuous electrospun fibers which, after a certain threshold is reached, results in poor jet emission due to blockage at the needle orifice [69]. For this reason, it is of paramount importance to identify if either particles or fibers are desired so that an optimum viscosity can be established during solution preparation.

The density of a solution is also considered as a vital physical property which determines the Taylor cone jet diameter and can be defined as the relative measurement by the ratio of mass to volume. As elaborated, viscosity plays a crucial role in the jet stability, however, this parameter is greatly dependent on the density of the formulation [64]. During ES, an increase in density has been associated with larger particle diameter sizes due to the influence of gravity on the conical droplet shape [46].

3.2 Processing parameters

Processing parameters such as the flow rate, voltage, working distance (needle exit to collector) and the needle diameter can also alter the functioning process of the EHDA set-up and determine the ability of the EHDA mode to produce spherical nanoparticles. Flow rate is the rate at which the liquid solution travels through the capillary needle and therefore a crucial parameter for optimising particulate structures. The variation in flow rate can impact the particle size, particle size distribution and morphology. Generally, a lower flow rate (≤ 1 mL/h) has been suggested for the ES mode of EHDA to achieve spherical monodisperse nanoparticles with narrow size distributions [66]. Coaxial EHDA, a further sophisticated EHDA variant, is a branch of ES in which two or more liquid solutions are passed through two or more concentrically located capillary needles. The flow rate of core and shell liquids must be optimised, where a suitable ratio between these should be established to achieve a successful coaxial ES system [68].

The applied voltage acts as a driving force for atomisation of the liquid and significantly impacts the various modes of ES, resulting in a number of conical sprays from initial dripping to stabilised Taylor cone formation and partially defines the overall outcome of the ES process

[70, 71]. The increase in applied voltage has shown to decrease the particle size of atomised formulations. However, after a certain threshold, the particles begin to morph into elongated or beaded fibers due to the presence of Coulomb forces and an increase in charge imposed on the droplet at the solution interface [16]. On the contrary, conflicting studies suggest that an increase in the applied voltage consequently results in an increased particle diameter size. For this reason, it is imperative that the applied voltage is chosen depending on the individual formulation and fine-tuned to accommodate the desired particle characteristics.

It is postulated that the working distance can considerably influence the capability of the EHDA process to engineer optimal particles. Typically, a shorter working distance results in a stronger electric field and therefore the fabrication of monodisperse particles [66]. As the collection distance increases, a higher voltage is required to provide a substantial electric field, otherwise, this may result in the failure of controlled atomised structures and potential material loss [72]. Too small a working distance equates to the inability of complete solvent evaporation, eventually forming a wet particulate system and possible particle aggregation on the grounded collection plate [73]. Therefore, an ideal working distance must be utilised during the ES mode, such optimisation could be achieved with adequate jetting maps [74]. The optimisation of the working distance in combination with jetting map experimentations can be used to predict the distance at which spheroid particles are formed. This is a useful technique, proving great flexibility during particle engineering in pharmaceutical manufacturing.

As well as the types of needles employed (Figure 1a), the diameter of the needle orifice, often expressed in gauge, also plays an instrumental role in the ES set-up and affects the properties of resultant structures [75]. The inner diameter of the nozzle also impacts the size and size distribution of fabricated droplets as it is heavily reliant on the development of the Taylor cone domain by means of its apex. Particles obtained using smaller needle gauges (small nozzle diameters) produce fine monodisperse particles and a stable ES mode [68]. In contrast, the utilisation of large needle gauges (large needle orifice diameters) demonstrates unstable sputtering, thereby generating particulates which display polydisperse behaviour [73].

3.3 Local environment engineering parameters

In addition to the significance of processing parameters and properties of materials used in ES for the effective production of particle engineering, the ambient environment is also of critical importance. For example, an increase in temperature results in accelerated evaporation of the

solvent medium, a decrease in polymer viscosity and a reduced particle size [70]. Additionally, a high humidity level influences the solidification process of ES and impacts the morphology of droplets due to condensation which could potentially lead to the diffusion of water into the particulate system as well as crystallisation [76]. For a more controllable atmosphere, the ES set-up can be placed in an isolated chamber where the cocooning nature of the chamber prevents contamination and allows the adjustability of ambient conditions [66].

3.4 Other considerations

Various modes of the ES phenomena can be obtained by optimising the parameters discussed earlier, including modes categorised into either dripping or jetting [77] (Figure 1b). This is based on the geometrical shape of the liquid droplet at the needle exit during the employment of an electric field and eventually the deformation of the droplet interface. There are several modes, these comprise of initial dripping, micro-dripping, spindle and multi-spindle modes, where the liquid solution forms fragments of the formulation. In jetting, however, a continuous jet is produced out of the capillary needle exit at a distance from the spinneret and includes cone-jet (Taylor cone), precession, oscillating-jet or multi-jet variations.

The choice of polymer and solvent employed during ES is crucial in determining the characteristics of fabricated particulates, including the weight, porosity and surface properties of the developed structures [78]. Various polymer properties should be considered (i.e. molecular weight, concentration, viscosity, surface tension, immunogenicity and biocompatibility) when streamlining the ES process and optimising solutions.

The solvent used during ES can also ultimately impact the particle morphology and the size of resultant structures. Highly volatile solvents positively correlate with greater evaporation rates and coincide with increased particle diameter [46]. Solvent properties must also include high miscibility with a large range of solutes [68] and high electrical conductivity, thus reducing the amount of applied voltage necessitated for the ES process [46]. The surface tension of the solvent is another essential criterion for determining the type of solvent material which should be employed during the atomisation process. In principle, solvents with high electrical conductivity and low surface tension are preferable to fabricate monodisperse particles [79].

The aforementioned processing and material parameters provide a detailed insight into the intricacies involved in EHDA and specifically ES technologies with respect to particle engineering. The broad selection of parameters indicates the ability to fabricate diverse

particles with multifunctional properties, which, in particular is an overarching challenge during drug delivery and related therapeutic (e.g. theranostic) applications.

4. Engineering various types of particulate structures using the electrospraying set-up.

Optimisation of the experimental arrangement, specifically the needles employed to fabricate particles have the potential to control a diverse range of functional structures that can be generated from ES (Table 1).

4.1 Pre-atomised formulations

Before the introduction of ES, various orthodox approaches can be utilised to prepare particulate systems including nanoprecipitation, emulsification and solid dispersion.

Nanoprecipitation, also known as agitated solvent displacement, involves the dissolution of the active in an aqueous polymer solution prior to the addition of the anti-solvent medium (containing distilled water or an aqueous buffer) [80]. Rapid desolvation of the polymeric material results in a precipitated polymer and drug entrapment in the polymer matrix. The nanoparticles which are ultimately formed can be explained by the Marangoni effect where interfacial turbulences between the two phases results in the fabrication of nanoparticles [81]. Instability in this phenomenon can cause coalescence and broad size distributions of polymeric nanoparticles [82].

Emulsion-based techniques have been widely employed for hydrophobic molecules, improving their release profiles for targeted drug delivery [83]. Emulsification methods can be further classified into single-emulsion (w/o, o/w and o/o) or double emulsion (w/o/w and o/w/o) disperse systems, where varying the proportions of oil and water molecules significantly impacts the emulsion blend and therefore the hydrophilic and lipophilic characteristics. For these methods, an aqueous solution of hydrophilic entities is thoroughly mixed with the appropriate polymeric formulation, forming microdroplets which are subsequently dried by solvent evaporation, eventually producing nanoparticles or microparticles [66]. The use of organic solvents is however accompanied with possible drug denaturation (due to loss of drug during emulsification or washing steps) leading to low drug encapsulation efficiencies [84].

Solid dispersion is a specific process referring to the dispersion of the drug at solid state into a polymeric solution [33]. The successful formation of particulate systems is achieved by establishing an optimum drug to polymer ratio as well as desired intermolecular interactions between the drug and the polymeric carrier matrix [85]. Although considered an advantageous method utilised to improve the bioavailability of poorly water-soluble drugs, polymers used in solid dispersions tend to absorb moisture, triggering a number of complications including phase separation, drug hydrolysis and crystal growth [86].

Due to the limitations associated with these particulate systems alone, it can be gathered that by combining these particulate formulations with ES can potentially avoid drawbacks related to conventional methods. Therefore, the introduction of ES in formulations can act as a progressive step following final solution preparation to circumvent any limitations and achieve highly monodisperse electrosprayed nanostructures.

4.2 Single-needle electrospraying

Single-needle ES is the most common and simple atomisation technique which employs a single spraying needle and can produce several types of particulate systems [87]. Upon mixing the polymer with an active agent (in a suitable solvent), a homogenous polymeric formulation is created, which, if complementary, forms a stable ES process and ideally a perfect Taylor cone, depending on the parameters discussed earlier (section 3). Single-needle ES shows great promise in various applications such as gene therapy [39], chemotherapeutic drug delivery [1] and targeted drug delivery [88].

4.3 Coaxial electrospraying

In addition to conventional Single-needle electrosprayed particles, further developments in the engineering field have meant that other assembled ES systems have been utilised to yield exceptionally functional particulate structures including various spinneret arrangements (Figure 1a). These include coaxial and triaxial ES systems (commonly three needles aligned in a co-axial configuration) as well as an array of different needle arrangements. In this review, triaxial ES is similar in arrangement to coaxial tri-needle unless stated otherwise. These complex ES techniques are used to prepare particulate systems with unique morphologies thus further expanding the opportunities to fabricate intricate particulate systems.

The coaxial EHDA mode provides an efficient technique which can be utilised to produce electrified liquid jets where possible resultant structures include core-shell, capsule and solid-shell solid-core formations. To assemble a coaxial ES arrangement, two concentric needles are employed in conjunction, in which an outer liquid encapsulates the inner formulation. This results in microparticulate or nanoparticulate systems characteristically possessing particles with near monodispersity [33, 89]. Capsulated structures are usually comprised of an aqueous core solution encased in a larger organic shell solution, in which both formulations are individually extruded through a bi-component needle system. The shell is usually made up of the polymeric solution while the drug is encapsulated within the polymer, where both flowing streams exit the dual needle configuration in an orifice. Various parameters can impact the experimental coaxial EHDA technique, in particular flow rates of both the core and shell formulations which ultimately determine morphological and surface characteristics of the resultant capsule system [66]. In addition, miscibility of the core and shell solution is a key characteristic which plays a vital factor in determining morphology of fabricated particles [90].

The successful application of coaxial EHDA has been reported by Lee *et al* for the fabrication of a monodisperse polymeric coated core-shell structure in which nearly 100% drug encapsulation efficiency was reported when coating Budesonide with poly(lactic-co-glycolic acid) (PLGA) [91]. Coaxial ES has been increasingly studied in recent years due to its attractive characteristics including its ability to protect sensitive actives (as core materials) *via* shell formation, improving drug encapsulation efficiencies, generating monodisperse particles, enhancing drug stability, and achieving controlled drug release patterns [79]. The applicability of this technique indicates the potential implementation of ES in various therapeutic or active delivery fields such as tissue engineering scaffolds, [92] anti-cancer theranostics, [93] coatings and biomedical imaging [51].

Multi-layered particles have also been fabricated by the ES mode where the transition to a coaxial set up entails a seamless arrangement of three coaxial needles [94]. A novel device containing this experimental set up was reported by Ahmad *et al*, where multi-layered bubbles, gas encapsulated threads and nanocapsules were generated and hence highlighted the numerous prospects of tri-needle coaxial spinnerets for drug delivery devices [95].

4.4 Other novel configurations of processing needles

Advancement in the realm of EHDA and specifically ES technology has allowed further modification of more sophisticated needle variations which can be developed to form a plethora of different microstructured or nanostructured particles.

Aligned needles have been designed with novel non-concentric spinnerets and angled tips at the needle outlet to form drug encapsulated Janus particles (particles that display multifunctional surfaces). Zhang *et al*, for instance, found that the utilisation of an aligned non-concentric angular nozzle resulted in minimal contact at the cone area (30°), whilst a nozzle angle of 60° produced stable co-jetting atomisation with continuous flow and eventual break-up at the needle apex. This led to the formation of Janus particles with desired morphological properties, where the model dye (Sudan Red G) and the drug (Indomethacin) were both successfully encapsulated into the Janus particulate system. The resultant particles displayed independent release patterns, hence demonstrating the potential for tailored combinatorial drug release [96]. In comparison to aligned needles, multiple needles can also be prepared possessing angular heads and a large variation of tips which can be modified to optimise drug encapsulation and release profiles, thereby indicating potential use for drug delivery applications.

In contrast to the utilisation of needles in the EHDA set-up, needleless approaches have also been investigated as a more cost-effective method to fabricate functional particulate systems. This type of process is reliant on jet formation from open liquid surfaces with the use of external forces [25]. In one study a two-layered formulation system was subjected to an electric field and additionally a magnetic field by the employment of a magnet or coil, resulting in solidified nanofibers [97]. A needleless electrospray apparatus was also prepared by Wang *et al* in which a spiral tower was employed to atomise polyvinylpyrrolidone (PVP)/ Fe_3O_4 ferrofluid. The addition of an external magnetic field in combination with an electric field formed multiple cone-jets. This ultimately resulted in a magnetic film composed of the PVP polymer in which Fe_3O_4 nanoparticles were homogenously distributed [98]. Alternatively, needleless devices can also include the utilisation of orifices. Bocanegra *et al* for example, drilled orifices in dielectric materials with hydrophobic properties. The hydrophobic characteristics of the dielectric materials provided an optimal Taylor cone-jet, successfully resulting in multi-electrosprays in up to 37 holes which displayed size distributions comparable to single needle ES [99]. Needleless approaches are therefore highly effective as they exhibit an extremely simplistic

process without the need of a feed unit and reduce complications associated with conventional tips (nozzle clogging within the ES set-up).

During the ES process, the application of an electrostatically charged jet at the nozzle exit results in the deposition of particles on a grounded substrate positioned under the tip of the capillary needle exit which serves as a collector [16]. Collecting substrates can be made up of a variety of materials including silicon, glass, aluminium and copper, where a conductive substrate is preferential as it limits the deposition of particles to the charged area [33]. The cone apex can break up into charged droplets, depending on the attraction of the formulation contained within the jet to the substrate collector [26].

It can therefore be concluded that various particulate structures can be engineered by fine-tuning the components of the ES set-up. Nozzle geometry as well as the use of multi-needle spinneret arrangements have a significant effect on the subsequent particle characteristics which can be modified for optimal drug delivery, targeting or imaging aspects. Substrate adjustments can also alter resultant particulates. Modifying these components can ultimately generate complex microparticles or nanoparticles for targeting specific anatomical sites, all of which will be discussed further in this review (section 8).

5. Engineered particulate structures

Manipulation of the EHDA system, (specifically for ES) to deposit microparticulate and nanoparticulate materials can potentially fabricate an array of specialised structures (Figure 3). The unique conformation of such particles including intricate features distinguish them from ordinary matrix based particulate structures. This is due to significant control over their shape, surface morphology, composition and overall particle chemistry. Technological advancement in a number of novel pharmaceutical applications has meant that the synthesis of desired nanoparticles of certain shapes and sizes are significant to complement drug delivery devices and enhance the particle engineering process [100]. The ES technique has been previously demonstrated as possessing the capability to produce smooth spherical particulates [101], [102], [103]. However, by the exploitation of various parameters and the adjustment of the spraying nozzles employed, a variety of decorated particulate structures can be fabricated [104]. These particulate systems can potentially exhibit several morphologies [105] including

donut shapes [106], nanocups [107], hollow microspheres [108] as well as cell-mimicking particles [109] as demonstrated in Table 1.

