IntechOpen

# New Topics in Electrospraying

*Edited by Weronika Smok, Pawel Jarka and Tomasz Arkadiusz Tański*

# New Topics in Electrospraying

*Edited by Weronika Smok, Pawel Jarka and Tomasz Arkadiusz Tański*

Published in London, United Kingdom

New Topics in Electrospraying
http://dx.doi.org/10.5772/intechopen.1001494
Edited by Weronika Smok, Pawel Jarka and Tomasz Arkadiusz Tański

Contributors
Bilgen Çeliktürk Kapar, Hatice Ibili, Kai Tang, Kausik Kapat, Mehmet Dasdemir, Mobina Bazari, Najmeh Najmoddin, Paweł Jarka, Pritiprasanna Maity, Rahul Sable, Serap Gamze Serdar, Tomasz Arkadiusz Tański, Weronika Smok, Yixin Zhu



First published in London, United Kingdom, 2025 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

New Topics in Electrospraying
Edited by Weronika Smok, Pawel Jarka and Tomasz Arkadiusz Tański
p. cm.
Print ISBN 978-1-83769-649-9
Online ISBN 978-1-83769-648-2
eBook (PDF) ISBN 978-1-83769-650-5

# Meet the editors

Weronika Smok is a researcher at the Department of Engineering Materials and Biomaterials of the Silesian University of Technology. In 2022, she defended her doctoral thesis, "Analysis of the structure and optical properties of one-dimensional SnO2 and In2O3 ceramic nanomaterials manufactured by the hybrid sol-gel technique and electrospinning from solution." She is a co-author of more than 40 publications and holds a patent for the invention "One-dimensional hybrid nanomaterial and the method of its production." She is a dedicated researcher with a focus on innovative nanomaterials, including semiconductor nanowires and thin films. Her work explores these advanced materials' synthesis, properties, and applications in cutting-edge technologies.

Paweł Jarka obtained his Ph.D. from the Department of Engineering Materials and Biomaterials, Silesian University of Technology in Poland, where he is currently employed. The subject of his doctoral thesis was "The optoelectronic properties of active layers obtained by PVD methods in organic photovoltaic devices." His scientific interests concern functional materials. During his scientific activity, he has participated in many international scientific conferences. He is the co-author of several dozen scientific papers and five chapters in monographs and co-editor of one book. He also has one patent to his credit.

Tomasz Tański is the head of the Department of Engineering Materials and Biomaterials at the Silesian University of Technology in Poland and a member of the Committee of Metallurgy of the Polish Academy of Sciences. He is also a specialist in nonferrous alloys, composite and nanostructured materials, and structural engineering materials. He has authored or co-authored more than 400 scientific publications worldwide, including 15 monographs and books. He has won 20 national and international awards and honours. He is and/or was a supervisor or contractor for more than 15 research and didactic projects in Poland and abroad. He is also a reviewer and promoter of numerous scientific papers, including eight doctoral research papers in the field of nanotechnology and materials.

# Contents

# Preface

The study of electrospraying, the process of atomizing liquids into fine droplets using an electric field, has witnessed remarkable growth in recent years. Its relevance spans an ever-expanding range of disciplines, including materials science, nanotechnology, medicine, and analytical chemistry. From its origins as a scientific curiosity to its current role in enabling breakthroughs in drug delivery, mass spectrometry, and advanced manufacturing, electrospraying has become an essential tool for addressing pressing scientific and technological challenges. This book, *New Topics in Electrospraying*, aims to study the latest advances and insights into this fascinating field, offering both foundational knowledge and a look into cutting-edge applications (**Figure 1**).

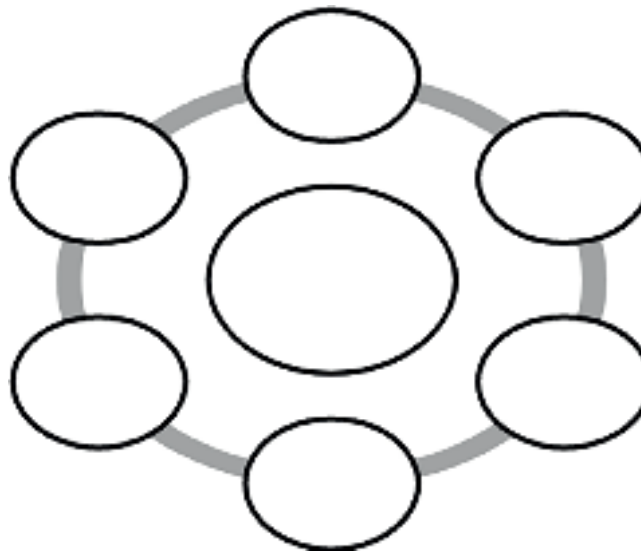

**Figure 1.**
*Application areas of electrospraying technique.*

The book is structured into five chapters, each designed to explore key topics in electrospraying, such as food industry use, drug delivery, and tissue engineering.

Chapter 1 introduces the reader to the principles of electrospraying and electrospinning processes and the use of nanoparticles and nanofibers manufactured in these processes for food preservation, food packaging, and even food enrichment. The following two chapters are devoted to the latest achievements in electrospraying in medicine, particularly tissue regeneration, which is extremely important for improving the comfort and health of patients with injuries, diseases, or degenerative conditions. In Chapter 4, you can find information on the development of nanoencapsulation of biocides using the coaxial electrospraying method. The authors of the

last chapter present a new protonation mechanism method and create very stable protonated small water clusters, which may exhibit interesting biological functions.

The book is a collection of knowledge for those looking for both basic information about the electrospraying process and the results of the latest research on this subject.

**Weronika Smok, Paweł Jarka and Tomasz Arkadiusz Tański**
Department of Engineering Materials and Biomaterials,
Silesian University of Technology,
Gliwice, Poland

Chapter 1

# Electrospraying and Electrospinning in Food Industry

*Weronika Smok, Paweł Jarka and Tomasz Arkadiusz Tański*

## Abstract

Due to the consumer's well-being, great attention is currently paid to the quality, safety and freshness of food, which is why smart and active food packaging technologies are being developed. There are many methods currently available for preparing active food packaging, but due to their high effectiveness, it is worth paying special attention to two closely related methods: electrospraying and electrospinning. These methods are universal and allow for the low-cost production of polymer and composite nanostructures of high quality and large specific surface area. The advantages of both technologies include simple equipment, easy control of process parameters, and the ability to produce structures with the desired morphology and physicochemical properties. Moreover, the multitude of varieties of these methods allows for the encapsulation of active ingredients inside nanostructures or modification of their surfaces, improving their functionality in food packaging. Therefore, the aim of this work is to present the application potential of the electrospinning and electrospraying methods in the modern food industry, with particular emphasis on the latest scientific reports.

**Keywords:** electrospraying, electrospinning, food industry, food packaging, encapsulation

## 1. Introduction

There is no doubt that caring for the high quality of the food eaten is extremely important for human health but also allows reducing food waste. Therefore, the food industry is developing many techniques aimed at extending shelf life, improving safety, increasing the bioactivity of food and improving taste, including active food packaging, food encapsulation, food filtration and separation, texture modification.

Currently, the food industry relies on plastics such as PET, LDPE, HDPE, PP, PE, etc. shaped using classic methods of injection molding, injection blow molding, and plastic extrusion. These materials must meet a number of stringent requirements to be considered food contact materials (FCMs), including: compounds that cannot penetrate and interact with the food, they must be thermally stable within the temperature range at which the food is stored or processed, and they must also be resistant to sunlight and gases used to protect the food (oxygen, nitrogen, carbon dioxide).

Current activities of engineers aim to improve or modify materials used in the food industry so that they can perform their functions even better or be able to perform additional functions. These modifications, however, must not result in deterioration of properties or failure to meet sanitary and hygienic guidelines.

The most frequently used material modification techniques in the food industry include sol-gel, casting, spray-drying, dip-coating, 3D printing. A serious disadvantage of the above-mentioned methods is the need to use high temperatures, which may lead to partial or complete degradation of the bioactive compounds. It is worth paying attention to electrohydrodynamic techniques that have been gaining popularity in recent years: electrospraying and electrospinning, as evidenced by the growing number of publications on this topic (**Figure 1**) [1].

### 1.1 Fundamentals of electrospinning and electrospraying

The main idea of electrospinning and electrospraying is based on an analogous principle. Hence, the equipment for carrying out both technologies has a similar construction. In both technologies, the solution is fed to the nozzle (by using a pump or e.g. a gravity feeder) [2], at the end of which a drop of solution appears. Due to the applied high voltage (HV), electrical charges are accumulated on the surface of the drop. After exceeding the critical voltage value, the charges overcome the surface tension of the polymer droplets and the jet is formed. Depending on how the process parameters are selected, fibers or particles are formed from the jet and fall on the collector (**Figure 2**). The main difference between electrospinning and electrospraying relies on the concentration of the used solution. In case of high concentration of solution, the jet is stabilized and the elongation occurs by the mechanism of whipping instability.

In case of low solution concentration, the jet is destabilized because of varicose instability which is the cause of forming the fine droplets The highly charged droplets are subject to self-dispersing, which is used for agglomeration and coagulation prevention

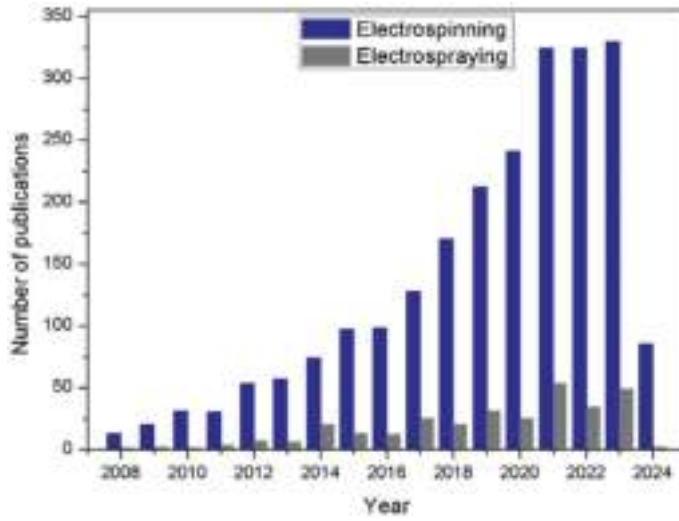


**Figure 1.**
*Number of publications in the field of application of electrospinning and electrospraying in food industry (Web of Science database, keywords: "electrospinning and food", "electrospraying and food").*

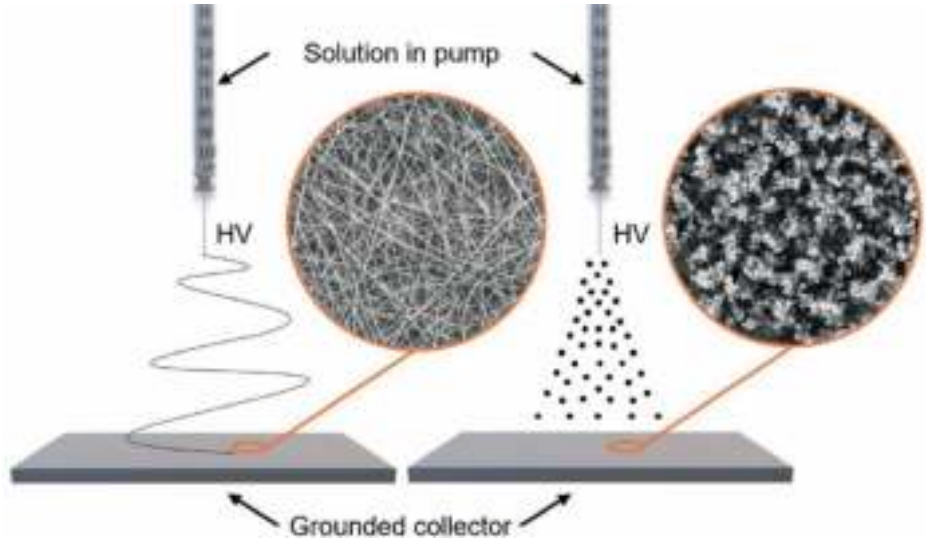


**Figure 2.**
*Schematic illustration of the electrospinning and electrospraying setup (in the case of solution).*

[3–5]. The electrospinning and electrospraying from solution methods have many advantages, including simplicity, the ability to carry out the process in room conditions, low costs of preparing high-quality materials, and the ability to produce materials with desired properties using precise control of process parameters (**Figure 3**). The process parameters are extremely important because they determine the morphology of the obtained nanostructures, including their porosity, shape and geometry.

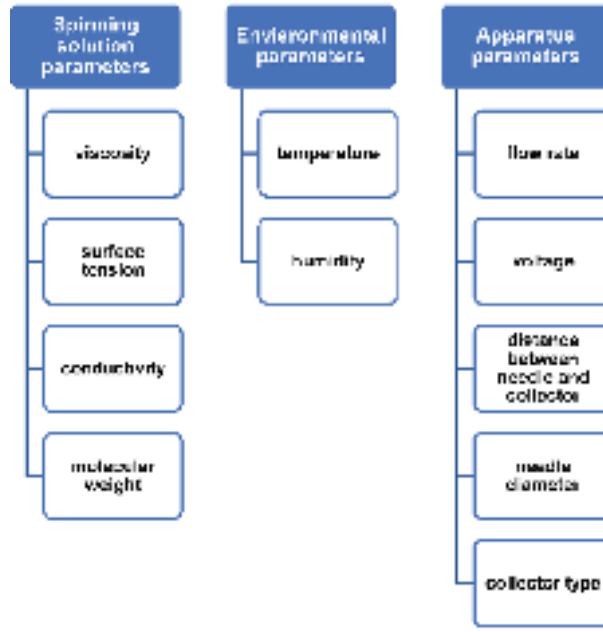


**Figure 3.**
*Common parameters of the electrospinning and electrospraying processes.*

It is worth paying special attention to the multitude of electrospinning and electrospraying modifications that may include the type of nozzle and collector. **Figure 4** shows the most popular types of collectors currently used, mainly in the electrospinning process, because they allow obtaining fibers arranged chaotically (plate) or aligned (drum, disc, dual). Modification of nozzles by using, for example, coaxial or multinozzle allows to obtain core-shell nanostructures or increases the volume of the obtained structures to an industrial scale.

Such a wide range of modification possibilities makes the electrospinning process extremely versatile and allows the production of polymer, composite and even metallic and ceramic nanofibers after appropriate thermal treatment. There is also great diversity in the morphology of fibers, both classic solid nanofibers and hollow, core-shell or high-porous nanofibers are produced, depending on the requirements (**Figure 5**).

There is also wide spectrum of ectrospraying (ESPR) techniques with different configurations (**Figure 6**) for fabrication the various material structures: layers [7] polymer or ceramic coated membranes [8], thin film composites [9], and nanoparticles surface [9]. In general, these techniques are based on the application of various spraying modes dependent on process conditions (flow rate and electric potential) and fluidic properties [10, 11]. It is assumed that according to the rate of the solution flow and used voltage, the electrospray process is classified into three modes: dripping, pulsating, and cone-jet [12]. The most frequently used is the cone-jet mode. The conical shape of it is formed as a result of parallelism of electric field (voltage) and surface tension. In comparison to other modes, cone-jet spraying demonstrates more stable working, achieves continuously produce homogeneous droplets, and indicate higher water concentration. In addition the external environment can easy modify the droplet characteristic [13]. The cone-jet mode is exploited in a wide range of applications, i.e. local-area cooling, nanofilm and nanowire manufacturing, mass spectrometry due to excellent spray pattern repeatability [14–16]. However, a certain limitation of the mod is the difficulty in precisely describing and planning the process due to the complexity of experimental and fluidic properties, which must be determined depending on various conditions. In practice, this most often means the need to select parameters experimentally [17].

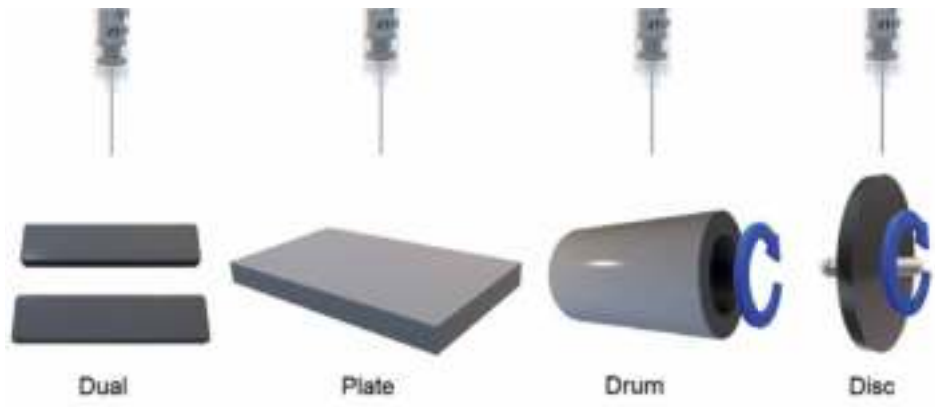


**Figure 4.**
*Popular types of electrospinning collectors.*


**Figure 5.**
*Possible morphologies of nanofibers obtained by electrospinning.*

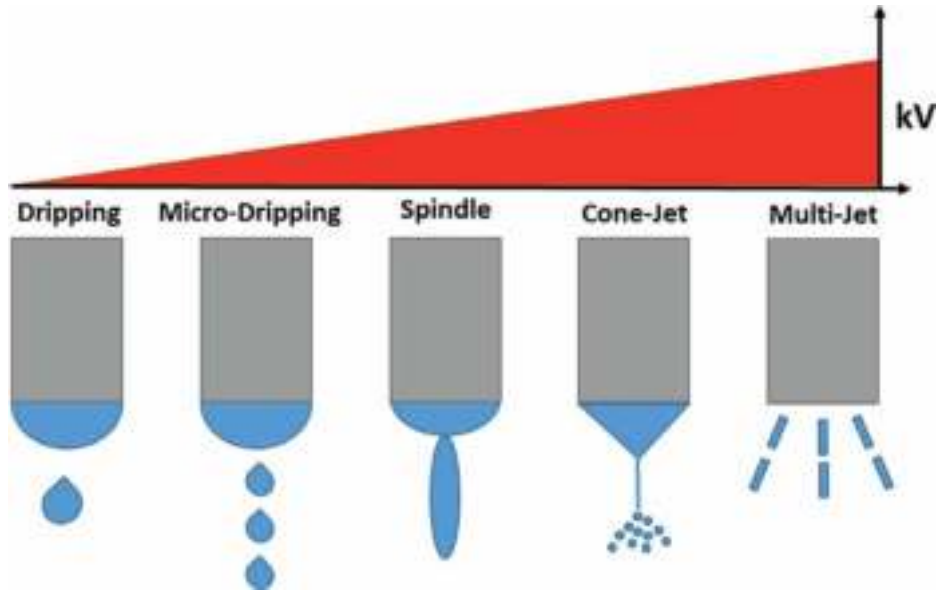


**Figure 6.**
*Spraying modes with potential difference increase [6].*

There are also more advanced varieties of electrospraying. One of them is the ring electrode system. In this system, the existing nozzle and substrate are attached to the ring electrode. While the process is ongoing, i.e. when voltage is being applied, the system of ring electrode can modulate the spray pattern by changing the external conditions, thereby adjusting the droplet size and rate which enhances the droplet distribution. Also, the ring electrode system can be used to overcome the low flow rate by increasing the liquid concentration. The ring electrode system is commonly utilized for local film coatings with high concentrations of fluid and areas where experimental parameters and fluidic characteristics are estimated [18, 19].

Another more advanced type of electrospraying is the nanoelectrospray laser deposition technique. It is a technique of applying layers of nanoparticles on two-dimensional and three-dimensional structures [20]. The technique uses a suspension of nanoparticles dispersed in water with an appropriate surfactant called aqueous ink increasing viscous at high concentrations [21–23]. Using this ink in nanoelectrospray enables microdroplets production which can be used for thin film deposition,

drop-on-demand printing, and microencapsulation [24–28] which provides easier manufacture of a variety of energy and electronic devices (solar cells, sensors) as well as masks for nanolithography, nanopillar arrays for photonic crystals, nanodot arrays for the plasmonic surface.

## 2. Electrospinning and electrospraying in food industry

High quality, the possibility of functionalization, and the multitude of available materials make nanofibers suitable for use as filtering membranes, scaffolds, drug delivery systems, intelligent textiles, face masks, and optical devices. It is also worth paying attention to the special role of electrospun nanofibers in the food industry.

Food packaging is intended to protect it from the harmful effects of microorganisms, oxidation and temperature during storage and transport. Currently, more and more attention is being paid to increasing the functionality of the packaging, which led to the creation of novel active food packaging technologies, which interact actively with the food product or its environment.

The basis of typical food packaging materials is plastics to which an active compound is added, which may be a moisture absorber, a substance that inhibits microbial growth or delays oxidation. Both nanofibers and nanoparticles work perfectly in this role, especially if they are based on biodegradable polymers such as polycaprolactone (PCL) and polylactide (PLA), natural polymers e.g. zein, gelatin, chitosan, gum Arabic, sodium alginate.

Zhang et al. [29] using the electrospinning method prepared composite PLA membranes with the addition of ZnO nanoparticles, which antimicrobial and optical properties study showed very good bactericidal activity against *Escherichia coli* and *Staphylococcus aureus* and excellent ultraviolet and visible light barrier performances, respectively. These features mean that the produced PLA/ZnO nanofibers can be used in packaging that protects food against the harmful effects of bacteria and light.

Liu et al. [30] showed that electrospun PLA/tea polyphenol nanofibers have very high antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and can potentially be used in food packaging to extend food shelf life.

PCL/lecithin/bacteriocin CAMT6 nanofiber films manufactured via emulsion electrospinning by Li et al. [31] effectively inhibited the growth of bacteria in salmon meat, slowed the oil oxidation and water loss in salmon meat, which indicates the multifunctionality of this packaging material.

Zhang et al. [32] prepared polycaprolactone/chitosan nanofibers with Chinese Yam Polysaccharide (CYP) as the active ingredient by electrospinning. The produced nanofibers were subjected to an in vitro CYP release test and an obvious antibacterial effect was demonstrated. Moreover, the result of using the PCL/Chitosan/CYP nanofiber packaging on cherry tomatoes was a delayed water loss, which indicates the possibility of using the nanofibers in active food packaging.

Using the needle-less electrospinning method Karim et al. [33] prepared zein nanofibers loaded with tetradecane and cinnamaldehyde as an antibacterial and antioxidant agent, which were to ensure protection of the sausage during temperature fluctuations and adequate protection against bacteria. The study showed that the nanofibers used perfectly prevented the growth of *Escherichia coli* and *Staphylococcus aureus*, and the packaging using them, when the refrigerator was turned off, retained the cool temperature four times longer than commercial packaging.

Gelatin/chitosan/3-phenylacetic acid nanofiber films were prepared via electrospinning by Liu et al. [34] to study their properties and antibacterial activity. It was shown that the obtained nanofibers were characterized by very good thermal stability and water vapor permeability, as well as effective antibacterial activity against *Salmonella enterica* Enteritidis and *Staphylococcus aureus*, which makes the gelatin/chitosan/3-phenylacetic acid nanofibers useful in active food packaging.

Wu et al. [35] developed edible food packaging gelatin/zein nanofibers loaded with cinnamaldehyde/thymol using electrospinning method. The produced nanofibers worked perfectly as strawberry packaging material, extending the freshness of the fruit from 2 to 7 days, which was attributed to high antibacterial activity and the ability to maintain appropriate humidity.

Bioactive coatings with antibacterial, antioxidant and pH-responsive activity are also produced using the electrospray method. Stoleru et al. [36] produced, using electrospraying technique, dual-bioactive layers in the form of PE surface with immobilized antimicrobial chitosan and antioxidant vitamin E. This kind of material has antifungal, antibacterial, biocompatibility, bioadhesivity, biodegradability properties derived from chitosan [37] and antioxidative activity as well as biological functions derived from vitamin E, and is very promising for the use of polyethylene as food packaging.

Electrospraying is used to produce active packaging material based on cellulose [38]. For this purpose, humic substances based on humic acid and fulvic acid (FA) were used. FA demonstrates antimicrobial and anti-inflammatory properties [39] and contains minerals and antioxidants. Finally, a biodegradable, sustainable, renewable, recyclable, and low-cost packaging material was produced by incorporating FA and carrier medium S on the packaging paper [40]. Due to their easy functionalization with various substances, nanofibers can not only protect against microorganisms but can also perform the function of monitoring the freshness of products by sensing pH changes, protecting consumers from health problems caused by the consumption of stale food.

In the work of Lafray et al. [40], it has been shown that the combination of electrospinning and electrospraying technologies enables the development of a new generation of super-repellent active biopapers with PLA, PCL, or PHBV coatings with incorporated hydrophobic silica microparticles with increased effectiveness for easy emptying food packaging to reduce waste of food. The research used: polylactide (PLA), poly(ε-caprolactone) (PCL), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), and hydrophobic silica microparticles ($SiO_2$), which were processed sequentially with use electrohydrodynamic processing (electrospinning and electrospraying). In the first stage, biopolymers were deposited onto a commercial food contact cellulose paper by electrospinning to create ultrathin fibers, next on the surface of fibers the nanostructured $SiO_2$ was electrosprayed sequentially. In the further part of the work, multilayer-coated papers have been annealed to intensify adhesion among the layers and the super-repellent properties. In the further part of the work, multilayer-coated papers have been annealed to intensify adhesion among the layers and the super-repellent properties.

Shavisi and Shahbazi [41] electrospun chitosan-gum Arabic nanofibers with encapsulated pH-sensitive *Rosa damascena* anthocyanins, which change color depending on the pH of the environment. It was shown that the produced nanofibrous mats easily responded to the pH change of chicken fillets during 8 days of refrigerated storage, changing their color from pale pink to dark khaki, corresponding to the spoilage of the chicken.

Guo et al. [42] manufactured double-layer mats composed of a layer of pullulan-purple sweet potato extract and a layer of zein-glycerol-carvacrol, which not only had good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* but also monitored the freshness of pork by changing the purple color sweet potato extract with the change of pH, as presented in **Figure** 7.

Multifunctional polylactide, butterfly pea flower extract and cinnamaldehyde nanofibers were obtained by Liu et al. [43] for tracking and maintaining beef freshness. Results showed that the nanofibers had hydrophobic, antioxidant and antibacterial properties and changed their color with the change in pH, which changed with the loss of freshness of the meat because the amount of volatile alkaline compounds resulting from amino acid deamidation reactions increased.

In the food industry, food encapsulation is also extremely important, as it enables the incorporation of vitamins, nutrients and bioactive compounds in food, supplements or packages that will improve the quality and safety of products [44].

Electrospinning and electrospraying methods are widely used for encapsulating active ingredients e.g. essential oils, primarily due to the use of efficient easy controlled low-temperature processes that are harmless to volatile bioactive compounds. These techniques can also protect these compounds against loss of stability caused by environmental conditions such as: light, oxygen, pH, humidity, and the effect of enzymes [45]. Research conducted, among others, by Coelho et al. indicates that in this case, the critical factor is the choice of the polymer for the encapsulation of active ingredients for manufacturing structures capable of improving and/or preserving its bioactive properties [1]. In particular, biodegradable and renewable polymers are being developed as encapsulating materials. In turn, lipids, carbohydrates, proteins (also in native state), and their mixtures are considered to be the most suitable food-encapsulating agents. However, currently, food proteins are of the greatest importance in encapsulation due to their biodegradability, and biocompatibility as well as technological functionalities (foaming, gelation, emulsification) ability to form various nanostructures (nanoparticles, nanofibers), surface activity, high ability to adsorb to the surfaces of colloidal particles and stabilization against aggregation. Proteins present the ability to interact strongly with both polar (e.g. water) and non-polar solvent molecules (e.g. hydrocarbons) and have different functional groups on surfaces, which are used to modify and create interaction with various bioactive molecules [46].

Weng et al. [47] encapsulated anti-inflammatory, antibacterial, antitumor and antioxidant camellia oil in core-shell zein nanofibers using emulsion electrospinning. It was shown that zein nanofibers are excellent controlled release systems for hydrophobic oil.

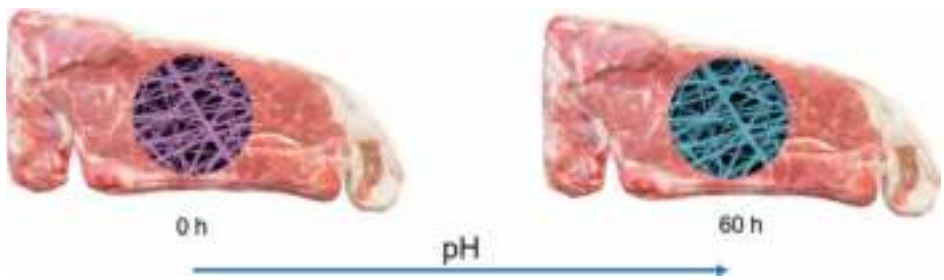


**Figure 7.**
*Pork freshness monitoring using pullulan-purple sweet potato extract and zein-glycerol-carvacrol nanofibers as pH indicator [own work].*

Vitamin E-loaded dextran nanofibers were obtained by Fathi et al. [48]. The produced nanofibers were used to fortify cheese, which indicates that they have great application potential in the development of modern functional foods.