5.1 Active polymer composite systems

ES is an advanced emerging technique for the preparation of microparticles or nanoparticles (including microspheres or nanospheres and microcapsules or nanocapsules) [110]. Some delivery systems only comprise two materials once the base solvent has evaporated: the polymeric matrix and API. The polymeric matrix acts as a carrier in which the active is dispersed or embedded. Ibuprofen has been embedded into zein microstructures using ES. Here, Li *et al* coated an epoxy resin around a traditional 20G needle tip for engineering. Conventional ES employing a standard 20G stainless-steel needle decreased the productivity of the process due to clogging. In comparison, implementing an epoxy coated spraying head prevented any clogging at the needle tip and ultimately generated microparticles exhibiting homogenous structures and a narrow size distribution. *In-vitro* dissolution tests of electrosprayed ibuprofen microstructures demonstrated a sustained release profile with a smaller burst release when compared to traditional ES (stainless-steel needle). Active-polymer loaded structures (into a single matrix material) can therefore be successfully developed to expand the applications of ES to novel drug delivery research [111]. Furthermore, ES paclitaxel as a polymeric particle system for the potential treatment of malignant glioma has been shown. In this study, Xie *et al* used paclitaxel and various polymer types (PCL and PLGA) as well as different polymer ratios to fabricate microparticles containing the active and a polymeric material. Cell cycling studies indicated promising results including an 80% encapsulation efficiency for all samples and a sustained release profile over a period of 30 days, therefore demonstrating ES as an efficient technique to fabricate an array of drug delivery systems based on a drug-polymer composite system [36].

5.2 Active-multi excipient composite systems

Composite structures comprising multiple excipients and the API can also be prepared *via* ES. These then transfer functional properties of excipients into nano or microparticulate systems. For instance, timolol maleate was encapsulated within various polymers and solidified chitosan was employed as a permeation enhancer, which when atomised using ES, resulted in the development of stable ocular lens coating. The flexibility to modify the polymeric formulations allowed controllable release of timolol maleate for improved permeation through the cornea and highlighted the advantage of ES for polymeric based dosages [112].

In addition, a study by Liu *et al* utilised the coaxial ES method, where an epoxy coated concentric spray was used for the fabrication of core-shell microparticles. In this, nanocomposites were formed from a PVP matrix and acyclovir distributed within the inner core, while the outer core consisted of sucralose and the organic compound sodium dodecyl sulfate which acted as a transmembrane enhancer. *In vitro* dissolution studies indicated that the solid dispersion composites rapidly released the active, thereby enhancing water solubility of acyclovir as well as increased permeation across porcine sublingual mucosa [113].

5.3 Multi-layered structures

ES as a technique for preparing multi-layered structures for drug delivery-based systems is a complex yet effective approach. This advanced technique can improve the size and morphology of polymeric matrix carriers, including the formation of either liquid or solid layers. Multi-layered structures can be prepared using the coaxial ES mode consisting of a triple-needle device. Labbaf *et al* successfully demonstrated the fabrication of spherical multi-layered particles by the employment of three liquid streams (containing the polymers PLGA, polymethylsilsesquioxane (PMSQ) and polycaprolactone (PCL)), which flowed simultaneously and resulted in a cone-jet at the needle orifice. A near monodisperse particulate system was achieved by the addition of three layers all combined in a single-step process. The prepared tri-layered system was found to have applications in sustained and prolonged drug release [114]. In addition to this, microencapsulation techniques have also been incorporated in the ES method to develop multi-shell capsules as shown by Kim *et al*. In this study, three immiscible flowing liquids (ethylene glycol, 4-hydroxybutyl acrylate and olive oil) when atomised using the ES set-up, resulted in multi-shell encapsulation [115]. Multi-layered encapsulated structures can provide valuable characteristics including homogenous monodisperse size distributions, tailored drug release patterns and a protective core in hostile environments (extreme pH levels, enzymatic degradation and temperature) for sensitive therapeutic agents.

5.4 Live-cell entrapment based structures

Living cells existing abundantly in nature can be entrapped within an ES set-up (more commonly referred to as bio-ES) and represents a revolutionary technique in the field of drug delivery research. The EHDA method requires ambient conditions of temperature, humidity and pressure, and is therefore a beneficial process for sensitive biomaterials and living cells [40]. This has been explored by Ma *et al* where pancreatic islet cells were isolated from

Sprague-Dawley rats and encased within core-shell hydrogel microcapsules by the coaxial ES mode. A simplistic atomisation step allowed islet cell entrapment within the core region of the microcapsules whilst masking the cells, resulting in immuno-protective properties and improved encapsulation [116]. Bio-ES can be employed for the fabrication of potential carriers such as biological materials including bacteria and viruses. In one study, adenovirus was encapsulated within alginate beads by the ES process. ES was shown to be an effective approach due to tuneable characteristics in which alginate concentrations, flow rate and voltage could be adjusted to optimise the fabrication of cross-linked adenovirus alginate beads [117]. As a result, adenovirus exhibited release in a controlled manner from the alginate carrier over a period of seven days to target cancerous cells. This study emphasised the role ES plays in improving protection and delivery for virion related release and could therefore have potential applications for advanced technologies in vaccine development [118].

5.5 Cell shaped particles

The unique approach of using polymeric particles that mimic cells can be utilised in biological research to reduce intracellular toxicity and cell variations.

The optimisation of several characteristics of solutions involved in the ES process (e.g., solvent evaporation rate, density and surface tension) can lead to the production of different particulate structures such as cell shaped particles. For instance, one study found a positive correlation between particle morphology and physical properties of the solution (e.g., solvent evaporation and polymer diffusion) [119]. In situations where the solvent rapidly evaporated from the droplet surface at the needle apex of the ES set-up, Lee *et al* found that the generated specialised structures possessed a biconcave discoidal shape comparable to that of the human red blood cell. Uniform red blood cell shaped particles were also fabricated during ES using the biopolymer chitosan [109]. Ju *et al* developed a stable process by monitoring solvent evaporation during ES, where they identified that the concave-like morphology of particulates was obtained due to solvent diffusion which occurred prior to the deposition of electrosprayed particles on the grounded substrate [109].

5.6 Spindle shaped particles

In addition to conventional spherical particle shapes, other shape of particulate structures can be fabricated *via* ES. ES can generate particles with novel structural configurations including anisotropic particles (particles that possess unique shapes with directional interactions) (e.g.

spindle-like structures) and non-anisotropic morphological particles (e.g. rod shaped particles). Spindle-shaped particles have been fabricated in the field of nutraceuticals by a study conducted by Khoshakhlagh *et al*. In this, researchers investigated the atomisation of an emulsion formed between D-limonene, Alyssum homolocarpum seed gum and Tween 20, which, when electrosprayed, under high electrical conductivity resulted in spindle-like morphologies and ultimately nanocapsules. These heterogenous structures demonstrated high encapsulation efficiencies (73.4%) and improved storage stability in harsh conditions [120].

5.7 Rod shaped particles

Other non-anisotropic morphological structures including rod, disc and toroidal shaped particulate systems can be prepared using ES. Biodegradable microparticles were fabricated by Bhaskar *et al* employing a side-by-side ES configuration. The prepared particles were generated by the co-jetting atomisation technique using PLGA polymers which resulted in distinct shapes including rods, discs and spheres. In particular, the formation of a Taylor cone at the needle apex resulted in rapid evaporation (solidification of the travelling jet) and consequently the fabrication of rod-shaped particles. These novel particles can be utilised for several multifunctional applications in drug delivery and medical imaging [121].

5.8 Donut shaped particles

Donut-like structures are another type of particulate system demonstrating unique morphology which can be engineered by the ES method. Xie *et al* used the EHDA system to prepare paclitaxel loaded polymeric microparticles for local and sustained delivery. Different solvents were utilised all of which when atomised, resulted in a variety of morphologies including donut shaped particles, spheres and corrugated particles. These particles inhibited the proliferation of C6 glioma cells, thereby reducing the malignant tumor growth rate. The resultant electrosprayed drug delivery devices demonstrated sizes ranging from 100 nm-10 µm and an encapsulation efficiency of ~80% hence finding applications in sustained delivery of anticancer therapeutics [36]. Single and coaxial ES were also employed for the preparation of Eudragit L100-5 polymeric nanoparticles loaded with prednisolone. When electrosprayed, toroidal donut-like structures were fabricated possessing a narrow size distribution. Dissolution studies revealed a site-specific release of prednisolone for the targeted treatment of inflammatory bowel disease and colorectal cancer [106].

5.9 Nanocups

Titania nanocups were engineered by the employment of titania isopropoxide (TIP) with polyvinyl acetate (PVAc) [107]. The combination of these materials when electrosprayed resulted in the formation of nanoparticles, where a depression formed in the solid structures generated a particulate system that displayed definitive cup-like morphology. The unique structures had a size distribution ranging from 200-500nm. Additionally, the model protein bovine serum albumin (BSA) was successfully loaded into the nanocups, where loading and release profiles of the nanocups demonstrated sustained release. Electrosprayed titania nanocups displayed higher protein adsorption and a gradual release compared to Titania nanoparticles and can therefore be considered as a novel system for protein drug delivery [107]. Similarly, Park *et al* also synthesised titanium oxide nanocups *via* ES using titanium tetraisopropoxide and the synthetic polymer polymethylmethacrylate (PMMA) as prospective drug delivery devices. The appearance of the particles demonstrated cup-like profiles where cavities in the structures were clearly observed, these were potentially generated during ES where solvent evaporation caused phase separation [122].

5.10 Hollow microspheres

ES can also be utilised to generate different types of hollow polymeric micropsheres. For example, Chang *et al* prepared PMSQ hollow polymeric microspheres which encapsulated the core liquid perfluorohexane (PFH). The close monitoring of certain parameters included in the ES set-up resulted in different particle properties including variations in particle diameter, shell thickness and particle uniformity. This demonstrated the capability of EHDA to successfully obtain a versatile range of hollow microspheres [108]. These types of particulate systems can be utilised in microencapsulation techniques for the protective coating of various molecules, to control their release as well as shielding them from extreme environments. Thus, these systems can be employed as controlled drug delivery systems for various applications in the medical and biological industry [123].

In a study by Zhou *et al*, coaxial ES was employed using a formulation consisting of PCL/chloroform (5 wt. %) (shell) and PEG/chloroform (15 wt.%) (core). In this, researchers reported the use of various solutions which when accompanied with the coaxial ES atomisation method, fabricated hollow particulate systems. Further experimentation using ethanol as a collecting substrate formed single-hole hollow microspheres due to solvent evaporation from the needle apex and eventual desolvation of the PCL carrier, consequently leading to hollow-

like structures. The research study highlighted the importance of hollow microparticle based systems for an array of applications. For example, hollow microspheres could function as tumor cell mimicking phantoms, designed to replicate characteristics of biological material and can therefore be identified as crucial structures for anti-cancer therapeutics [124].

5.11 Porous microcarriers

In addition, other structural conformations which can be fabricated by ES include particles which display a well-connected network of pores on the solidified surface [125]. Wu *et al* investigated a novel ES technique, where they studied the influence of applying various atmospheric pressures in contrast to the conventional ES method which employs ambient pressure conditions. By tuning the ES parameters discussed earlier (section 3), solvent evaporation and subsequently the solidification process, resulted in PCL polymeric particles which exhibited porous geometry on the surface. Researchers deduced that the porous structure of the particles was a result of phase separation at the spinneret which contained an electrostatically charged jet. It was concluded that adjusting the atmospheric pressure utilised during ES can be seen as a vital parameter and is particularly useful for sensitive drug delivery methods [126]. In addition, porous particulate systems can also be fabricated for other applications, for example, ES was utilised as a versatile technique for the preparation of inhalable porous microspheres. In this study, an anti-inflammatory drug (oridonin) was loaded into polymer coated microspheres which resulted in porous-like structures during ES. The porous structures demonstrated high anti-cancer effects (attributed to high lung deposition of oridonin-loaded electrosprayed porous microspheres) due to their geometry. The delivery system demonstrated high efficiency of inhalable drugs deep into the lungs and the subsequent rapid release of them to the surroundings. Inhalable porous microcarriers could therefore be utilised for applications in targeted drug delivery for the treatment of lung carcinomas [37].

5.12 Janus particles

Janus particles are types of unique microparticles or nanoparticles which contain anisotropic structures where the surface is exposed to the environment. Due to the surface chemistry of these particles, Janus shaped structures have an array of functional characteristics which can be applied to a diverse range of particle engineering fields. Rahmani *et al* designed nanoparticles with controlled characteristics using electrohydrodynamic co-jetting. Fabricated Janus particles exhibited a mean diameter of 105.7 nm and contained additional functional groups on the particle surface for further modification. Hence, bicompartmental nanoparticles

like Janus structures can be successfully applied as drug delivery carriers as well as diagnostic imaging agents [127]. Others have gone on to further strengthen the claims of utilising Janus particles for use as imaging agents in biomedical applications. Uniformly sized compartmentalised Janus particles containing both rose Bengal dye and the anti-cancer drug carmofur in separate compartments were prepared using side by side ES. The particles demonstrated a narrow size distribution, and the great potential these particulate systems exhibit since their surfaces can be modified to include several sections, which are tailored for segmented targeting for a number of drug delivery vehicles and photodynamic therapy [128].

5.13 Other unique morphologies

5.13.1 Strawberry shaped particles

A novel method for the preparation of particulates was also reported in which silver nanoparticle-doped SiO_2 microspheres were generated using the EHDA technique [129]. Scanning electron microscopy images revealed peculiar morphologies of particles which were strawberry-like and mirrored aggregates of achenes found on the surface of strawberries. The engineered particles demonstrated efficacious inhibitory effects against the *Escherichia coli* bacterium due to the silver nanoparticles embedded within the microspheres. This resulted in increased antibacterial activity of the fabricated structures, these structures could therefore be utilised as antibacterial materials for biomedical applications.

5.13.2 Fibril shaped particles

Particles resembling fibril-like shapes can also be prepared using the ES method as shown by Khanum *et al*. In this study, an array of solvents and a thiophene derivative (7,9-di(thiophen-2-yl)-8H-cyclopenta[a]acenaphthylen-8-one) (DTCPA) were used in the ES process to generate spike-spheres and spike morphologies which imitated fibril particulate structures. The study brought to light how the ES process can be potentially employed for the novel fabrication of organic molecules, and thus could be applied to photoactive materials for biomedical applications [130].

5.13.3 Yolk-shell particles

Recently a novel EHDA system consisting of a tri-needle coaxial device was developed. By the use of ES, Zhang *et al* fabricated a multicomponent particulate system consisting of particles that displayed unique yolk-shell like morphology. These were comprised of magnetic

nanoparticles, silicone oil and a polymeric core. Multiple model probes when co-encapsulated into these particulate systems demonstrated an array of release profiles which could be fine-tuned *via* the employment of an external auxiliary magnetic field. Owing to their compartmentalised structure, the engineered magnetic polymeric yolk-shell particles could be implemented for multiple drug loading as well as applications in dual imaging modality [131]. The delicate interplay of several parameters involved in the ES technique allows the precise control of the size, size distribution, chemical compositions and morphological characteristics of the particulate system. Effects of parameters such as material modification, nozzle design, types of substrates used, voltage, flow rate and working conditions (e.g., temperature, humidity and pressure) all contribute to the several types of architectural particulate structures that can be developed. It can therefore be concluded that ES provides an exceptional platform to fabricate an array of elegant structures all of which have been thoroughly discussed above as well as their applications in a plethora of drug delivery fields. Hence, ES is elucidated as a prospective facile technique for the future development of opportunistic particulate systems for addressing significant challenges faced by the pharmaceutical engineering industry.

6. Active agent selection

Microparticles and nanoparticles are attractive drug delivery systems that can be employed for a wide range of applications. They offer interesting advantages including a high surface area, ability to encapsulate large amounts of active molecules, biodegradability and capability of achieving controlled release [132]. The use of ES to produce microparticulate and nanoparticulate structures provides a plethora of advantages as it is a one-step, versatile system which operates under ambient conditions. This allows an increased number of actives and biomaterials (e.g. proteins) [133] and other APIs which are sensitive to elevated temperatures or shear stress to be processed and encapsulated within a system [134]. EHDA and specifically ES, have a significant impact on API entrapment, solubility and release which will be discussed.

6.1 Choice of active pharmaceutical ingredient and other molecules

The aqueous solubility of APIs is critical for drug efficacy, specifically when the drug is to be orally administered. Low solubility results in inconsistent absorption, decreased bioavailability and slower onset, thus limiting their use in pharmaceutical applications. A handful of methods have been developed over the years to improve solubility through modification at either the

molecular, colloidal or particulate level. ES works by modification on a particulate level and more specifically *via* nanonisation and amorphisation. Drugs with a low solubility are often processed into microparticle or nanoparticle formulations to enhance their low bioavailability. A substantial number of APIs are poorly water soluble, however through use of ES in combination with a formulation consisting of a stable emulsion (a hydrophilic polymer, surfactant and water-insoluble API), it is possible to produce microparticles with improved solubility [68]. Moreover, ES is an interesting technique with great capability of enhancing the solubility of poorly water-soluble actives *via* amorphisation. Through the use of electrical forces, formulation containing the API is atomised upon which the solvent rapidly evaporates, and the droplets almost immediately solidify. APIs are left in an amorphous form, partially due to the rapid solvent evaporation. When in an amorphous form, APIs have increased free energy as well as a larger surface area due to the submicron particles, thus improving the solubility of poorly soluble molecules [66]. For example, Bohr *et al* loaded the poorly soluble drug Celecoxib into PLGA microspheres using ES. Resultant microspheres were near-monodisperse (1-5 μm) and displayed a smooth morphology. Differential scanning calorimetry confirmed that the Celecoxib was entrapped in an amorphous form in electrosprayed PLGA microspheres. Celecoxib loaded microspheres showed a bi-phasic release pattern (initial burst release followed by sustained release). This research demonstrates the potential use of ES in entrapping poorly soluble drugs into particulate drug delivery systems and as a result improving their solubility [135].

6.2 Stability of biomolecules and larger entities

Therapeutic proteins for pharmaceutical applications are problematic to process due to their specific properties and functions. Often methods (e.g. primary emulsion) of manufacturing and processing proteins result in protein denaturation and aggregation as they deploy harsh conditions. In contrast, ES encapsulates actives under ambient temperature and pressure making it a favourable method for processing sensitive drugs or biomolecules (proteins, peptides and cells).

For example, Coaxial ES was used in several studies to encapsulate different types of proteins into biodegradable polymeric microcapsules under ambient conditions, overcoming the limitations associated with the primary emulsion technique [136], [133] and [137]. ES was used by Yu Fuki *et al* to electrospray a sodium alginate aqueous solution directly into a 0.5 wt % chitosan aqueous solution resulting in the fabrication of polyelectrolyte complex

microcapsules. The produced microcapsules exhibited a narrow size distribution and controlled diameters ranging from 80-230 μm. The research group were successful in encapsulating protein, dextran and a polymeric microsphere individually within the polyelectrolyte complex with encapsulation efficiencies >99%. Yeast was also encapsulated in the polyelectrolyte microparticles *via* ES, where it was found that the encapsulated yeast preserved its activity. This shows the possible advantage of using ES for the encapsulation of physiologically active substrates as their activity is not hindered during the process [138]. Enzymes are other sensitive biomolecules that can be encapsulated using ES in order to improve their stability and bioavailability. For example, Fung *et al* successfully electrosprayed Coenzyme Q10 loaded Copovidone microparticles (3–5 μm) to improve Coenzyme Q10 solubility and bioavailability. *In-vitro* studies showed a reduction in both the crystallinity and particle size which could improve bioavailability. Dissolution studies and *in-vivo* oral bioavailability were assessed using a murine model, where the electrosprayed particles demonstrated improved dissolution properties and oral bioavailability respectively when compared to both the raw materials and physical mixture [139].

6.3 Encapsulation of small and volatile/sensitive actives

Nanoencapsulation is the process by which a bioactive compound is loaded or entrapped into a carrier matrix or material in the nano range [140]. ES allows for increased encapsulation efficiency owing to the absence of an external medium which permits migration or dissolution of water-soluble molecules [137]. Thus, ES is a useful technique in producing a drug loaded particulate based delivery systems with high payloads.

Loading of APIs into mesoporous materials (such as silica) has been a favourable technique to improve drug solubility, permeability and bioavailability [141]. However, conventional loading techniques that are used to load drugs into a mesoporous matrix usually lead to low entrapment efficiency. Recently, ES was explored as a loading technique to incorporate poorly water-soluble drugs into a mesoporous silica matrix. Sayed *et al* utilised ES to encapsulate KAZ3; a novel poorly water soluble chalcone with anticancer properties, into mesoporous materials (SBA-15 and MCM-41). This loading method was compared with the conventional common solvent impregnation loading process. It was found that through using the ES technique, KAZ3 was successfully loaded within the pores of the silica particles with a high encapsulation efficiency in an amorphous form. Solvent impregnated formulations indicated a lower encapsulation efficiency and partial crystallinity of the loaded drug. In addition, drug

dissolution studies demonstrated a 30-fold improvement in drug dissolution for the ES loaded particles when compared to the pure drug. Moreover, *ex-vivo* studies showed formulations to have increased the permeability across murine intestine in comparison to the solvent impregnated formulations or pure drug. This research, for the first time, highlights the ability of ES to produce loaded mesoporous silica particles with high payloads, an improved dissolution profile and enhanced permeability across the intestine [1].

6.4 Drug loading

Limited drug delivery efficiency occurs as a result of poor encapsulation, however, ES has been used as a prospective technique to increase encapsulation efficiency whilst protecting drugs from degradation [63]. Often, polymeric and lipid nanoparticles are used as a means of transport as well as encapsulating APIs and proteins, largely because they possess the capacity to protect the active from environmental conditions which often results in degradation [132]. In a study by Zamani *et al*, BSA was used as a model protein and loaded into PLGA nanoparticles *via* two different ES methods; co-axial ES and emulsion ES. It was found that the solvent, PLGA molecular weight, and PLGA concentration influenced the particle diameter, producing particles between 3.0-5.5 μm. The electrosprayed particles displayed a core-shell structure, where the encapsulation efficiency of structures fabricated *via* co-axial ES was found to be significantly higher (69.2% and 71.8%) than those electrosprayed using emulsion ES (53.5% and 46.7%) for both high and low molecular weight PLGA respectively. In the initial 24 hours, a small burst release of 8-12% was displayed in emulsion electrosprayed particles, in comparison, those which were co-axially electrosprayed had a core-shell structure which demonstrated a release of 24-27%. This study highlights the capability of the ES process to fabricate protein particles which display both a uniform size distribution and high encapsulation efficiency making them advantageous for use in the drug delivery remit [142].

6.5 Release kinetics

ES has shown to control the shape, size and morphology of fabricated particles, all of which heavily impact the rate of degradation, drug diffusion and therapeutic effect of actives [143]. Formulation scientists have employed various strategies to control the active release and dissolution in order to improve their solubility, to sustain their release or to target the site of action. These strategies include the use of amorphous solid dispersions [144], inclusion into mesoporous silica [1], nanosizing [145], and ES [146]. Occasionally, a targeted regiospecific release is required, for example, when site specific administration is necessary in the

gastrointestinal tract. Coaxial ES is advantageous in the processing of such formulations as it fabricates monodisperse micro sized or nano sized amorphous solid dispersion particles. These particles can be subsequently coated with a responsive polymer, ultimately dissolving and releasing the encapsulated active at the required site. For example, Smeets *et al*, utilised coaxial ES to fabricate core-shell microparticles in which an amorphous solid dispersion of hydroxypropyl methylcellulose (HPMC) or PVP based matrix was used. The antiviral drug Darunavir was coated using a gastro-resistant polymeric coating (co-polymer poly(methacrylic acid-co-methyl methacrylate)) which is insoluble at a low pH of the stomach but dissolves at higher pHs of the intestine. Thus, the polymeric coating prevented the premature release of the drug in the stomach, protecting it from degradation and ensuring delivery of the active to the site of absorption (intestine). This research demonstrates the applicability of coaxial ES in the fabrication of core-shell particles as a delayed release dosage form [144].

6.6 *In-vitro* and *In-vivo* performance of actives

Aforementioned, through careful adjustment of process and solution parameters, numerous particulate systems with different structures can be obtained [40]. Hao *et al*, employed coaxial ES for the fabrication of aspirin loaded nanoparticles with an enteric coating for sustained release. A pH responsive polymer (Eudragit L100-55) was used as an outer coating while a sustained release polymer (Eudragit RS) was chosen for the inner core. Through altering the flow rate of both the inner and outer solutions, a maximum loading capacity of 23.66% was observed for the nanoparticles and an entrapment efficiency close to 100% was achieved. *In-vitro* release was carried out *via* a method previously used by Hosny *et al*, [147]. *In-vitro* release studies demonstrated both pH sensitive and sustained release which occurred with less than 5% of the aspirin being released in the gastric simulated fluid. A change in the pH to 6.8 resulted in a substantially increased release rate. Sustained release was observed in the simulated intestinal fluid where more than 95% of the aspirin was released from the nanoparticles over 120 hours (5 days). This study demonstrated how coaxial ES can be exploited to produce core–shell nanoparticles with an enteric coating to provide sustained release whilst also protecting the stomachs delicate lining from the side effects of aspirin [148]. A site-specific delivery system consisting of gambogic acid nanoparticles was produced using ES. A poly(D, L-Lactic Acid (PDLLA) matrix was used and gambogic acid was successfully encapsulated. *In-vivo* liver distribution studies were carried out alongside pharmacokinetic profiles and anti-tumor efficacy tests for hepatocellular carcinoma. Results highlighted the advantages of using ES to fabricate gambogic acid particles to attain substantial antitumor efficacy as well as overcoming

limitations associated with gambogic acid such as poor water solubility and side effects due to its pharmacological toxicity [149].

Through the above-mentioned studies, ES can be demonstrated as a versatile technique which can be used to process several APIs and sensitive biomolecules. Thus, the ES process enables particle sizing and structure enhancement to improve solubility of APIs, therefore improving bioavailability as well as obtaining desirable drug release profiles. In addition to this, ES can be used to encapsulate sensitive and toxic molecules to protect them from the biological environment and minimise adverse drug reactions respectively.

7. Dosage design

Dosage design plays a key role in developmental and engineering processes for active particulate systems. This entails the consideration of several physiochemical and biological characteristics of the drug as well as their ultimate targeted application when fabricating drug delivery systems. Establishing the required dosage design during ES involves the careful monitoring of relevant parameters (e.g., polymer matrix concentration, solution type, flow rate and applied voltage) which ultimately define the physiochemical characteristics of the resultant particulates. ES can serve as a prospective technique where the interplay between parameters can fabricate both nanostructures and microstructures with improved biopharmaceutical performance, ultimately spanning several targeted therapies. These can include an arsenal of different systems such as matrix, multi-layer encapsulation and bio-responsive strategies.

7.1 Matrix systems

Matrix drug delivery particulate systems generally implies delivery devices in which the API is homogenously dispersed either molecularly or in the solid state into a polymeric carrier. Formulating drugs as a matrix type dosage form can aid in attaining different release profiles and is particularly useful for drugs with short half-lives which are eliminated from the bloodstream at a quicker rate. For instance, a study by Cavalli *et al* investigated the use of the ES technique to fabricate spherical poly(amidoamine) (PAA) PAA-cholesterol nanoparticles by carefully fine-tuning the applied voltage and flow rate parameters. In addition, tamoxifen, an anti-cancer therapeutic for the treatment of breast cancer was molecularly dispersed within the PAA-cholesterol conjugate matrix. The nanoparticles exhibited a drug loading efficiency

of 40% and a prolonged drug release profile. The study highlighted the effectiveness of ES as a credible technique for fabricating matrix-based system nanoparticles. More specifically, the work was conducted in a single-step without additional excipients and therefore concluded as a cost-effective method which can be implemented in the commercial industry [150].

7.2 Layered particulate systems

Dosage forms designed with layered particles can provide a vast array of advantages; a protective shell to sensitive bioagents embedded within the core and encapsulating drugs within a layered system to enable a variety of release kinetics. The coaxial ES method can be utilised for the production of core-shell particulate structures in the nanometre or micrometre size range [151]. These innovative structures play an important role in designing dosage forms with required attributes for the pharmaceutical industry. Core-shell particles can aid in formulating dosage forms for several purposes including; protecting actives from degradation (stomach acidity) , an array of release profiles for example, immediate release [152], targeted release [153], and sustained release [154] or co-delivery of different actives [155]. For instance, a fast onset dosage form can be designed by ensuring an immediate release of the active by coating it with a thin layer of fast-dissolving polymeric shell [152]. In contrast, a sustained dosage form can be obtained using a slowly degrading solid lipid shell [154]. Moreover, a delayed onset dosage form with a targeted therapeutic action can be attained by coating the core structure with a responsive polymer that only dissolves at the required site of action under the response of physiological stimuli [144]. Layered particulate structures are also promising in combining different actives in a single dosage form, thus capable of achieving delivery of different types of actives simultaneously [155].

Yu *et al* electrosprayed helicid loaded nanoparticles in order to improve its poor aqueous solubility, bioavailability and onset of action. A core-shell structure was fabricated consisting of the biopolymer shellac at the core enveloped by a PVP-helicid shell, ultimately resulting in solid composite nanoparticles. *In-vitro* studies demonstrated a faster dissolution of helicid from the core-shell particles due to the presence of the active which exhibited amorphous characteristics within the PVP layer. Moreover, the presence of a thin solid nanocoating on the particles enabled immediate release of helicid (one minute) compared to raw helicid where only 11.4 ± 4.2% of the active dissolved into the dissolution medium (after a period of 30 minutes). The results of the study indicated that the core-shell matrix composites displayed

characteristics of rapidly dissolving dosage forms and could therefore alleviate symptoms at a faster rate when processed using ES, specifically employing a dual concentric spray nozzle [152].