Drosou et al. [49] encapsulated β-carotene into pullulan/whey protein isolate nanofibers via coaxial electrospinning, providing more than 90% encapsulation efficiencies. The study showed that the nanofibers showed the function of protecting β-carotene against oxidation by molecular oxygen, which may open new avenues for the development of carotenoid supplements.

It should be mentioned that the conventional ESPR techniques have some limitations in food encapsulation due to the physical parameters of the used solution (mainly viscosity and surface tension): difficulties of organic solvent eliminating by evaporation, limited range of operation (working window). In turn, the parameters of the solution depend strictly on molecular weight of the polymer and its concentration in the solution. All of this influences on general low productivity of the electrospraying process among others in the food and food packaging industry (few milliliters per hour per single emitter) [50]. Thus, ongoing work on the development of ESPR technology in the food processing industry is necessary. One of the most common perspective is "Supercritical assisted electrospraying" SCF, combining high electric voltage and pneumatic spray biomass disruption, extraction and encapsulation, microbial/enzymatic inactivation [51].

One of the varieties of this technology is proposed by Bioinicia S.L. The "Electrospraying assisted by pressurized gas" (EAPG). The introduction of EAPG is particularly important for increasing the efficiency of the ESPR process to enable mass production. The idea of EAPG is to modernize the classical electrospray atomization method through the addition of supercritical $CO_2$ to the liquid polymer solution creating an expanded liquid. This ensures an increase in the production rate of micro- and nanometric particles with controlled size and distribution up to 100 times in comparison traditional ESPR process due to the reduction of surface tension and viscosity [52].

This technique can be used for particle (micro- and nanoparticles) producing from a very high molecular weight supercritical fluid (mainly SC-$CO_2$), supercritical antisolvent (SAS), supercritical assisted atomization (SAA) and supercritical emulsion extraction (SEE) [53–57].

Modification of ESPR has been extensively explored in encapsulation technology. Gómez-Mascaraque et al. described excellent encapsulation profiles and stabilities of zein and gelatin by containing b-Carotene [58], epigallocatechin gallate (EGCG) [59], extract of green tea (catechins) [58], saffron extract [60], chanar extract [61], and curcumin [62]. Using ESPR ensured the appropriate particle size in this case: micron and nanoscale, good stability of core material and properties of the active compound. Good structural properties combined with an appropriate level of protection against oxidation, high thermal stability, and controlled release make the manufactured materials suitable for food applications.

ESPR technology was also used by authors who worked on microencapsulating probiotics like *Lactiplantibacillus plantarum* and *Bifidobacterium lactis* [63–65] i.e. for double-layer co-encapsulation.

ESPR was also used in the study by Human et al. regarding the nanoencapsulation of aspalatin (hydrophilic bioactive dihydrochalcone). Aspalathin has poor bioavailability and stability, which significantly limits its use as an active food ingredient. The solution to this problem was encapsulation using a synthetic polymer as a wall material [66].

In the encapsulation process, another modification of ESPR is often used, which involves the use of a special nozzle structure (coaxial nozzle). This element is built by the outer needle and an inner needle. During the process two liquids (immiscible), eventually liquid and gas, are injected into the outer and the inner needles and the flow rates is controlled by two syringe pumps. There are a number of advantages of coaxial electrospray in comparison with other techniques of encapsulation: high efficiency, effective protection of bioactivity, uniform size distribution, but the control of the process is complex due to the multiphysical nature of the processes involved and the complex interplay of multiple material and technology parameters [67].

Coaxial electrospraying encapsulation is successfully used for bioactive agents for pharmaceutical applications [68, 69] as well as for food-grade materials, in which: alginate-pectin [70], dextran and whey protein concentrate (WPC) [71] and WPC-gelatin [72] have been used as matrix materials.

Many literature reports also concern the use of combining electrospinning and electrospraying methods to encapsulate essential oil with the use of natural polymer material such as protein (soy, whey), zein, hordein, gliadin, casein, protein, and gelatin [1, 44, 73, 74].

## 3. Conclusions

This review presents the basics of electrospinning and electrospraying techniques and summarizes the latest achievements in their use in the food industry. The use of electrospinning and electrospraying techniques in the food industry brings many benefits, such as extending the shelf life of products, the ability to monitor the quality and freshness of food, and even improving the nutritional value of food. At the same time, these methods are simple, versatile and do not generate high costs. The use of these techniques in the food industry still requires refinement and close cooperation of scientists, because more studies are needed in particular to investigate the threats to human health that may appear with the use of nanomaterials in contact with food and to study the behavior of nanomaterials in contact with protective gases used during packaging. This is very important because materials in the food industry must meet very strict requirements. It is also worth to pay attention to the repeatability of research and increasing the efficiency of nanomaterial production so that they can meet the requirements of the industry. In addition, more attention should be paid to an ecological approach in the selection of materials, increasing the emphasis on the development of biopolymers in the food industry.

Both methods have great potential to revolutionize food industry, but for this to happen, a lot of work of scientists on this subject is needed.

## Conflict of interest

The authors declare no conflict of interest.

*Electrospraying and Electrospinning in Food Industry*
*DOI: http://dx.doi.org/10.5772/intechopen.1008438*

## Author details

Weronika Smok*, Paweł Jarka and Tomasz Arkadiusz Tański
Department of Engineering Materials and Biomaterials, Silesian University of Technology, Gliwice, Poland

*Address all correspondence to: weronika.smok@polsl.pl

IntechOpen

## References

[1] Castro Coelho S, Nogueiro Estevinho B, Rocha F. Encapsulation in food industry with emerging electrohydrodynamic techniques: Electrospinning and electrospraying – A review. Food Chemistry. 2021;**339**:127850. DOI: 10.1016/j.foodchem.2020.127850

[2] Muthukrishnan L. An overview on electrospinning and its advancement toward hard and soft tissue engineering applications. Colloid & Polymer Science. 2022;**300**(8):875-901. DOI: 10.1007/S00396-022-04997-9

[3] Brandenberger H, Nüssli D, Piech V, Widmer F. Monodisperse particle production: A method to prevent drop coalescence using electrostatic forces. Journal of Electrostatics. 1999;**45**(3):227-238. DOI: 10.1016/S0304-3886(98)00052-7

[4] Jaworek ATSA, Sobczyk AT. Electrospraying route to nanotechnology: An overview. Journal of Electrostatics. 2008;**66**(3):197-219. DOI: 10.1016/j.elstat.2007.10.001

[5] Vetcher AA, Gearheart R, Morozov VN. Polyacrylamide-based material for electrospun humidity-resistant, water-soluble nanofilters. Polymers for Advanced Technologies. 2008;**19**(9):1276-1285. DOI: 10.1002/PAT.1124

[6] Saallah S, Lenggoro IW. Nanoparticles carrying biological molecules: Recent advances and applications. Kona Powder and Particle Journal. 2018;**35**(35):89-111. DOI: 10.14356/KONA.2018015

[7] Vatanpour V, Kose-Mutlu B, Koyuncu I. Electrospraying technique in fabrication of separation membranes: A review. Desalination. 2022;**533**:115765. DOI: 10.1016/j.desal.2022.115765

[8] Go Y, Lee JH, Shamsudin IK, Kim J, Othman MR. Microporous ZIF-7 membranes prepared by in-situ growth method for hydrogen separation. International Journal of Hydrogen Energy. 2016;**41**(24):10366-10373. DOI: 10.1016/j.ijhydene.2015.09.060

[9] Ma XH, Yang Z, Yao ZK, Guo H, Xu ZL, Tang CY. Interfacial polymerization with electrosprayed microdroplets: Toward controllable and ultrathin polyamide membranes. Environmental Science & Technology Letters. 2018;**5**(2):117-122. DOI: 10.1021/ACS.ESTLETT.7B00566/SUPPL_FILE/EZ7B00566_SI_001.PDF

[10] Kim JY, Lee SJ, Lee MH, Hong JG. Experimental investigation of electrospraying properties based on ring electrode modification. ACS Omega. 2024;**9**(1):1125-1133. DOI: 10.1021/ACSOMEGA.3C07402/ASSET/IMAGES/LARGE/AO3C07402_0013.JPEG

[11] Chen X, Jia L, Yin X, Cheng J, Lu J. Spraying modes in coaxial jet electrospray with outer driving liquid. Physics of Fluids. 2005;**17**:3. DOI: 10.1063/1.1850691/314126

[12] Kim MJ, Song JY, Hwang SH, Park DY, Park SM. Electrospray mode discrimination with current signal using deep convolutional neural network and class activation map. Scientific Reports. 2022;**12**(1):1-10. DOI: 10.1038/s41598-022-20352-y

[13] Wu Y, Mackay JA, Mcdaniel JR, Chilkoti A, Clark RL. Fabrication of elastin-like polypeptide nanoparticles for drug delivery by electrospraying.

Biomacromolecules. 2009;**10**(1):19-24. DOI: 10.1021/BM801033F

[14] Yuill EM, Sa N, Ray SJ, Hieftje GM, Baker LA. Electrospray ionization from nanopipette emitters with tip diameters of less than 100 nm. Analytical Chemistry. 2013;**85**(18):8498-8502. DOI: 10.1021/AC402214G/SUPPL_FILE/AC402214G_SI_001.PDF

[15] Määttä KR, Kamal-Eldin A, Riitta TA. High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: Ribes species. Journal of Agricultural and Food Chemistry. 2003;**51**(23):6736-6744. DOI: 10.1021/JF0347517

[16] Wu X, Prior RL. Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: Vegetables, nuts, and grains. Journal of Agricultural and Food Chemistry. 2005;**53**(8):3101-3113. DOI: 10.1021/JF0478861

[17] Takáts Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science. 2004;**306**(5695):471-473. DOI: 10.1126/SCIENCE.1104404

[18] Jiang Z, Gan Y, Ju Y, Liang J, Zhou Y. Experimental study on the electrospray and combustion characteristics of biodiesel-ethanol blends in a meso-scale combustor. Energy. 2019;**179**:843-849

[19] Kuwahata Y, Takehara H, Ichiki T. Comprehensive study on electrospray deposition in the single Taylor cone-jet mode by changing the spatial electric potential using a ring-shaped ternary electrode. AIP Advances. 2020;**10**:4. DOI: 10.1063/1.5142317

[20] Castillo-Orozco E, Kar A, Kumar R. Electrospray mode transition of microdroplets with semiconductor nanoparticle suspension. Scientific Reports. 2017;**7**:1. DOI: 10.1038/S41598-017-05175-6

[21] Milanova D, Kumar R. Role of ions in pool boiling heat transfer of pure and silica nanofluids. Applied Physics Letters. 2005;**87**(23):1-3. DOI: 10.1063/1.2138805/329976

[22] Davanlou A, Kumar R. Thermally induced collision of droplets in an immiscible outer fluid. Scientific Reports. 2015;**5**(1):1-8. DOI: 10.1038/srep09531

[23] Davanlou A, Lee JD, Basu S, Kumar R. Effect of viscosity and surface tension on breakup and coalescence of bicomponent sprays. Chemical Engineering Science. 2015;**131**:243-255. DOI: 10.1016/j.ces.2015.03.057

[24] Jaworek A. Micro- and nanoparticle production by electrospraying. Powder Technology. 2007;**176**(1):18-35. DOI: 10.1016/j.powtec.2007.01.035

[25] Cascio AJ, Lyon JE, Beerbom MM, Schlaf R, Zhu Y, Jenekhe SA. Investigation of a polythiophene interface using photoemission spectroscopy in combination with electrospray thin-film deposition. Applied Physics Letters. 2006;**88**:6. DOI: 10.1063/1.2172069/331728

[26] Loscertales IG, Barrero A, Guerrero I, Cortijo R, Marquez M, Gañán-Calvo AM. Micro/Nano encapsulation via electrified coaxial liquid jets. Science (80-). 2002;**295**(5560):1695-1698. DOI: 10.1126/SCIENCE.1067595

[27] Choi J, Kim YJ, Lee S, Son SU, Ko HS, Nguyen VD, et al. Drop-on-demand

printing of conductive ink by electrostatic field induced inkjet head. Applied Physics Letters. 2008;**93**:19. DOI: 10.1063/1.3020719/336481

[28] Marín ÁG, Loscertales IG, Márquez M, Barrero A. Simple and double emulsions via coaxial jet electrosprays. Physical Review Letters. 2007;**98**:1. DOI: 10.1103/PHYSREVLETT.98.014502

[29] Zhang R, Lan W, Ji T, Sameen DE, Ahmed S, Qin W, et al. Development of polylactic acid/ZnO composite membranes prepared by ultrasonication and electrospinning for food packaging. LWT. 2021;**135**:110072. DOI: 10.1016/j.lwt.2020.110072

[30] Liu Y, Liang X, Wang S, Qin W, Zhang Q. Electrospun antimicrobial polylactic acid/tea polyphenol nanofibers for food-packaging applications. Polymers. 2018;**10**:5. DOI: 10.3390/POLYM10050561

[31] Li Q, Liang W, Lv L, Fang Z, Xu D, Liao J, et al. Preparation of PCL/lecithin/bacteriocin CAMT6 antimicrobial and antioxidant nanofiber films using emulsion electrospinning: Characteristics and application in chilled salmon preservation. Food Research International. 2024;**175**:113747. DOI: 10.1016/j.foodres.2023.113747

[32] Zang C, Zhang Y, Yang W, Hu Y. Polycaprolactone/chitosan electrospun nanofibrous membranes loaded Chinese yam polysaccharide for active food packaging. LWT. 2024;**198**:115985. DOI: 10.1016/j.lwt.2024.115985

[33] Karim M, Fathi M, Soleimanian-Zad S, Spigno G. Development of sausage packaging with zein nanofibers containing tetradecane produced via needle-less electrospinning method. Food Packaging and Shelf Life. 2022;**33**:100911. DOI: 10.1016/j.fpsl.2022.100911

[34] Liu Y, Wang D, Sun Z, Liu F, Du L, Wang D. Preparation and characterization of gelatin/chitosan/3-phenylacetic acid food-packaging nanofiber antibacterial films by electrospinning. International Journal of Biological Macromolecules. 2021;**169**:161-170. DOI: 10.1016/j.ijbiomac.2020.12.046

[35] Wu X, Liu Z, He S, Liu J, Shao W. Development of an edible food packaging gelatin/zein based nanofiber film for the shelf-life extension of strawberries. Food Chemistry. 2023;**426**:136652. DOI: 10.1016/j.foodchem.2023.136652

[36] Stoleru E, Munteanu SB, Dumitriu RP, Coroaba A, Drobotă M, Zemljic LF, et al. Polyethylene materials with multifunctional surface properties by electrospraying chitosan/vitamin E formulation destined to biomedical and food packaging applications. Iranian Polymer Journal. 2016;**25**(4):295-307. DOI: 10.1007/S13726-016-0421-0/METRICS

[37] Stefanowska K, Woźniak M, Dobrucka R, Ratajczak I. Chitosan with natural additives as a potential food packaging. Maternité. 2023;**16**(4):1579. DOI: 10.3390/MA16041579

[38] Ormanli E, Amca Uluturk B, Bozdogan N, Bayraktar O, Tavman S, Kumcuoglu S. Development of a novel, sustainable, cellulose-based food packaging material and its application for pears. Food Chemistry. 2023;**429**. DOI: 10.1016/J.FOODCHEM.2023.136719

[39] Van Rensburg CEJ, Malfeld SCK, Dekker J. Topical application of oxifulvic acid suppresses the cutaneous immune response in mice. Drug Development

Research. 2001;**53**(1):29-32. DOI: 10.1002/DDR.1166

[40] Lafraya A, Prieto C, Pardo-Figuerez M, Chiva A, Lagaron JM. Super-repellent paper coated with electrospun biopolymers and electrosprayed silica of interest in food packaging applications. Nanomaterials. 2021;**11**(12):3354. DOI: 10.3390/NANO11123354/S1

[41] Shavisi N, Shahbazi Y. Chitosan-gum Arabic nanofiber mats encapsulated with pH-sensitive Rosa damascena anthocyanins for freshness monitoring of chicken fillets. Food Packaging and Shelf Life. 2022;**32**:100827

[42] Huang Z, Gao H, Wang Q, Zhao Y, Li G. Fabrication of amorphous SnO2@C nanofibers as anode for lithium-ion batteries. Materials Letters. 2017;**186**:231-234

[43] Liu X, Song X, Gou D, Li H, Jiang L, Yuan M, et al. A polylactide based multifunctional hydrophobic film for tracking evaluation and maintaining beef freshness by an electrospinning technique. Food Chemistry. 2023;**428**:136784

[44] Lim LT. Encapsulation of bioactive compounds using electrospinning and electrospraying technologies. In: Nanotechnology and Functional Foods: Effective Delivery of Bioactive Ingredients. Hoboken, New Jersey, U.S.: Wiley; 2015. pp. 297-317. DOI: 10.1002/9781118462157.CH18

[45] Pires JB, Santos FN d, Costa IH d L, Kringel DH, Zavareze E d R, Dias ARG. Essential oil encapsulation by electrospinning and electrospraying using food proteins: A review. Food Research International. 2023;**170**:112970

[46] Mohammadian M, Waly MI, Moghadam M, Emam-Djomeh Z, Salami M, Moosavi-Movahedi AA. Nanostructured food proteins as efficient systems for the encapsulation of bioactive compounds. Food Science and Human Wellness. 2020;**9**(3):199-213

[47] Weng J, Zou Y, Zhang Y, Zhang H. Stable encapsulation of camellia oil in core–shell zein nanofibers fabricated by emulsion electrospinning. Food Chemistry. 2023;**429**:136860

[48] Fathi M, Nasrabadi MN, Varshosaz J. Characteristics of vitamin E-loaded nanofibres from dextran. International Journal of Food Properties. 2017;**20**(11):2665-2674. DOI: 10.1080/10942912.2016.1247365

[49] Drosou C, Krokida M, Biliaderis CG. Encapsulation of β-carotene into food-grade nanofibers via coaxial electrospinning of hydrocolloids: Enhancement of oxidative stability and photoprotection. Food Hydrocolloids. 2022;**133**:107949. DOI: 10.3390/foods13121933

[50] Torres-Giner S. Electrospun nanofibers for food packaging applications. In: Multifunctional and Nanoreinforced Polymers for Food Packaging. Sawston, UK: Woodhead Publishing; 2011. pp. 108-125. DOI: 10.1533/9780857092786.1.108

[51] Baldino L, Cardea S, Reverchon E. A supercritical $CO_2$ assisted electrohydrodynamic process used to produce microparticles and microfibers of a model polymer. Journal of CO2 Utilization. 2019;**33**:532-540. DOI: 10.1016/j.jcou.2019.08.013

[52] Busolo MA, Torres-Giner S, Prieto C, Lagaron JM. Electrospraying assisted by pressurized gas as an innovative high-throughput process for the microencapsulation and stabilization of docosahexaenoic acid-enriched fish oil in

zein prolamine. Innovative Food Science and Emerging Technologies. 2019;**51**:12-19. DOI: 10.1016/j.ifset.2018.04.007

[53] Baldino L, Cardea S, Reverchon E. Supercritical assisted electrospray: An improved micronization process. Polymers. 2019;**11**(2):244. DOI: 10.3390/POLYM11020244

[54] Wang Q, Guan YX, Yao SJ, Zhu ZQ. Controllable preparation and formation mechanism of BSA microparticles using supercritical assisted atomization with an enhanced mixer. Journal of Supercritical Fluids. 2011;**56**(1):97-104. DOI: 10.1016/j.supflu.2010.12.002

[55] Reverchon E. Supercritical-assisted atomization to produce micro- and/or nanoparticles of controlled size and distribution. Industrial and Engineering Chemistry Research. 2002;**41**(10):2405-2411. DOI: 10.1021/IE010943K

[56] Furlan M, Kluge J, Mazzotti M, Lattuada M. Preparation of biocompatible magnetite–PLGA composite nanoparticles using supercritical fluid extraction of emulsions. Journal of Supercritical Fluids. 2010;**54**(3):348-356

[57] Falco N, Reverchon E, Della PG. Injectable PLGA/hydrocortisone formulation produced by continuous supercritical emulsion extraction. International Journal of Pharmaceutics. 2013;**441**(1-2):589-597. DOI: 10.1016/J.IJPHARM.2012.10.039

[58] Gómez-Mascaraque LG, Hernández-Rojas M, Tarancón P, Tenon M, Feuillère N, Vélez Ruiz JF, et al. Impact of microencapsulation within electrosprayed proteins on the formulation of green tea extract-enriched biscuits. LWT - Food Science and Technology. 2017;**81**:77-86

[59] Shi M, Shi YL, Li XM, Yang R, Cai ZY, Li QS, et al. Food-grade encapsulation systems for (−)-epigallocatechin gallate. Molecules. 2018;**23**:2. DOI: 10.3390/MOLECULES23020445

[60] Alehosseini A, Gómez-Mascaraque LG, Ghorani B, López-Rubio A. Stabilization of a saffron extract through its encapsulation within electrospun/electrosprayed zein structures. LWT. 2019;**113**:108280. DOI: 10.1016/j.lwt.2019.108280

[61] Costamagna MS, Gómez-Mascaraque LG, Zampini IC, Alberto MR, Pérez J, López-Rubio A, et al. Microencapsulated chañar phenolics: A potential ingredient for functional foods development. Journal of Functional Foods. 2017;**37**:523-530. DOI: 10.1016/j.jff.2017.08.018

[62] Alehosseini A, Gómez-Mascaraque LG, Martínez-Sanz M, López-Rubio A. Electrospun curcumin-loaded protein nanofiber mats as active/bioactive coatings for food packaging applications. Food Hydrocolloids. 2019;**87**:758-771. DOI: 10.1016/j.foodhyd.2018.08.056

[63] Coghetto CC, Brinques GB, Siqueira NM, Pletsch J, Soares RMD, Ayub MAZ. Electrospraying microencapsulation of Lactobacillus plantarum enhances cell viability under refrigeration storage and simulated gastric and intestinal fluids. Journal of Functional Foods. 2016;**24**:316-326. DOI: 10.1016/j.jff.2016.03.036

[64] Gómez-Mascaraque LG, Ambrosio-Martín J, Perez-Masiá R, Lopez-Rubio A. Impact of acetic acid on the survival of *L. plantarum* upon microencapsulation by coaxial electrospraying. Journal of Healthcare Engineering. 2017;**2017**:4698079. DOI: 10.1155/2017/4698079

[65] Zaeim D, Sarabi-Jamab M, Ghorani B, Kadkhodaee R. Double layer co-encapsulation of probiotics and prebiotics by electro-hydrodynamic atomization. LWT. 2019;**110**:102-109. DOI: 10.1016/j.lwt.2019.04.040

[66] Human C, De Beer D, Van Der Rijst M, Aucamp M, Joubert E. Electrospraying as a suitable method for nanoencapsulation of the hydrophilic bioactive dihydrochalcone, aspalathin. Food Chemistry. 2019;**276**:467-474. DOI: 10.1016/j.foodchem.2018.10.016

[67] Zhang L, Huang J, Si T, Xu RX. Coaxial electrospray of microparticles and nanoparticles for biomedical applications. Expert Review of Medical Devices. 2012;**9**(6):595-612. DOI: 10.1586/ERD.12.58

[68] Kim W, Kim SS. Multishell encapsulation using a triple coaxial electrospray system. Analytical Chemistry. 2010;**82**(11):4644-4647. DOI: 10.1021/AC100278C

[69] Wu Y, Fei Z, Lee LJ, Wyslouzil BE. Coaxial electrohydrodynamic spraying of plasmid DNA/polyethylenimine (PEI) polyplexes for enhanced nonviral gene delivery. Biotechnology and Bioengineering. 2010;**105**(4):834-841. DOI: 10.1002/BIT.22583

[70] Koo SY, Cha KH, Song DG, Chung D, Pan CH. Microencapsulation of peppermint oil in an alginate–pectin matrix using a coaxial electrospray system. International Journal of Food Science and Technology. 2014;**49**(3):733-739. DOI: 10.1111/IJFS.12358

[71] Pérez-Masiá R, López-Nicolás R, Periago MJ, Ros G, Lagaron JM, López-Rubio A. Encapsulation of folic acid in food hydrocolloids through nanospray drying and electrospraying for nutraceutical applications. Food Chemistry. 2015;**168**:124-133. DOI: 10.1016/j.foodchem.2014.07.051

[72] Gómez-Mascaraque LG, Perez-Masiá R, González-Barrio R, Periago MJ, López-Rubio A. Potential of microencapsulation through emulsion-electrospraying to improve the bioaccesibility of β-carotene. Food Hydrocolloids. 2017;**73**:1-12. DOI: 10.1016/j.foodhyd.2017.06.019

[73] Ansarifar E, Moradinezhad F. Encapsulation of thyme essential oil using electrospun zein fiber for strawberry preservation. Chemical and Biological Technologies in Agriculture. 2022;**9**:1. DOI: 10.1186/S40538-021-00267-Y

[74] Falsafi SR, Topuz F, Esfandiari Z, Can Karaca A, Jafari SM, Rostamabadi H. Recent trends in the application of protein electrospun fibers for loading food bioactive compounds. Food Chemistry X. 2023;**20**:100922. DOI: 10.1016/j.fochx.2023.100922

Chapter 2

# Applications of Electrospraying in Tissue Repair and Regeneration

*Rahul Sable, Pritiprasanna Maity and Kausik Kapat*

## Abstract

Electrospraying (ES) is becoming popular in tissue engineering owing to its ability to produce customized micro- or nanoscale particles for delivering bioactive molecules (e.g., growth factors, genes, enzymes, and therapeutic molecules possessing antimicrobial, anti-inflammatory) and living cells aimed at skin, bone, cartilage, and neural tissue repair and regeneration. Compared to conventional delivery methods, ES significantly reduces the denaturation of growth factors (such as BMP-2, BMP-7, VEGF, PDGF, and SDF-1) because of the limited exposure to organic solvents. Bioelectrospraying (BES) allows the encapsulation of living cells, including stem cells, fibroblasts, ligament cells, epithelial and endothelial cells, etc. Electrospray nanocarriers containing cells and other bioactive compounds can be further integrated into intricate three-dimensional (3D) constructs intended for implantation into defects to achieve targeted delivery and tissue regeneration. The chapter highlights ES's principles, advantages, and significant applications in tissue repair and regeneration and outlines the key challenges and limitations.

**Keywords:** electrospraying, bioelectrospraying (BES), repair and regeneration, cell encapsulation, drug delivery

## 1. Introduction

The process of repairing a wound involves the replacement of injured tissue with new tissue through collagen synthesis, epithelialization, and angiogenesis. During natural wound healing, fibroblasts and other reparative cells cause random deposition of the collagen matrix, often associated with scar tissue formation. Tissue regeneration involving three key elements—reparative cells, growth factors (GFs), or other signaling molecules like chemokines, cytokines, etc., and biomaterials or scaffolds, also known as tissue engineering TRIAD, could be a better strategy for complete wound recovery [1]. Scaffolds provide several epitopes that facilitate the formation of an extracellular milieu, support cell homing, and steer intracellular signaling pathways linked to cell motility, proliferation, and differentiation [2, 3]. The conventional scaffold manufacturing techniques, namely solvent casting, particulate leaching, freeze drying, electrospinning, gas foaming, etc., often involve cytotoxic solvents and sometimes encounter challenges like long processing time, inadequate strength, pore interconnectivity, and irregular pore

size [4]. Advanced 3D printing techniques based on photopolymerization involve large monomers, often subjected to high-temperature treatment for efficient conversion into polymeric structures.

The electrohydrodynamic (EHD) techniques, namely electrospinning and electrospraying (ES), are the other advanced methods of producing nano-/micro fibrous scaffolds and particles of different shapes and sizes, respectively, utilizing electrostatic forces (**Figure 1**) [5]. They principally differ in terms of (a) collectors, a liquid bath in wet ES and a solid plate in dry electrospinning; (b) nozzles: triaxial, coaxial, and uniaxial; and (c) final product: particles vs. nano/microfibers. In electrospinning, a syringe pump propels a polymer solution through a needle, forming a Taylor cone at the needle tip. As the repulsion force exceeds surface tension and viscoelastic forces, the polymer jet subsequently erupts into fragmented droplets, which are then drawn toward the collector-generating nanofibers. On the other hand, ES produces micro/nanodroplets at ambient temperature and pressure, which are then converted into hollow spheres, nano cups, Janus particles, porous, cell-shaped/core-shell, and multilayered micro- or nanospheres with a narrow size distribution. In ES, a polymer solution containing drugs or bioactive molecules is loaded into the syringe and sprayed through a capillary or small-bore stainless steel needle at a constant rate using a syringe pump and applied voltage of 1–30 kV (mostly in DC mode) [6]. The solvent evaporates from the droplets as they separate from the Taylor cone. It creates dense, solid particles collected on a rotating drum collector or grounded flat plate positioned 7–30 cm apart from the capillary. Polycaprolactone (PCL), chitosan, polylactide (PLA), poly(lactic-co-glycolic acid) (PLGA), etc., are frequently used to encapsulate a variety of hydrophilic and hydrophobic model drugs/proteins [7]. Optimization of electrosprayed particle characteristics (particle size distribution, encapsulation efficacy, % loading, and release profile) is a complex process since it involves the manipulation of process variables, such as voltage, tip-to-collector distance, needle diameter, viscosity, flow rate, nature of drug/polymer/solvent, drug or protein-to-polymer ratio, organic-to-aqueous solvent ratio, surfactant, and conductivity [8].