A novel EHDA technique was proposed by Labbaf *et al* but rather consisting of a four-needle device in comparison to the conventional single conductive needle utilised during ES. A four-layered particulate system was developed simultaneously, in which each layer was comprised of different organic polymers, namely: PLGA, PCL, PMSQ and PEG. When electrosprayed, particles displayed a smooth spherical morphology, a mean particle size of 620 ± 150 nm and a polydispersity index of 26%. To determine if four layered structures had potential significance in the pharmaceutical remit, dye release studies were conducted, revealing different release rates due to a variation in thickness of each polymeric layer. It was elucidated from the study that modern therapeutics could benefit from a multilayer delivery system as a controlled release dosage form could be generated by adjusting the ES processing parameters [101].

Similarly, multi-layered structures were generated using a triaxial electrospray system by Kim *et al*. In this study, triple layered capsulated shells were prepared with the use of acetonitrile and trifluoroethanol solutions containing biocompatible polymers (PLGA and PDLLA) in a single-step process. The assembled system consisted of a triaxial nozzle where the generation of a stable Taylor cone at the needle apex resulted in multi-shell capsules. The anti-cancer drugs Doxorubicin and Paclitaxcel were loaded in the innermost and intermediate shell respectively, with a high entrapment efficiency (>80%). *In-vitro* release profiles for both drugs from fabricated capsules demonstrated zero-order release kinetics with minimal burst release. The retention of both anti-tumor drugs within the shell of the capsulated structures could be manipulated by varying the shell thickness and mean diameter. This was achieved by fine-tuning the flow rate parameter of the ES set-up as well as the concentration of the polymeric formulation. Thus, it was gathered from the study that ES is an amenable process where tailoring the solution (polymer concentration) and process (flow rate) parameters can result in multi-layered structures for applications in controlled drug release [156].

7.3 Targeted systems

Different active agents can be utilised during pharmaceutical engineering to generate dosage forms with a plethora of therapeutic effects. For an efficient dosage form design, other

pharmaceutical excipients (e.g., penetration enhancers, targeting ligands or solubility enhancers) can be incorporated within the API to achieve desired pharmacological characteristics. ES can serve as a viable technique to fabricate particulate systems by refining the operating parameters of the ES mode and hence load actives in conjugation with different types of excipients in a single-step. Thus, ES is capable of improving the *in-vivo* performance of drug moieties involved, for example, improving their onset of action [134], cellular uptake [157], bioavailability [152] and targeting ability [158].

Hyaluronic acid-ceramide and solubility enhancer Soluplus® were electrosprayed to form nanocomposites for tumor targeted drug delivery. Lee *et al* fabricated nanostructures where the anti-cancer stibenoid resveratrol was enveloped within the nanoparticulate system and when atomised demonstrated a narrow size distribution (230 nm). Drug entrapment studies calculated a high entrapment efficiency where 80% of the active resveratrol was successfully entrapped within the nanocomposite. Here, hyaluronic acid-ceramide was employed as a targeting moiety to interact with the CD44 receptor, a cell-surface glycoprotein upregulated in breast cancer. *In-vivo* studies revealed that the electrosprayed resveratrol-Soluplus® nanocomposites displayed a higher cellular uptake and cancer targeting ability when compared to the raw resveratrol-Soluplus® nanocomposite. From this it was confirmed that the tumor targeting drug delivery system benefitted from utilising the ES process as atomised composites demonstrated decreased *in-vivo* clearance, sustained drug release patterns and extended blood circulation times of resveratrol, all which can be deemed favourable for tumor targeted drug therapy [158].

Grafting targeting ligands (e.g functional groups or monoclonal antibodies) on the surface of engineered particulate delivery systems is a unique approach to target specific tissues or cells where the therapeutic action of an active is required [159]. Grafting ligands on preformed nanoparticles usually requires a chemical conjugation reaction which is time consuming, expensive and sometimes leads to loss of the loaded active. However, ES is a unique procedure that can attach ligands on drug loaded particles under ambient conditions *via* a single-step, thereby designing a dosage form with an improved therapeutic action and a reduced toxicity profile [160]. These particles are capable of selectively binding to structures (antigen or receptors) that are upregulated only in diseased tissues, hence enhancing cellular uptake and protecting healthy tissues from the toxicity of actives.

Aptamers can also be utilised to promote and augment the targeting ability of drugs by binding to specific target molecules. Docetaxel, also known as Taxotere is a clinically established chemotherapeutic drug used for several treatments including cancers of the lungs, stomach and ovaries. It does however come with complications owing to its poor aqueous solubility, low bioavailability and increased toxicity, thus limiting its use in anti-cancer therapeutics [161]. ES was employed by Ghasammi *et al* to potentially overcome drawbacks associated with Docetaxel and improve its physiochemical properties. Docetaxel loaded nanoparticles were prepared using Ecoflex and PEG 6000 at a flow rate of 1mL/h and were subsequently attached to a HER-2-specific aptamer (for site-specific delivery), eventually yielding aptamer targeted Ecoflex nanoparticles. During analysis, the electrosprayed delivery system displayed higher cellular uptake and anti-cancer efficacy in comparison to non-targeted Taxotere loaded nanoparticles. ES was therefore concluded as an efficient technique for preparing targeted drug delivery systems, in particular, those involving aptamer-based technology [157].

7.4 Systems hosting toxic agents

ES has also shown its potential for the delivery of cytotoxic agents where employing the novel technique can fabricate particulate systems with reduced toxicity, therefore establishing suitable drug safety profiles for clinical applications. Cytotoxic drugs exert unselective toxicity against both cancerous cells as well as healthy living tissue [40]. Moreover, during ES, solvents play a fundamental role in the formulation process, however toxic solvents can damage biomolecules (genes, enzymes and proteins). ES can be fine-tuned to produce particulate-based dosage systems where issues associated with toxic drugs and solvents can be overcome whilst maintaining therapeutic effects.

Cisplatin is a standard anticancer drug used in chemotherapeutic treatments for several malignant tumors. However, due to its significant toxicity it can trigger a number of lethal side effects including kidney dysfunction [162] and thus, its therapeutic use in a number of applications is hindered. ES can be employed as a potential technique to fabricate delivery systems for cytotoxic agents such as cisplatin, thereby diminishing clinical limitations associated with cytotoxic drugs. Parhizkar *et al*, for example, utilised single-needle ES for the atomisation of PLGA polymer and cisplatin into spherical particles exhibiting smooth surface morphology and a high encapsulation efficiency (>70%). Through the use of ES, cisplatin was encapsulated within a polymeric matrix, thus protecting it from possible degradation within the biological environment. In addition to this, particles produced *via* ES which are in the nano

range often have prolonged circulation times. Initial sharp release is undesirable for cytotoxic drugs as this often does not reduce side effects related to premature delivery. Drug release kinetic studies revealed that nanoparticles with reduced sizes demonstrated a biphasic release profile; initial burst release followed by sustained release. This is beneficial for cytotoxic drugs as dosage forms must be designed to reduce the risk of adverse events which could affect patient safety. Moreover, nanoparticles produced using a 10 wt% formulation displayed a release of 14% after 4 hours whereas a formulation of 5 wt% released 45% of the loaded cisplatin. Using ES, where the flow rate and applied voltage could be manipulated, resulted in tailored controlled release of drug particles. ES can therefore be considered as a potential approach to reduce toxicity of anti-cancer drugs as well as improving encapsulation capacities and hence can be applied to an array of chemotherapeutic treatments [102].

Organic solvents are essential in most techniques that aim to encapsulate actives or biomolecules into particulate systems. However, some of these biological entities (e.g., cells, nucleotides, enzymes) are sensitive to solvents and denature or degrade upon contact with them. It is therefore crucial to develop a delivery system where toxic organic solvents may be used, however, are encased within a protective layer for safe drug delivery systems. Co-axial ES is a prospective technique to protect these biomolecules or cells from degradation by solvents during the encapsulation process *via* the use of concentric needles. Esfahani *et al* for example fabricated core-shell microcapsules consisting of PLGA and chloroform/dimethylformamide by co-jetting ES technology, resulting in the successful encapsulation of live cells. Although the solvents displayed high toxicity against viable cells, the coaxial system provided an effective approach to shield biomolecules from the solvent formulation, mitigating toxicity issues and consequently providing a safe environment for encapsulating live cells. The development of such microcapsules is an innovative technology that can be useful in different pharmaceutical applications such as regenerative medicine, tissue engineering and cell-based drug delivery platforms [163].

7.5 Stimuli-responsive systems

Stimuli responsive drug delivery systems allow a site-specific release and/or targeting action. These types of systems can release their contents upon the response of different derived stimuli including external (ultrasound, light, electrical and magnetic fields) or internal (temperature, pH, ionization and enzymes) [164] stimuli. Engineering of stimuli responsive drug delivery devices using ES can enable dosage forms with high bioavailability, exceptional targeting

ability and on-demand release profiles, thus these delivery systems can be utilised as an evolutionary approach for several clinical applications. ES can be employed to design dosages with tailored drug release by enveloping the active as a core within microcapsules nanocapsules where the shell acts a responsive polymer and dissolves only in the presence of a specific trigger.

For example, a coaxial device was developed by Cao *et al* which contained a formulation consisting of Silk fibroin protein (outer needle), polyvinyl alcohol (PVA) polymer (inner needle) and the anti-cancer drug Doxorubicin (1% w/w) encapsulated within the PVA core. When infused into a coaxial system under relevant processing parameters, the formulation generated a variety of smooth core-shell structures by modifying the PVA concentration (0.3-0.5 wt%) and applied voltage (12-20kV) of the ES process. The electrosprayed core-shell particles demonstrated encapsulation efficiencies over 90%, whilst release profiles indicated an initial burst release followed by sustained release of Doxorubicin. Low intensity focused ultrasound was employed as an external trigger for tailored drug release, where groups treated with the stimulation technique displayed twice the cell apoptosis rate in comparison to the control group (no stimulus). The utilisation of compatible polymers to coaxially ES core-shell structures was concluded as a suitable method, particularly modifying the PVA/silk-fibroin ratio and the addition of ultrasound for tumor related therapy, which resulted in stimuli-responsive structures displaying double the amount of apoptotic activity in human breast cancer cell lines [153]. Atomized stimuli responsive core-shell delivery systems can therefore be a successful approach to enhance the cytotoxicity of anticancer drugs thereby reducing their adverse effects.

7.6 Bio-responsive systems

Bio-responsive systems or environmentally responsive systems are a promising stimuli responsive delivery system that release their contents upon change in the biological surrounding or in certain cellular micro-environment. These intelligent drug delivery systems deliver their cargo upon the effect of a biological stimulus (e.g., enzymes, redox, antibodies, hormones and pH), triggering a diverse range of responses which can find applications for clinical and biomedical routes. The construction of these drug delivery devices in combination with ES can enable dosage forms which can generate specific targeting and therefore elicit efficacious therapeutic doses when optimising engineered drug delivery platforms, particularly those displaying a stimuli-responsive nature.

ES can be utilized to engineer responsive drug delivery systems by dispersing the drug in a polymeric nanoparticle where the polymer can dissolve only under biological stimuli. For example, Wu *et al* designed a novel concept to fabricate bioresponsive controlled particulates using an elastin-like polypeptide polymeric solution and doxorubicin, co-dissolved in a mixture of solvents with the use of single-needle ES. The developed nanoparticles were spherical in shape and displayed smooth morphology with particle diameters within the range of 150-570 nm, influenced by the processing (applied voltage and flow rate) and solution (polymer concentration and molecular weight) parameters. Doxorubicin loaded particles (20 w/w%) demonstrated pH responsive behaviour where drug release followed pH-dependant solubility of the bioresponsive polymer (elastin-like polypeptide), impacting drug release profiles due to the nature of the polymer [165]. Polymeric carriers that trigger specific stimuli for enhanced control of drug release profiles could therefore provide a suitable platform for stimuli-responsive particulate systems when employing ES in conjugation with bioresponsive materials.

7.7 Systems hosting biopharmaceuticals

Biological entities such as ligands, nucleotides or antibody-drug conjugates can provide an exceptional platform for biopharmaceutical applications. As ES is a simplistic single-step procedure under ambient conditions, it can combine the convectional solution consisting of the polymer and solvent but with additional therapeutic agents, biological entities and targeting ligands, simultaneously. Thereby generating dosage designs which can deliver drugs and biological molecules with high specificity to diseased cells.

Wu *et al* engineered a transferrin grafted lipolex nanoparticulate system loaded with oligodeoxynucleotide using coaxial ES. Lipoplex molecules are classified as synthetic carriers of API molecules or genetic material. They are therefore beneficial owing to their encapsulation properties within the hydrophobic bilayers or in the hydrophilic core of the particulate system. After co-axial atomisation, fabricated nanoparticles displayed a mean particle size of 190 ± 39 nm as well as an encapsulation capacity of 90 ± 6% (measured using gel electrophoresis). In addition, the glycoprotein transferrin was conjugated to lipoplex nanoparticles to improve nanoparticle targeting and cellular uptake. Transferrin-conjugated lipoplex nanoparticles demonstrated efficient delivery to human leukaemia cells. As a result, this downregulated the bcl-2 protein expression by 57 ± 3%, resulting in effective treatment of

leukaemia cancer cells and thereby decreasing their multidrug resistance [166]. This study clearly shows the immense potential of ES in encapsulating molecules such as nucleotides, siRNA and plasmid DNA into suitable particulate systems. Thus, designing a delivery system that can successfully play a role in treating genetic based diseases such as sickle cell anaemia, crohn's disease and cancer.

The employment of biological material which can undergo further modification such as conjugation with other actives or targeting ligands is a valuable approach in biopharmaceutical applications. Furthermore, this concept can be expanded to include dosage designs utilising drug-conjugates which can be successfully translated into gene drug delivery or tissue engineering applications.

7.8 Theranostic systems

Theranostic technology is an advanced approach in the field of pharmaceutical drug delivery and research referring to an amalgamation between the use of therapeutic agents and diagnostic applications [167]. It can include the incorporation of targeting moieties in conjugation with nanomedicines, hence leading to the development of a smart dosage system. Rasekh *et al*, for example developed an ES system incorporating a concentric design for the fabrication of a potential theranostic agent to be used in combined imaging and therapy. The co-flow system included a formulation consisting of superparamagnetic iron oxide nanoparticles (SPIONs), PEG polymer, genistein (model drug) and a fluorescent dye, which were all encapsulated within the triglyceride derivate, tristearin. Although SPIONS display exceptional applications as contrast agents for magnetic resonance imaging, the characteristics of these nanoparticles tend to form aggregates. To overcome these limitations, the combination of materials in the formulation when coaxially sprayed, resulted in encapsulated SPIONS (diameter 0.65-1.2µm). The release profiles of composite tristearin-SPIONs (single-needle electrospray) and encapsulated SPIONs (co-axial electrospray) were investigated, where encapsulated genistein exhibited a triphasic release profile, useful for controlled drug release where drugs may be required for a longer duration. The co-axial technique was found to be a facilitative approach as atomised microparticles could reduce aggregates formed on SPIONS by adjusting processing parameters and encapsulating these SPIONs with active components for magnetic resonance imaging related multimodular theranostic applications [2].

7.9 Sensor systems

ES has also been utilised to spray biologically active substances to include dosage designs for gene expression, for the detection of chromosomal aberrations in cancer therapy or to identify specific protein biomarkers [168]. Here, ES can be employed to create microarrays for the detection of several biomolecules including DNA and proteins and in turn reduce limitations associated with conventional techniques including poor flow rates. For example, Morozov *et al* electrosprayed solutions of biological molecules (proteins, DNA and dyes) through an array of voids presented in a dielectric mask, ultimately depositing multiple dots of biological material onto various grounded substrates. Fabricated microdots demonstrated a uniform deposition on the collecting substrate, displaying a mean size of 2-6 μm for resultant structures. ES was found to be a promising technique where the shifting of the di-electric mask generated a multi-component matrix and in turn enhanced the rate of deposition. In addition, biological molecules maintained their functional activity, where ultimately the atomisation process could be employed for gene profiling as well as clinical diagnostic devices for applications in diseased states [169].

In summary, intelligently prepared dosage designs focus on efficient drug uptake, release mechanisms, biocompatibility of involved materials as well as the incorporation of API which display complementary characteristics. By tailoring the solution and processing parameters, the ES technique can engineer particulate delivery systems with uniform particle dimensions, higher encapsulation efficiencies, increased cellular uptake and improved drug targeting, thus fabricating exceptional dosage forms with highly efficacious therapeutic value. ES displays clear cut advantages over existing platforms at ambient environments with all processing assembled into a single-step, eliminating complicated pre-preparation during the manufacturing process. ES therefore offers an extraordinary approach to address the emerging requirements of the pharmaceutical industry by developing smart dosage designs for multi-functional applications in theranostics, targeted therapy and stimulus-based approaches.

8. Applications of electrosprayed particulate systems

Over the years, research has been heavily focused on utilising ES for both pharmaceutical and biomedical applications. ES has been used to prepare different types of drug delivery systems such as nanoemulsions [170], nanoparticles [171] and liposomes [172], using different materials including polymers, lipids and inorganic materials [66]. Nanoparticles fabricated *via*

ES have the ability to encapsulate large amounts of drug and act as specific drug carriers owing to their active surface absorption, binding or complexation, where the size of the nanoparticles produced is critical in the therapeutic remit [173]. The chosen route of administration (ROA) heavily influences therapeutic efficacy, and each ROA presents unique advantages and drawbacks, thus often resulting in the need for a specific delivery vehicle. Electrosprayed engineered particles have been used in different drug delivery systems including; oral, transdermal, parenteral, ocular, pulmonary, buccal and topical (Table 2, Figure 4).

8.1 Oral drug delivery systems

Oral drug delivery is a favourable ROA owing to its ease of administration (increased patient compliance), and the potential of having a solid dosage form with an extended shelf life. However, the oral ROA presents a number of barriers such as degradation of drugs due to the acidic nature of the stomach. In addition to this, oral drug delivery is also limited due to the poor solubility and bioavailability of a number of drugs, low systemic availability and reduced efficacy [174] [175]. ES has been used to process a number of drugs, the incorporation of a polymeric carrier has shown to improve drug release characteristics *via* the encapsulation of drugs, thus leading to different release patterns including sustained release [173]. Through using ES for the coating or encapsulation of molecules, improved bioavailability [176] and solubility of poorly water-soluble drugs [177] can be achieved. PLGA nanoparticles have been used as a carrier for Naproxen; Yang Cao *et al*, used coaxial ES to fabricate nanoparticles of PVP/PLGA and PCL/PLGA with a distinct core–shell structure in which PLGA was used for the outer shell each time. A single-step process was employed in which both rhodamine B (hydrophilic) and Naproxen (hydrophobic) were encapsulated. A high encapsulation efficiency was observed; greater than 85% Rhodamine B encapsulated in the inner PCL core, whilst naproxen was encapsulated in the outer PLGA shell. Different release patterns were observed which can be attributed to dual drug encapsulation and the distinctive core-shell (drug and polymeric matrix interaction) structure of the fabricated nanoparticles. In addition, release kinetics were investigated and demonstrated a dual drug release profile. Through the use of ES and modification of the polymeric matrix, it was possible to achieve the desired drug release patterns, highlighting the use of coaxial ES for co-delivery of actives [178].

In another study by Mehmood *et al*, novel nanospherules were fabricated *via* ES to improve the aqueous solubility and oral bioavailability of the poorly soluble drug fenofibrate. PVP and Labrafil M 2125 (non-ionic surfactant) were used as carriers for fenofibrate. The nanospherules

were less than 200 nm in size and fenofibrate was entrapped in an amorphous state. An improved solubility of fenofibrate (32.5% µg/mL) and a high dissolution rate of 85% within 10 minutes was observed. In addition, oral bioavailability was seen to be 2.5-fold better in comparison to the free drug, thus highlighting the possibility of using ES for nanospherule production for the improvement of solubility and oral bioavailability of the drug. This study proved ES to be a promising technique for the fabrication of drug delivery systems for the oral administration of poorly water-soluble drugs [179].

A study by Mai *et al*., 2017 used ES to fabricate curcumin loaded poly-lactic acid (PLA) microcapsules. Curcumin is a therapeutic molecule and has anti-septic, analgesic and anti-inflammatory properties, however, it possesses poor water solubility which, in turn, causes a decrease in bioavailability, thus limiting its applications. In addition, it is unstable in a number of physical and chemical surroundings and therefore electrosprayed PLA microspheres can be used to encapsulate therapeutic agents such as curcumin whilst providing high encapsulation efficiency and sustained release profiles. Atomized PLGA microcapsules were found to be monodispersed and spherical with a diameter in the range of 3.8 µm to 4.4 µm. Microcapsules with a 15% loading of curcumin showed a release of 67.6% after 24 hours and followed a Ritger-Peppas model which indicated sustained release following an initial burst release at 12 hours. It could be concluded that spherical microcapsules fabricated *via* ES have a broad range of applications specifically for oral drug delivery [103].

8.2 Systemic drug delivery systems

Parenteral administration offers many advantages over other routes such as oral administration as it provides enhanced bioavailability, reliable dosing and also avoids first pass metabolism [180]. However, disadvantages of the parenteral route include the requirement of sterile conditions, patient compliance as well as water soluble API for a feasible ROA [181]. ES has been used to fabricate different types of nanoparticles which have been used for various applications such as cancer targeting [36] and vaccine delivery [182]. ES is advantageous for the processing of proteins compared to other techniques such as emulsions due to the use of decreased shear forces and reduced contact times with solvents [39]. Protein antigens have been encapsulated in polymeric microparticles using emulsion techniques which often results in non-neutralising antibodies. Coaxial ES was used in a study by Gallovic *et al*, to produce a microparticulate anthrax vaccine in which the recombinant protective antigen and the adjuvant resiquimod were encapsulated in either the same or separate acetalated dextran microparticles.

This study highlighted the advantage of using ES for the encapsulation of proteins, with arguably the most significant advantage being the ability of the protein to maintain its bioactivity following microparticulate fabrication. In addition to this, research by this group was the first to show that through using specially engineered delivery vehicles in which the vaccine was encapsulated, a greater degree of protection was demonstrated [183].

ES has been used to process a number of anticancer drugs such as paclitaxel, etoposide and cisplatin. Zhang *et al* used coaxial ES for the production of Paclitaxel and etoposide loaded PLGA microspheres. It was found that the atomized microspheres exhibited a core-shell structure, a high entrapment efficiency of 85.8% and a size range of 1-4 μm. Controlled release of both drugs was achieved as follows; after 24 hours, 17% of paclitaxel was released, whereas 22% of etoposide was released, following this, after 120 hours 31% of paclitaxel was released compared to 33% for etoposide. The paclitaxel and etoposide loaded electrosprayed microspheres demonstrated an improved cytotoxic effect on saos-2 osteosarcoma cells in comparison to the pure drugs individually, thus highlighting the application of electrosprayed microspheres for combinatorial drug therapy in the medical remit [155].

8.3 Transdermal drug delivery systems

Transdermal drug delivery is an alternative ROA compared to oral and parenteral administration. This method overcomes the challenges associated with the aforementioned ROA, such as poor absorption and degradation of drugs occurring in the gastrointestinal tract or the liver, whilst providing a sustained release of drugs with a single application. It should be noted, only a selective number of medications can be delivered *via* the transdermal route in therapeutic amounts, however microneedles can be employed to improve transdermal delivery [184-186]. Microneedles work by creating micropunctures in the skin through which drugs and nutrients can be transported into deeper layers within the skin. In addition, the use of microneedles is convenient, inexpensive, non-invasive, painless, and can be self-administrated thus improving patient compliance [187]. The ES process has the ability to fabricate controlled particle coatings for stainless-steel microneedles. This improves drug delivery, specifically for sensitive biomolecules such as peptides and proteins which remain stable during the ES process but cannot be delivered orally. The ES method is favourable over others such as dip coating due to the fact that it allows a controlled coating for only the microneedle tips as opposed to the whole microneedle substrate, as is the case with dip coating [188]. Serdar Tort *et al*,

produced loaded (drug and insulin) nanoparticle coatings for microneedles *via* ES, insulin loaded nanoparticles with a size in the range of 522 ± 261 nm were produced. Following this, the effectiveness of the electrosprayed insulin coated microneedles was tested on diabetic rats, where the study observed a significant decline in fluctuating blood glucose levels when compared to subcutaneous injections [189].

8.4 Pulmonary drug delivery systems

Pulmonary drug delivery systems are often utilised for both systemic and local drug delivery applications. Through use of inhaled drug delivery, the drug is able to directly target the site of action, thus allowing the use of a reduced dose whilst maintaining an increased drug concentration at the site of action and minimized systemic side effects [190]. Often, the use of particulate pulmonary drug delivery systems is limited by the size polydispersity of the particles. In order to provide successful pulmonary drug delivery, particle size and uniformity is critical, with an aerodynamic diameter in the range of 1-5 μm considered suitable for drug delivery devices. By controlling particle size, it is possible to regulate drug absorption and bioavailability. Through the use of ES, monodispersed particles with a narrow particle size distribution can be fabricated [191]. ES allows tight control over both particle size and polydispersity, thus making it desirable for the fabrication of inhalable pharmaceuticals [192].

ES has been used for a number of pulmonary applications in which targeted drug delivery *via* inhalation is required, an example of these is asthma or cystic fibrosis. When in the cone-jet mode, the ES technique gives rise to the production of droplets which are a few micrometers in diameter with a very narrow size distribution. Droplet size can be altered *via* strict control over the process parameters, where the ability to control droplet size is key in maximising distal lung deposition and thus the electrospray must be appropriate to target drug inhalation. BatellePharma developed novel electrospray inhalers which were able to nebulise fine drug loaded electrosprayed droplets which reached the distal regions of the lung alveoli and bronchioles [68, 193]. Youliang hong *et al*, successfully loaded PLGA with Rifampicin, forming microparticles *via* a modified ES technique. Particles produced had a mean diameter, with a range of 2-5 μm, making it suitable for pulmonary drug delivery applications [194]. Yaqoubi *et al*, employed single-needle ES to produce a dry powder inhalation formulation of montelukast and budesonide, individually and in conjugation for pulmonary drug delivery. This formulation was processed using ES, in which montelukast behaved as both an API and a carrier for budesonide in the excipient free formulation, producing an inhalable dry powder

formulation for the treatment of asthma. Research demonstrated that the therapeutic efficiency in controlling asthmatic episodes was significantly enhanced when budesonide was inhaled in combination with orally administered montelukast. This method of administration was found to be as effective as doubling the dose of budesonide would. It would therefore be reasonable to produce a formulation consisting of both montelukast and budesonide for dry powder inhalation *via* ES [190].

8.5 Nasal drug delivery systems

The nasal pathway has been a favourable route of administration for a number of years due to the effect of a significant amount of drugs being more prominent when delivered *via* this pathway. The use of nanotechnology for nasal drug delivery has shown to have a number of advantages including targeted drug delivery, increased bioavailability and decreased toxicity [195]. ES has been used to process asthmatic drugs, Midhun *et al*, utilised ES for the production of Budesonide loaded PCL nanobeads. The effectiveness of Budesonide is restricted due to its rapid elimination, thus encapsulation into a biodegradable polymer to obtain sustained release is necessary. Particles with a size of 116.1 ± 19 nm and a drug encapsulation efficiency of 75 ± 2.4 % were achieved using ES under optimised conditions. Controlled drug release was obtained *in-vitro* at pH 7.4 and 5.6 [196]. Through the employment of ES, high drug encapsulation efficiencies and sustained release profiles were demonstrated, thereby forming efficient nanocarriers for the treatment of inflammatory disorders.

8.6 Ocular drug delivery systems

Ocular delivery is an advancing area within the drug delivery remit, largely due to the challenges associated with delivery to the eye irrespective of the ease of access [197]. The eye possesses several anatomical and physiological barriers which protect it from foreign objects. As a result, it is vital that a delivery system is produced to overcome these barriers and allow targeting to specific ophthalmic tissues for the control and treatment of ocular diseases [134]. Conventional drug delivery methods such as eye-drops are disadvantageous as a significant amount of the drug (>90%) is lost upon administration *via* tear production and drainage mechanisms [198]. In addition to this, drug bioavailability is poor due to reduced residence time in the eye. ES overcomes these barriers when used as a coating method for contact lenses. Mehta *et al*, encapsulated timolol maleate into three different polymeric matrixes; PVP, poly(Nisopropylacrylamide) (PNIPAM) and PVP:PNIPAM (50:50%w/w) from which both fibrous and particulate samples were generated. Over 52% of the fabricated structures for all

formulations were less than 200 nm in diameter. *In-vitro* release studies found a biphasic release for all formulations where the PNIPAM and timolol maleate coating released 89.8% of the drug after a period of 24 hours. *In-vitro* studies suggested through the combination of a fast-dissolving polymer (PVP) and a sustained dissolving polymer (PNIPAM), a polymeric coating which offers controlled prolonged release could be achieved *via* ES [199].

8.7 Other routes of administration

Electrosprayed particles for other ROAs such as buccal, topical and brain offer a number of possibilities [200, 201]. Buccal mucosa is a promising route for systemic drug delivery, owing to its convenience, accessibility and high vasculature. ES has been used to process mucoadhesive biopolymers for applications in buccal drug delivery. ES is a favourable method for the fabrication of drug loaded multi-layered membrane mucoadhesive patches [202]. The skin acts as a mechanical barrier, preventing the penetration of several drug substances, however, this aside, the skin is an advantageous site for drug delivery. Often, products which are formulated for topical use are categorised into two groups; those which are used for local drug delivery or systemic drug delivery systems [203]. Najme Hazeri *et al* used ES for sericin (an antibacterial agent) and a resultant nano-powder was formed with a mean particle size of 25 nm. The sericin nanoparticles were tested for their moisture absorption, where a moisture regain of 56.2% was reported, thus indicating the potential use of these nanoparticulates in topical applications such as moisturisers and sun creams [200]. Nie *et al* used co-axial ES to produce paclitaxel and suramin loaded core-shell microspheres for the treatment of brain tumors. Both drugs possessed varying hydrophilic characteristics and were encapsulated into Poly(L-lactide) PLLA (core) and PLGA (shell) microspheres. Furthermore, two different types of microspheres (10 – 20 μm) were produced with swapped distributions as follows; sample A consisted of paclitaxel encapsulated in the core and suramin in the shell whereas sample B consisted of suramin encapsulated in the core and paclitaxel in the shell. *In-vitro* studies were carried out and sample B demonstrated enhanced apoptotic activity in comparison to sample A and drug controls. In addition, sample A and B were superior in inducing apoptosis in comparison to single drugs, therefore highlighting the advantages of using dual drug delivery to inhibit U87 cell growth. Similarly, *in-vivo* studies demonstrated tumor inhibition of U87 glioma in nude mice, in which samples A and B showed substantial decrease in the number of tumor cells, again highlighting the advantages of using ES to fabricate dual drug delivery systems [204].