Electrospray nano/micro-particles are useful in encapsulating sensitive biomolecules (growth factor, peptide) and living cells with high encapsulation efficiency, occupying significant space in wound healing and tissue regeneration [5, 9–11]. The factors involved in ES are not fully known and require a systematic study [12].

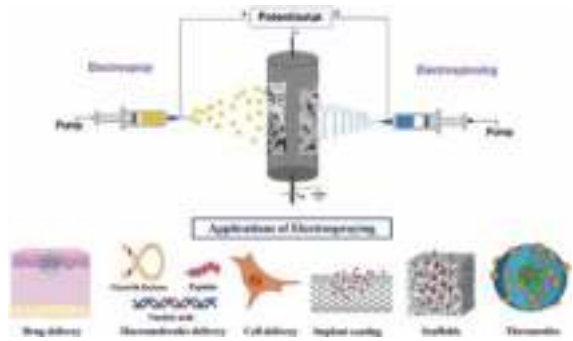

**Figure 1.**
*Principle and applications of electrospraying and electrospinning.*

## 2. ES principle

The principles of electrospraying, established by Rayleigh, Zeleny, and Taylor, are based on the capacity of an electric field to deform the liquid drop interface by generating an electric charge (electrostatic of Coulomb force) inside the droplet, which contests with the intrinsic cohesive force to the liquid droplet [8, 13]. High-voltage external electrical fields and columbic repulsion are necessary for electrospraying [14]. The phenomenon commences at the Taylor Cone, where the unstable, charged macro-droplet is gradually contracted into a cone, causing the expulsion of the smaller charged droplets as the Coulomb force dominates over the surface tension. Depending on the process parameters, micro- or nanometre-sized droplets are produced when the electrostatic force supersedes the cohesive force. "Rayleigh limit" refers to this breakdown point (LR). After disintegration, the liquid droplets quickly desolvate and are deposited on the collector as solid micro- and nanosized particles [5].

## 3. ES for wound repair and regeneration

### 3.1 Delivery of therapeutics

By adjusting the operational parameters (flow rate, external voltage, and polymer concentration), ES has proven to be one of the most efficient, quick, and adaptable methods for creating nanofibrous membranes and micro-/nano-particles with variable shape and sizes that influence their cellular or tissue uptake. During ES, the biomolecules in the spray liquid get entrapped and randomly dispersed inside the polymer matrix as the solvent evaporates from the liquid droplet [15, 16]. Drugs and other biomolecules have been successfully encapsulated and delivered via ES [17].

#### *3.1.1 Drugs*

##### *3.1.1.1 Antimicrobial drugs*

Curcumin is well known for its anti-inflammatory, anti-cancer, antibacterial, and antilipidemic properties. However, conventional delivery systems failed to achieve adequate bioavailability because of their limited water solubility and heat, pH, and light sensitivity. Several drug delivery systems, such as ES, have been investigated to enhance bio-, cyto-, and hemo-compatibility, besides cell activity.

As shown in **Figure 2(i)**, curcumin as the model drug was successfully entrapped into the polylactic acid (PLA)-based microcapsules prepared by ES the mixture of drugs with the polymer solution, displaying entrapment efficiency over 95%, in vitro drug release over 200 h, significant biocompatibility, besides excellent antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* (**Table 1**) [18]. In a similar study, curcumin-loaded PLGA microparticles were prepared by coaxial ES, and the same exhibited optimal in vitro drug release profiles compared to burst release from conventional microparticles [22]. Coaxial jet electrospray was used to encapsulate *Mentha piperita* oil with broad-spectrum antimicrobial activity. The micro-nanocapsules core containing peppermint oil was entrapped in the alginate shell using Tween 20 as an emulsifier. The same exhibited 100% inhibition of *S. aureus* and *E. coli*, similar to pure peppermint oils [23].

AgNP-laden alginate beads were produced by ES silver nanoparticles (AgNPs) and sodium alginate solution into a calcium chloride bath. These beads were then

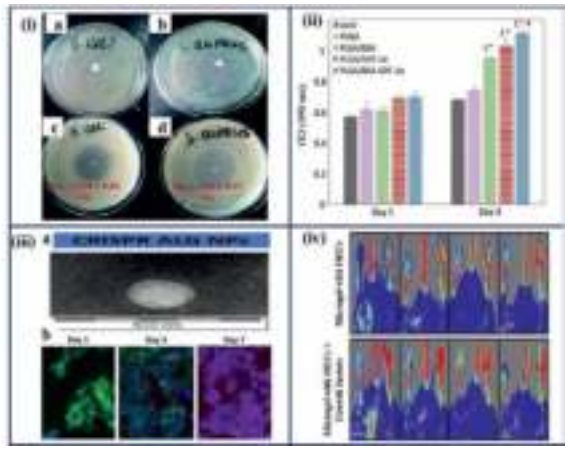

**Figure 2.**
*Application of electrospraying for the delivery of drugs, growth factors, nucleic acid, and live cells (i) 10% curcumin-loaded microcapsules showing bactericidal activities (c and d) against E. coli and S. aureus as compared to no zone of inhibition (a and b) in absence and the drug. Reproduced with permission from Mai et al. [18] Copyright© 2017 The Royal Society of Chemistry. (b) Coaxially electrosprayed PLGA particles loaded with bovine serum albumin (BSA), stromal-derived factor-1α (SDF-1α), and BSA-SDF-1α significantly enhanced mesenchymal stem cells (MSCs) proliferation compared to PLGA particles after 3 days of culture. Reproduced with permission from Maedeh et al. [19]. Copyright© 2015 Elsevier Inc. (c) Live cell imaging of HepG2 cells transfected with CRISPR plasmids encapsulated into coumarin-6 labeled ALG NPs showed successful RFP expression after 1, 2, and 7 days after initial localization in cytosol followed by degradation of nanoparticles and subsequent release of the plasmid. (Blue = nuclei, red = expressed Cas9-RFP in cytosol, green = coumarin-6-labeled nanoparticles). Most of the cells expressed Cas9-RFP on day 7, without any traces of nanoparticles in cytosol. Reproduced with permission from Alallam et al. [20] Copyright© 2020 by the authors. Licensee MDPI, Basel, Switzerland. (d) Laser-doppler flowmeter imaging revealed complete recovery of blood flow perfusion of the ischemic hindlimb in mice after 4 weeks of treatment with RGD-alginate microgels with outgrowth endothelial progenitor cells (OECs) and vascular endothelial growth factor (VEGF), compared to that of OECs only and RGD-alginate microgels with OECs. Reproduced with permission from Kim et al. [21]. Copyright© 2014 The Authors. Published by Elsevier B.V.*

embedded into gelatin scaffolds and showed more potent antibacterial activity against *S. aureus* than *E. coli*. Additionally, they demonstrated noncytotoxic effects on normal human dermal fibroblasts (NHDF) cells, suggesting their potential as wound dressing materials [24]. Cefoxitin, a cephalosporin antibiotic used to treat postoperative infections, was electrosprayed as nanoparticles (551 ± 293 nm) interconnected with nanofibers (61 ± 13 nm in diameter) of hyaluronic acid (HA) thin films. Their effectiveness against *Klebsiella pneumoniae*, *S. aureus*, and *Listeria monocytogenes*, with zones of inhibition of 24.3 ± 0.5, 13 ± 1, and 1.1 ± 0.2 mm, respectively, indicates that they could be used as nanofibrous scaffolds in surgical dressings to control postoperative infections [25]. Ciprofloxacin, a poorly soluble drug, was added to electrosprayed biodegradable poly-butyl-succinate (PBS) microparticles that demonstrated excellent antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa*, which are frequently implicated in diabetic foot, venous leg ulcers, and nonhealing surgical wounds. Additionally, the microparticles demonstrated the ability to counteract *P. aeruginosa* biofilm formation implicated in chronic wounds [26].

### *3.1.1.2 Anti-inflammatory drugs and antioxidants*

Alpha-lipoic acid, known for its anti-inflammatory and antioxidant properties, was encapsulated in single-capillary electrosprayed poly(ethylene oxide)-chitosan nanoparticles (707 ± 66.7 nm) with a zeta potential of 57.7 ± 0.5 mV. Due to an

| Delivery | Category | Carrier | Particle type | Outcome | Ref. |
|---|---|---|---|---|---|
| (a) Therapeutics | | | | | |
| Curcumin | Antimicrobial | PLA | Micro-capsules | ↓ *E. coli* and *S. aureus* | [18] |
| Peppermint oil | Antimicrobial | Alginate | Micro/nano-capsules | ↓ *E. coli* and *S. aureus* | [23] |
| Silver nanoparticles | Antimicrobial | Sodium alginate | Nanoparticles | ↓ *E. coli* and *S. aureus* | [24] |
| Cefoxitin | Antibiotic | HA | Nanoparticles (551 ± 293 nm) | ↓ *Klebsiella pneumoniae*, *S. aureus*, *Listeria monocytogenes* | [25] |
| Ciprofloxacin | Antibiotic | PBS | Micro-particles | ↓*S. aureus*, *P. aeruginosa*, biofilm formation | [26] |
| Alpha-lipoic acid | Anti-inflammatory | Poly(ethylene oxide)–chitosan | Nanoparticles (707 ± 66.68 nm) | ↓ Nitrite production in macrophages | [27] |
| Ranibizumab | Anti-inflammatory | PLGA | Microparticles | ↓ Microglial activity, apoptosis | [28] |
| Curcumin | Anti-inflammatory | Alginate | Microcapsules | ↓ Fibrotic overgrowth, ↑ glycaemic control | [29] |
| (b) Biomacromolecules | | | | | |
| SDF-1α | Chemotactic growth factor | PLGA | Core-shell particles | ↑ MSCs migration, proliferation, cardiac regeneration | [19] |
| rhBMP-2, BSA | Osteogenic growth factor | PLGA | Microspheres (2.5–8 μm) | ↑ BMSCs proliferation | [30] |
| VEGF, BMP-2 | Angiogenic growth factor | PLGA/PDLA | Microparticles | ↑ Endothelial cell proliferation, osteogenic differentiation | [31] |
| VEGF, BMP-7 | Angiogenic growth factor | PLGA/poly(ethylene glycol) | Micro-particles | Osteogenic differentiation | [32] |
| Collagen type II | Extracellular matrix protein | hyaluronic acid/chondroitin sulfate | Nanoparticles (<10 nm) | ↑ Chondrogenic gene expression | [33] |
| BMP-2, SDF-1 | Osteogenic and chemotactic growth factor | Alginate, chitosan | Micro-spheres | ↑ Osteogenic differentiation | [34] |
| PDGF | Osteogenic growth factor | PDLLA–PLGA | Micro-spheres | ↑ Osteogenesis | [35] |

| Delivery | Category | Carrier | Particle type | Outcome | Ref. |
|---|---|---|---|---|---|
| Angiotensin II | Peptide | Tristearin, N-octyl-O-sulphate chitosan | Nanoparticles (100–300 nm) | Triphasic activity on cells | [36] |
| Elastin-like polypeptides (ELPs) | Peptide | — | Nanoparticles (300–400 nm) | Stimuli-responsive nanocarrier | [37] |
| Daidzein | Phytoestrogen | PHBV | Micro-spheres | ↓ Osteoporosis | [38] |
| CRISPR plasmids | Plasmid | Na-alginate | Nanoparticles | ↑ Cytocompatibility | [20] |
| pET30a | Gene | Gold nanoparticles | Nanoparticles | GFP-expressing bacterial colonies | [39] |
| Recombinant self-inactivating lentiviral vectors | Plasmid | PLGA-polyethyleneimine | Nanoparticles | ↑ Transfection HEK293T cells | [40] |
| (c) Cells | | | | | |
| BMSC | Live cells | Direct spraying | — | Retention of differentiation capacity | [41] |
| MSCs | Live cells | Direct spraying | — | Survival, proliferation, plasticity, or unaltered immuno-phenotypic profile | [42] |
| ESCs | Live cells | Direct spraying | — | Retention of pluripotency | [43] |
| OECs | Live cells | RGD-conjugated alginate | Microgels | ↑ HUVEC proliferation, viability, tube formation, ex vivo sprouting of rat aorta | [21] |
| Bacteria (*Lactobacillus plantarum*) | Probiotic | Chitosan-coated alginate | Microparticles (60–1300 μm) | Gastric protection | [44] |
| (d) Implant coating | | | | | |
| Montelukast | Anti-inflammatory | PLGA | Nanoparticle | ↓ Neointimal hyperplasia | [45] |
| Vitamin E | Antioxidant | Polyethylene (PE) | Coating on polyethylene | ↑ Antioxidant, antibacterial properties | [46] |
| Cathelicidin-2 | Peptide | PDLG, GelMA | Coating on titanium implants | ↓ *E. coli* and *S. aureus* | [47] |

| Delivery | Category | Carrier | Particle type | Outcome | Ref. |
|---|---|---|---|---|---|
| DEX | Anti-inflammatory | PLA | Coating on medical implants | ↑ HUVEC survival | [48] |
| β-FGF | Growth factor | PLGA | Coating on titanium implants | ↑ Osseo-integration | [49] |
| (e) Scaffold fabrication | | | | | |
| Insulin | Hormone | SF | Micro-particles | ↑ Vasculari-zation, wound closure | [50] |
| Collagen | ECM protein | PCL | Scaffold | ↑ Neurogenesis | [51] |
| BSA | Protein | PLGA | Nano-spheres | ↑ Neurogenesis | [52] |
| Collagen | ECM protein | Nano-apatite | Scaffold | ↑ Cell proliferation | [53] |
| (f) Theranostics | | | | | |
| Fe3O4<br>DOX | MRI contrast Model drug | PVA | Micro-spheres | Integrated imaging and therapy | [10] |
| BODIPY (Dye)<br>Genistein<br>SPIONs | Fluorophore<br>model drug,<br>MRI contrast | Tri-stearin | Nanoparticles | Integrated imaging and therapy | [36] |
| Tantalum nanoparticles<br>DOX | X-ray/CT contrast,<br>model drug | Alginate | Micro-spheres | Integrated imaging and therapy | [54] |

*PLA: polylactic acid; PLGA: poly(lactic-co-glycolic acid); HA: hyaluronic acid; PBS: poly-butyl-succinate; DOX: doxorubicin; SPIONs: superparamagnetic iron oxide nanoparticles; Ta@CaAlg: calcium alginate microspheres; SDF-1α: stromal derived factor-1α; MSCs: mesenchymal stem cells; rhBMP-2: recombinant human bone morphogenetic protein-2; BSA: bovine serum albumin; PDGF: platelet-derived growth factor; PHBV: poly(3-hydroxybutyrate-co-3-hydroxyvalerate); ESCs: mouse embryonic stem cells; OECs: outgrowth endothelial cells; PE: polyethylene; PDLG: poly(D,L-lactide-co-glycolide); GelMA: gelatin methacryloyl; β-FGF: fibroblast growth factor; DEX: dexamethasone; SF: silk fibroin; PCL: polycaprolactone; ECM: extracellular matrix; the upward and downward arrows indicate an increase and decrease in activity, respectively.*

**Table 1.**
*Potential of electrospraying in wound repair and monitoring.*

effective electrostatic interaction with cell-surface molecules, the attached particles were quickly endocytosed by the lipopolysaccharide (LPS)-treated Raw 264.7 macrophages, demonstrating the anti-inflammatory effects of nanoparticles [27].

PLGA microparticles encapsulating ranibizumab, a nonsteroidal anti-inflammatory drug (NSAID), were prepared by coaxial ES. The same resulted in 70% encapsulation efficacy, higher stability, and sustained release of the drug from microparticles for over a month without significant loss of bioactivity. The absence of any long-term microglial activity or apoptotic effects was observed after an intravitreal injection of 200 μg microparticles [28].

Transplantation of encapsulated islets can be a better choice for functional recovery of the damaged pancreas in diabetic patients. However, to maximize graft life and prevent fibrotic growth from the host response, the encapsulated islets need to be immuno-isolated. In a study by Dang et al., in a chemically-induced type I diabetic mouse model, electrosprayed alginate microcapsules co-encapsulating antioxidant and anti-inflammatory curcumin with pancreatic rat islets significantly decreased fibrotic overgrowth and enhanced glycemic control [29].

Doxorubicin (DOX)-loaded chitosan nanoparticles (300–570 nm) were created by electrospray ionization utilizing a 26-gauge needle, 13 kV voltage, 0.5 mL/h flow rate, with 8 cm working distance, and tripolyphosphate as the stabilizer. Their drug encapsulation efficiency ranged from 63.4 to 67.9% at 1–0.25% DOX loading with a prolonged drug release for 7 hours [55].

### *3.1.2 Biomacromolecules*

A variety of bioactive proteins (such as hormones and growth factors), peptides, and nucleic acids intended for tissue regeneration and wound healing have been effectively delivered by ES.

#### *3.1.2.1 Growth factor*

Because of the limited stability, short half-life, high inactivation rate, and risk of overdose that can lead to cancer, in vivo growth factor delivery in tissue engineering encounters severe challenges in promoting cell migration, proliferation, and differentiation. In this context, electrosprayed microcarriers were better for delivering growth factors [11].

Numerous studies have demonstrated the potential benefits of administering stromal-derived factor-1α (SDF-1α) in treating myocardial infarction (MI) by drawing native stem cells to the injured myocardium. PLGA core-shell particles prepared by coaxial ES with SDF-1α incorporated resulted in a controlled release of the growth factor for more than 40 days, along with 38% higher migration (chemotactic activity) and proliferation of mesenchymal stem cells (MSCs), and the ability to inject the particles for in situ cardiac regeneration (**Figure 2(ii)**) [19]. The recombinant human bone morphogenetic protein-2 (rhBMP-2) and bovine serum albumin (BSA) as stabilizer were loaded into core-shell PLGA microspheres with a size of 2.5–8 μm, prepared by coaxial ES. In vitro release studies showed a stable, prolonged rhBMP-2 release for up to 21 days after a burst release within the first 6 hours with little chance of protein denaturation. Particle size substantially impacted the release rates in relation to rhBMP-2 loading. Cell proliferation was steadily increased for 7 days with minimal cytotoxicity within the studied dosage range when bone marrow-derived MSCs (BMSCs) were cultured with rhBMP-2-loaded PLGA microspheres, which

showed great promises for bone tissue regeneration [30]. Electrosprayed PLGA core/PDLA shell spheres that were loaded with angiogenic VEGF to stimulate endothelial cell proliferation and osteogenic BMP-2 to stimulate osteogenic differentiation showed an initial burst release with ~80% release of the VEGF in the first 10 days and up to 30 days of stable, sustained release of BMP-2. BMSCs cultured with VEGF/BMP-2 spheres for 14 days significantly increased osteogenic-related gene (ALP, OPN, and BMP-2) expression. Micro-computed tomography and histological analysis of critical-sized rat cranial defects treated with VEGF/BMP-2 spheres showed faster bone regeneration with vascular tissue ingrowth [31].

Similarly, pre-osteoblasts (MC3T3-E1) cultured with electrosprayed PLGA microparticles encapsulating VEGF, bone morphogenetic protein 7 (BMP-7), and stabilizers for 3 weeks significantly induced osteogenic differentiation [32]. Electrosprayed nanoparticles (size <10 nm) loaded with collagen type II specific to cartilage and either hyaluronic acid or chondroitin sulfate enhanced the synthesis of cartilage-specific proteins after culturing with chondrocytes. After internalizing the particles by nonspecific pinocytosis, the expression of chondrogenic genes (transforming growth factor-beta 1, collagen type II, and aggrecan) was significantly increased. The patella grooves of the male New Zealand rabbits implanted with pellets containing chondrocytes and polymeric nanoparticles exhibited signs of early closure of the injured cartilages and neo-tissue formation after 8 weeks of implantation [33].

Electrospray technology was used to manufacture core/shell double-layered microspheres in two steps: first, an alginate core loaded with BMP-2 was prepared, followed by a chitosan shell loaded with SDF-1. The in vitro release study revealed differential release of SDF-1 and BMP-2, with an initial burst release of SDF-1 (~80%) in the first 6 hours and 50% of the latter released in 4 hours without sacrificing bioactivity. The microspheres also demonstrated chemotactic migration of MC3T3-E1 pre-osteoblast cells and in vitro osteogenic differentiation of mice-derived BMSCs as evidenced by enhanced ALP activity after 3 days, development of mineralized modules after 14 days, and increased expression of osteogenic-related genes (Runx2, OCN, Osterix) and Smad signaling genes (Smad 1, 5, 8) after 7 days of osteogenic induction [34]. Platelet-derived growth factor (PDGF), an early mitogenic factor and simvastatin, a late osteogenic inducer were administered to promote dentoalveolar regeneration. PDGF and simvastatin were encapsulated in a double-walled PDLLA–PLGA microspheres using the coaxial electrohydrodynamic atomization technique. Simvastatin (core)/PDGF (shell) microspheres exhibited a faster release of simvastatin than PDGF; however, PDGF (core)/simvastatin (shell) microspheres revealed 60% release of both PDGF and simvastatin concurrently at day 10. When PDGF/simvastatin-loaded microspheres were injected into the maxillary first molar (M1) defect formed in the post-extraction ridge of the Sprague Dawley rats, osteogenesis level at day 14 and BV/TV, TMD, Tb.Th, and Tb.N values at day 28 was higher than control [35].

#### *3.1.2.2 Peptides and hormones*

Angiotensin II, a model peptide, was encapsulated using single or coaxial electrospray methods into tristearin and N-octyl-O-sulphate chitosan nanocarriers, respectively. The same resulted in peptide entrapment in the particle matrix or a core-shell structure, depending on the single or coaxial format. The 100–300 nm-sized electrosprayed nanoparticles showed encapsulation efficiencies of about 92 ± 1.8%; nevertheless, cytotoxicity tests indicated that an ideal peptide loading concentration

of about 1 mg/ml would result in a triphasic activity slow in the beginning, fast afterward, and diffusive at the end [36].

Genetically modified elastin-like polypeptides (ELP) were used to develop a biodegradable, biocompatible, and bioresponsive polymeric drug nanocarrier by ES technique. DOX-loaded ELP nanoparticles (300–400 nm in diameter) prepared in this way exhibited 20 w/w% drug loading without affecting the particle morphology and a pH-dependent drug release that was correlated with the pH-dependent solubility of the ELPs, indicating their potential as a stimuli-responsive drug nanocarrier [37].

Since estrogen and progesterone have several adverse effects, phytoestrogens like daidzein may be a viable substitute for hormone therapy in the treatment of osteoporosis. ES was used to create daidzein-loaded microspheres based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), which showed a 7.6% drug release at 1 hour followed by prolonged release over nearly 3 days. As a result, these microspheres may find potential applications in bone tissue engineering and osteoporosis treatment [38].

*3.1.2.3 Nucleic acids*

The success of gene/plasmid DNA delivery relies on a safe and effective delivery system that can cross the biological barriers of the target cells. Viral or non-viral carrier systems typically deliver plasmid DNA because naked DNA is too big, negatively charged, hydrophilic, and prone to enzymatic destruction to penetrate cell membranes. Non-viral delivery methods (lipid/polymer/inorganic nanocarriers, electroporation, nucleofection, hydrodynamic injection, and microinjection) have a lower risk of immunogenicity and carcinogenicity than viral vectors (adeno-associated virus, lentivirus, and adenovirus), despite having a lower delivery efficiency. Electrospray can be used to develop excellent gene or plasmid delivery carriers [20].

CRISPR plasmids loaded in alginate nanoparticles were developed by ES. Their mean size, encapsulation efficiency, and zeta potential were measured at 228 nm, >99.0%, and −4.42 mV, respectively, without loss of payload integrity (**Figure 2(iii)-a**). The encapsulated CRISPR plasmids retained adequate cytocompatibility and integrated with HepG2 cells [20]. pET30a-green fluorescent protein (GFP) plasmid and gold nanoparticles were electrosprayed as a thin layer over *E. coli*, allowing rapid cellular uptake and effective bacterial transformation evidenced by the formation of GFP-expressing bacterial colonies on an agar plate (**Figure 2(iii)-b**) [39].

BES helps deliver a genetic construct into living cells to increase plasmid DNA stability and transfection efficiency. Human Embryonic Kidney 293T (HEK293T) cells transduced with recombinant self-inactivating lentiviral vectors expressing green fluorescent protein (GFP) were subsequently manipulated by bio-electrospraying (BES). The combined technique offers the creation of live therapeutic constructs that allow for the precise and regulated release of cells or genes, apart from cell or gene therapies [56]. GFP plasmid was electrosprayed onto chick embryos to achieve localized GFP expression. Co-jetting/ES dye-loaded PLGA-polyethyleneimine and imaging/pH-responsive siRNA solutions could result in bicompartmental particles, a viable alternative to bacterial/cell transformation [40]. Nucleic acid-based therapies were also evaluated for treating lung disease. Nucleic acids (siRNA-FITC, luciferase DNA, and mRNA) were locally delivered to pig tracheal tissue and the whole lung ex vivo using a bronchoscopic electrospray administration method [57].

### 3.2 Cell delivery

BES became an attractive tool for delivering living cells or complete organisms in scaffolds for tissue engineering applications [58]. Many cells, such as fibroblasts, adipose-derived stem cells (ADSCs), bone marrow-derived mesenchymal stem cells, umbilical vascular endothelial cells, gastric epithelial cells, periodontal ligament cells, and retinal pigment epithelial cells have been electrosprayed with low current in the nanoampere range and voltage up to several kilovolts, without significantly affecting the cell morphology, viability, and proliferation [59, 60].

ES of BMSC suspension was accomplished at 6 mL/h flow rate and 7.5–15 kV voltages, with 88% cell survival and a proliferation rate comparable to native BMSCs. An unstable ES with decreased cell viability was noted at higher voltages because of the thermal or electrical shock to the cells. The effective differentiation of BMSCs, electrosprayed at 7.5 kV, into adipogenic, chondrogenic, and osteogenic lineages while maintaining their multipotency, suggests that BES is a safe method for delivering progenitor/stem cells for regenerative purposes [41]. Using 15 kV, 0.46 ml/h flow rate, 4 cm distance, and 15 and 60 minutes spray time, a steady and continuous stream of BES could be formed without affecting the MSC's survival, proliferation, plasticity, or immunophenotypic profile. DNA damage occurred when the BES time exceeded 30 minutes, but it underwent self-recovery within 5 hours [42]. Similarly, the BES of mouse embryonic stem cells (ESCs) demonstrated that pluripotency retention was established by an alkaline phosphatase assay and gene expression profile [43].

A strategy combining therapeutic stem/progenitor cells and angiogenic proteins is attractive for treating vascular disease. Injectable multifunctional microgels comprising arginine-glycine-aspartic acid (RGD)-conjugated alginate encapsulated with outgrowth endothelial cells (OECs), VEGF, and hepatocyte growth factor (HGF) were developed by ES, which showed a time-dependent growth factor release, improved cell proliferation, viability, and human umbilical vein endothelial cells (HUVECs)-mediated tube formation and ex vivo sprouting of rat aorta. Mice treated with RGD-microgel containing OECs and other growth factors for 1 week showed an increased level of angiogenesis and capillary density in the subcutaneous pocket of the abdomen, while an enhanced blood flow perfusion into a hindlimb ischemia model (**Figure 2(iv)**) [21].

Extended gastrointestinal retention of probiotics is crucial for enhancing their functional efficacy. Mucoadhesive probiotic formulations (spherical with a 60–1300 μm diameter) containing *Lactobacillus plantarum* based on resistant starch-reinforced and mucoadhesive chitosan-coated alginate microparticles were developed using an electrospray approach. When the in vitro wash-off mucoadhesion of the formulations was evaluated using fluorescence microscopy, the alginate-starch electrosprayed formulations showed better protection against simulated gastric fluid than the alginate ones, though not as good as the chitosan-coated ones [44].

### 3.3 Implant coating

Drug-eluting stents (DESs) are more effective in preventing in-stent restenosis in injured arteries following percutaneous coronary intervention (PCI), which is frequently associated with increased leukotrienes [61]. Montelukast, an anti-inflammatory and anti-proliferative drug, has often been used to treat various

inflammatory diseases. ES montelukast/PLGA particles onto DES could effectively prevent the development of neointimal hyperplasia, which is the cause of in-stent restenosis, without affecting the healing of the stented vessel [45]. The bioactive coating of antioxidant vitamin E and antimicrobial chitosan onto the polyethylene (PE) surface, accomplished by electrosprayed, significantly inhibited bacterial growth and exerted potent antioxidant and pH-responsive properties [46]. An antibacterial coating was created on titanium surfaces by loading the cationic and amphipathic peptide chicken cathelicidin-2 between layers of poly(D,L-lactide-co-glycolide) (PDLG) and gelatin methacryloyl (GelMA), which were electrosprayed in a layer-by-layer (LbL) assembly fashion. The coating was biocompatible with hMSCs and macrophages while exhibiting potent antibacterial activity against *E. coli* and *S. aureus* for 4 days. Such coating provides a suitable platform for preventing peri-implantitis (**Figure 3(i)**) [47].

Using the ES technique, the PLA loaded with dexamethasone (DEX), an anti-inflammatory drug, was coated onto medical implants. Compared to spin-coated stents, electrosprayed stents demonstrated a suitable drug release profile and more remarkable HUVEC survival after 1 and 4 days [48]. Similarly, titanium implants coated with fibroblast growth factor ($\beta$-FGF)-loaded PLGA by ES showed enhanced osseointegration (bone-to-implant contact) following implantation in rabbit tibial defects [49].

### 3.4 Scaffold fabrication

Coaxial ES was used to create bioactive insulin-encapsulated silk fibroin (SF) microparticles to treat chronic wounds. The SF sponge loaded with microparticles was a bioactive wound dressing assessed for in vivo therapeutic effects on dorsal full-thickness wounds in diabetic Sprague–Dawley rats. The result demonstrated evidence

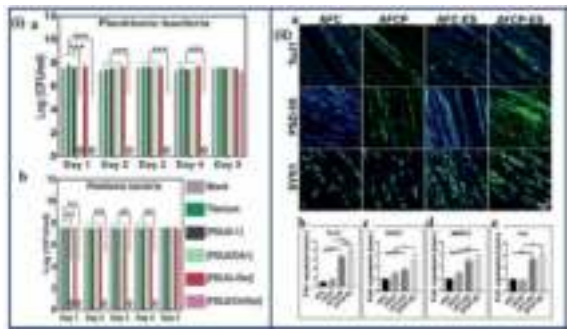

**Figure 3.**
*Electrosprayed particles used for implant coating and scaffold fabrication. (a) Among the samples containing etched titanium, etched titanium electrosprayed with (poly(D,L-lactide-co-glycolide)) (PDLG) [PDLG/–/–], PDLG with chicken cathelicidin-2 (CATH-2) [PDLG/CA/–], PDLG and gelatin methacryloyl (GelMA) [PDLG/-Gel] and PDLG, CATH-2 and GelMA [PDLG/CA/Gel], only [PDLG/CA/Gel] killed 100% of bacteria for 4 days, while [PDLG/CA/–] was void of any antimicrobial response against, (b) S. aureus and (c) E. coli. Reproduced with permission from Keikhosravani et al. [47] Copyright© 2014 Wiley-VCH GmbH. (b) Immunofluorescence staining of neurogenic proteins (TuJ1, SYN1, PSD-95) expressed by PC12 cells cultured on microfibrous scaffolds for 7 days exhibited significantly higher expression of proteins on electrosprayed aligned fibers coated with collagen (AFC-ES) as well as both collagen and polypyrrole (AFCP) compared to scaffolds without electrospraying (n = 3, **p < 0.01, *p < 0.05). Reproduced with permission from Tang et al. [51] Copyright© 2020 American Chemical Society.*

of vascularization, collagen deposition, and faster wound closure, which considerably facilitated the healing process [50].

Biomimetic scaffolds comprising aligned PCL microfibrous scaffolds co-sprayed with collagen or conductive polypyrrole nanoparticles were developed for simultaneously delivering topographic cues and electrotransduction by integrating ES with electrospinning. While PC12 cells were oriented and elongated along the fibers' direction on collagen-coated PCL microfibrous scaffold, more functional expressions, including elongation, gene expression, and protein expression, were observed on PPy-coated aligned fibers, which led to increased neurogenesis [51]. Electrosprayed bovine serum albumin (BSA)-loaded core-shell PLGA nanospheres produced by coaxial ES were collected on an electrically grounded highly aligned electrospun PCL microfibrous mat. The study suggested that when cultured with rat pheochromocytoma (PC-12) and astrocyte cell lines, the nanocomposite scaffold was promising for directing neural tissue growth along the fibers and regeneration (**Figure 3(ii)**) [52]. A composite scaffold prepared by ES of nano-hydroxyapatite on electrospun collagen significantly increased the proliferation of human dermal fibroblasts, keratinocytes, and hMSCs differentiation in addition to inhibiting bacterial adhesion. The scaffold exhibited no adverse reactions following implantation in a rat subcutaneous pocket, indicating its potential for application as a dressing material or skin wound regeneration [53].

## 4. Delivery of theranostics

Theranostic agents were created using the electrospray technology, which allowed for the simultaneous administration of therapeutic and diagnostic compounds [62]. In situ synthesized magnetic iron oxide ($Fe_3O_4$) nanoparticles were encapsulated in PVA microspheres by one-step ES to be used as an MRI contrast agent. The PVA matrix was further cross-linked to facilitate embolization. In vivo evaluation of Fe3O4@PVA microspheres in the rabbit renal artery revealed increased MRI contrast, an embolic effect with excellent biocompatibility. On the other hand, the ability of doxorubicin (DOX)-loaded $Fe_3O_4$@PVA microspheres to release DOX over an extended period indicates its potential as a theranostic agent [10]. Superparamagnetic iron oxide nanoparticles (SPIONs), which may be steered by external magnetic fields to create localized hypothermia to damage targeted cells, have been widely investigated as MRI contrast agents due to their capacity to reduce drastically spin–spin relaxation (T2) time. In a fluorophore (BODIPY) integrated tristearin core-shell system, a model drug, genistein, an isoflavonoid, and SPIONs (10–15 nm) were encapsulated with a 92% drug encapsulation efficiency utilizing coaxial ES. These particles showed low toxicity and were internalized by the cells in 4 hours, indicating the possibility of developing multimodal particles for integrated imaging and therapy [36]. One-step ES was used to create calcium alginate microspheres (Ta@CaAlg) loaded with tantalum nanoparticles and DOX, which not only allowed for a maximum 97.3 mg DOX loading per mL of beads and a pH-dependent release profile but also demonstrated embolic effects as revealed by digital subtraction angiography. The microspheres also produced good X-ray/CT contrast, allowing their real-time monitoring at embolized sites for up to 4 weeks (**Figure 4(i)**) [54].

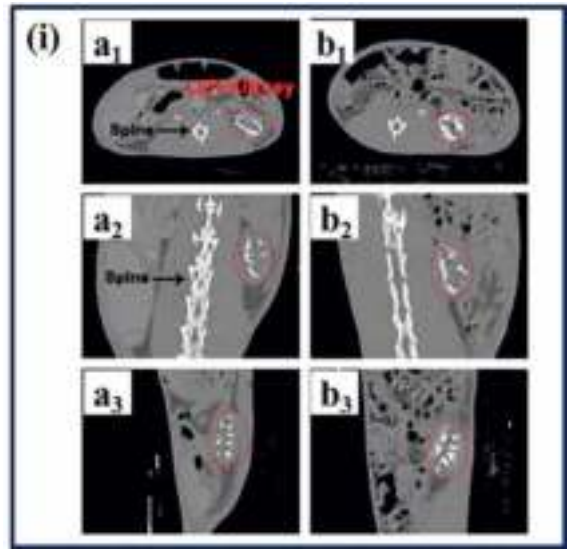


**Figure 4.**
*Theranostic applications of electrosprayed nanoparticles. (i) CT images of rabbit abdomens after embolization (marked with red circles) of the left kidney with tantalum nanoparticles loaded calcium alginate microspheres (Ta@CaAlg) for (a) 1 week and (b) 4 weeks, where 1, 2, and 3 representing transverse, coronal, and sagittal views, respectively. Reproduced with permission from Zeng et al. [54] Copyright© 2024 Ivyspring International Publisher.*

## 5. Challenges and future perspectives

ES can quickly and easily prepare nano/micro-carriers with varying sizes, shapes, and encapsulation efficiency; however, low throughput is one of the major disadvantages of ES, mainly when using the cone-jet mode [63]. The technique is well ahead of industrial-scale production since only about 300 mg of particles could be created after 24 hours of spraying. Alternative approaches by applying a flute-like multipore emitter device [64], customized multi-hole spinneret [65], and needleless apparatus with microchannels made of two parallel glass plates sharpened and grooved at their edges forming multiple spraying jets under high electric potential [66] were suggested to increase production rate through ES. There has been limited success in enhancing yield through multiplex electrospraying devices, which have several emitters fitted with a cone-jet at each emitter. Maintaining a constant electrical field across the emitters to produce equal jets is challenging [67]. BES has the potential to cause electrical stress on biomacromolecules and cells, perhaps leading to detrimental effects on their bioactivity. Although preliminary research suggests that ES does not influence the stability of proteins, enzymes, nucleic acids, and cells, further research is needed to determine the long-term implications of organic solvents and high voltages involved in ES [16]. More attention is required to create environmentally friendly ES methods that use aqueous-based solvents, as organic solvents are mostly hazardous and can harm intracellular components, payloads, and the viability of cells [68]. Integrating BES with the techniques to fabricate an organ-on-a-chip can eventually replace animal testing for determining the toxicity of therapeutic substances toward cells and tissues.

## Acknowledgements

NIPER Kolkata and the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Government of India supported the work.

## Conflict of interest

The authors declare no conflict of interest.

## Author details

Rahul Sable[1], Pritiprasanna Maity[2*] and Kausik Kapat[1*]

1 Department of Medical Devices, National Institute of Pharmaceutical Education and Research Kolkata, Kolkata, West Bengal, India

2 Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC, USA

*Address all correspondence to: maity@musc.edu
and kausik.kapat@niperkolkata.ac.in

IntechOpen

## References

[1] Battafarano G, Rossi M, De Martino V, Marampon F, Borro L, Secinaro A, et al. Strategies for bone regeneration: From graft to tissue engineering. International Journal of Molecular Sciences. 2021;**22**(3):1128

[2] Zhao Q, Zhou Y, Wang M. Three-dimensional endothelial cell incorporation within bioactive nanofibrous scaffolds through concurrent emulsion electrospinning and coaxial cell electrospraying. Acta Biomaterialia. 2021;**123**:312-324

[3] Xu Y, Peng J, Richards G, Lu S, Eglin D. Optimization of electrospray fabrication of stem cell–embedded alginate–gelatin microspheres and their assembly in 3D-printed poly (ε-caprolactone) scaffold for cartilage tissue engineering. Journal of Orthopaedic Translation. 2019;**18**:128-141

[4] Adel IM, ElMeligy MF, Elkasabgy NA. Conventional and recent trends of scaffolds fabrication: A superior mode for tissue engineering. Pharmaceutics. 2022;**14**(2):306

[5] Kurakula M, Naveen NR. Electrospraying: A facile technology unfolding the chitosan based drug delivery and biomedical applications. European Polymer Journal. 2021;**147**:110326

[6] Bhushani JA, Anandharamakrishnan C. Electrospinning and electrospraying techniques: Potential food based applications. Trends in Food Science & Technology. 2014;**38**(1):21-33

[7] Arun Y, Ghosh R, Domb AJ. Biodegradable hydrophobic injectable polymers for drug delivery and regenerative medicine. Advanced Functional Materials. 2021;**31**(44):2010284

[8] Bock N, Woodruff MA, Hutmacher DW, Dargaville TR. Electrospraying, a reproducible method for production of polymeric microspheres for biomedical applications. Polymers. 2011;**3**(1):131-149

[9] Khan MKI, Nazir A, Maan AA. Electrospraying: A novel technique for efficient coating of foods. Food Engineering Reviews. 2017;**9**:112-119

[10] Wang J, Jansen JA, Yang F. Electrospraying: Possibilities and challenges of engineering carriers for biomedical applications—A mini review. Frontiers in Chemistry. 2019;**7**:258

[11] Moreira A, Lawson D, Onyekuru L, Dziemidowicz K, Angkawinitwong U, Costa PF, et al. Protein encapsulation by electrospinning and electrospraying. Journal of Controlled Release. 2021;**329**:1172-1197

[12] Feng K, Huangfu L, Liu C, Bonfili L, Xiang Q, Wu H, et al. Electrospinning and electrospraying: Emerging techniques for probiotic stabilization and application. Polymers. 2023;**15**(10):2402

[13] Kasoju N, Ye H. Biomedical Applications of Electrospinning and Electrospraying. Cambridge, MA, United States: Woodhead Publishing; 2021

[14] He T, Jokerst JV. Structured micro/nano materials synthesized via electrospray: A review. Biomaterials Science. 2020;**8**(20):5555-5573

[15] Gómez-Mascaraque LG, Sanchez G, López-Rubio A. Impact of

molecular weight on the formation of electrosprayed chitosan microcapsules as delivery vehicles for bioactive compounds. Carbohydrate Polymers. 2016;**150**:121-130

[16] Boda SK, Li X, Xie J. Electrospraying an enabling technology for pharmaceutical and biomedical applications: A review. Journal of Aerosol Science. 2018;**125**:164-181

[17] Booysen E, Bezuidenhout M, van Staden ADP, Dimitrov D, Deane SM, Dicks LM. Antibacterial activity of vancomycin encapsulated in poly (DL-lactide-co-glycolide) nanoparticles using electrospraying. Probiotics and Antimicrobial Proteins. 2019;**11**:310-316

[18] Mai Z, Chen J, He T, Hu Y, Dong X, Zhang H, et al. Electrospray biodegradable microcapsules loaded with curcumin for drug delivery systems with high bioactivity. RSC Advances. 2017;**7**(3):1724-1734

[19] Maedeh Z, Prabhakaran MP, San TE, Ramakrishna S. Controlled delivery of stromal derived factor-1? from poly lactic-co-glycolic acid core-shell particles to recruit mesenchymal stem cells for cardiac regeneration. Journal of Colloid and Interface Science. 2015;**451**:144-152

[20] Alallam B, Altahhan S, Taher M, Mohd Nasir MH, Doolaanea AA. Electrosprayed alginate nanoparticles as CRISPR plasmid DNA delivery carrier: Preparation, optimization, and characterization. Pharmaceuticals. 2020;**13**(8):158

[21] Kim P-H, Yim H-G, Choi Y-J, Kang B-J, Kim J, Kwon S-M, et al. Injectable multifunctional microgel encapsulating outgrowth endothelial cells and growth factors for enhanced neovascularization. Journal of Controlled Release. 2014;**187**:1-13

[22] Yuan S, Lei F, Liu Z, Tong Q, Si T, Xu RX. Coaxial electrospray of curcumin-loaded microparticles for sustained drug release. PLoS One. 2015;**10**(7):e0132609

[23] Ghayempour S, Mortazavi S. Antibacterial activity of peppermint fragrance micro–nanocapsules prepared with a new electrospraying method. Journal of Essential Oil Research. 2014;**26**(6):492-498

[24] Pankongadisak P, Ruktanonchai UR, Supaphol P, Suwantong O. Development of silver nanoparticles-loaded calcium alginate beads embedded in gelatin scaffolds for use as wound dressings. Polymer International. 2015;**64**(2):275-283

[25] Ahire JJ, Dicks LM. Antimicrobial hyaluronic acid–cefoxitin sodium thin films produced by electrospraying. Current Microbiology. 2016;**73**:236-241

[26] Puleo G, Terracina F, Catania V, Sciré S, Schillaci D, Licciardi M. Electrosprayed poly-butyl-succinate microparticles for sustained release of ciprofloxacin as an antimicrobial delivery system. Powder Technology. 2024;**432**:119152

[27] Bai M-Y, Hu Y-M. Development of alpha-lipoic acid encapsulated chitosan monodispersed particles using an electrospray system: Synthesis, characterisations and anti-inflammatory evaluations. Journal of Microencapsulation. 2014;**31**(4):373-381

[28] Zhang L, Si T, Fischer AJ, Letson A, Yuan S, Roberts CJ, et al. Coaxial electrospray of ranibizumab-loaded microparticles for sustained release of anti-VEGF therapies. PLoS One. 2015;**10**(8):e0135608

[29] Dang TT, Thai AV, Cohen J, Slosberg JE, Siniakowicz K, Doloff JC,

et al. Enhanced function of immuno-isolated islets in diabetes therapy by co-encapsulation with an anti-inflammatory drug. Biomaterials. 2013;**34**(23):5792-5801

[30] Zhang M, Ma Y, Li R, Zeng J, Li Z, Tang Y, et al. RhBMP-2-loaded poly (lactic-co-glycolic acid) microspheres fabricated by coaxial electrospraying for protein delivery. Journal of Biomaterials Science, Polymer Edition. 2017;**28**(18):2205-2219

[31] Wang Y, Wei Y, Zhang X, Xu M, Liu F, Ma Q, et al. PLGA/PDLLA core–shell submicron spheres sequential release system: Preparation, characterization and promotion of bone regeneration in vitro and in vivo. Chemical Engineering Journal. 2015;**273**:490-501

[32] Bock N, Dargaville TR, Kirby GT, Hutmacher DW, Woodruff MA. Growth factor-loaded microparticles for tissue engineering: The discrepancies of in vitro characterization assays. Tissue Engineering Part C: Methods. 2016;**22**(2):142-154

[33] Yang S-W, Ku K-C, Chen S-Y, Kuo S-M, Chen I-F, Wang T-Y, et al. Development of chondrocyte-seeded electrosprayed nanoparticles for repair of articular cartilage defects in rabbits. Journal of Biomaterials Applications. 2018;**32**(6):800-812

[34] Xu C, Xu J, Xiao L, Li Z, Xiao Y, Dargusch M, et al. Double-layered microsphere based dual growth factor delivery system for guided bone regeneration. RSC Advances. 2018;**8**(30):16503-16512

[35] Chang P-C, Chong LY, Dovban AS, Lim LP, Lim JC, Kuo MY-P, et al. Sequential platelet-derived growth factor–simvastatin release promotes dentoalveolar regeneration. Tissue Engineering Part A. 2014;**20**(1-2):356-364

[36] Rasekh M, Ahmad Z, Cross R, Hernández-Gil J, Wilton-Ely JD, Miller PW. Facile preparation of drug-loaded tristearin encapsulated superparamagnetic iron oxide nanoparticles using coaxial electrospray processing. Molecular Pharmaceutics. 2017;**14**(6):2010-2023

[37] Wu Y, MacKay JA, McDaniel JR, Chilkoti A, Clark RL. Fabrication of elastin-like polypeptide nanoparticles for drug delivery by electrospraying. Biomacromolecules. 2008;**10**(1):19-24

[38] Scheithauer EC, Li W, Ding Y, Harhaus L, Roether JA, Boccaccini AR. Preparation and characterization of electrosprayed daidzein–loaded PHBV microspheres. Materials Letters. 2015;**158**:66-69

[39] Lee Y-H, Wu B, Zhuang W-Q, Chen D-R, Tang YJ. Nanoparticles facilitate gene delivery to microorganisms via an electrospray process. Journal of Microbiological Methods. 2011;**84**(2):228-233

[40] Misra AC, Bhaskar S, Clay N, Lahann J. Multicompartmental particles for combined imaging and siRNA delivery. Advanced Materials. 2012;**24**(28):3850-3856

[41] Sahoo S, Lee WC, Goh JCH, Toh SL. Bio-electrospraying: A potentially safe technique for delivering progenitor cells. Biotechnology and Bioengineering. 2010;**106**(4):690-698

[42] Braghirolli D, Zamboni F, Chagastelles P, Moura D, Saffi J, Henriques J, et al. Bio-electrospraying of human mesenchymal stem cells:

An alternative for tissue engineering. Biomicrofluidics. 2013;**7**(4):1-12, 044130

[43] Abeyewickreme A, Kwok A, McEwan JR, Jayasinghe SN. Bio-electrospraying embryonic stem cells: Interrogating cellular viability and pluripotency. Integrative Biology. 2009;**1**(3):260-266

[44] Ta LP, Bujna E, Kun S, Charalampopoulos D, Khutoryanskiy VV. Electrosprayed mucoadhesive alginate-chitosan microcapsules for gastrointestinal delivery of probiotics. International Journal of Pharmaceutics. 2021;**597**:120342

[45] Zamani M, Prabhakaran MP, Varshosaz J, Mhaisalkar PS, Ramakrishna S. Electrosprayed Montelukast/poly (lactic-co-glycolic acid) particle based coating: A new therapeutic approach towards the prevention of in-stent restenosis. Acta Biomaterialia. 2016;**42**:316-328

[46] Stoleru E, Munteanu SB, Dumitriu RP, Coroaba A, Drobotă M, Zemljic LF, et al. Polyethylene materials with multifunctional surface properties by electrospraying chitosan/vitamin E formulation destined to biomedical and food packaging applications. Iranian Polymer Journal. 2016;**25**:295-307

[47] Keikhosravani P, Jahanmard F, Bollen T, Nazmi K, Veldhuizen EJ, Gonugunta P, et al. Antibacterial CATH-2 peptide coating to prevent bone implant-related infection. Advanced Materials Technologies. 2023;**8**(18):2300500

[48] Rahvar M, Ahmadi Lakalayeh G, Nazeri N, Marouf BT, Shirzad M, Najafi T, et al. Assessment of structural, biological and drug release properties of electro-sprayed poly lactic acid-dexamethasone coating for biomedical applications. Biomedical Engineering Letters. 2021;**11**:393-406

[49] Lee SY, Koak JY, Heo SJ, Kim SK, Lee SJ, Nam SY. Osseointegration of anodized titanium implants coated with poly (lactide-co-glycolide)/basic fibroblast growth factor by electrospray. International Journal of Oral & Maxillofacial Implants. 2010;**25**(2):315-320

[50] Li X, Liu Y, Zhang J, You R, Qu J, Li M. Functionalized silk fibroin dressing with topical bioactive insulin release for accelerated chronic wound healing. Materials Science and Engineering: C. 2017;**72**:394-404

[51] Tang J, Wu C, Chen S, Qiao Z, Borovskikh P, Shchegolkov A, et al. Combining electrospinning and electrospraying to prepare a biomimetic neural scaffold with synergistic cues of topography and electrotransduction. ACS Applied Bio Materials. 2020;**3**(8):5148-5159

[52] Zhu W, Masood F, O'Brien J, Zhang LG. Highly aligned nanocomposite scaffolds by electrospinning and electrospraying for neural tissue regeneration. Nanomedicine: Nanotechnology, Biology and Medicine. 2015;**11**(3):693-704

[53] Ribeiro N, Sousa A, Cunha-Reis C, Oliveira AL, Granja PL, Monteiro FJ, et al. New prospects in skin regeneration and repair using nanophased hydroxyapatite embedded in collagen nanofibers. Nanomedicine: Nanotechnology, Biology and Medicine. 2021;**33**:102353

[54] Zeng J, Li L, Zhang H, Li J, Liu L, Zhou G, et al. Radiopaque and uniform alginate microspheres loaded with tantalum nanoparticles for real-time imaging during transcatheter arterial embolization. Theranostics. 2018;**8**(17):4591

[55] Songsurang K, Praphairaksit N, Siraleartmukul K, Muangsin N.

Electrospray fabrication of doxorubicin-chitosan-tripolyphosphate nanoparticles for delivery of doxorubicin. Archives of Pharmacal Research. 2011;**34**:583-592

[56] Ward E, Chan E, Gustafsson K, Jayasinghe SN. Combining bio-electrospraying with gene therapy: A novel biotechnique for the delivery of genetic material via living cells. The Analyst. 2010;**135**(5):1042-1049

[57] Riddell P, Gilbert JL, Molloy EL, Finnegan S, Egan JJ, O'Dea S. Delivery of nucleic acids to ex vivo porcine airways using electrospray. Experimental Lung Research. 2018;**44**(8-9):405-416

[58] Jayasinghe SN. Biojets in regenerative biology & medicine. Materials Today. 2011;**14**(5):202-211

[59] Xin Y, Chai G, Zhang T, Wang X, Qu M, Tan A, et al. Analysis of multiple types of human cells subsequent to bioprinting with electrospraying technology. Biomedical Reports. 2016;**5**(6):723-730

[60] McCrea Z, Arnanthigo Y, Cryan S-A, O'Dea S. A novel methodology for bio-electrospraying mesenchymal stem cells that maintains differentiation, immunomodulatory and pro-reparative functions. Journal of Medical and Biological Engineering. 2018;**38**:497-513

[61] Bi J. Utility of Intravascular Imaging among Coronary Artery Disease Patients Undergoing Percutaneous Coronary Intervention: The Chinese University of Hong Kong. Shatin, NT, Hong Kong SAR: The People's Republic of China; 2021

[62] Ali A, Zaman A, Sayed E, Evans D, Morgan S, Samwell C, et al. Electrohydrodynamic atomisation driven design and engineering of opportunistic particulate systems for applications in drug delivery, therapeutics and pharmaceutics. Advanced Drug Delivery Reviews. 2021;**176**:113788

[63] Huang S, Mansouri J, Le-Clech P, Leslie G, Tang CY, Fane AG. A comprehensive review of electrospray technique for membrane development: Current status, challenges, and opportunities. Journal of Membrane Science. 2022;**646**:120248

[64] Zhang C, Chang M-W, Ahmad Z, Hu W, Zhao D, Li J-S. Stable single device multi-pore electrospraying of polymeric microparticles via controlled electrostatic interactions. RSC Advances. 2015;**5**(107):87919-87923

[65] Zhang T, Liu YY, Yu HC, Li S, Chen HP, Hu QX. Bio-electrospraying of human umbilical vein endothelial cells with a customized multi-hole spinneret. Key Engineering Materials. 2016;**705**:291-296

[66] Jordahl JH, Ramcharan S, Gregory JV, Lahann J. Needleless electrohydrodynamic cojetting of bicompartmental particles and fibers from an extended fluid interface. Macromolecular Rapid Communications. 2017;**38**(1):1600437

[67] Nguyen DN, Clasen C, Van den Mooter G. Pharmaceutical applications of electrospraying. Journal of Pharmaceutical Sciences. 2016;**105**(9):2601-2620

[68] Agarwal S, Greiner A. On the way to clean and safe electrospinning—Green electrospinning: Emulsion and suspension electrospinning. Polymers for Advanced Technologies. 2011;**22**(3):372-378

Chapter 3

# Advancements and Applications of Electrospray Methods in Skin Tissue Regeneration

*Mobina Bazari and Najmeh Najmoddin*

## Abstract

Skin tissue engineering, a critical area within regenerative medicine, focuses on creating functional replacements for damaged or diseased skin. Electrospray ionization has emerged as a promising method in this field due to its precision in biomaterial and bioactive molecule deposition. This chapter discusses electrospray's role in revolutionizing scaffold fabrication, cell encapsulation, and therapeutic delivery in skin engineering. Electrospray allows for the production of scaffolds that mimic the skin's extracellular matrix, enhancing cell adhesion, proliferation, and differentiation. It also enables efficient encapsulation of growth factors, promoting sustained release at targeted sites to improve wound healing and skin regeneration. Electrospray-assisted fabrication of scaffolds has shown superior biocompatibility and structural features over traditional methods. Furthermore, the technology's capability for directing cell and therapeutic delivery to wound sites introduce personalized treatment options for various skin conditions, making a significant advancement toward the clinical use of engineered skin tissues.

**Keywords:** electrospray, skin, tissue engineering, regeneration, wound healing

## 1. Introduction

During the last few years, interesting progress has been made in the field of tissue engineering, particularly in the development of innovative methods for skin tissue regeneration. In this vein, electrospray (ES) techniques have emerged as a pivotal technology, offering new possibilities for the precise deposition of bioactive materials and cells. This article seeks to explore the significant progress and applications of ES methods in skin tissue regeneration, highlighting the fusion of technology and biology that aims to enhance therapeutic outcomes [1–3].

Electrospray ionization (ESI) is a technique that has found novel applications in the biomedical field, especially for the fabrication of tissue scaffolds and the encapsulation of cells and drugs. The method involves the creation of an aerosol by applying a high-voltage to a liquid, which is then used to create fine particles or fibers. This ability to generate micro- and nanoparticles (NPs) with controlled size and distribution makes ES a valuable tool in tissue engineering [4, 5].

In recent advancements, the ES technique has been utilized for the co-deposition of multiple materials, including polymers, cells, and bioactive molecules, in a spatially controlled manner. For instance, researchers have successfully used the ES technique to create layered structures that mimic the natural architecture of human skin. Such structures are crucial for the successful integration of ES-engineered scaffolds with native tissues and for promoting wound healing [1, 6].

Young et al. [7] investigated the creation of synthetic hydrogel microspheres using submerged electrospray combined with UV photopolymerization, targeting cell encapsulation applications. The electrospray technique allows precise control over microsphere size from 50 to 1500 μm by manipulating the flow rate and voltage, which is beneficial for specific biomedical applications, such as drug delivery and cell therapy. The microspheres revealed cell viability higher than 90% after 24 h, underscoring the technique's compatibility with sensitive biological entities like cells and proteins.