9. Emerging applications utilising electrohydrodynamic atomisation

Further advancements in biomedical and pharmaceutical research areas have resulted in the need for more sophisticated therapies, such as regenerative medicine bioactive cargo carriers, scaffolds for tissue engineering, implantables, coatings and sensing devices.

9.1 Tissue Engineering

ES is beneficial when used for tissue engineering applications in the regenerative medicine remit [205]. Commonly, scaffolds have been utilised to support; bone cell growth and tissue regeneration, as well as promoting bone-forming cells through natural proteins and growth factors. Often porous surface topographies are fabricated to mimic the extracellular matrix which in turn induces restoration of damaged bone tissues [10]. Bio-ES is a promising technique in tissue engineering for cell delivery in scaffolds owing to the low current used making it safe to process cells. Previously a number of cells have been processed *via* bio-ES including bone marrow derived mesenchymal stem cells [206], embryonic stem cells [207] and cardiac cells [208]. In recent years, artificial 3D constructs have been explored as a means of direct delivery of mesenchymal cells for tissue engineering. Limitations with these constructs have arisen due to the lack of cell infiltration, reduced cell functioning and minimal diffusion of essential nutrients and oxygen through the scaffold. For feasible tissue engineering, a uniform occupation of cells is critical [209]. ES and electrospinning were used in combination to overcome some of these limitations, for example, Braghirolli *et al* combined poly(lactide-co-glycolide) fibers produced *via* electrospinning with the bio-ES of a suspension of mesenchymal stem cells, as a means of directly integrating cells into fibers. The scaffolds were cultivated and following this, cells remained viable. Confocal imaging confirmed cell adaptation and spreading between fibers and scanning electron microscopy assessed the morphology of the scaffolds, which indicated the presence of a significant number of cells within the scaffold structure. Through combining bio-ES and electrospinning, uniform cellular distribution was promoted across the 3D structures. The data attained highlights the possibility of combining electrospinning and bio-ES for the successful fabrication of 3D cell-integrated scaffolds which increase the development of tissues and is therefore favourable for use in regenerative medicine [210].

One of the main challenges in tissue engineering is the localised delivery of growth factors where the administration of these molecules can result in potential inflammation and ectopic

bone formation in soft tissues. ES as a means of producing delivery systems has shown great promise in engineering microparticles to deliver biomolecules (e.g., proteins, enzymes and growth factors) to bones whilst maintaining their bioactivity throughout the manufacturing process [211]. Bock *et al* utilized the ES technique to encapsulate growth factors (vascular endothelial growth factor and bone morphogenetic protein 7 (BMP-7)) into microparticles of PLGA, PEG and trehalose composite. Here, the growth factor loaded PLGA particles were fabricated and combined with a protein stability enhancer (PEG and trehalose) in a single-step. Electrosprayed particles loaded with BMP-7 were cultured with preosteoblasts, where substantial cell differentiation into osteoblasts was seen up to 3 weeks in culture. This research highlighted the successful delivery of active growth factors specific to bone tissue engineering through the use of electrosprayed microparticles. Moving forward it would be useful to assess the way in which microparticulate systems, specifically those consisting of sensitive molecules such as growth factors are analysed [212].

Through continuously evolving research in the tissue engineering remit, products have been developed to enhance the regenerative medicine sector with advances in scaffold production for bone, tendon and ligament repair *via* EHDA. By using bio-ES in combination with electrospinning, there is an opportunity to fabricate complex living 3D architectures which have potential uses in regenerative medicine. Bio-ES is an evolving technique which allows the encapsulation of cells directly into scaffolds as well as having potential uses in the advancement of organs-on-chip technologies. Currently, the ES process works on a small scale which is not sufficient for commercial use, however through employing a number of needles, the ES process is becoming closer to being used on a much larger scale [40]. In another study conducted by Shokraei *et al*, nanocomposites for use in cardiac tissue engineering were fabricated using electrospinning (polyurethane fibers) and ES (multiwall carbon nanotubes) simultaneously. Carbon nanotubes were electrosprayed onto a rotating collector and polyurethane fibers were electrospun at the same time from the opposite side of the collector. The resulting scaffolds demonstrated cytocompatibility for cardio myoblasts, inducing cardio myoblast attachment and proliferation on the novel conductive nanocomposite patches. This study emphasised the potential use of electrosprayed scaffolds in cardiac tissue engineering moving forward [3].

9.2 Implant device coatings

Aforementioned, the ES process is a favourable technique for the fabrication of polymeric nanoparticles as drug carriers. Implant failure often occurs as a result of infections following implantation. With the ever-evolving area of implants, it is vital to propose an approach to prevent surgical site infections. Here, Tsiapla *et al* used ES to produce biodegradable nanoparticles as drug carriers for the treatment of orthopaedic infections. Titanium metal implants were coated with vancomycin loaded PCL nanoparticles using ES. Drug release was investigated, and a bi-phasic release was observed with an initial burst release of 57.9% followed by sustained release over a 61-day period. The initial burst release from the nanoparticles is desirable for treating the onset of infections specifically following surgery. A subsequent sustained release pattern is also important, as often the administration of high doses of antimicrobial agents is required in the infected area over a period of 6 – 8 weeks. Emerging research following this study could focus on exploiting these electrosprayed nanoparticles for use in cytotoxicity studies as well as researching their interactions with different cell types such as mesenchymal stem cells or bone marrow stromal cells [213].

ES has been used for the fabrication of polymer coatings on implant surfaces, thus allowing tight control over the surface texture. Guo *et al*., produced a number of surface micro-topographies using polyhedral oligosilsesquioxane thermoplastic polyurethane as a coating for stainless steel coronary stents. Upon altering the electric field over a range of voltages, surface coatings of three different morphologies; smooth, roughened and fibrous were achieved at 1.5 kV, 1.6 – 1.7 kV and 1.8 kV, respectively. The control over process parameters in ES is advantageous as it allows the manipulation of coating topographies which in turn regulates the integration of implant devices with tissue in the surrounding environment [214]. Due to the adherence between the charged droplets and the conductive metallic stent (collector), coatings produced *via* ES for metallic stents have proved to be superior in robustness and uniformity in comparison to those formed *via* conventional methods [215]. Drug eluting stents are used for the treatment of acute symptoms caused by coronary artery disease. By using ES to produce dual action stent coatings, it is possible to enhance the therapeutic potential by providing more personalised treatment approaches. This can be achieved through tight control over drug loading and release kinetics due to ES deposition. McKittrick *et al* developed a novel coronary stent coating for the controlled release of sirolimus from accelerate essentially combining an anti-proliferative agent and a bioactive polymer coating which promotes re-endothelialisation to form a dual action coating. The coating was deposited *via* ES which allowed strict control

over coating thickness, roughness, drug loading and release kinetics providing a significant therapeutic potential. The study demonstrated an improvement in the attachment of primary porcine endothelial cells to the surface. Further advancements in this area would benefit from investigating the effect of the ES process on drug loading and release kinetics on the performance of drug eluting stents [216].

9.3 Sensing Devices

Novel devices have also incorporated sensors for drug delivery systems, where the addition of ES has found to be an attractive approach. Ruecha *et al* developed a solution consisting of graphene, PVP and polyaniline (PANI), which, when atomised using ES, produced nanocomposites onto a grounded substrate consisting of a paper-based biosensor and a rotating drum. Fabricated nanocomposites displayed a mean particle size of 160 ±1.02 nm. The modification of the biosensor by the ES technique also led to nanoparticulates exhibiting high conductivity and a large surface area, subsequently improving electrochemical sensitivity for cholesterol detection. Thus, ES was found to provide a diverse platform for improving the detection of sensing devices for biologics, applicable in medical diagnostic therapies [217].

9.4 Eco-friendly and green technologies

The timely strategic expansion of the pharmaceutical industry and more specifically the advancement of such emerging technologies indicates the potential to address unmet clinical needs is promising. Furthermore, beyond the scope of this review is the adaptation of such technologies in more eco-friendly and ‘green’ channels. Employing organic solvents in engineering processes can be potentially harmful and consequently raise issues of cell toxicology and environmental safety [40]. As ES methods have shown extended applications in tissue engineering, biomedical therapeutics and theranostic systems it is of critical importance to utilise non-toxic solvents when fabricating various particulate structures [218]. ‘Green ES’ is therefore a prospective technique which, if adopted in the pharmaceutical industry, could potentially improve the environmental profile of such processes.

Conclusion and future perspectives

The successful control and subsequent targeted drug release of therapeutic moieties is of overarching importance with regards to the pharmaceutical industry. Currently, conventional particle fabrication methodologies although well established, are accompanied with several shortfalls including poor drug loading, inefficient encapsulation capacities, unstable therapeutic compounds as well as undesirable drug release kinetics. The ES method can therefore be adopted as a facilitative approach for particle engineering to address these limitations. These include upscaling manufacture of desired drug delivery systems, enabling batch-batch reproducibility, improving stability as well as cost efficiency, all of which can be implemented into the industry to yield highly efficacious drug delivery systems. The interplay of processing parameters involved in the ES technique can have an immense impact on the fabricated particulates which can exhibit highly functional structures, thus providing tremendous scope in the field of pharmaceutics and drug delivery. Nevertheless, bottle necks for the ES method can be assigned to its poor throughput due to low flow rates, particularly in the Taylor cone-jet mode owing to its stable geometry. As a result, rendering a particulate system with long production times and therefore insufficient to be utilised during scale-up. To further refine the electrospray method, multiple cone-jet systems have been considered as a feasible approach to tackle these issues where multi-tip emitters can be employed each possessing separate capillaries (which individually display the steady cone-jet mode). These can generate monodisperse particles with a controllable spray plume, thus allowing effective deposition of particles on the grounded substrate. This in turn increases the yield of resultant particles for continuous production in the commercial industry. In addition, “Green ES” has been used to further enhance the safety of the ES technique, by limiting the use of organic solvents and their associated toxicity with biological entities (genes, peptides and enzymes) and alternatively using aqueous solvents which are non-toxic in nature.

Furthermore, the employment of the ES technique in the pharmaceutical remit to fabricate nanoparticles has shown great potential. This competent method can improve drug efficacy at the site of interest demonstrating higher loading capacities and payload release in addition to minimal side effects and toxicity. ES also provides a versatile technique to enhance the solubility and bioavailability when encapsulating therapeutic compounds into drug carriers. Following particle engineering, extensive in-depth analysis on the relationship between generated particulate systems and biological structures is of paramount importance. This involves the consideration of permeability, pharmacokinetics and physiochemical stability

which are critical factors in the development of desired particles for therapy, diagnosis and targeted drug delivery. Further research in the realm of EHDA should focus on diagnosing and monitoring the therapeutic response of efficacious delivery systems, particularly in animal models and clinical trials, making it a robust method for industrial implementation and hence translating particle engineering from small scale manufacture into commercial clinical practice.

On a final note, the industrialisation of EHDA technologies hinges on several aspects ranging from industrial perspectives (e.g., business models, regulatory matters, production scale, multi-mode testing and product validation) to the actual implementation within the clinic. It is well known that the pharma remit is resilient to change and therefore adopting new technologies must be of significantly higher value than those currently deployed. The current state of the art has shown EHDA technologies to provide multiple benefits (process, material and space) when compared to established protocols. In fact, the versatility of this method makes it promising for numerous ROA and target sites. At present several SMEs and indeed large corporate pharma have shown a luke-warm interest indicating the need to scale-up of such systems with emphasis on control, management, utilisation and product reproducibility on a larger scale. This is the first major challenge and quite possibly the biggest challenge. The next few stages then move towards *in-vivo* models and then potentially small-scale clinical trials.

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Figure 1: Schematic diagram of the EHDA process

a) Spinneret needle designs: **a1)** Single-needle elecctrospraying **a2)** Coaxial, **a3)** Multi-tip emitter, **a4)** triple-needle coaxial [95] **a5)** angular nozzle arrangements [96] **a5i)** θ = 30°, **a5ii)** θ = 60°, **a6)** needleless approach using an external magnetic field [98] **a7)** needleless approach using multiple orifices [99].

b) Jetting modes: **b1)** no flow, **b2)** initial dripping, **b3)** microdripping, **b4)** oscillating jet, **b5)** unstable jetting, **b6)** stable jetting (Taylor-cone), **b7)** unstable multi-jet, **b8)** stable multi-jet.

Figure 2: Schematic diagram of forces acting in the liquid cone at the tip of the capillary needle

Figure 3: Various types of particulate structures engineered using the electrospraying process:

a) 6% PCL matrix active-polymer microspheres loaded with paclitaxel [36] **b)**Titanium oxide nanocups produced *via* electrospraying nitromethane solution of 8 wt% PMMA and titanium tetraisopropoxide [122] **c)** Triple layered PLGA–PCL–PMSQ nanoparticles [114] **d)** Islet cells encapsulated in core-shell hydrogel microcapsules using a concentric nozzle and coaxial jetting [116] **e)** 5 w/v % PCL in DCM polymeric porous carrier particles at an externally applied pressure of -150 mmHg [126] **f)** PVP-RBC (5%w/v PVP in ethanol, 0.1% w/v Rose Bengal and 0.86% w/v Carmofur) Janus particles *via* side-by-side electrospraying [128] **g)** Spindle particles obtained from electrospraying D-limonene (20%) with Alyssum homolocarpum seed gum (0.75%) and Tween 20 (0.1%) [120] **h)** microrods generated from co-jetting of a 3.4%

w/w solution (in 95:5 v/v chloroform: dimethylformamide (DMF)) of each polymer (PLGA 85:15 and PLGA 50:50) and triethylamine (3.6 vol% of solvent) [121] **i)** Red-blood-cell-shaped chitosan microparticles with evaporable ethanol (20 vol%) and diffusible DMSO (30 vol%) [109] **j)** Fibril spike-like structures from electrosprayed DTCPA (1.5 wt%) in Chlorobenzene [130] **k)** 3% Ethylene/vinyl acetate copolymer (EVAC) toroidal-like donut shaped microparticles loaded with anti-cancer drug paclitaxel [36] **l)** Disc shaped biphasic particles fabricated from co-jetting of 1.3% w/w solution (in 95:5 v/v chloroform: dimethylformamide (DMF)) of each polymer (PLGA 85:15 and PLGA 50:50) [121] **m)** 0.5g Silver nanoparticle-doped SiO_2 strawberry-like microspheres [129] **n)** Adenovirus encapsulating cross linked alginate (0.5% wt) beads [117] **o)** PCL/chloroform (5 wt. %) + PEG/chloroform (15 wt.%) hollow microspheres with single surface hole collected in an ethanol bath substrate by coaxial spraying [124] **p)** 2%ibuprofen/12%zein microparticles coated with an epoxy resin needle [111] **q)** Magnetic yolk shell particles prepared by coaxial electrospraying using 20% w/v PCL in glacial acetic acid with magnetic nanoparticles and model probes. Nile blue/PCL (outer shell), silicone oil/Sudan Red G (central layer) and Acridine yellow/PCL (inner layer) [131] **r)** Four layered structure prepared with a four-needle coaxial device, electrospraying various dyes in the polymeric layers of PCL, PEG, PMSQ and PLGA [101].