Moreover, the adaptability of ES technology has been broadened to include the incorporation of bioactive agents and cellular growth factors that enhance the regeneration of skin tissues. For example, a study conducted by Chen et al. [8] explored the use of microfluidic ES in creating a drug delivery system based on natural polysaccharides, demonstrating its effectiveness in promoting wound healing. This technique enhances skin tissue repair by ensuring targeted, sustained release of therapeutic agents directly to wound sites, thus accelerating cellular processes essential for skin regeneration.

The combination of ES techniques with other technological progressions like 3D printing and nanotechnology additionally unlocks fresh opportunities for developing intricate tissue structures. This integrative approach can lead to the development of highly functional and biomimetic tissues, which are vital for the treatment of severe burns and chronic wounds [5, 9].

As the field continues to evolve, further studies are required to optimize these techniques, particularly in the areas of material compatibility, the long-term viability of deposited cells, and the scaling of the technology for clinical applications. Ongoing research and development are expected to further refine the precision and efficacy of ES methods, making them indispensable in the realm of regenerative medicine [1, 5].

In conclusion, the advancements in ES technology are not only enhancing our understanding of tissue engineering but are also paving the way for groundbreaking applications in skin tissue regeneration. As this field progresses, it holds the promise of significantly improving patient outcomes in skin repair and regeneration.

## 2. Overview of skin tissue engineering

Skin tissue engineering is a rapidly advancing field aimed at developing effective treatments for severe skin injuries and diseases. Recent advancements focus on creating bioengineered skin substitutes that integrate both dermal and epidermal components, which are all-important for mimicking the natural structure and function of human skin.

One of the notable developments in this area is the use of biodegradable scaffolds, such as the NovoSorb™ Biodegradable Temporizing Matrix (BTM), which are designed to temporize wounds and promote the integration and establishment of dermal elements while resisting infection. These scaffolds are advantageous because they are inexpensive, easy to handle, and can be produced in large sheets, providing essential support for cellular growth and eventual wound closure [10].

Another critical aspect of skin tissue engineering is the interplay between different cell types. For example, the communication between fibroblasts and keratinocytes is essential for basement membrane synthesis, which is crucial for the stability and protection of engineered skin substitutes. This synergy supports the molecular bonding necessary for attaching the epidermis to the dermis [10].

Engineered Skin Substitutes (ESS) represent a significant breakthrough, combining autologous keratinocytes and fibroblasts within a bovine collagen-glycosaminoglycan scaffold. This model has shown promising clinical outcomes, especially in treating extensive burns, by reducing the need for donor skin grafts and improving survival rates [10].

Recent research also explores the incorporation of various cell types, such as melanocytes, microvascular endothelial cells, and even hair follicles into these engineered constructs, enhancing their functional and esthetic outcomes. The field continues to face challenges, particularly in terms of the immune response to xenogeneic materials (like bovine or porcine collagens) used in some dermal-epidermal substitutes. Synthetic scaffolds and autologous cell approaches are being explored to minimize these risks [10].

Additionally, the remodeling phase of healing, which involves the maturation of scars and the replacement of type III collagen with type I collagen, is critical for the long-term success of these treatments. Innovations in fetal tissue engineering also show potential for scarless healing, suggesting new directions for future research [11].

Overall, the field of skin tissue engineering holds great promise for improving patient outcomes in wound healing and skin replacement. Ongoing research aims to make these technologies more user-friendly, commercially viable, and effective in clinical settings [12].

### 2.1 Importance of skin regeneration

Skin regeneration, a vital aspect of dermatological science, involves intricate processes aimed at restoring the functional integrity and structure of skin after injuries such as burns, wounds, or surgical interventions. The regenerative capability of the skin is primarily facilitated by the interplay of skin stem cells and various signaling pathways that govern cell proliferation, differentiation, and migration. Recent studies highlight the indispensable role of mesenchymal stem cells (MSCs) due to their potent immunomodulatory effects, ability to differentiate into multiple cell types relevant to the skin, and secretion of paracrine factors that significantly accelerate wound healing [13–16].

Technological progressions in tissue engineering have bolstered methods for skin regeneration. One instance is the refinement of collagen-based scaffolds to bolster cellular activities crucial for tissue repair. These scaffolds offer a conducive matrix to the effective integration and regeneration of skin tissues [17]. Nanomaterials have also been employed to improve these outcomes by fine-tuning the mechanical properties and degradation rates of scaffolds, which can be critical for matching the dynamic nature of skin tissue repair [18].

Moreover, the use of hydrogels in skin regeneration has seen significant advancements. Hydrogels are particularly effective due to their high-water content and soft tissue-like consistency, which makes them ideal for interacting with the natural skin environment. They promote cell adhesion, proliferation, and migration—all critical for tissue regeneration [19]. Moreover, it has been suggested that biomimetic methods capable of mirroring the structure of the extracellular matrix improve the healing process by offering signals that guide cell behavior and tissue growth [20].

The interplay between mechanical forces and cellular responses, known as mechanotransduction, has also been recognized as a key element in skin regeneration. Mechanical signals can alter cell behavior, influencing wound healing and potentially reducing scar formation by modulating fibrotic responses [21]. This highlights the need for a comprehensive understanding of both biological and mechanical factors in developing therapeutic strategies for skin repair and regeneration.

Overall, the combination of stem cell technology, advanced biomaterials, and an understanding of cellular mechanobiology provides a robust framework for enhancing skin regeneration. This integrated approach not only promises improved healing outcomes but also opens avenues for personalized therapeutic strategies tailored to individual healing needs and conditions [13, 17–21].

### 2.2 Challenges in skin tissue engineering

Expanding on the challenges in skin tissue engineering, one of the primary objectives remains the replication of the skin's multilayered structure and its complex functions. Each layer of the skin—epidermis, dermis, and hypodermis—plays a specific role, and successful tissue engineering must replicate these to restore full functionality. This involves not only mimicking the physical barrier of the skin but also its sensory and thermoregulatory properties [12, 22].

The scalability of producing tissue-engineered skin also presents a significant challenge. The methods must not only be effective but also economically viable for widespread clinical use. Advances in manufacturing technologies, such as 3D printing and automated bioreactors, are being explored to scale up the production of skin substitutes that are consistent in quality and function [23, 24].

Infection control is another critical issue, particularly for wounds susceptible to bacterial contamination. Incorporating antimicrobial agents or designing materials that naturally reduce bacterial growth without harming the regenerative cells or the patient's tissues is a key area of ongoing research [22, 25].

## 3. Fundamentals of electrospray technique

### 3.1 Principle of electrospray technology

ES technology, also known as electrohydrodynamic spraying, serves as a useful technique in various fields such as tissue engineering, drug delivery, and gene delivery systems. The process fundamentally relies on the dispersion of a liquid into fine droplets via an electric field, employing the principle of Coulombic repulsion to achieve this dispersion. As charges within the liquid repel, the liquid disintegrates into smaller droplets, a process crucial for the precise delivery mechanisms required in these fields [26, 27].

The operational mechanics of ES are highlighted by the formation of a Taylor cone at the liquid's surface when subjected to a high-voltage. This conical shape results from the equilibrium between the electric field's force and the liquid's surface tension. Once the electric field's strength exceeds a critical threshold, it disrupts the surface tension, initiating the ejection of a liquid jet from the cone's apex. This jet then undergoes several instabilities, ultimately breaking into droplets whose sizes can vary from a few nanometers to several micrometers, suitable for targeted applications in medicine and engineering. Central to the ES setup are its core components, which include a syringe pump for controlled liquid flow, a nozzle that allows for the adjustment of droplet size,

and a high-voltage power supply that charges the droplets. These droplets are subsequently collected after solvent evaporation. The ability to control droplet formation is vital for the success of the ES system, ensuring the achievement of specific particle characteristics necessary for the targeted applications, thereby underscoring the technique's versatility and effectiveness in precise particle generation [27–31].

Structured micro/nanomaterials synthesized via ES offer unique properties and functionalities, making them valuable in biomedical applications. By controlling the composition and processing conditions, researchers can tailor the properties of the resulting materials to meet specific requirements for tissue engineering scaffolds, drug delivery carriers, and biosensors [27, 32].

By understanding the principles underlying ES technology and optimizing its parameters, researchers can harness its potential to advance various biomedical applications, from tissue engineering to drug delivery systems, offering innovative solutions to address complex healthcare challenges.

### 3.2 Parameters influencing the electrospray process

ES is a versatile technique widely employed in various fields, including tissue engineering, for its ability to precisely control the size, morphology, and composition of particles or fibers. Several parameters play crucial roles in determining the outcome of the ES process, as elucidated by recent studies [33].

Firstly, the choice of solvent(s) significantly impacts the ES process. The polarity, viscosity, and surface tension of the solvent affect the formation and stability of the Taylor cone, which is all-important for generating fine droplets or fibers. The study by Xu et al. delves into this aspect, highlighting how different solvents influence the properties of electrospray particles, such as size and morphology. Understanding solvent-solute interactions is imperative for optimizing ES parameters [34, 35].

Secondly, the flow rate of the solution being sprayed plays a crucial role in controlling droplet or fiber size and uniformity. Variations in flow rate affect the breakup dynamics of the jet emanating from the Taylor cone, thereby influencing the size distribution of the resulting particles or fibers. This parameter is extensively discussed in the study by Li et al., wherein the impact of flow rate on the morphology and encapsulation efficiency of electrospray particles is investigated. Additionally, the nozzle design significantly impacts the particle distribution and size. The size of the droplets is controlled through nozzle geometry and flow rate, further emphasizing the importance of mechanical configuration in achieving desired outcomes [28, 36].

Moreover, the choice of voltage applied during ES significantly influences the process. Voltage affects the surface charge density of the solution, thereby influencing the formation and stability of the Taylor cone and subsequent droplet or fiber generation. Molecular dynamics simulations, as discussed in the study by Xu et al., provide valuable insights into how different voltage conditions affect the dynamics of ES [37].

Lastly, parameters such as the distance between the needle tip and the collector, ambient humidity, and temperature also play crucial roles in the ES process, albeit to varying extents depending on the specific application and materials used [35].

### 3.3 Materials used in electrospray in tissue engineering

Materials like natural polymers (e.g., alginate, collagen) and synthetic ones (e.g., PLGA) are preferred due to their biocompatibility and controlled degradation rates, supporting the integration of engineered tissues [29–31, 38, 39].

Polymers used in ES techniques include both natural and synthetic materials. Natural polymers like alginate and collagen are particularly valuable due to their biocompatibility and biodegradability. They are used to create scaffolds that support cell proliferation and differentiation, mimicking the extracellular matrix. For instance, the gelatin-alginate composite has demonstrated remarkable utility in promoting wound healing, burn treatment, and other biomedical applications [40]. Synthetic polymers like PLGA offer versatility, providing customizable degradation rates and mechanical properties, which are critical for drug delivery systems. Advances in composite materials and the combination of natural and synthetic polymers have been highlighted by various research groups for their adaptability and compatibility [38, 40]. Injectable gels and hydrogels have also been explored for their potential in regenerative medicine, particularly using ES and related techniques for their preparation. These materials can be loaded with cells, growth factors, or drugs, and then precisely deployed to wound sites, providing a moist environment that facilitates tissue growth and repair. The versatility of these systems allows for the incorporation of various bioactive components that can be tailored to specific clinical needs [41].

## 4. Advancements in electrospray techniques

Recent advancements in ES techniques have significantly expanded their applicability in various scientific and technological fields, enhancing precision and efficiency. ES has been refined to create micro and NPs with controlled size and morphology, which is vital for applications such as drug delivery systems. This is achieved through precise manipulation of electrospray parameters such as voltage, flow rate, and needle size, allowing the production of tailored microspheres suitable for targeted drug delivery. For example, alginate microspheres have been developed for controlled release and encapsulation of therapeutic agents, capitalizing on alginate's biocompatibility and biodegradability [42].

Further, ES has been combined with other technologies to improve outcomes in fields such as tissue engineering and vascularization. The electrospray bioprinting method developed by Jack et al. was pivotal in enhancing vascularization by rapidly generating hydrogel microspheres encapsulating vascular endothelial growth factor (VEGF)-overexpressing HEK293T cells. These microspheres effectively promoted angiogenesis in a mouse hind-limb ischemia model. The controlled release of VEGF from the microspheres induced collateral vessel formation, vital for therapeutic angiogenesis aimed at treating limb ischemia. The study demonstrated that applying increased voltage using the electrospray method not only refined microsphere size but also ensured the targeted delivery and sustained release of VEGF, enhancing vascularization and tissue repair in ischemic conditions [43].

Additionally, the integration of ES with nanospray desorption techniques has fostered advancements in mass spectrometry, enabling the direct, in-depth analysis of metabolites from biological samples. This combination facilitates a minimal sample preparation approach, maintaining the integrity and original distribution of the metabolites on sample surfaces [44].

In biomedical engineering, ES is used in conjunction with other encapsulation techniques to enhance the delivery and efficacy of probiotics and other therapeutic agents. This includes the development of novel biomaterials that can protect active ingredients against harsh gastrointestinal environments, thereby increasing the survival and delivery efficiency of therapeutic microbes [44].

## 5. Electrospray techniques in skin tissue engineering

### 5.1 Application of electrospray in skin cell deposition

The application of ES in skin cell deposition is gaining traction due to its capacity to create micro- and nano-scale structures beneficial for skin tissue engineering. ES offers a highly controlled method for depositing cells and biomaterials onto various substrates, which is essential for the precise construction of skin layers.

For instance, the deposition of fibroblasts using ES has shown promising results in maintaining cell viability and function, essential for effective skin regeneration [45, 46]. Zheng et al. have explored the use of ES for depositing zinc oxide (ZnO) thin films, which are utilized in sensors but have potential applications in skin tissue engineering due to their biocompatibility and microbial resistance [45].

Moreover, the precision of ES allows for the deposition of cells in defined patterns, mimicking the natural extracellular matrix and thus promoting better cell adhesion and proliferation. This technique surpasses traditional methods like manual cell seeding or random fibroblast spraying, which lack control over cell distribution and density [46, 47].

Recent advancements have also explored combining ES with other technologies. For example, combining ES and bioprinting has been proposed to fabricate complex tissue structures by precisely placing cells and scaffolds layer by layer, further enhancing the mimicry of natural skin architecture [45].

ES deposition represents a promising frontier in skin tissue engineering, offering advancements in how cells and materials are layered and integrated into functional tissue constructs. This approach could significantly improve the outcomes of skin regeneration therapies, making them more efficient and effective.

### 5.2 Role in the fabrication of skin tissue scaffolds

ES is a beneficial method for producing scaffolds for skin tissue, allowing for the generation of particles at micro- and nano-scales. Unlike electrospinning, which focuses on fiber production, ES uses electrical forces to produce droplets of polymer solutions. These particles can be functionalized with bioactive agents like growth factors or drugs, promoting wound healing and providing an environment conducive to tissue regeneration [48, 49].

In the context of skin tissue engineering, electrosprayed particles loaded scaffolds support cell attachment and proliferation, essential processes in skin regeneration. Their high surface area-to-volume ratio enhances the scaffolds' ability to deliver therapeutic compounds efficiently. For instance, scaffolds can be loaded with growth factors, which stimulate cell growth, differentiation, and migration, aiding wound healing [25].

A significant advantage of the ES technique is its compatibility with different materials. Both synthetic and natural polymers can be used, allowing for the design of scaffolds with customizable properties such as mechanical strength, degradation rate, and biological functionality. These customizable features can be tailored to meet specific clinical needs, such as treating diabetic ulcers or extensive burn injuries [50].

ES is a versatile and promising tool for producing innovative scaffolds in skin tissue engineering. By enabling precise control over the delivery of bioactive agents and scaffold architecture, it offers significant potential for developing effective, personalized treatments in regenerative medicine.

### 5.3 Delivery of growth factors and bioactive molecules

Growth factors play an important role in wound healing and tissue regeneration by promoting cell proliferation and migration, modulating immune responses, and enhancing the formation of extracellular matrix. Alginate, combined with other biopolymers like sericin and platelet lysate, has been used to create bioactive wound dressings that release growth factors in a controlled manner, thereby accelerating the healing process in skin lesions. This approach uses the ES technique to encapsulate these molecules within biodegradable materials, which can then be applied directly to wound sites for improved healing outcomes [51].

The use of scaffolds in skin tissue engineering is another significant area where ES techniques are beneficial. Scaffolds provide a 3D structure that supports cell adhesion, growth, and differentiation. Materials like chitosan and polylactic-co-glycolic acid (PLGA) have been utilized to create nanofibrous scaffolds that mimic the natural extracellular matrix of the skin. These scaffolds can be functionalized with growth factors like basic fibroblast growth factor (bFGF) and transforming growth factor beta 1 (TGF-β1), which are critical for promoting skin regeneration and reducing scarring [52, 53].

ES technique is important in advancing wound dressing technology by enabling precise deposition of bioactive molecules on fabrics, which plays a crucial role in controlled drug release and targeted therapeutic actions. Zhang et al. [54] utilized the electrospray technique to apply a polydimethylsiloxane (PDMS) coating on one side of a chitosan-loaded cotton fabric, creating a Janus bandage with hydrophilic and hydrophobic properties. This unique structure facilitated unidirectional moisture management, crucial for maintaining optimal wound moisture, during the healing process. The bandage showed a one-way transport index of 1068%, indicating highly efficient moisture transfer away from the wound. Moreover, the bioactive coating ensured sustained release of antimicrobial agents, significantly enhancing fibroblast proliferation and reducing infection risks at the wound site, thus accelerating the healing process.

## 6. Case studies: success stories in skin regeneration

### 6.1 Detailed accounts of successful applications

In the realm of tissue engineering, the ES technique has been harnessed successfully to foster advancements in skin regeneration. Below are several illustrative case studies from recent years that highlight these developments:

Jayarajan et al. [55] demonstrated that bio-electrospray could effectively reconstruct 3D organotypic human skin tissues with high cell viability using a hydrogel mixed with human fibroblasts. The results showed that bio-electrospray facilitated the formation of skin tissues with varying epidermal thickness. Specifically, manually seeded constructs had an epidermal thickness of approximately 30 μm, while bio-electrospray constructs displayed an inconsistent thickness due to the impact of larger cell-bearing droplets. These findings underscore the potential of bio-electrospray in tissue engineering, suggesting its capability to handle and integrate high concentrations of cells into complex tissue structures effectively.

Another significant study explored the use of ES to apply shrimp and mushroom-derived chitin nanofibrils onto cellulose tissues, targeting skin contact applications.

The findings showed that this application significantly enhanced the anti-inflammatory properties of the cellulose, with cytokine inhibition efficiency reaching up to 67%. Moreover, the ES technique maintained over 90% viability for human dermal keratinocytes, emphasizing its suitability for creating biocompatible and functional skincare materials. These results highlight the potential of ES in developing innovative, bio-based products for skin care applications [56]. **Figure 1** revealed that HaCaT cells (human epidermal keratinocyte line) were viable in all the electrosprayed cellulosic tissues. Only a few dead cells were observed, with no differences between the different samples.

In the innovative study, Luo et al. [57] employed a microfluidic electrospray technique to produce discal microparticles (DMPs) loaded with therapeutic agents for enhanced wound healing. The effectiveness of this method was notably evident in the significant results observed in the angiogenesis process, crucial for wound repair. Specifically, the DMPs+drugs group demonstrated a marked increase in the formation of new blood vessels, as indicated by the measurement of "tube length." This term refers to the length of tube-like structures formed by endothelial cells, which are used as a quantitative indicator of new blood vessel growth. The results revealed an impressive 63% increase in tube length, with the treated group achieving an average tube length of 1.8 mm compared to just 1.1 mm in the control group. These findings clearly highlight the electrospray's capacity to enhance therapeutic delivery and efficacy, accelerating the healing process by promoting more robust and rapid angiogenesis.

Research has explored the regenerative and anti-aging capacities of adipose-derived stem cells (ADSCs) loaded with growth differentiation factor 11 (GDF11) using the ES technique. This combination has been found to enhance skin integrity, density, and strength, while also reducing wrinkles. The study indicates that ADSCs, when electrospray with bioactive factors like GDF11, can significantly contribute to skin rejuvenation and repair processes [58].

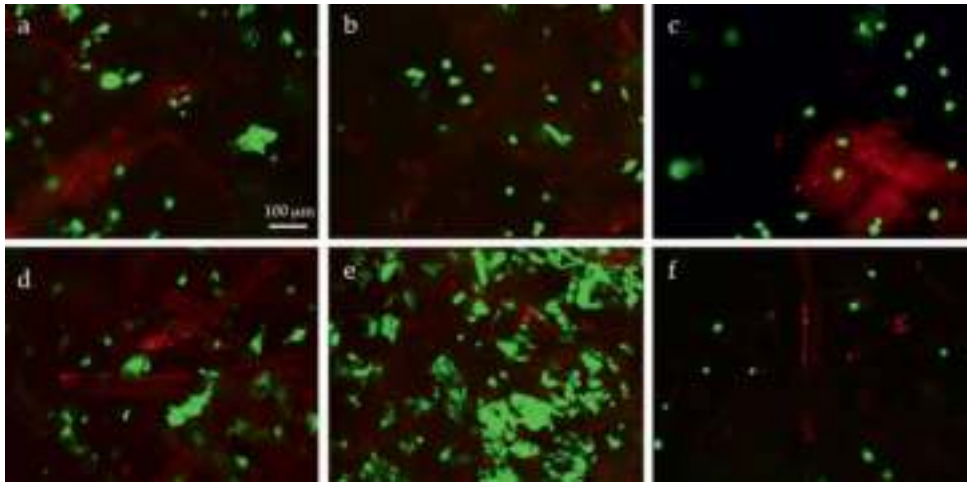


**Figure 1.**
*Direct cytotoxicity test: Live/Dead viability test performed on HaCaT cell line seeded on cellulose tissues electrospray with (a) shrimp chitin nanofibrils (sCNs) (water); (b) sCNs (water/acetic acid); (c) sCNs (water/HFIP); (d) mushroom chitin nanofibrils (mCNs) (water); (e) mCNs (water/acetic acid). (f) Pristine cellulose tissue. Viable cells are stained in green, dead cells are stained in red, and the cellulosic tissue shows autofluorescence mainly in the red channel. Adopted with permission [56].*

A study documented the use of ES to deposit silver nanoparticles (AgNPs) on radiosterilized porcine skin to prevent infections in deep burn wounds help maintain cell viability and promote cell growth. This innovative approach used an ES technique to apply a solution of AgNPs directly to the skin, enhancing its antimicrobial properties and thus reducing the risk of infection in severe burn injuries. This method highlights the potential of combining NP technology with the ES technique to improve outcomes in critical care scenarios [59]. **Figure 2** provides an insightful overview of the impact of AgNPs on fibroblast viability, with micrographs and quantitative data demonstrating the distinctions between live and dead cells and offering a comparative assessment of their counts and proportions. It reveals that while low concentrations of AgNPs maintain high cell viability in human dermal fibroblasts, increasing the concentration leads to significant reductions in both the number and viability of cells. At the lowest concentration (0.055 M), viability is at 99%, but it decreases to 85% at the highest concentration (0.500 M). This indicates a concentration-dependent cytotoxic effect of AgNPs on cell viability and number.

These case studies exemplify how the ES technique is being utilized to create and optimize bioactive scaffolds that enhance skin regeneration. By manipulating various biomaterials and cellular components, researchers can tailor properties that meet specific therapeutic needs, paving the way for advanced solutions in skin repair and regeneration.

### 6.2 Discussion on the impact of electrospray techniques on patient outcomes

The impact of ES technique in skin tissue engineering is a burgeoning area of research that promises significant advancements in patient outcomes, particularly in wound healing and skin regeneration. ES and related techniques, such as electrospinning, have been essential in the development of nanofibrous scaffolds that mimic the extracellular matrix, thereby enhancing tissue integration and cellular interactions.

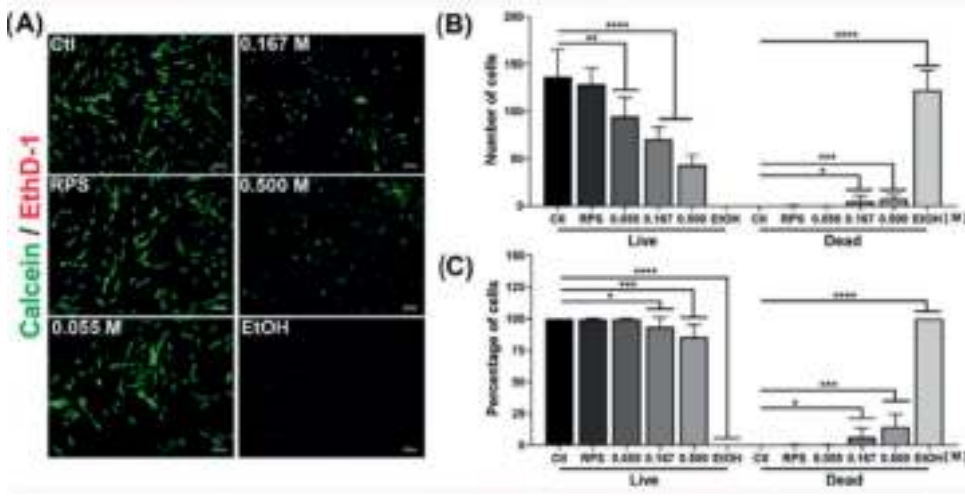


**Figure 2.**
*Viability of fibroblasts exposed to electrospray AgNPs. The effect of AgNPs from porcine skin on fibroblast viability was measured. (A) Micrographs of viable cells (calcein/green) and dead cells (ethidium homodimer/red); (B) number of living and dead cells; and (C) percentage of living and dead cells. Adopted with permission [59].*

ES deposition, for instance, is utilized to create fine mists of collagen particles, which are then directed onto target surfaces. This technique is especially valuable in the pharmaceutical and biomedical fields due to its precision and the ability to maintain the stability and activity of the sprayed particles. The resultant collagen nanostructures are ideal for drug delivery applications and are used to enhance the healing processes in tissue engineering [60].

In the realm of skin tissue engineering, the structural properties of scaffolds created via ES and electrospinning are of paramount importance. These scaffolds provide a three-dimensional matrix that supports cell attachment, proliferation, and differentiation, all of which are essential for effective tissue regeneration. Recent advancements have highlighted the role of NP additives in these processes, such as ZnO and graphene, which confer additional antibacterial properties and enhance the mechanical integrity of the scaffolds [61, 62].

The versatility of ES techniques extends to the creation of hybrid scaffolds that combine both synthetic and natural polymers, improving the overall functionality of the scaffolds. These hybrid materials demonstrate improved mechanical properties, stability, and bioactivity, which are critical for their performance *in vivo* [61].

Furthermore, the use of nature-derived polymers in ES applications, such as cellulose and its derivatives, has been explored for their biocompatibility and environmental stability. These materials offer the potential for controlled degradation, which is beneficial for temporary scaffolds that need to support tissue formation and then degrade safely within the body [61].

ES techniques significantly impact patient outcomes in skin tissue engineering by providing sophisticated tools for creating advanced biomaterials that support wound healing and tissue regeneration. The ongoing development of these technologies suggests a promising future for their application in regenerative medicine and other biomedical fields.

### 6.3 Comparative analysis with traditional skin regeneration methods

ES and traditional methods of skin regeneration each bring unique approaches and technologies to the field of tissue engineering. The adoption of ES techniques, particularly in the generation of nano and microscale fibers, presents an innovative path that diverges significantly from more conventional methods like grafting or the use of biomaterial scaffolds.

Traditional skin regeneration methods have largely relied on direct transplantation and the application of skin grafts, including autografts, allografts, and xenografts. These methods, while effective in many contexts, often face limitations due to donor site scarcity, potential for immune rejection, and infection risks. Additionally, the mechanical properties and integration of these grafts can vary, potentially complicating the healing process [41, 63].

In contrast, ES technology offers a highly versatile platform for creating tailored tissue scaffolds that can enhance cell attachment, proliferation, and differentiation. The technique allows for the precise deposition of polymers, cells, and active compounds in an organized manner, enabling the development of structures that closely replicate the natural architecture of skin tissue. This level of control is particularly beneficial for promoting proper tissue integration and function [63, 64].

Recent advancements have demonstrated the potential of ES in delivering bioactive molecules effectively. For instance, research has shown that ES can be used to encapsulate and subsequently release growth factors in a controlled manner, which

is crucial for modulating wound healing processes. This method ensures sustained release, which is often necessary for the prolonged durations required in tissue regeneration scenarios [64].

Furthermore, the integration of ES with other technologies like 3D bioprinting has been explored, resulting in hybrid techniques that leverage the strengths of both approaches. For example, the combination of 3D printing and ES has been utilized to create layered structures that offer mechanical support and active biological functionality, which are essential for effective skin regeneration [41].

While traditional skin regeneration techniques continue to be valuable, ES and related advanced manufacturing technologies represent a significant step forward. These methods provide enhanced capabilities for creating more complex, functional, and personalized tissue constructs that could lead to improved patient outcomes in skin repair and regeneration. The ongoing development and refinement of these technologies hold promise for addressing the limitations of existing methods and expanding the possibilities within the field of tissue engineering.