Figure 4: Applications of EHDA engineered particles as established and emerging technologies

a) Paclitaxel and suramin loaded core-shell microspheres for glioma treatment [204] **b)** Montelukast and budesonide microparticles for treatment of asthma [190] **c)** Cisplatin encapsulated nanoparticles as chemotherapeutic agents [102] **d)** Prednisolone loaded toroidal microstructures for treatment of inflammatory bowel disease and colorectal cancer [106] **e)** Gambogic acid loaded nanoparticles to treat hepatocellular carcinoma [149] **f)** Sericin nanoparticles with antibacterial properties [200] **g)** Uniform nanoparticle coatings for microneedles [189] **h)** Nanospherules for improved oral bioavailability of fenofibrate [179] **i)** Timolol maleate contact lens coating for glaucoma treatment [199]

STRUCTURE	NOZZLE GEOMETRY	NOZZLE SIZE (MM)	VOLTAGE (kV)	MATRIX /CARRIER	ACTIVE /INNER MATERIAL	PARTICLE SIZE (μm)	APPLICATIONS	KEY DETAILS	REFEREENCE
Active-polymer composite microspheres	Single-needle	0.91	Nozzle-8.8 Ring-7.1	PCL	Paclitaxel	17	Anti-cancer drug delivery	Formulation filled with 6% PCL matrix solid microparticles and loaded with Paclitaxel. Using a flow rate of 3mL/h fabricated matrix solid structures.	[36]
Active-polymer composite structure	Single-needle	ID- 0.60mm OD-0.91mm	20	-	Ibuprofen and Zein	1.78±0.31	Controlled release drug delivery platforms	Formulation consisted of 2% ibuprofen and 12% zein (w/v) microparticles in 85% ethanol. Epoxy resin was rapidly coated around the needle tip. Particles were collected onto an aluminium foil substrate at a distance of 15 cm.	[111]
Triple layered structures	Coaxial (triple needle device)	Inner- OD (0.5mm) ID (0.2mm) Central- OD (1.5mm) ID (1.0mm) Outer- OD (2.6mm) ID (2.0mm)	6.9-7.9	PLGA (outer) PCL (central) PMSQ (inner)	-	0.2(±0.008)-0.32 (±0.08)	Used for a variety of controlled release drug delivery systems	Spherical multi-layered nanoparticles were generated using a combination of polymers: 5wt% PLGA in DMC, 6wt% PCL in DCM and 12wt% EtOH. Varying flow rates were used 300-(outer)50(central)-5(inner) μL/h, which when atomised were collected at a working distance of 15cm.	[114]
Red blood cell shaped	Single-needle	Not specified	5-6	-	Chitosan	Various sizes but as small as 5 (similar to red blood cells)	Autofluorescence imaging for biomedical applications	Particles mimicking red blood cells were fabricated containing chitosan, evaporable ethanol (20vol%) and diffusible DMSO (30%vol%).Collecting distance was kept at 5cm as well as a flow rate of 120 μl/h.	[109]
Spindle shaped	Single-needle	Not specified	15	Alyssum homolocarpum seed gum	D-limonene Tween 20	Various sizes below 0.5	Nutraceutical stability	Spindle-like particles were obtained by emulsion Electrospraying. D-limonene (20%) with Alyssum homolocarpum seed gum (0.75%) and Tween 20 (0.1%). A flow rate of 0.05ml/h- 0.1ml/h was utilised, and particles were collected at working	[120]

								distance of 15cm.	
Rod shaped	Dual capillary needle system	Not specified	6 ±-0.1	PLGA DMF Triethylamine	-	Mean lengths of 18.37 ±6.17	Drug delivery and cell targeting	Microrods were generated using a 3.4% w/w solution (in 95:5 v/v chloroform: dimethylformamide (DMF)) of each polymer (PLGA 85:15 and PLGA 50:50). A flow rate of 0.45ml/h and a collection distance of 28-33cm, yielded rod-shaped microparticulates.	[121]
Fibril shaped	Single-needle	Not specified	15	Chlorobenzene.	(7, 9-di (thiophen-2-yl)-8H-cyclopenta[a]acenaphthylen-8-one) (DTCPA)	6-8	Photoactive materials for biomedical imaging	Fibril shaped structures from DTCPA (1.5wt%) in Chlorobenzene. Flow rate of 1.5 ml/hr is used as well as an aluminium plate with a collection distance of 12cm from the nozzle.	[130]
Donut shaped	Single-needle	0.34mm	Nozzle-8.8 Ring-7.1	Ethylene/vinyl acetate copolymer (EVAC)	Paclitaxel	Around 10 in size	Anti-cancer therapeutics	A formulation consisting of 3% EVAC and loaded with paclitaxel fabricated toroidal-like donut shapes at a flow rate of 3ml/h.	[36]
Strawberry shaped	Single-needle	Not specified	15	SiO_2	Ag-NPs	0.95	Antibacterial materials for biomedical applications	0.5g Silver nanoparticle(Ag-NPs)-doped SiO_2 strawberry shaped microspheres were fabricated at a flow rate of 0.2ml/h, where particles were collected at a distance of 20cm. Ag-NPs were doped inside the SiO_2 microspheres as well as embedded on the surface, therefore appearing strawberry-like.	[129]
Hollow microspheres	Co-electrospraying	Not specified	9	PCL/Chloroform (shell)	PEG/ Chloroform (core)	Below 15	Tumour cell mimicking phantoms for anti-cancer drug	PCL/chloroform (5 wt. %) and PEG/chloroform (15 wt.%) were co-electrosprayed and collected in an ethanol bath substrate using a flow	[124]

							delivery	rate of 1.0/3.0ml/h (core/shell) and. collected at a distance of 20cm.	
Nanocups	Single-needle	Not specified	12	Polymethylmethacrylate (PMMA) Titanium tetraisopropoxide	Nitromethane	Diameter of around 0.5	Applications in photocatalysis and drug delivery systems	Titanium oxide nanocups were produced by electrospraying nitromethane, 8% PMMA and titanium tetraisopropoxide . A flow rate of 2ml/h was used as well as a collection distance of 10cm.	[122]
Porous microcarriers	Single-needle	Not specified	12	PCL	-	3.5 ±0.4	Biomolecules, drug encapsulation and inhalable drug delivery platforms	5 w/v % PCL in DCM when electrosprayed fabricated porous microcarrier particles using an externally applied pressure of -150 mmHg. A flow rate of 0.5ml/h was applied, and particles were collected at a working distance of 30cm on an aluminium disc (additional 0.5kV).	[126]
Four layered structure	Coaxial (four needle)	-Needle 1 outermost (OD 4mm, ID 3.2mm) -Needle 2 second outermost (OD 2.6mm, ID 2.0mm) -Needle 3 second innermost (OD 1.5mm, ID 1.0mm) -Needle 4- innermost (OD 0.5mm, ID 0.2mm)	9-12	PCL PEG PMSQ PLGA	Various dyes (Evans blue, Pyronin B, Pinacyanol chloride, Hematoxylin)	0.62 ± 0.15	Multi-layered delivery system as a controlled release dosage form for combinatorial drug targeting	A four layered particulate system was developed : DCM:PEG 90:10 DCM:PLGA 95:5 DCM:PCL 97:3 EtOH:PMSQ 88:12. Additionaly a flow rate of 50-50-25-10 (μl min−1) and a working distance of 10cm was utilised.	[101]
Janus	Side-by-side	Two 0.6mm	17	PVP	Rose Bengal	0.607 ±	Photochemo-	PVP-RBC particles were fabricated	[128]

	electrosprayin g	ID spinnerets			Carmofur	0.191	therapy and imaging agents in biomedical applications	*via* side-by-side electrospraying using 5%w/v PVP in ethanol, 0.1% w/v Rose Bengal and 0.86% w/v Carmofur. Janus particles contained Rose Bengal in one compartment whilst Carmofour was in the other. Flow rate was at 0.5ml/h where particles were collected at a working distance of 20cm.	
Yolk-shell particles	Coaxial Electrosprayin g (triple needle device)	Inner needle- OD (0.50 mm) ID (0.31mm). Central needle- OD (1.60 mm) ID (1.07 mm) Outer needle- OD (2.85 mm) ID (2.26 mm)	10	PCL Silicone oil	Multiple probes	13.5 ± 1.74	Theranostic applications requiring multi-drug release	Magnetic yolk shell particles were prepared using 20% w/v PCL in glacial acetic acid with magnetic nanoparticles and model probes. Nile blue/PCL (outer shell), silicone oil/Sudan Red G (central layer) and Acridine yellow/PCL (inner layer). Flow rates varied for each layer: 10ml/h (outer shell), 2.5ml/h (central layer) and 1.6ml/h (inner layer).	[131]
Disc shaped	Dual capillary needle system	Not specified	6 ±0.1	PLGA DMF	-	3.41± 0.72	Biomedical imaging and drug delivery platforms	Disc shaped particles were fabricated using a 1.3% w/w solution (in 95:5 v/v chloroform: dimethylformamide (DMF)) of polymers (PLGA 85:15 and PLGA 50:50). A flow rate of 0.15ml/h was used, and discoid-biphasic particles were collected at a working distance of 28-33 cm.	[121]

Table 1

APPLICATIONS	EXPERIMENTAL DETAILS	STRUCTURE AND SIZE	COMMENTS	REFERENCE
Brain Delivery of paclitaxel and suramin microspheres for the treatment of brain tumors	Co-axial ES to produce microspheres with PLLA core and PLGA shell as a dual drug delivery system	Core-shell microspheres 10 – 20 μm	Electrosprayed microspheres were superior in inducing apoptosis compared to single drugs. *In-vivo* studies showed tumor inhibition of U87 glioma.	[204]
Ocular ES as a coating for contact lenses in the treatment of glaucoma	ES used to encapsulate timolol maleate into polymeric matrixes; PVP, PNIPAM and PVP:PNIPAM (50:50%w/w)	Fibrous and particulate structures of dimensions <200 nm were formed.	*In-vitro* release studies found a biphasic release for all formulations where the PNIPAM and timolol maleate coating released 89.8% of the drug after a period of 24 hours	[199]
Nasal Budesonide for the treatment of asthma	Single needle ES for the loading of Budesonide into PCL	Nanobeads with a Size of 116.1 ± 19 nm	Through ES drug encapsulation efficiency of 75 ± 2.4 % was achieved. Controlled drug release was obtained *in-vitro* at pH 7.4 and 5.6	[196]
Oral ES to improve the aqueous solubility and oral bioavailability of fenofibrate	Single needle ES to fabricate novel nanospherules. PVP and Labrafil M 2125 were used as a carrier for fenofibrate.	Nanospherules <200 nm in size and fenofibrate was in an amorphous state	2.5-fold improvement in oral bioavailability. Solubility of 32.5% μg/mL and a dissolution rate of 85% within 10 minutes was observed	[179]
Pulmonary ES to fabricate dry powder inhalation of montelukast and budesonide for the treatment of asthma	An excipient free formulation was used in single needle ES; montelukast behaved as both an API and a carrier for budesonide	Smooth spherical particles 1 – 5 μm suitable for respirable particles	Therapeutic efficiency in controlling asthmatic episodes was significantly enhanced when budesonide was inhaled in combination with orally administered montelukast thus highlighting the advantage of producing a formulation consisting of both montelukast and budesonide for dry powder inhalation	[190]

Topical Sericin with antibacterial properties for moisturisers and sun creams.	Single-needle ES (needle diameter 0.6 mm) Applied voltage of 15kV was used whilst Concentration, flow rate and collection distance were varied	Uniform spheroidal nanoparticles Mean size < 80nm	Decreased concentration and feed rate with increased collection distance caused reduced particle size. Absorbance increased by 6x for sericin nanoparticles compared to sericin sponge.	[200]
Transdermal ES loaded (dye or insulin) nanoparticle coatings for microneedles	Single needle ES used to fabricate drug-loaded nanoparticles as uniform coatings for microneedles	Spherical particles produced with a particle size of: Dye: 515 nm Insulin: 522nm	Microneedles with the optimised coating demonstrated a >70% transfer into porcine skins. *In-vivo* studies of coated microneedles on diabetic rats demonstrated a decrease in blood glucose levels fluctuations, compared to subcutaneous injections	[189]
Parenteral ES of cisplatin; anticancer drug used in chemotherapeutic treatments	Single needle ES for producing cisplatin encapsulated PLGA nanoparticles. Effect of applied voltage, flow rate and concentration of cisplatin on particle size was assessed.	Spherical particles with smooth surface morphology in the 550nm range	When using a flow rate of 5µl/min, applied voltage of 16kV, decreased PLGA concentration (2%w/w) and increased cisplatin concentration (0.2%w/w) particle diameter was reduced from 1.2µm to 550nm.	[102].
Liver A site-specific delivery system of gambogic acid for the treatment of hepatocellular carcinoma	Single needle ES was used to encapsulate gambogic acid into a PDLLA matrix. Particles were collected using ultrapure water and residual solvent was removed *via* vacuum drying. Preparation parameters were varied thus producing different sized particles *In-vivo* liver studies were carried out	Optimal nanoparticles were spherical and were 185.6 nm in size	Upon increasing particle size from the nanoscale to the microscale, gambogic acid release rate sharply decreased. 2 weeks after administration, hepatocellular carcinoma mice treated with the particles demonstrated lower degree of tumor invasion and cell lesions as well as recovered liver function.	[149].