## 7. Challenges and limitations

ES technology holds significant promise for skin tissue engineering, particularly in the encapsulation and controlled release of bioactive molecules. However, several challenges and limitations must be addressed to optimize its efficacy. One major issue is the precise control over droplet size and distribution, which is crucial for creating uniform particles and fibers. Inconsistent droplet formation can lead to nonuniform scaffold structures, adversely affecting cell growth and tissue regeneration outcomes. Achieving and maintaining the stability of the Taylor cone, especially with solutions of varying viscosities and conductivities, requires meticulous fine-tuning of parameters such as voltage and flow rate, which can be time-consuming and labor-intensive [65].

Moreover, the integration of ES with other techniques like electrospinning to fabricate hybrid scaffolds introduces additional complexity. Ensuring material compatibility and maintaining the structural integrity of combined scaffolds are significant engineering challenges. Hybrid scaffolds must achieve desired mechanical properties while retaining the bioactivity of incorporated molecules, necessitating extensive optimization and iterative testing. This complexity often involves a trial-and-error approach to balance mechanical robustness and biological functionality [66].

Scaling up the ES process for clinical applications presents another substantial challenge. Laboratory-scale experiments show potential, but translating these findings to large-scale production involves addressing batch-to-batch variability, equipment scalability, and compliance with stringent regulatory standards. Ensuring that ES-based products meet quality and safety standards essential for medical use is a demanding task, requiring rigorous validation and quality control measures [67].

## 8. Future directions

ES is emerging as a promising technique in skin tissue engineering due to its ability to generate finely controlled particles and droplets. Its future directions lie in integrating with advanced technologies like nanotechnology, 3D printing and biofabrication, enabling more complex and functional skin tissue structures.

The convergence of ES with nanotechnology holds significant potential for enhancing biofabrication strategies. Nano-biomaterials can be embedded into bio-inks via ES, providing targeted delivery of growth factors or drugs and mimicking the extracellular matrix structure to trigger specific cell responses. For instance, embedding nano-biomaterials within bio-inks has demonstrated success in delivering growth factors like TGF-β1 for cartilage regeneration. These nano-carriers protect and sustainably release their cargo, which could be crucial for controlled differentiation in skin tissue engineering [68].

The ability of ES to produce particles of controlled sizes complements 3D printing technologies by enhancing the layering of bio-inks and ensuring uniform particle distribution in engineered tissues. Advanced biofabrication techniques can leverage this to produce more complex, multilayered structures that closely mimic native skin. For instance, research on cardiac tissue engineering illustrates how ES can contribute to improved bio-ink formulations, enhancing the layering and integration of cells and scaffolds within 3D-printed constructs [69].

Despite promising advancements, challenges like bio-ink printability, cell viability, and material compatibility need resolution. Future research aims to optimize bio-ink formulations with tailored mechanical and chemical properties, making them suitable for ES processing. Moreover, integrating ES with real-time monitoring systems could refine the printing process to achieve more precise control over the tissue structures produced [68, 69].

Overall, the future of ES in skin tissue engineering appears promising, especially when combined with these advanced technologies. The interdisciplinary approach will be a key to unlocking new potentials and overcoming current limitations.

## 9. Conclusion

The ES technique is a valuable tool in skin tissue engineering, offering precise fabrication of cell-seeded scaffolds and controlled delivery of bioactive molecules. This precision enhances cell adhesion and growth, while its compatibility with various biomaterials makes it suitable for diverse tissue scaffolds. The technology shows promise for personalized skin grafts and sophisticated wound healing through multilayered structures and controlled release of therapeutic agents.

Moving forward, ES's integration with emerging technologies like bioprinting and improvements in operational parameters can lead to significant clinical applications. Collaboration among researchers, clinicians, and industry will be crucial to overcoming technical challenges and accelerating its transition to clinical use. With a concerted effort, ES technology could revolutionize skin regeneration and treatment.

## Author details

Mobina Bazari[1] and Najmeh Najmoddin[2*]

1 Biomedical Engineering Department, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran

2 Department of Biomedical Engineering, Medical Engineering and Biology Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Address all correspondence to: najmoddin@srbiau.ac.ir

IntechOpen

## References

[1] Chi H, Qiu Y, Ye X, Shi J, Li Z. Preparation strategy of hydrogel microsphere and its application in skin repair. Frontiers in Bioengineering and Biotechnology. 2023;**11**:PMC10405935. DOI: 10.3389/fbioe.2023.1239183

[2] Data Bridge Market Research on Tissue Regeneration Market [Internet]. [cited 2024 Apr 27]. Available from: https://www.databridgemarketresearch.com/reports/global-tissue-regeneration-market

[3] Prabhu GRD, Williams ER, Wilm M, Urban PL. Mass spectrometry using electrospray ionization. Nature Reviews Methods Primers. 2023;**3**(1):23. DOI: 10.1038/s43586-023-00203-4

[4] Medtronic's Durepair Dura Regeneration Matrix [Internet]. [cited 2024 Apr 27]. Available from: https://www.medtronic.com/covidien/en-us/products/hernia-repair/durepair-regeneration-matrix.html

[5] Qin M, Qian Y, Huang L, Zhong C, Li M, Yu J, et al. Extractive electrospray ionization mass spectrometry for analytical evaluation and synthetic preparation of pharmaceutical chemicals. Frontiers in Pharmacology. 2023;**14**:1110900. DOI: 10.3389/fphar.2023.1110900

[6] Coppola D, Lauritano C, Palma Esposito F, Riccio G, Rizzo C, de Pascale D. Fish waste: From problem to valuable resource. Marine Drugs. 2021;**19**(2):116. DOI: 10.3390/md19020116

[7] Young CJ, Poole-Warren LA, Martens PJ. Combining submerged electrospray and UV photopolymerization for production of synthetic hydrogel microspheres for cell encapsulation. Biotechnology and Bioengineering. 2012;**109**(6):1561-1570. DOI: 10.1002/bit.24430

[8] Chen C, Wang Y, Zhang D, Wu X, Zhao Y, Shang L, et al. Natural polysaccharide based complex drug delivery system from microfluidic electrospray for wound healing. Applied Materials Today. 2021;**23**:101000. DOI: 10.1016/j.apmt.2021.101000

[9] Midha MK, Kapil C, Maes M, Baxter DH, Morrone SR, Prokop TJ, et al. Vacuum insulated probe heated electrospray ionization source enhances microflow rate chromatography signals in the Bruker timsTOF mass spectrometer. Journal of Proteome Research. 2023;**22**(7):2525-2537. DOI: 10.1021/acs.jproteome.3c00305

[10] Dearman BL, Boyce ST, Greenwood JE. Advances in skin tissue bioengineering and the challenges of clinical translation. Frontiers in Surgery. 2021;**8**:640879. DOI: 10.3389/fsurg.2021.640879

[11] Nourian Dehkordi A, Mirahmadi Babaheydari F, Chehelgerdi M, Raeisi DS. Skin tissue engineering: Wound healing based on stem-cell-based therapeutic strategies. Stem Cell Research & Therapy. 2019;**10**(1):111. DOI: 10.1186/s13287-019-1212-2

[12] Vig K, Chaudhari A, Tripathi S, Dixit S, Sahu R, Pillai S, et al. Advances in skin regeneration using tissue engineering. International Journal of Molecular Sciences. 2017;**18**(4):789. DOI: 10.3390/ijms18040789

[13] Wu S, Sun S, Fu W, Yang Z, Yao H, Zhang Z. The role and prospects of mesenchymal stem cells in skin repair

and regeneration. Biomedicine. 2024;**12**(4):743. DOI: 10.3390/biomedicines12040743

[14] Jo H, Brito S, Kwak BM, Park S, Lee MG, Bin BH. Applications of mesenchymal stem cells in skin regeneration and rejuvenation. International Journal of Molecular Sciences. 2021;**22**(5):2410. DOI: 10.3390/ijms22052410

[15] Shadman-Manesh V, Gholipour-Kanani A, Najmoddin N, Rabbani S. Preclinical evaluation of the polycaprolactone-polyethylene glycol electrospun nanofibers containing egg-yolk oil for acceleration of full thickness burns healing. Scientific Reports. 2023;**13**(1):919. DOI: 10.1038/s41598-023-28065-6

[16] Saghafi Y, Baharifar H, Najmoddin N, Asefnejad A, Maleki H, Sajjadi-Jazi SM, et al. Bromelain- and silver nanoparticle-loaded Polycaprolactone/chitosan Nanofibrous dressings for skin wound healing. Gels. 2023;**9**(8):672. DOI: 10.3390/gels9080672

[17] Mbese Z, Alven S, Aderibigbe BA. Collagen-based nanofibers for skin regeneration and wound dressing applications. Polymers (Basel). 2021;**13**(24):4368. DOI: 10.3390/polym13244368

[18] Bellu E, Medici S, Coradduzza D, Cruciani S, Amler E, Maioli M. Nanomaterials in skin regeneration and rejuvenation. International Journal of Molecular Sciences. 2021;**22**(13):7095. DOI: 10.3390/ijms22137095

[19] Arabpour Z, Abedi F, Salehi M, Baharnoori SM, Soleimani M, Djalilian AR. Hydrogel-Based Skin Regeneration. International Journal of Molecular Sciences. 2024;**25**(4):1982. DOI: 10.3390/ijms25041982

[20] Hama R, Reinhardt JW, Ulziibayar A, Watanabe T, Kelly J, Shinoka T. Recent tissue engineering approaches to mimicking the extracellular matrix structure for skin regeneration. Biomimetics. 2023;**8**(1):130. DOI: 10.3390/biomimetics8010130

[21] Kuehlmann B, Bonham CA, Zucal I, Prantl L, Gurtner GC. Mechano-transduction in wound healing and fibrosis. Journal of Clinical Medicine. 2020;**9**(5):1423. DOI: 10.3390/jcm9051423

[22] Tavakoli S, Klar AS. Bioengineered skin substitutes: Advances and future trends. Applied Sciences. 2021;**11**(4):1493. DOI: 10.3390/app11041493

[23] De Chiara F, Ferret-Miñana A, Fernández-Costa JM, Ramón-Azcón J. The tissue engineering revolution: From bench research to clinical reality. Biomedicine. 2024;**12**(2):453. DOI: 10.3390/biomedicines12020453

[24] Przekora A. A concise review on tissue engineered artificial skin grafts for chronic wound treatment: Can we reconstruct functional skin tissue In vitro? Cells. 2020;**9**(7):1622. DOI: 10.3390/cells9071622

[25] Riha SM, Maarof M, Fauzi MB. Synergistic effect of biomaterial and stem cell for skin tissue engineering in cutaneous wound healing: A concise review. Polymers (Basel). 2021;**13**(10):1546. DOI: 10.3390/polym13101546

[26] Morais AÍS, Vieira EG, Afewerki S, Sousa RB, Honorio LMC, Cambrussi ANCO, et al. Fabrication of polymeric microparticles by electrospray: The impact of experimental parameters. Journal of Functional Biomaterials. 2020;**11**(1):4. DOI: 10.3390/jfb11010004

[27] Tycova A, Prikryl J, Kotzianova A, Datinska V, Velebny V, Foret F. Electrospray: More than just an ionization source. Electrophoresis. 2021;**42**(1-2):103-121. DOI: 10.1002/elps.202000191

[28] He T, Jokerst JV. Structured micro/nano materials synthesized via electrospray: A review. Biomaterials Science. 2020;**8**(20):5555-5573. DOI: 10.1039/D0BM01313G

[29] Serafim V, Tiugan DA, Andreescu N, Mihailescu A, Paul C, Velea I, et al. Development and validation of a LC–MS/MS-based assay for quantification of free and Total omega 3 and 6 fatty acids from human plasma. Molecules. 2019;**24**(2):360. DOI: 10.3390/molecules24020360

[30] Vasala A, Hytönen VP, Laitinen OH. Modern tools for rapid diagnostics of antimicrobial resistance. Frontiers in Cellular and Infection Microbiology. 2020;**10**:308. DOI: 10.3389/fcimb.2020.00308

[31] Pluhařová K, Leontovyčová H, Stoudková V, Pospíchalová R, Maršík P, Klouček P, et al. "Salicylic acid mutant collection" as a tool to explore the role of salicylic acid in regulation of plant growth under a changing environment. International Journal of Molecular Sciences. 2019;**20**(24):6365. DOI: 10.3390/ijms20246365

[32] Kurakula M, Raghavendra NN. Electrospraying: A facile technology unfolding the chitosan based drug delivery and biomedical applications. European Polymer Journal. 2021;**147**:110326. DOI: 10.1016/j.eurpolymj.2021.110326

[33] Joaquim A, Paul O, Ibezim M, Johnson D, Falconer A, Wu Y, et al. Electrospray deposition of Polyvinylidene fluoride (PVDF) microparticles: Impact of solvents and flow rate. Polymers (Basel). 2022;**14**(13):2702. DOI: 10.3390/polym14132702

[34] Aminoroaya A, Khorasani SN, Bagheri R, Talebi Z, Malekkhouyan R, Das O, et al. Facile encapsulation of cyanoacrylate-based bioadhesive by electrospray method and investigation of the process parameters. Scientific Reports. 2024;**14**(1):5389. DOI: 10.1038/s41598-024-56008-2

[35] Tanhaei A, Mohammadi M, Hamishehkar H, Hamblin MR. Electrospraying as a novel method of particle engineering for drug delivery vehicles. Journal of Controlled Release. 2021;**330**:851-865. DOI: 10.1016/j.jconrel.2020.10.059

[36] Zhang J, Cai G, Liu X, He B, Wang W. Molecular dynamics simulation of ionic liquid electrospray: Revealing the effects of interaction potential models. Acta Astronautica. 2021;**179**:581-593. DOI: 10.1016/j.actaastro.2020.11.018

[37] John JV, McCarthy A, Wang H, Chen S, Su Y, Davis E, et al. Engineering biomimetic nanofiber microspheres with tailored size, predesigned structure, and desired composition via gas bubble–mediated coaxial electrospray. Small. 2020;**16**(19):1907393. DOI: 10.1002/smll.201907393

[38] Chelu M, Musuc AM. Advanced biomedical applications of multifunctional natural and synthetic biomaterials. PRO. 2023;**11**(9):2696. DOI: 10.3390/pr11092696

[39] Siraj MA, Rahman MS, Tan GT, Seidel V. Molecular docking and molecular dynamics simulation studies of triterpenes from Vernonia patula with the cannabinoid type 1 receptor. International Journal of Molecular

Sciences. 2021;**22**(7):3595. DOI: 10.3390/ijms22073595

[40] Ha TLB, Quan TM, Vu DN, Si DM. Naturally derived biomaterials: Preparation and application. In: Andrades JA, editor. Regenerative Medicine and Tissue Engineering. Rijeka: IntechOpen; 2013. DOI: 10.5772/55668

[41] Omidian H, Chowdhury SD. Advancements and applications of injectable hydrogel composites in biomedical research and therapy. Gels. 2023;**9**(7):533. DOI: 10.3390/gels9070533

[42] Ciarleglio G, Russo T, Toto E, Santonicola MG. Fabrication of alginate/Ozoile gel microspheres by electrospray process. Gels. 2024;**10**(1):52. DOI: 10.3390/gels10010052

[43] Shen J, Ji Y, Xie M, Zhao H, Xuan W, Yin L, et al. Cell-modified bioprinted microspheres for vascular regeneration. Materials Science and Engineering: C. 2020;**112**:110896. DOI: 10.1016/j.msec.2020.110896

[44] Cha S, Jun G, Park Y, An SJ, Lee D. Toward depth-resolved analysis of plant metabolites by Nanospray desorption electrospray ionization mass spectrometry. Molecules. 2022;**27**(21):7582. DOI: 10.3390/molecules27217582

[45] Li W, Lin J, Wang X, Jiang J, Guo S, Zheng G. Electrospray deposition of ZnO thin films and its application to gas sensors. Micromachines (Basel). 2018;**9**(2):66. DOI: 10.3390/mi9020066

[46] Lapomarda A, Acutis A De, Maria C De, Vozzi G. Pectin-based scaffolds for tissue engineering applications. In: Masuelli MA, editor. Pectins. Rijeka: IntechOpen; 2021. DOI: 10.5772/intechopen.101521

[47] Mozafari M, Mehraien M, Vashaee D, Tayebi L. Electroconductive nanocomposite scaffolds: A new strategy into tissue engineering and regenerative medicine. In: Ebrahimi F, editor. Nanocomposites. Rijeka: IntechOpen; 2012. DOI: 10.5772/51058

[48] Zhang S, Chen X, Shan M, Hao Z, Zhang X, Meng L, et al. Convergence of 3D bioprinting and nanotechnology in tissue engineering scaffolds. Biomimetics. 2023;**8**(1):94. DOI: 10.3390/biomimetics8010094

[49] Da LC, Huang YZ, Xie HQ, Zheng BH, Huang YC, Du SR. Membranous extracellular matrix-based scaffolds for skin wound healing. Pharmaceutics. 2021;**13**(11):1796. DOI: 10.3390/pharmaceutics13111796

[50] Krishani M, Shin WY, Suhaimi H, Sambudi NS. Development of scaffolds from bio-based natural materials for tissue regeneration applications: A review. Gels. 2023;**9**(2):100. DOI: 10.3390/gels9020100

[51] Nardini M, Perteghella S, Mastracci L, Grillo F, Marrubini G, Bari E, et al. Growth factors delivery system for skin regeneration: An advanced wound dressing. Pharmaceutics. 2020;**12**(2):120. DOI: 10.3390/pharmaceutics12020120

[52] Flores-Rojas GG, Gómez-Lazaro B, López-Saucedo F, Vera-Graziano R, Bucio E, Mendizábal E. Electrospun scaffolds for tissue engineering: A review. Macromolecules. 2023;**3**(3):524-553. DOI: 10.3390/macromol3030031

[53] Chaudhari AA, Vig K, Baganizi DR, Sahu R, Dixit S, Dennis V, et al. Future prospects for scaffolding methods and biomaterials in skin tissue engineering: A review. International Journal of

Molecular Sciences. 2016;**17**(12):1974. DOI: 10.3390/ijms17121974

[54] Zhang Z, Wang T, Chen S, Lv C, Zhuang X, Wang S. Fabrication of antibacterial Janus bandages with high wound healing performances by facile single-side electrospray PDMS coating. Surfaces and Interfaces. 2022;**34**:102392. DOI: 10.1016/j.surfin.2022.102392

[55] Jayarajan V, Auguste JO, Gene KA, Auguste L, Nunez C, Marcinowski B, et al. Bio-electrospraying 3-D Organotypic human skin cultures. Small. 2024;**20**(7):2304940. DOI: 10.1002/smll.202304940

[56] Azimi B, Ricci C, Fusco A, Zavagna L, Linari S, Donnarumma G, et al. Electrosprayed shrimp and mushroom Nanochitins on cellulose tissue for skin contact application. Molecules. 2021;**26**(14):4374. DOI: 10.3390/molecules26144374

[57] Luo Z, Cai L, Bian F, Zhao Y. Microfluidic electrospray of multifunctional Discal microparticles for wound healing. ACS Materials Letters. 2024;**6**(1):27-35. DOI: 10.1021/acsmaterialslett.3c01182

[58] Mazini L, Rochette L, Malka G. Adipose-derived stem cells (ADSCs) and growth differentiation factor 11 (GDF11): Regenerative and antiaging capacity for the skin. In: Choudhery MS, editor. Regenerative Medicine. Rijeka: IntechOpen; 2020. DOI: 10.5772/intechopen.91233

[59] Pérez-Díaz MA, Alvarado-Gómez E, Martínez-Pardo ME, José Yacamán M, Flores-Santos A, Sánchez-Sánchez R, et al. Development of Radiosterilized porcine skin Electrosprayed with silver nanoparticles prevents infections in deep burns. International Journal of Molecular Sciences. 2022;**23**(22):13910. DOI: 10.3390/ijms232213910

[60] Makkithaya KN, Nadumane S, Zhuo GY, Chakrabarty S, Mazumder N. Nanoparticle based collagen biomaterials for wound healing. In: Mazumder N, Chakrabarty S, editors. Collagen Biomaterials. Rijeka: IntechOpen; 2022. DOI: 10.5772/intechopen.104851

[61] Bacakova L, Zikmundova M, Pajorova J, Broz A, Filova E, Blanquer A, et al. Nanofibrous scaffolds for skin tissue engineering and wound healing based on synthetic polymers. In: Stoytcheva M, Zlatev R, editors. Applications of Nanobiotechnology. Rijeka: IntechOpen; 2019. DOI: 10.5772/intechopen.88744

[62] Liu S, Lin R, Pu C, Huang J, Zhang J, Hou H. Nanocomposite biomaterials for tissue engineering and regenerative medicine applications. In: Sharma A, editor. Nanocomposite Materials for Biomedical and Energy Storage Applications. Rijeka: IntechOpen; 2022. DOI: 10.5772/intechopen.102417

[63] Antezana PE, Municoy S, Álvarez-Echazú MI, Santo-Orihuela PL, Catalano PN, Al-Tel TH, et al. The 3D bioprinted scaffolds for wound healing. Pharmaceutics. 2022;**14**(2):464. DOI: 10.3390/pharmaceutics14020464

[64] Zsikó S, Csányi E, Kovács A, Budai-Szűcs M, Gácsi A, Berkó S. Methods to evaluate skin penetration In vitro. Scientia Pharmaceutica. 2019;**87**(3):19. DOI: 10.3390/scipharm87030019

[65] Singh V, Marimuthu T, Makatini MM, Choonara YE. Biopolymer-based wound dressings with biochemical cues for cell-instructive wound repair. Polymers

(Basel). 2022;**14**(24):5371. DOI: 10.3390/polym14245371

[66] Alven S, Aderibigbe BA. Chitosan and cellulose-based hydrogels for wound management. International Journal of Molecular Sciences. 2020;**21**(24):9656. DOI: 10.3390/ijms21249656

[67] Doberenz F, Zeng K, Willems C, Zhang K, Groth T. Thermoresponsive polymers and their biomedical application in tissue engineering – A review. Journal of Materials Chemistry B. 2020;**8**(4):607-628. DOI: 10.1039/C9TB02052G

[68] Di Marzio N, Eglin D, Serra T, Moroni L. Bio-fabrication: Convergence of 3D bioprinting and Nano-biomaterials in tissue engineering and regenerative medicine. Frontiers in Bioengineering and Biotechnology. 2020;**8**:326. DOI: 10.3389/fbioe.2020.00326

[69] Wu CA, Zhu Y, Woo YJ. Advances in 3D bioprinting: Techniques, applications, and future directions for cardiac tissue engineering. Bioengineering. 2023;**10**(7):842. DOI: 10.3390/bioengineering10070842

Chapter 4

# Exploring the Formation of Gum Arabic-Poly(Vinyl Alcohol)/Irgasan Nanocapsules through Coaxial Electrospraying

*Mehmet Dasdemir, Serap Gamze Serdar, Hatice Ibili and Bilgen Çeliktürk Kapar*

**Abstract**

Gum Arabic, known for its natural, biodegradable, and non-toxic attributes, holds significant promise in encapsulation. Despite the limited capacity of its natural form to create particles or fibers, this study aimed to produce nanocapsules through co-axial electrospraying, employing a solution of Gum Arabic/poly(vinyl alcohol) for the shell and Irgasan for the core. Additionally, process and solution parameters during co-axial electrospraying have been optimized. Solvent concentrations, total feed rates of shell/core solutions, needle tip to collector distance, electric field, and needle diameter have been studied in detail as a part of this optimization. Their effects on nanocapsule formation were observed through SEM images for morphological analyses and TEM images for observing capsule wall formation. The study thoroughly examines the properties of the resulting nanocapsules, reporting successful acquisition in the nano size range and monodispersity. This highlights the co-axial electrospraying method's potential for the nanoencapsulation of Gum Arabic and Irgasan.

**Keywords:** Gum Arabic, PVA, Irgasan, nanoencapsulation, co-axial electrospraying

## 1. Introduction

Gum Arabic (GA) has been widely utilized for its certain advantages such as wide availability, cost-effectiveness, and biocompatibility; furthermore, it offers exceptional encapsulation properties such as high efficiency, controlled release, and protection against compound degradation. The most common type is derived from *Acacia senegal*—Senegal trees [1]. GA is typically characterized as neutral or mildly acidic and primarily constitutes a branched complex polysaccharide structure, enriched with mineral combinations of potassium, magnesium, and calcium salts [2].

Currently, GA finds extensive application across the food and pharmaceutical sectors, especially in food processing to enhance texture and consistency. GA can

be employed in lower-calorie candies [3], serving as a coating agent [4, 5], pigment stabilizer [3, 6], emulsifier [3, 7], and texture enhancer [7] in various products such as candies, jellies, soups, and dessert mixes [1]. GA serves multiple roles in cosmetics, including stabilizing lotions and protective creams by enhancing viscosity and providing a smooth texture. It also acts as an adhesive in blushers and a foam stabilizer in liquid soaps. Furthermore, GA functions as a dispersant in paints ensuring uniform distribution of pigments and active ingredients. Within the textile sector, it serves as a thickening agent in printing pastes for dyeing cellulose fabrics [3]. Microcapsules are obtained by encapsulating very small liquid droplets, solid particles, or gases with a continuous film or polymer material [1]. Common encapsulation methods include spray drying [8, 9], electro-spraying [10], and coacervation [11].

There are numerous studies on microencapsulation, with GA being commonly used in most of them [12]. GA is typically used as an auxiliary material or viscosity enhancer in the electrospraying process [13]. In the study conducted by Stinjman et al. [14] a total of 17 natural polymers were divided into three groups to examine their electrospinning capabilities: polysaccharides obtained from microorganisms, polysaccharides obtained from algae, and polysaccharides obtained from plant cells and plant exudates. GA was included in the third group, and a 50% concentration was prepared and subjected to the electrospinning process. During the process, droplets were observed at the needle tip, but electrospinning could not be achieved. In another study, researchers aimed to explore the impact of combining synthetic and natural biopolymers with GA on the encapsulation of probiotics. The utilization of GA enhanced the viability of cells within the electrosprayed capsules [15]. GA was also used as a nanocomposite scaffold and demonstrated promising potential [16]. It contains low protein content in its structure, is one of the most important hydrocolloids with a high solubility in water, and solutions can be prepared up to concentrations of 50–55%. This property of GA stems from its highly branched and complex structure [17].

GA is water-soluble (except below pH 3.0), exhibits compatibility with solids, and demonstrates low viscosity in Newtonian fluid foods at below 40% concentrations [17]. Because of being water soluble, GA treated products have low washing stability which makes these products disposable. While insoluble in fats and many organic solvents, GA can dissolve in aqueous ethanol solutions. Additionally, it has limited solubility in glycerol, ethylene glycol, acetate esters, and acetate-alcohol mixtures [17]. GA has been widely utilized in encapsulating due to its numerous benefits, including its widespread availability, affordability, non-toxic nature, and encapsulation properties such as high efficiency, controlled release. Despite its advantages, the biodegradability of GA may vary based on processing conditions and cross-linking agents used. Combining GA with other polymers is suggested to enhance the mechanical properties of microcapsules [1]. Several studies have been conducted on GA utilization for different purposes. Recent studies such as those by Zaeim et al. [10] and Koh et al. [18] reported on the significance, optimization potential, and positive impact of GA on the stability and viability of microencapsulated probiotics.

Polyvinyl alcohol (PVA) stands as a significant polymer, holding pivotal importance within the biodegradable counterparts PVA, a synthetic polymer, is non-toxic and biodegradable, possessing superb compatibility and film-forming capabilities [19]. It is commonly employed as a stabilizing agent in the fabrication of nanoparticles, facilitating the production of small, homogeneous particles [20]. In a study, nanocapsules were synthesized using the emulsion diffusion technique,

wherein PVA served as a stabilizer in conjunction with polylactic acid (PLA) in the external phase and plasmid DNA in the internal phase [19]. Ganti et al. [21] employed PVA as a cryoprotectant material during the manufacture of nanocapsules. Similarly, in the study conducted by Yadav et al. [22] nanocapsules were prepared via the salting-out method, with PVA utilized once again as a stabilizing agent.

Biodegradable polymers widely utilized in various applications, Irgasan is one of the potent agents known for its broad-spectrum effectiveness. It is a non-ionic, broad-spectrum antimicrobial agent known as Triclosan, demonstrates rapid and lasting antibacterial efficacy, and is extensively utilized across various personal care products such as toothpaste, deodorant, soaps, detergents, dish washing liquid, cosmetics [23–25]. Subsequently, Irgasan has been used for skincare applications and oral care products. Irgasan serves to inhibit bacterial growth on various plastic items used in households, bathroom accessories, flooring materials, and toys [24, 25]. Several studies have reported on Irgasan utilization for antibacterial applications such as coating plasma-modified polyethylene [26] and active antibacterial medical-grade polyvinyl chloride for medical purposes [23], using in drug delivery applications for increasing the efficiency [27].

The objective of this study was to produce GA/PVA-based nanocapsules using the coaxial electrospraying technique, with GA/PVA solution serving as the shell and Irgasan (triclosan) solution as the core. The investigation centered on analyzing the impact of process parameters such as flow rate, needle size, working distance, and voltage on the formation of nanocapsules. Additionally, the effects of solvent concentration of Irgasan core solution on nanocapsule formation were explored. The properties of the nanocapsules were assessed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

## 2. Materials and methods

### 2.1 Materials

Irgasan (72,779 ≥ 97%), GA, and ethanol (CAS No:64-17-5) were all purchased from Sigma Aldrich.

25/75 v/v% GA/distilled water solutions were prepared at 50°C. Poly(vinyl alcohol) (PVA) solutions were prepared weight/volume concentration (wt/vol%) as 2 wt/vol% at 40–80°C. For electrospraying, 1:1 GA/PVA solution was prepared.

Irgasan solutions were prepared weight/volume concentration (wt/vol%) with ethanol solvent. Three different solvent concentration (25%, 50%, and 100% ethanol) were prepared using distilled water. Irgasan solution was prepared at 1 wt/vol% concentration.

### 2.2 Methods

GA/PVA solution is fed from the outer needle tip while Irgasan solution is fed from inner needle tip through dual syringe pumps via micro pumps (New Era NE-1000X). The coaxial needle tip is connected to the high DC voltage (Gamma High Voltage Series ES100P). The samples were collected on an aluminum foil covering the grounded plate. In each experiment, GA/PVA and Irgasan solutions at each mixing ratio were subjected to a 30-minute electrospraying process. Electrospraying parameters are presented in **Table 1**. Temperature and humidity were measured as 20–28°C and 20–25%, respectively.

| Solvent concentration (% wt/vol) | Working distance (cm) | Electric field (kV cm$^{-1}$) | Flow rate (µl min$^{-1}$) | Needle size (Gauge) |
|---|---|---|---|---|
| 25/75%; 50/50% water/ethanol; 100% ethanol | 15 | 10 | 11/9 | 22–26 |
| 50/50% water/ethanol | 5–20 (5*) | 10 | 11/9 | 22–26 |
| 50/50% water/ethanol | 15 | 5–20 (5*) | 11/9 | 22–26 |
| 50/50% water/ethanol | 15 | 10 | 5/15; 11/9; 10/10; 15/5 | 22–26 |
| 50/50% water/ethanol | 15 | 10 | 11/9 | 21–22; 21–24; 21–26; 22–26 |
| 50/50% water/ethanol | 15 | 10 | 11/9 | 22–26 |

*Increment.

**Table 1.**
*Investigated material and process parameters (temp. 20–28°C, hum. 20–25%, time 30 min).*

The surface morphology of electrosprayed GA/PVA nanocapsules is examined using a scanning electron microscope (SEM) (JEOL JSM-6390). The SEM images were captured at magnifications ranging from 1000 to 10,000 at an acceleration voltage of 5–20 kV. Transmission electron microscope (TEM) (JEOL JEM 100C) was used for high-resolution imaging to determine nanocapsule formation. Nanocapsules produced by electrospraying were collected onto copper grids placed on the upper surface of the foil during application. Samples on these grids were characterized using TEM.

## 3. Results and discussion

### 3.1 Effects of total flow rate on nanocapsulation formation

Flow rates of solutions frequently serve as critical parameters for regulating the formation of structures through the electrospray method [28]. Coaxial electrospraying was conducted at different total flow rates, including 5 µl min$^{-1}$, 10 µl min$^{-1}$, 20 µl min$^{-1}$, and 40 µl min$^{-1}$. Electrospraying process was conducted at fixed applied voltage and distance as presented in **Table 1**. The ratio of needle tip area of the core and the shell was calculated as 55/45%. This ratio was used to arrange the flow rates of the core and the shell. According to this arrangement, the flow rates of the core and the shell are given in **Table 2**.

The pictures of the samples are presented in **Figure 1**. Dripping was observed in all samples across all flow rates. Upon comparing the effects of flow rate, an increase in the

| Total flow rate | 5 µl min$^{-1}$ | 10 µl min$^{-1}$ | 20 µl min$^{-1}$ | 40 µl min$^{-1}$ |
|---|---|---|---|---|
| Core flow rate | 2.75 µl min$^{-1}$ | 5.5 µl min$^{-1}$ | 11 µl min$^{-1}$ | 22 µl min$^{-1}$ |
| Shell flow rate | 2.25 µl min$^{-1}$ | 4.5 µl min$^{-1}$ | 9 µl min$^{-1}$ | 18 µl min$^{-1}$ |

**Table 2.**
*Flow rate ratios of core and shell solutions.*

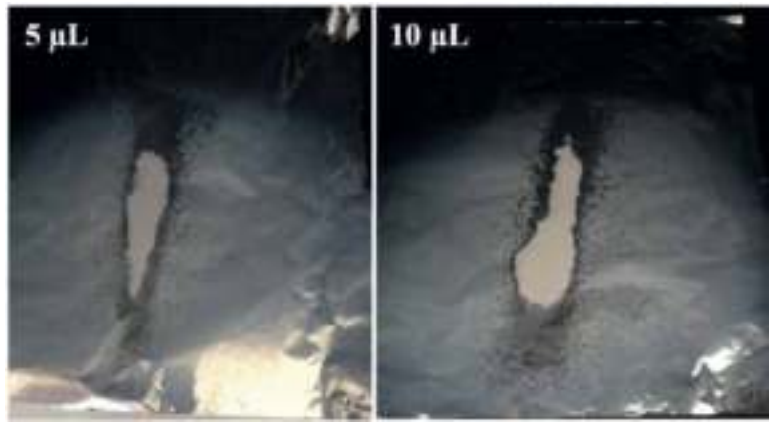


**Figure 1.**
*Images of samples.*

flow rate of the shell solution corresponded to an increase in dripping. Particularly, at total flow rates of 20 and 40 µl $min^{-1}$, dripping was much more intense.

SEM images were captured to observe the formation of nanocapsule (**Figure 2**). Electrospraying was observed at all flow rates. However, at flow rates of 5 µl $min^{-1}$ and 10 µl $min^{-1}$, film formation occurred, obscuring the nanocapsules underneath. At flow rates of 20 µl $min^{-1}$ and 40 µl $min^{-1}$, film formation also occurred, but the capsules were more clearly visible. A monodisperse distribution was observed at 20 µl $min^{-1}$ and 40 µl $min^{-1}$, indicating successful encapsulation on a nanoscale, with no fibril formation.

Due to issues with dripping and film formation, the coaxial electrospraying setup was isolated, and the electrospraying process was repeated using the same total flow rates. In the isolated setup, in all samples (at all flow rates), dripping was observed. However, this dripping was less than previous samples.

Isolated environment provided electrospray with much less dripping. In the isolated setup, dripping was observed in all samples at all flow rates, though to a lesser extent compared to previous samples. The isolated environment resulted in significantly reduced dripping during the electrospray process (**Figure 3**).

SEM images confirmed the formation of nanospheres with a monodisperse distribution, and no fibril formation was observed, indicating successful encapsulation on a nanoscale (**Figure 4**). The quantity of nanocapsules increased with the rising flow rate, while the particle size enlarged between 5 and 10 µL $min^{-1}$. Several studies reported that higher flow rates produced larger particles [29–32]. Increased flow rates led to the ejection of more solution, leading to the formation of larger particles, whereas lower rates produced small, highly charged droplets, resulting in small particles through Coulomb fission [31]. At higher flow rates, the particle size remained similar. Based on the SEM analysis, a total flow rate of 20 µL $min^{-1}$ was chosen considering the quantity and morphology of the nanocapsules.

### 3.2 Effects of solvent concentration

The effects of solvent concentration on electrospray were investigated. 25/75% water/ethanol, 50/50 water/ethanol, and 100% ethanol solvents were used to solve Irgasan. Electrospraying was observed in all solvent concentration. SEM images were captured to ensure nanocapsule formation through electrospraying (**Figure 5**).

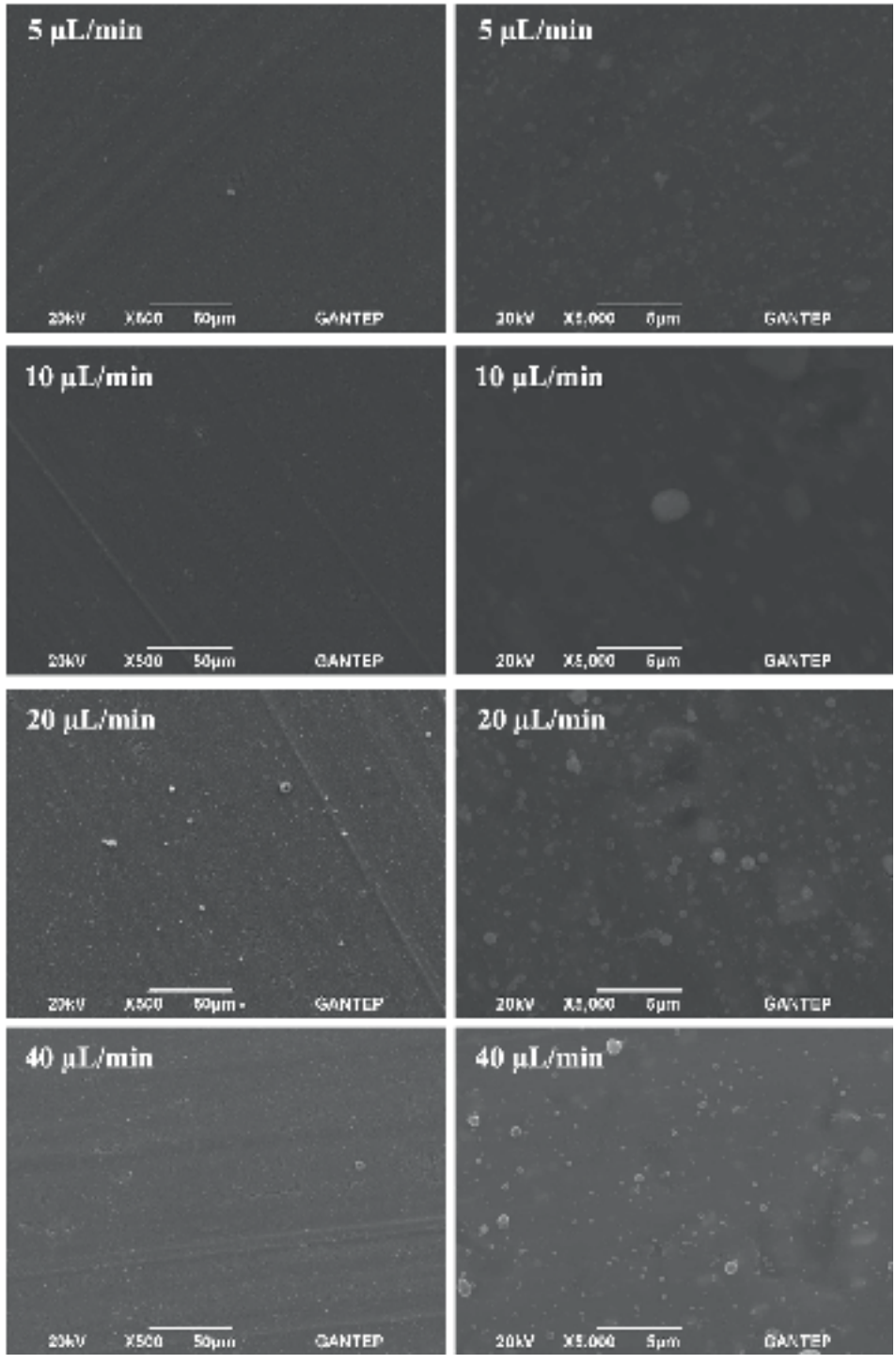


**Figure 2.**
*SEM images of samples electrosprayed at different total flow rate.*

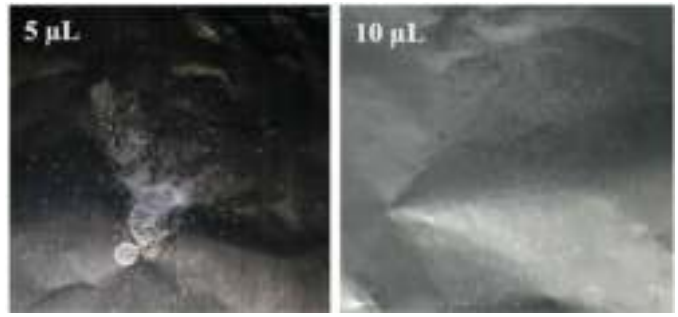


**Figure 3.**
*Images of samples in isolated environment.*

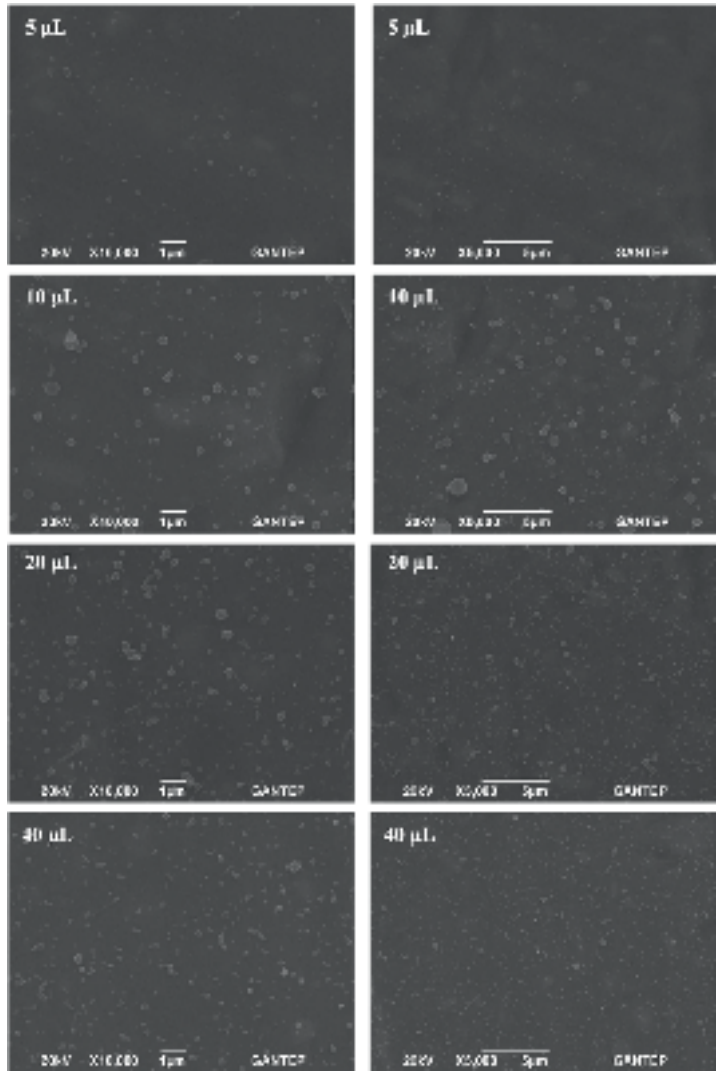


**Figure 4.**
*SEM images of samples electrosprayed at different total flow rate in isolated environment.*

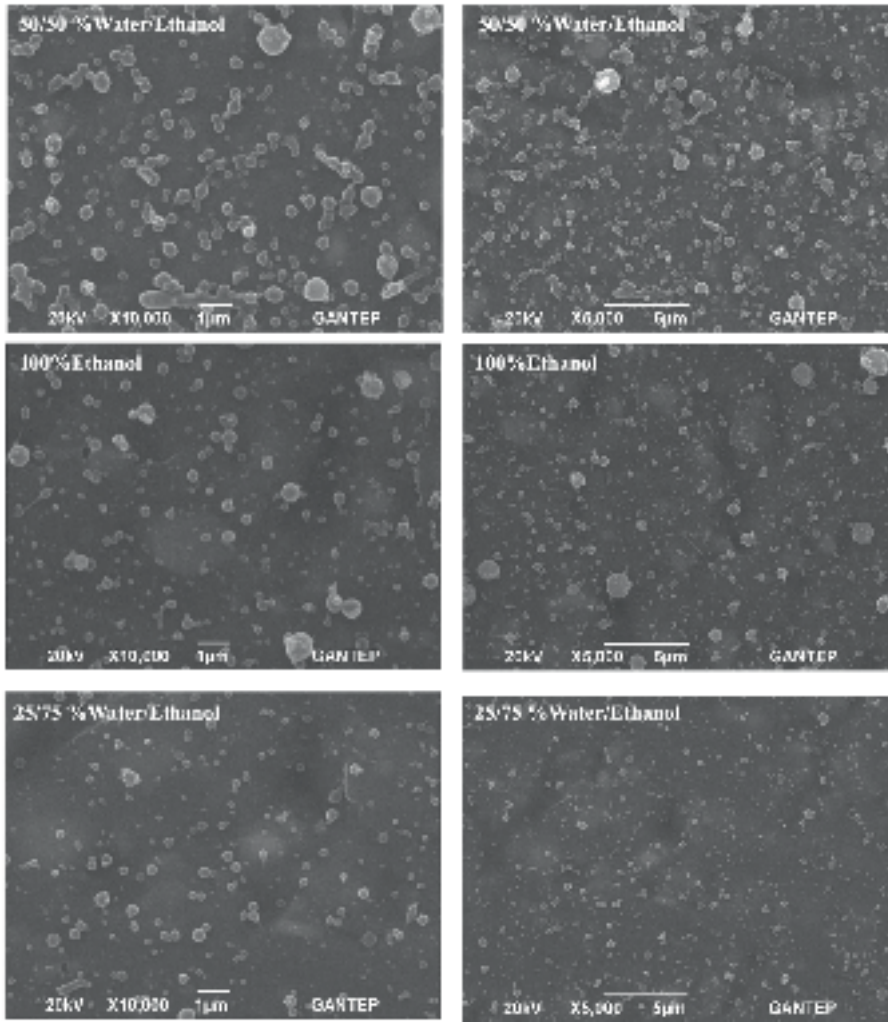


**Figure 5.**
*SEM images of samples electrosprayed at different solvent concentration.*

The nanocapsules displayed a spherical shape with uniform distribution and no fibril formation across all solvent concentrations. The solvent evaporation in electrospraying plays a key role for achieving particles that are monodispersed and uniform in size [33]. At 25/75% water/ethanol and 100% ethanol solvent concentrations, the size of nanocapsules was reduced; however, the number of nanocapsules was decreased too. Employing 100% ethanol solvent resulted in the presence of nanocapsules of various sizes. At 50/50% water/ethanol solvent, nanocapsules were slightly larger, yet displayed the most uniform distribution, with the highest number observed. Therefore, subsequent applications were conducted using a 50/50% water/ethanol solvent.

### 3.3 Effects of working distance

The effect of distance between the needle and the collector on electrospraying was investigated. Electrospraying was conducted at four different distances as

5 cm, 10 cm, 15 cm, and 20 cm to determine the optimum distance between the needle and the collector. 50/50% water/ethanol solvent was used. The total flow rate was set to 20 μl $min^{-1}$. SEM images of electrosprayed surfaces were captured to ensure nanocapsule formation (**Figure 6**). Nanocapsules were in form of sphere

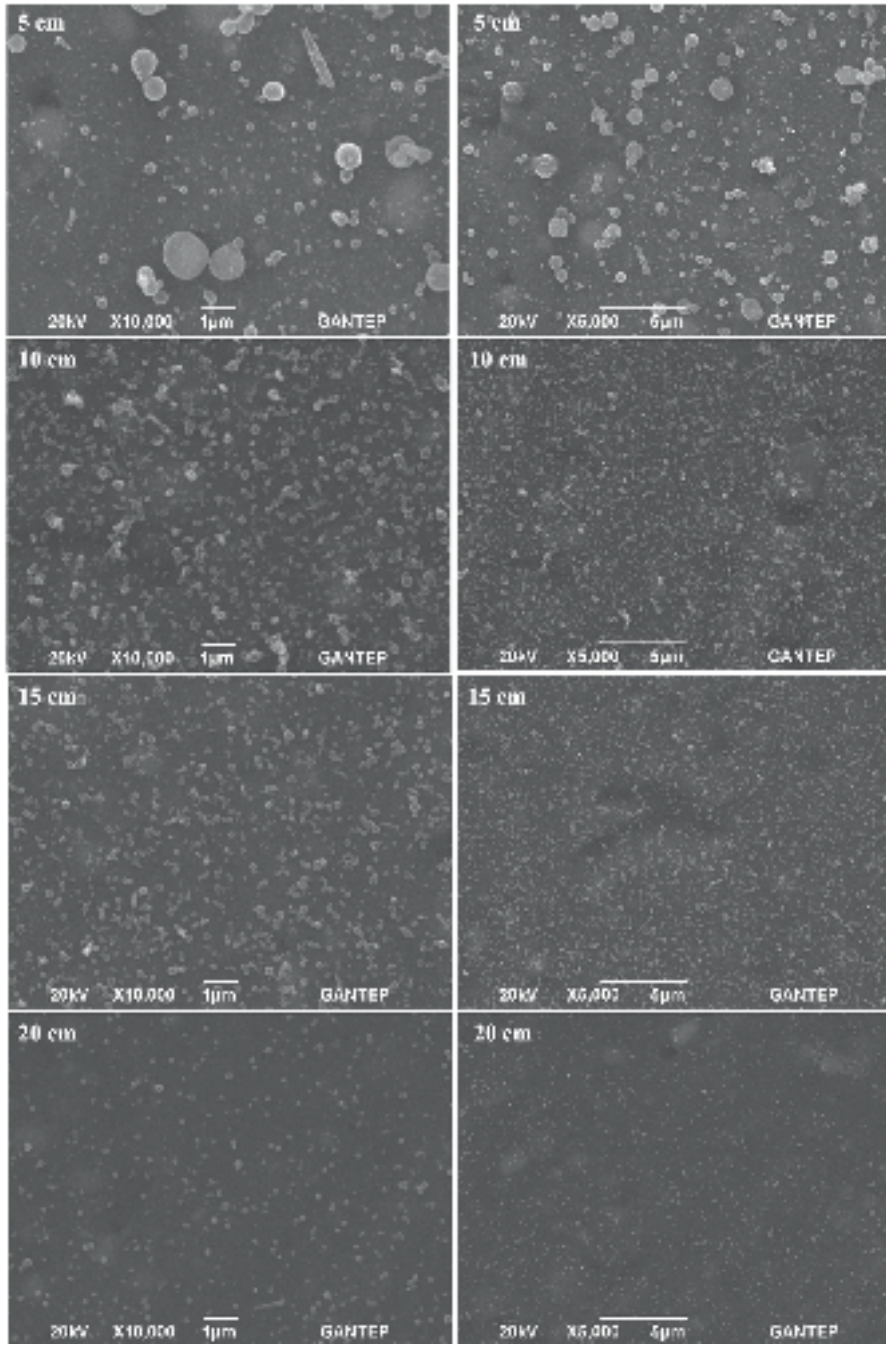


**Figure 6.**
*SEM images of samples electrosprayed at various distance.*

and had monodisperse distribution without fibril formation. Encapsulation is achieved on a nanoscale.

SEM images in **Figure 6** revealed that with increasing distance, the distribution of capsules became more uniform. At a distance of 5 cm, a wide size distribution was observed. At short distances, the solvent did not fully evaporate before reaching the collector, leading to an increase in particle size [34, 35]. The size has also decreased proportionally, but the capsules at a distance of 20 cm are so small that they cannot even be observed with SEM images properly. Also at 20 cm, the number of nanocapsules was reduced. Although increasing the distance allows for longer evaporation time and enhances cone-jet stability, it also results in a decrease in the electric field [29]. Determining the optimal distance is crucial for electrospraying. **Figure 6** shows that nanocapsules formed at a distance of 15 cm exhibited uniform distribution

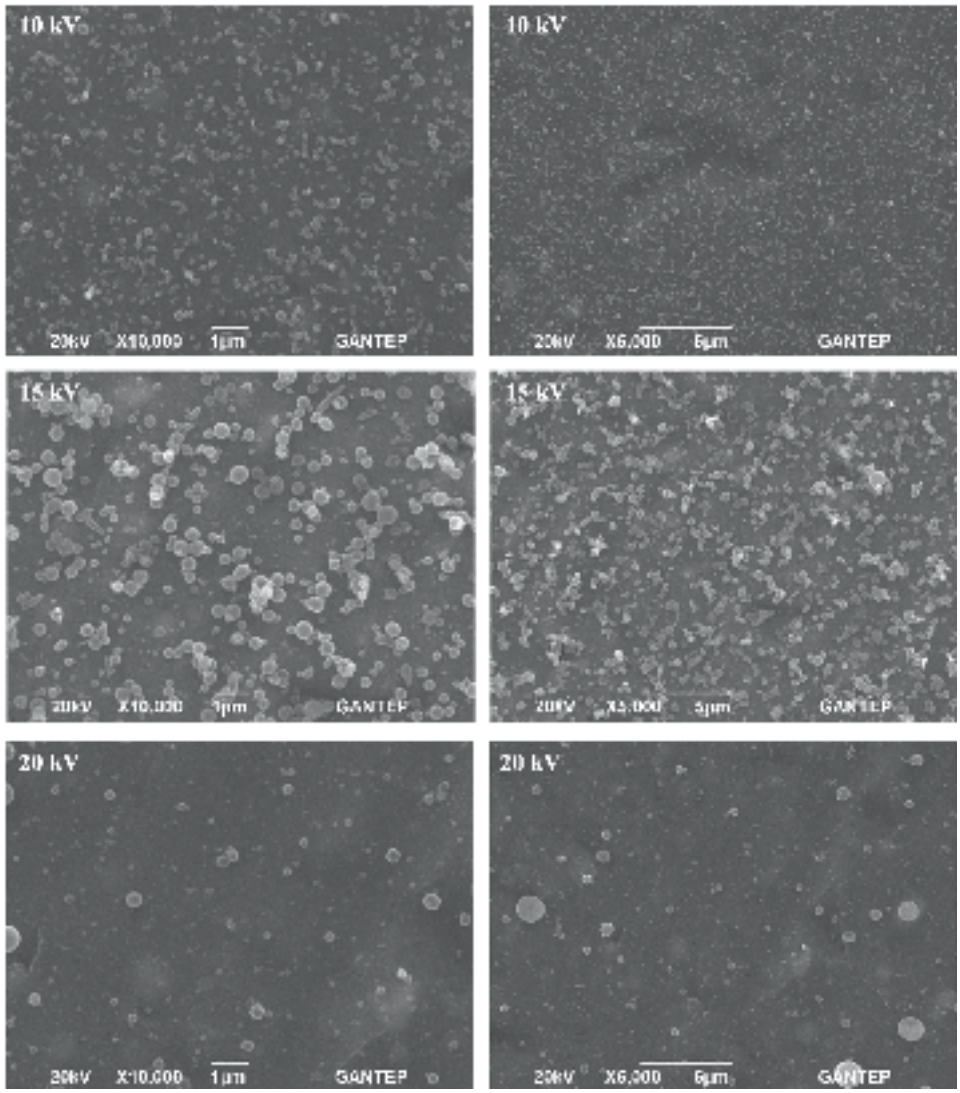


**Figure 7.**
*SEM images of samples electrosprayed at various voltage.*

and smaller diameter compared to those at 5 and 10 cm. This is because as the distance increases, drying occurs more effectively. Also at 15 cm, the number of nanocapsules was higher compared to those at 20 cm. Therefore, the distance of 15 cm was selected for optimum process parameter.

### 3.4 Effects of electric field

The effect of electric field on electrospray process was investigated. At this stage, electrospray was conducted at four different voltages as 5 kV, 10 kV, 15 kV, and 20 kV. Other process parameters were set as 20 μl $min^{-1}$ total flow rate, 15 cm distance, 50/50% water/ethanol solvent. SEM images of electrosprayed surfaces were captured to ensure nanocapsule formation (**Figure 7**). Electrospraying was not achieved at all voltages. At 5 kV voltage, only dripping occurred. However, electrospraying was occurred at 10 kV, 15 kV, and 20 kV. Nanocapsules were in form of sphere and had monodisperse distribution without fibril formation. Encapsulation is achieved on a nanoscale.

Voltage was known as a fundamental parameter for electrospraying process [33]. Lowering the voltage could result in a reduced electrical field, potentially leading to the formation of non-spherical droplets. The uniform shape was related to higher voltage [32, 33]. According to the SEM images, nanocapsules formed at a voltage of 10 kV were smallest in diameter. At higher voltage the diameter of nanocapsules was getting larger. At 20 kV voltage, the number of the nanocapsules was reduced significantly. Therefore, a voltage of 10 kV is selected as the optimum voltage.

### 3.5 Effects of flow rate

The effect of flow rate of shell and core solutions on electrospraying was investigated. Total flow rate was kept constant at 20 μl $min^{-1}$. Coaxial electrospraying of GA/PVA as shell and 1% Irgasan solution as core was conducted at four different core/shell solution flow rate as 25/75% (5–15 μl $min^{-1}$) core/shell, 50/50% (10–10 μl $min^{-1}$) core/shell, 75/25% (15–5 μl $min^{-1}$) core/shell, and 55/45% (11–9 μl $min^{-1}$) core/shell.