Colon ES of prednisolone for applications in inflammatory bowel disease and colon cancer	Coaxial ES was used to fabricate Eudragit L100-55 microparticles containing prednisolone. Flow rate was different for inner and outer needles; 6	Toroidal donut-like structures The outer diameter and inner diameter were 1.7 µm and 0.6 µm respectively	Dissolution studies revealed site-specific release of prednisolone for the targeted treatment of inflammatory bowel disease and colorectal cancer.	[106]

Table 2

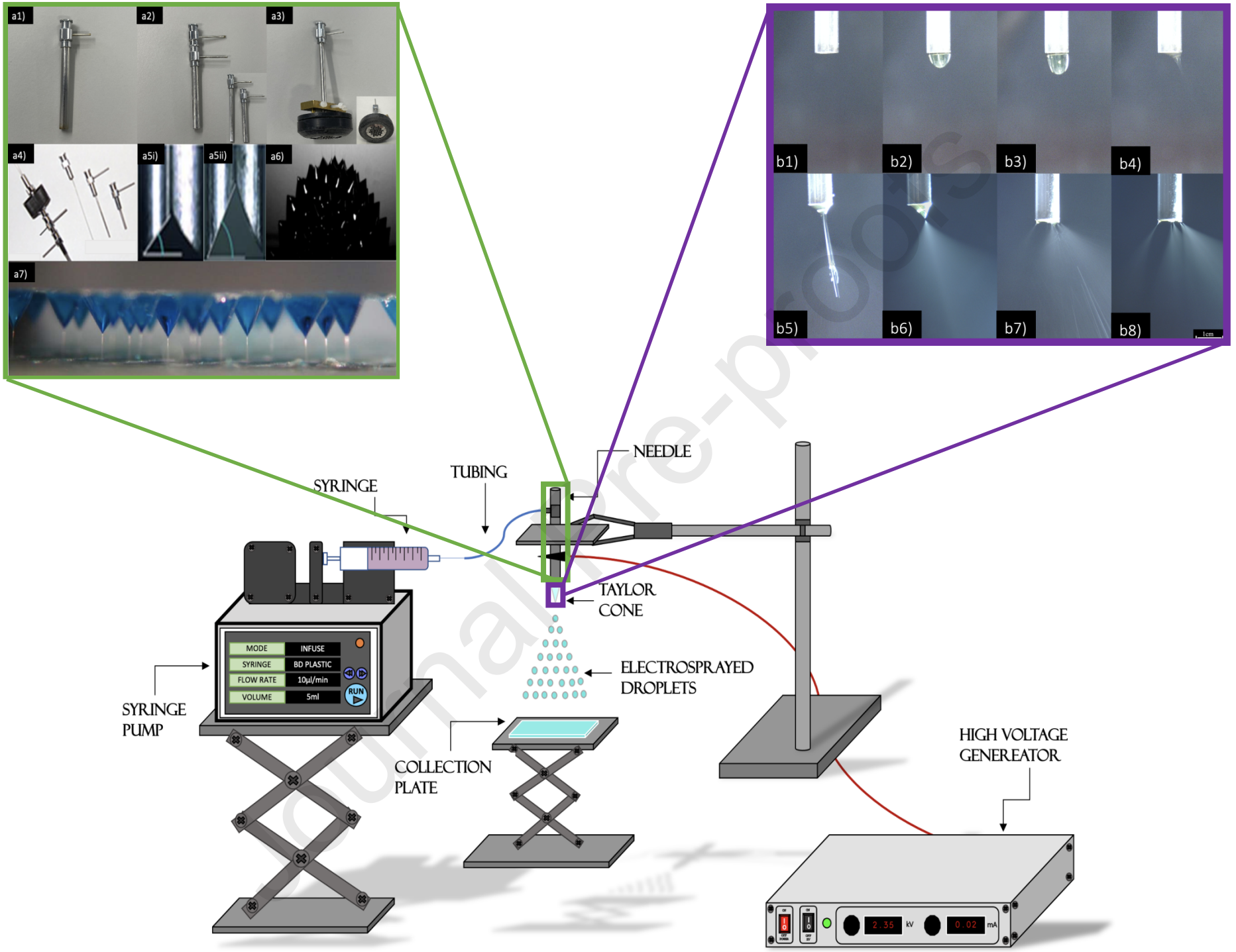
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Figure 1

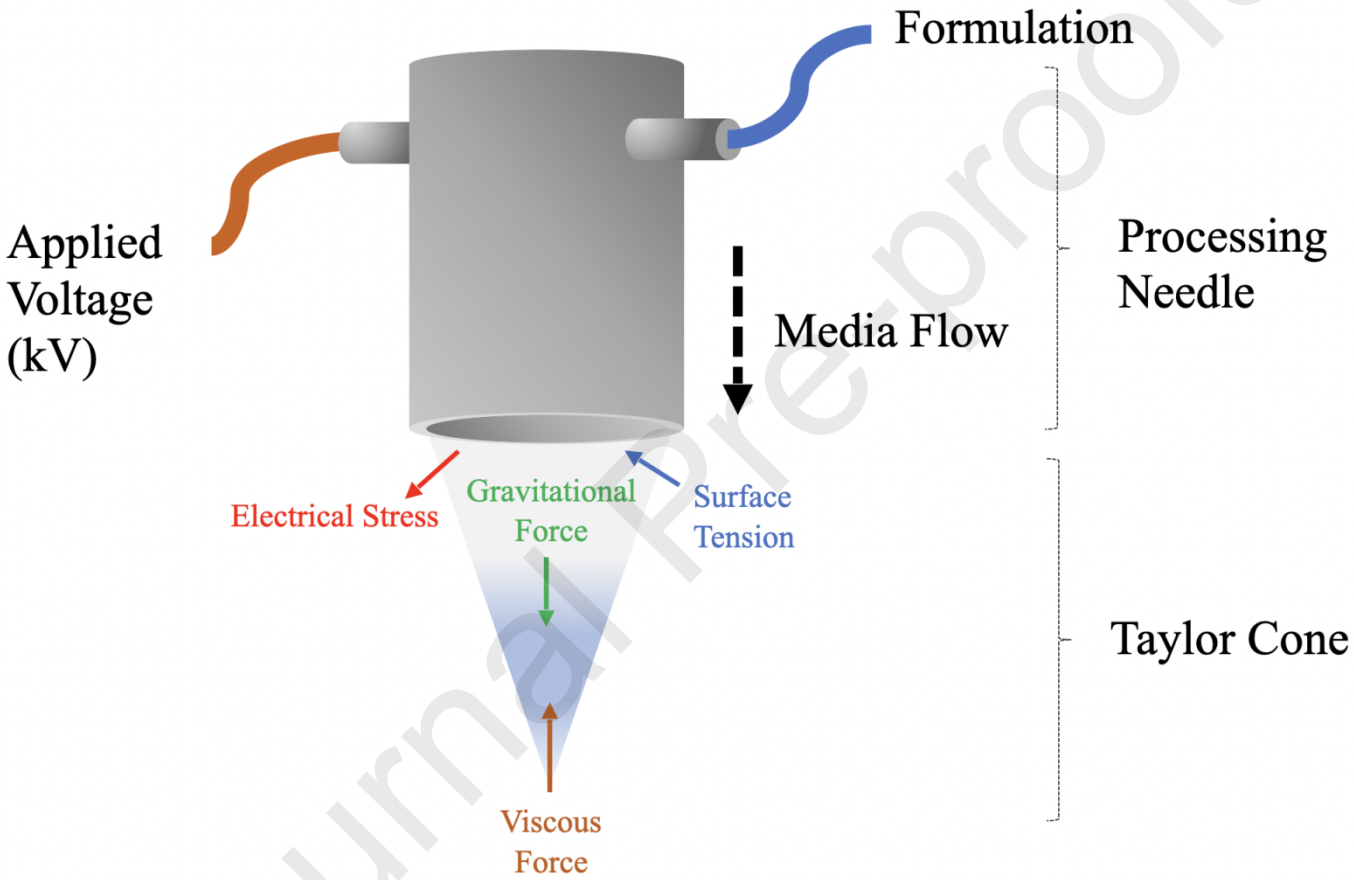

Figure 2

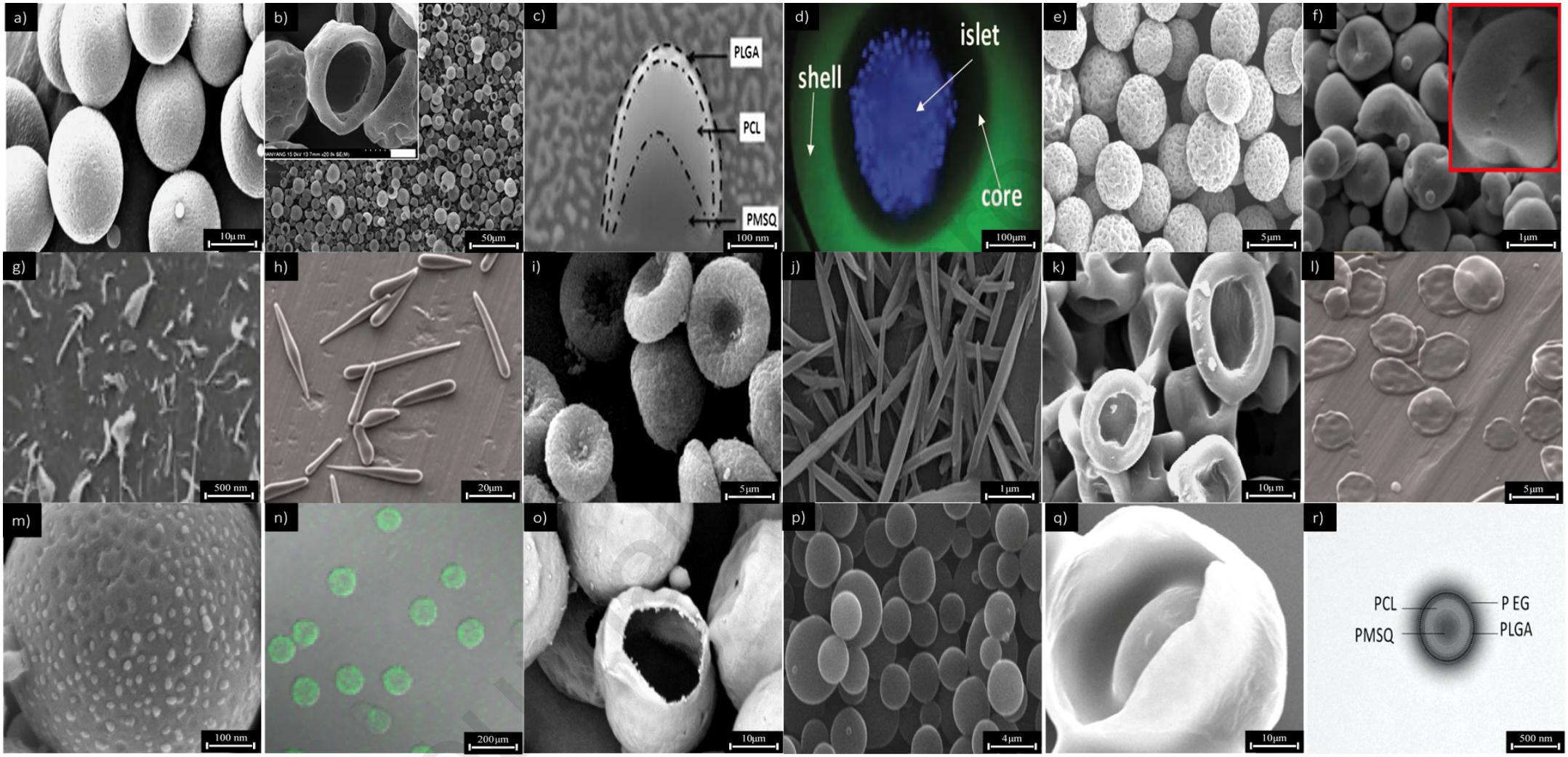
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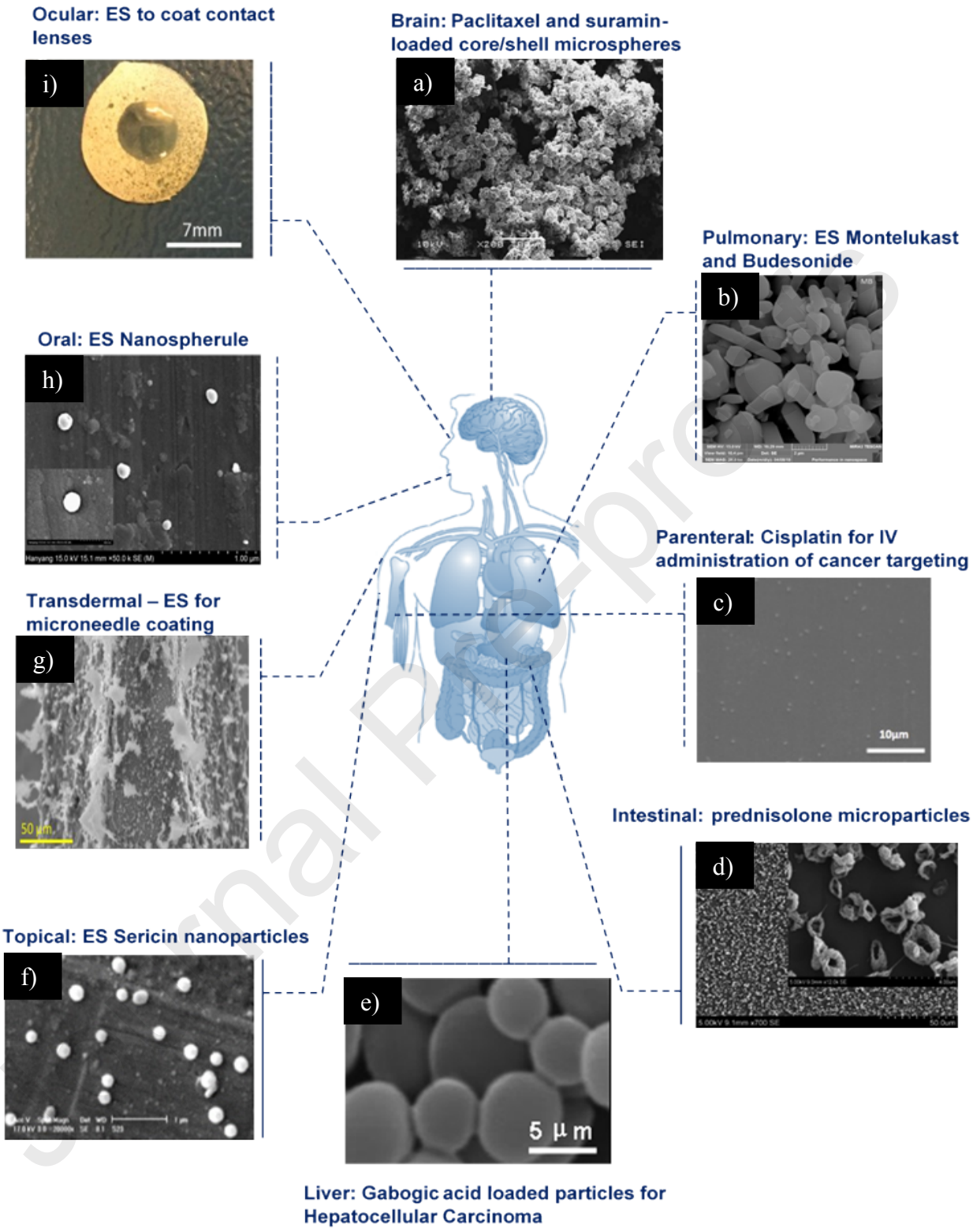
Figure 3


Ocular: ES to coat contact lenses
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7mm
Brain: Paclitaxel and suramin-loaded core/shell microspheres
a)
Pulmonary: ES Montelukast and Budesonide
b)
Oral: ES Nanospherule
h)
Parenteral: Cisplatin for IV administration of cancer targeting
c)
10μm
Transdermal – ES for microneedle coating
g)
50 μm
Intestinal: prednisolone microparticles
d)
Topical: ES Sericin nanoparticles
f)
e)
5 μm
Liver: Gabogic acid loaded particles for Hepatocellular Carcinoma

Figure 4