SEM and TEM images of electrosprayed surfaces were captured to ensure nanocapsule formation (**Figures 8** and **9**).

SEM images have shown that electrospraying was achieved with no fibrillation at all flow rate ratios (**Figure 8**). A distinctive core/shell structure was observed at all flow rates. The lower shell solution flow rate resulted in the failure of the shell material to cover the core material, whereas higher shell solution flow rates facilitated capsule formation [36]. Xie et al. studied on encapsulation of protein inside PLGA (poly(DL-lactide-co-glycolide)) microparticles by coaxial electrospray and found that when the flow rate of the shell solution was significantly higher than that of the core solution, the distribution of droplet diameters tended to be more uniform [32]. Yet, the most uniform distribution was achieved at the flow rate 11–9 μL $min^{-1}$ core/shell ratio in this study. At 15–5 and 5–15 μL $min^{-1}$ core/shell ratios, the size of the nanocapsules was seen smaller in diameter. However, TEM images of electrosprayed surfaces proved that at the 11–9 μL $min^{-1}$ core/shell ratio, this flow rate gave the best morphology (**Figure 9**). Therefore, the optimum flow rate was determined as 11 μL $min^{-1}$ for 1% Irgasan core and 9 μL $min^{-1}$ for GA/PVA shell. In TEM images, the inner and outer phases of the formed capsules can be clearly observed (**Figure 9**).

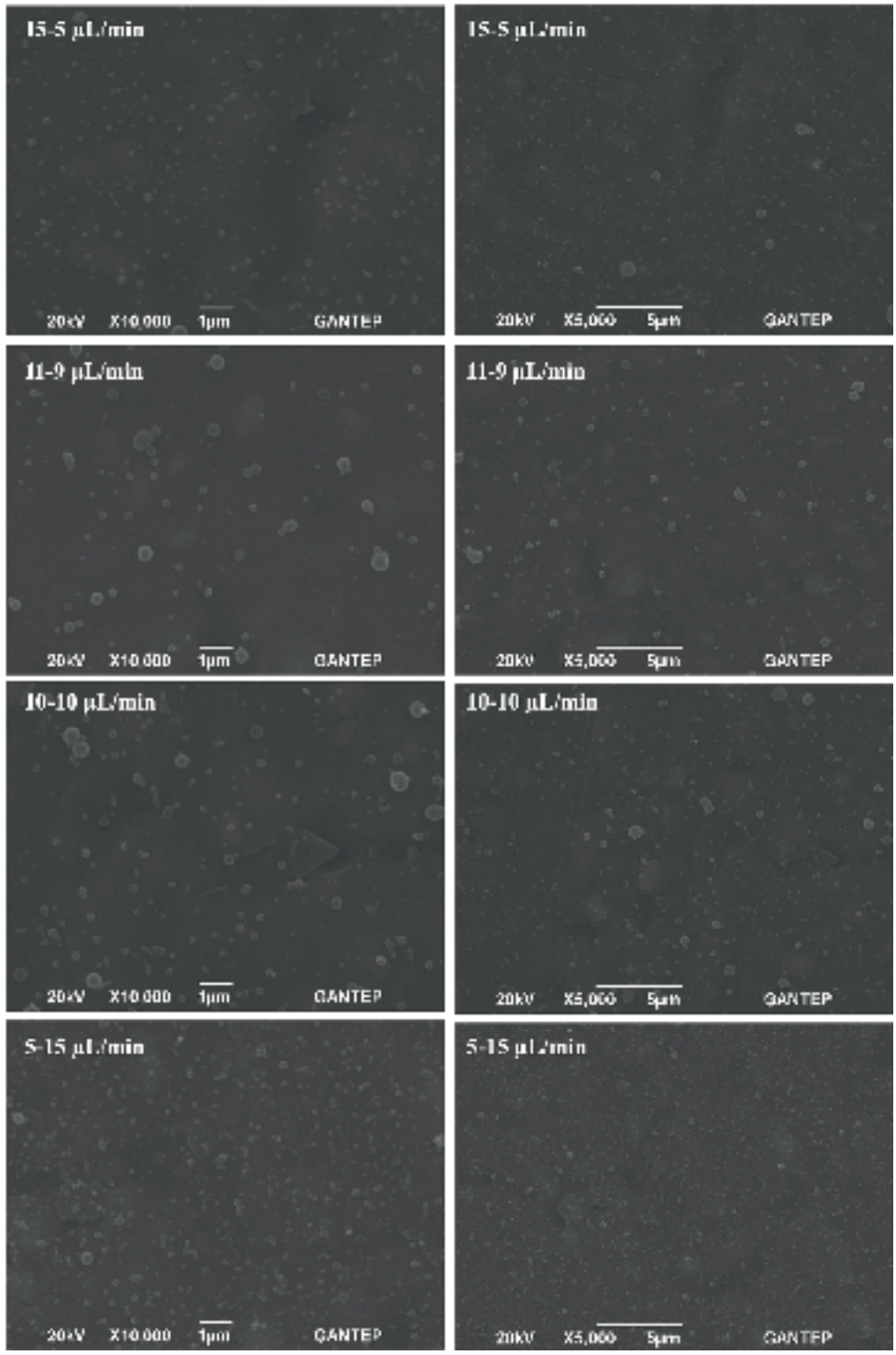


**Figure 8.**
*SEM images of samples electrosprayed at various flow rate.*

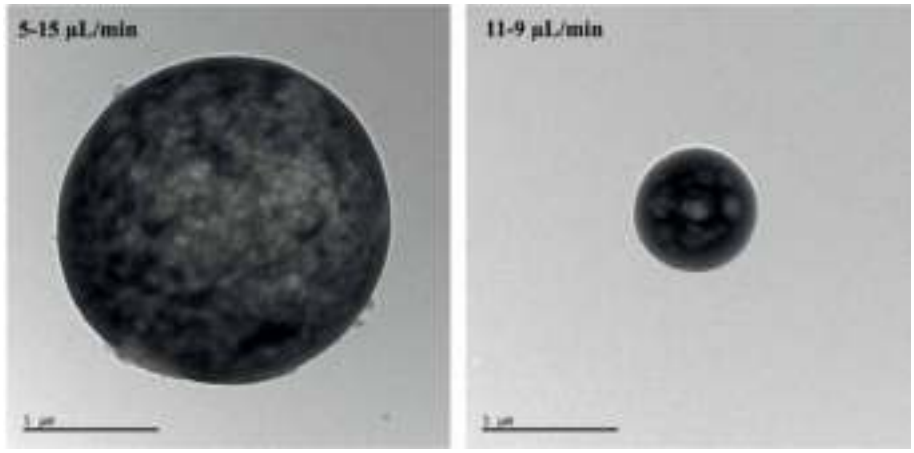


**Figure 9.**
*TEM images of samples electrosprayed at 5–15 and 11–9 µL min$^{-1}$ core/shell ratio flow rate.*

### 3.6 Effects of needle size

The needle size may influence the electrospraying process, particle size and distribution [35]. The effect of needle size on electrospraying was investigated. Four different needle size combinations for core/shell construction were used for coaxial electrospraying. By this way, flow rate ratios of core/shell solutions were changed, and its effect on electrospraying and nanocapsule formation were investigated. 21, 24, and 26 G needle utilization implied a decrease in core flow rate.

SEM images of coaxial electrosprayed surfaces were captured to ensure nanocapsule formation (**Figure 10**). Electrospraying was achieved with all needle size combinations. Nanocapsules were in form of sphere with no fibril formation.

Some studies found no significant relation between the needle size and particle size [37, 38]. Larger particles may be produced by utilizing larger needle due to higher flow rate [26]. The effect of the needle size was found more significant especially at lower flow rates [28]. At the constant flow rate, with 21–24 G and 21–26 G needle combinations, smaller core needle caused decrease in the size of the nanocapsules compared to that of 21–22 G needle combination. Also, the number of the nanocapsules was higher (**Figure 10**). 21–26 G needle combination resulted to form larger and less nanocapsules. TEM images of coaxial electrosprayed surfaces were captured to ensure nanocapsule formation (**Figure 11**). In TEM images, the inner and outer phases of the formed capsules can be clearly observed. With 22–26 G needle combination, the nanocapsules were formed at more uniform distribution.

## 4. Conclusion

This study investigated the formation of nanocapsules through the coaxial electrospraying method employing GA/PVA solutions for the shell and Irgasan solution for the core. The effects of ethanol solvent concentration, flow rate and total flow rate, needle size, working distance, and electric field were analyzed to see compatibility these parameters on the electrospraying process. Following the electrospraying

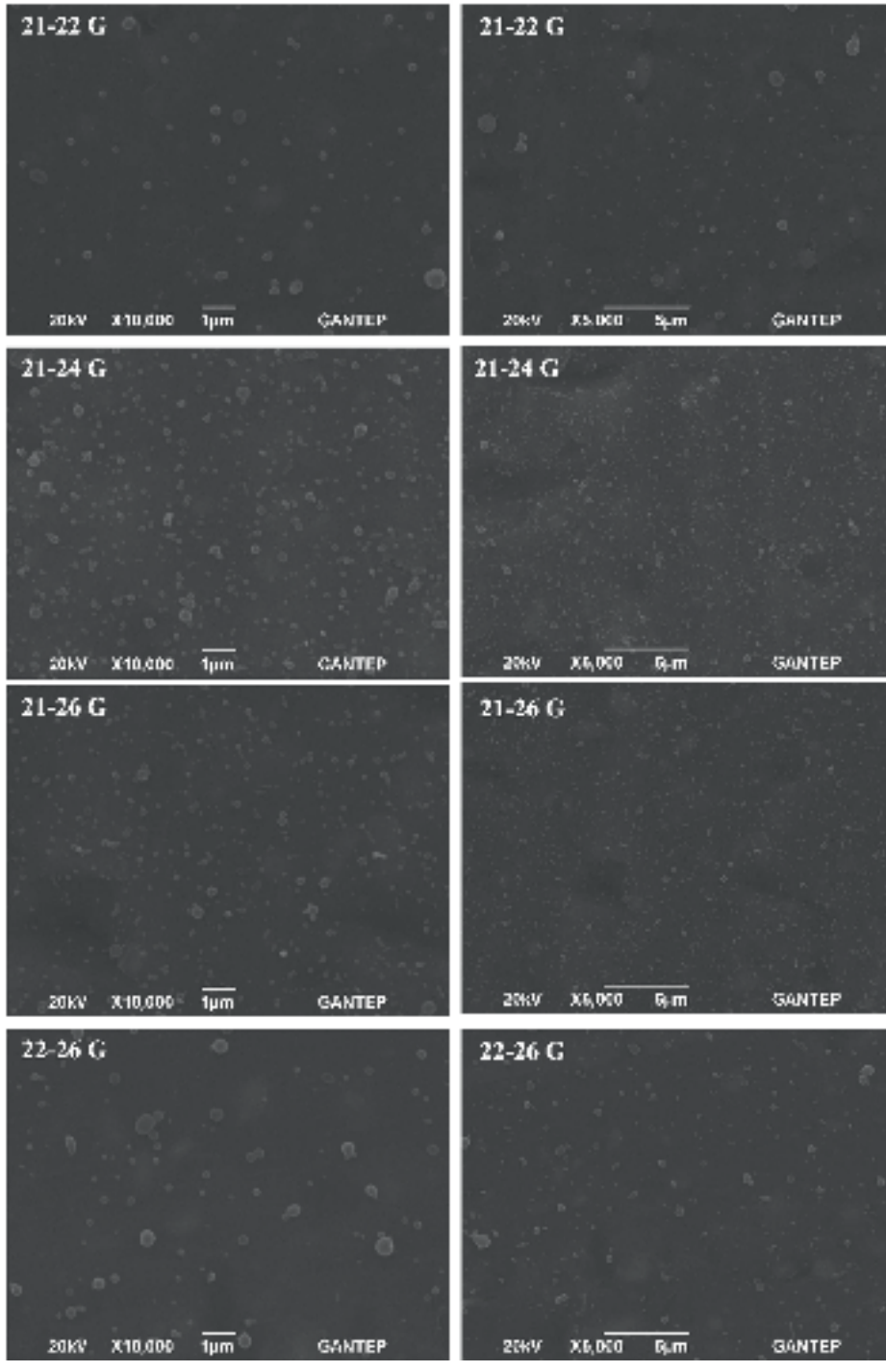


**Figure 10.**
*SEM images of samples electrosprayed with various needle sizes.*

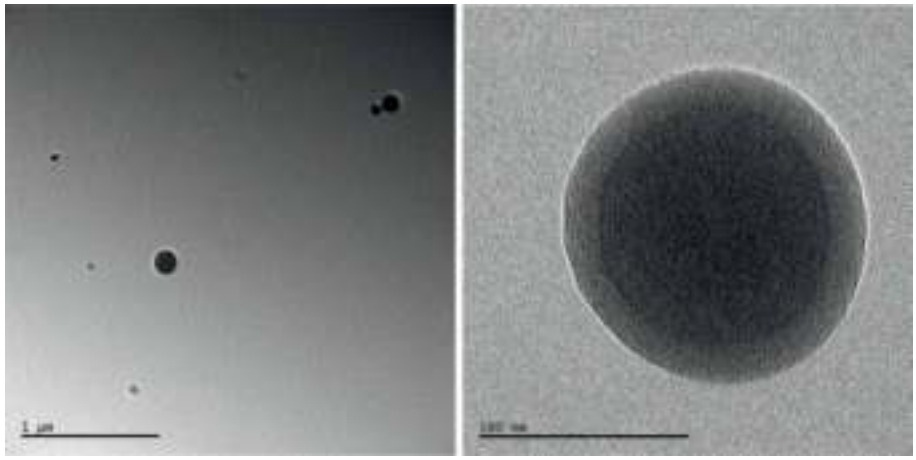

**Figure 11.**
*TEM images of samples electrosprayed with 21–26 G needle size combination.*

process, SEM and TEM images were utilized to discuss the size, morphology, and distribution of produced particles. Our findings revealed that the solvent concentration influenced the nanocapsule size and the distribution. At 50/50% water/ethanol solvent, nanocapsules were displayed the most uniform distribution, with the highest number observed. Furthermore, working distance and electric field play crucial roles in determining nanocapsule size and morphology. The electrostatic field affected the balance between coulombic forces and flight time, resulting in smaller particles at higher values up to a threshold point. Our findings also indicate that electrospraying distance has similar working mechanism and optimum working distance was found as 15 cm. The other significant outcome of our study was lower flow rate of the shell solution led to inadequate coverage of the core material by the shell material. As a result of this study, desired nanocapsules comprising GA/PVA shell and Irgasan core were achieved. Optimum electrospraying parameters and concentration rates were found to be key for achieving a well-formed shell-core structure from GA and Irgasan. These findings suggest that the electrospraying process of the GA/PVA-Irgasan nanocapsules can be a valuable reference for future encapsulation applications.

## Acknowledgements

The authors are thankful to The Scientific and Technological Research Council of Turkey (MAG-113M517) for funding this work.

## Conflict of interest

The authors declare no conflict of interest.

## Author details

Mehmet Dasdemir[1], Serap Gamze Serdar[2*], Hatice Ibili[2] and Bilgen Çeliktürk Kapar[2]

1 The Nonwovens Institute, North Carolina State University, Raleigh, NC, USA

2 Department of Textile Engineering, Gaziantep University, Gaziantep, Turkey

*Address all correspondence to: sgserdar@gantep.edu.tr

IntechOpen

## References

[1] Al-Hamayda A, Abu-Jdayil B, Ayyash M, Tannous J. Advances in microencapsulation techniques using Arabic gum: A comprehensive review. Industrial Crops and Products. 2023;**205**:117556

[2] Masuelli MA. Hydrodynamic properties of whole arabic gum. American Journal of Food Science and Technology (Science and Education Publishing). 2013;**1**(3):60-66

[3] Dauqan. Utilization of gum Arabic for industries and human health. American Journal of Applied Sciences. 2013;**10**(10):1270-1279

[4] Esmaeili F, Hashemiravan M, Eshaghi MR, Gandomi H. Encapsulation of *Arctium lappa* L. root extracts by spray-drying and freeze-drying using maltodextrin and gum Arabic as coating agents and it's application in synbiotic orange-carrot juice. Journal of Food Measurement and Characterization. 2022;**16**(4):2908-2921

[5] Tiamiyu QO, Adebayo SE, Yusuf AA. Gum Arabic edible coating and its application in preservation of fresh fruits and vegetables: A review. Food Chemistry Advances. 2023;**2**:100251

[6] Mariod AA, editor. Gum Arabic: Structure, Properties, Application and Economics. London, United Kingdom: Academic Press; 2018

[7] Sulieman AME-H. Gum Arabic as thickener and stabilizing agents in dairy products. In: Gum Arabic. London, United Kingdom: Academic Press; 2018. pp. 151-165. DOI: 10.1016/B978-0-12-812002-6.00013-0

[8] Tonon RV, Grosso CRF, Hubinger MD. Microencapsulation of flaxseed oil by spray drying: Influence of process conditions and emulsion properties. In: 11th International Congress on Engineering and Food, Athens, Greece. 2011. pp. 1-6

[9] Tirgar M, Jinap S, Zaidul ISM, Mirhosseini H. Suitable coating material for microencapsulation of spray-dried fish oil. Journal of Food Science and Technology. 2015;**52**(7):4441-4449

[10] Zaeim D, Sarabi-Jamab M, Ghorani B, Kadkhodaee R, Tromp RH. Electrospray-assisted drying of live probiotics in acacia gum microparticles matrix. Carbohydrate Polymers. 2018;**183**:183-191

[11] Jun-xia X, Hai-yan Y, Jian Y. Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. Food Chemistry. 2011;**125**(4):1267-1272

[12] Sri SJ, Seethadevi A, Prabha KS, Muthuprasanna P, Pavitra P. Microencapsulation: A review. International Journal of Pharmaceutical and Biological Science. 2012;**3**(1):509-531

[13] Kuo TY, Jhang CF, Lin CM, Hsien TY, Hsieh HJ. Fabrication and application of coaxial polyvinyl alcohol/chitosan nanofiber membranes. Open Physics. 2017;**15**(1):1004-1014

[14] Stijnman AC, Bodnar I, Hans TR. Electrospinning of food-grade polysaccharides. Food Hydrocolloids. 2011;**25**(5):1393-1398

[15] Ma J, Li T, Wang Q, Xu C, Yu W, Yu H, et al. Enhanced viability of probiotics encapsulated within synthetic/natural biopolymers by the addition of gum Arabic via electrohydrodynamic

processing. Food Chemistry. 2023;**413**:135680

[16] Pedram Rad Z, Mokhtari J, Abbasi M. Fabrication and characterization of PCL/zein/gum Arabic electrospun nanocomposite scaffold for skin tissue engineering. Materials Science and Engineering: C. 2018;**93**:356-366

[17] Basal G, Karagonlu S. Preparation of antimicrobial agent loaded microcapsules for medical textiles. Pamukkale University Journal of Engineering Science. 2013;**19**(4):174-178

[18] Koh WY, Lim XX, Tan TC, Kobun R, Rasti B. Encapsulated probiotics: Potential techniques and coating materials for non-dairy food applications. Applied Sciences. 2022;**12**(19):10005

[19] Liu Y, Wang S, Lan W. Fabrication of antibacterial chitosan-PVA blended film using electrospray technique for food packaging applications. International Journal of Biological Macromolecules. 2018;**107**:848-854

[20] Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations☆. Advanced Drug Delivery Reviews. 2006;**58**(15):1688-1713

[21] Ganti V, Mengesha A, Marszalek J, Youan BB. Statistical analysis of low molecular mass heparin nanoencapsulation. Acta Pharmaceutica. 2010;**60**(3):281-293

[22] Nagavarma BV, Yadav HK, Ayaz AVLS, Vasudha LS, Shivakumar HG. Different techniques for preparation of polymeric nanoparticles: A review. Asian Journal of Pharma and Clinical Research;**5**(3):16-23

[23] Asadinezhad A, Novák I, Lehocký M, Sedlařík V, Vesel A, Junkar I, et al. A physicochemical approach to render antibacterial surfaces on plasma-treated medical-grade PVC: Irgasan coating. Plasma Processes and Polymers. 2010;**7**(6):504-514

[24] Wong-Wah-Chung P, Rafqah S, Voyard G, Sarakha M. Photochemical behaviour of triclosan in aqueous solutions: Kinetic and analytical studies. Journal of Photochemical and Photobiological Chemistry. 2007;**191**(2-3):201-208

[25] Lu J, Hill MA, Hood M, Greeson DF, Horton JR, Orndorff PE, et al. Formation of antibiotic, biodegradable polymers by processing with Irgasan DP300R (triclosan) and its inclusion compound with β-cyclodextrin. Journal of Applied Polymer Science. 2001;**82**(2):300-309

[26] Zhang W, Chu PK, Ji J, Zhang Y, Fu RKY, Yan Q. Antibacterial properties of plasma-modified and triclosan or bronopol coated polyethylene. Polymer. 2006;**47**(3):931-936

[27] Igor M. An increased efficiency of Triclosan delivery by novel acrylate-based nanoparticles. Journal of Nanomedicine and Nanotechnology. 2013;**04**(04):1-8. Available from: https://www.omicsonline.org/an-increased-efficiency-of-triclosan-delivery-by-novel-acrylate-based-nanoparticles-2157-7439.S11-001.php?aid=6070

[28] Chang MW, Stride E, Edirisinghe M. Controlling the thickness of hollow polymeric microspheres prepared by electrohydrodynamic atomization. Journal of Royal Society Interface. 2010;**7**(suppl_4):451-460. Available from: https://www.royalsocietypublishing.org/doi/10.1098/rsif.2010.0092.focus

[29] Li Z, Wang C. Effects of working parameters on electrospinning. In:

One-Dimensional nanostructures. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. pp. 15-28. Available from: http://link.springer.com/10.1007/978-3-642-36427-3_2

[30] Dastourani H, Jahannama MR, Eslami-Majd A. A physical insight into electrospray process in cone-jet mode: Role of operating parameters. International Journal of Heat and Fluid Flow. 2018;**70**:315-335

[31] Nguyen Vu LV. Electrospray method: Processing parameters influence on morphology and size of PCL particles. Vietnam Journal of Science and Technology. 2018;**55**(1B):209

[32] Xie J, Ng WJ, Lee LY, Wang CH. Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray. Journal of Colloid and Interface Science. 2008;**317**(2):469-476

[33] Tasci ME, Dede B, Tabak E, Gur A, Sulutas RB, Cesur S, et al. Production, optimization and characterization of polylactic acid microparticles using electrospray with porous structure. Applied Sciences. 2021;**11**(11):5090

[34] Morais AÍS, Vieira EG, Afewerki S, Sousa RB, Honorio LMC, Cambrussi ANCO, et al. Fabrication of polymeric microparticles by electrospray: The impact of experimental parameters. Journal of Functional Biomaterials. 2020;**11**(1):4

[35] Faramarzi AR, Barzin J, Mobedi H. Effect of solution and apparatus parameters on the morphology and size of electrosprayed PLGA microparticles. Fibers and Polymers. 2016;**17**(11):1806-1819

[36] Ibili H, Dasdemir M, Çankaya İİT, Orhan M, Güneşoğlu C, Arabacı AS. Investigation of poly(lactic acid) nanocapsules containing the plant extract via coaxial electrospraying method for functional nonwoven applications. Journal of Industrial Textiles. 2022;**51**(3_suppl):5304S-5327S

[37] Arya N, Chakraborty S, Dube N, Katti DS. Electrospraying: A facile technique for synthesis of chitosan-based micro/nanospheres for drug delivery applications. Journal of Biomedical Materials Research. Part B, Applied Biomaterials. 2009;**88B**(1):17-31

[38] Jafari-Nodoushan M, Barzin J, Mobedi H. Size and morphology controlling of PLGA microparticles produced by electro hydrodynamic atomization. Polymers for Advanced Technologies. 2015;**26**(5):502-513

Chapter 5

# On the Origin of Protons in Electrospray Ionization

*Yixin Zhu and Kai Tang*

## Abstract

In electrospray ionization (ESI), analyte molecules are protonated in the positive ion mode to form molecular ions. However, little is known about the origin of these protons. To investigate, an enclosed ESI ion source was used to control the type of gas introduced into the source. First, when only inert gas such as $N_2$ or $CO_2$ was used, protonated molecular ions disappeared. Second, when gas phase $D_2O$ was used, $[M+D]^+$ became the predominant peak in the mass spectrum. However, when gas phase $H_2O$ was used, $[M+H]^+$ became dominant even though caffeine was dissolved in pure $D_2O$. Furthermore, when mixtures of $D_2O/H_2O$ with different ratios were used in the gas phase, the percentages of $[M+D]^+$ peak calculated from the mass spectra strictly follow the percentages of $D_2O$ in the gas phase mixture. Based on these observations, we conclude that the source of protonation in ESI could originate entirely from the gas phase $H_2O$ molecules, independent of the solvent the analyte molecules are dissolved in. When pure $H_2O$ was sprayed out, protonated small water clusters (PSWC) were formed, which comprise of a single proton surrounded by 2 to 5 water molecules. These PSWC may have a wide range of biological functions.

**Keywords:** electrospray ionization (ESI), protons, gas phase molecules, $D_2O$, gas phase molecules (e.g. $H_2O$) surrounding the ESI tip, protonated small water clusters

## 1. Introduction

Since its introduction in 1989 [1], electrospray ionization (ESI) has become the dominant ionization method for biological mass spectrometry, with exciting applications in proteomics, genomics, metabolomics and drug discovery. However, the fundamental mechanisms involved in ESI remain elusive. It is commonly agreed that electrospray produces charged droplets [2], and droplets shrink due to evaporation as they move along the heated capillary entrance of the mass spectrometer and eventually result in solvent-free ions [3]. However, it is not clear how the droplets are charged and where the charges come from. We can divide the ESI process into three stages:

Stage I: the generation of charged droplets from a needle held at high voltage,

Stage II: the shrinkage of the droplets as they are moving along the heated capillary, and

Stage III: the generation of charged molecular ions from minimized charged droplets.

The end result in Stage III is clear—the analyte molecules are protonated, which is revealed by the observation of $[M+nH]^{n+}$ ions in mass spectrometry. Various mechanisms have been proposed to describe processes II and III [4, 5], mainly based on the charged residue model (CRM) of Dole [6] and the ion evaporation model (IEM) of Iribarne and Thomson [7]. A chain ejection model (CEM) was also introduced by the Konermann group to describe disordered polymer/protein ion generation from charged droplets [8, 9]. Many excellent reviews have been published over the years [10–12], representing a progressive view of our understanding of these two stages.

In the upstream of Stage I, the generation of charged droplets starts with the formation of the Taylor cone and the ejection of a liquid jet from its apex. The process has been photographed with fast time-lapse imaging [13] and comprehensively reviewed by Fernandez de la Mora [14]. The surface of the Taylor cone is positively charged when a positive high voltage is applied to the spray needle. It is commonly believed that charging of the sprayed droplets originates from the excess positive charges on the surface of the Taylor cone, which in turn originate from the acid added as a mobile phase modifier or from electrochemical reaction at the metal surface interfacing with the mobile phase through which high voltage is applied [15, 16]. However, the electrochemical theory of protonation in ESI was not without controversy [17]. It is also known that ESI could happen without any acid modifier in the mobile phase and regardless of solution pH [18].

In this report, we try to trace the origin of protons that forms molecular ions $[M+H]^+$ with analyte molecules by using $D_2O$ in solution and in gas phase. Caffeine was chosen as a model compound as it does not have exchangeable hydrogens. Experimental evidence strongly suggest that the source of protons could originate from the surrounding gas phase $H_2O$ around the Taylor cone.

## 2. Methods

Caffeine, $D_2O$ (99.9%) and deuterated acetic acid ($H_3CCOOD$, 99.0%) were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). Acetonitrile was from Sigma-Aldrich.

Caffeine was dissolved in pure water or acetonitrile without adding any acid. The working concentration was 1 μM. ESI was performed using a syringe pump running at 2 μl/min. An enclosed nano ESI source (CEESI ion source, Zhejiang Haochuang Biotech Co. Ltd., Hangzhou, China) with carrier gas flow was used to control the atmosphere around the spray tip (**Figure 1**). Ultra-pure $N_2$ or $CO_2$ was used as carrier gas. A tubing from the carrier gas tank was fed through an inlet port of an HPLC mobile phase bottle and reached near the bottom. The outlet of the HPLC solvent bottle was connected to the inlet of the CEESI ion source via a low-pressure gas regulator. When carrier gas alone was introduced, no liquid was present in the bottle. When the bottle was partially filled with liquid, such as $H_2O$, $D_2O$ or a mixture of them with a certain ratio, gas phase molecules of the same kind(s) present in the vapor were introduced into the CEESI ion source with the carrier gas. The overall gas pressure in the spray chamber was monitored and kept at the same level throughout the experiments. The CEESI ion source was an enclosed nano/micro flow ion source with an axial capillary spray tip. Since the CEESI ion source was not open to the atmosphere, the gas phase

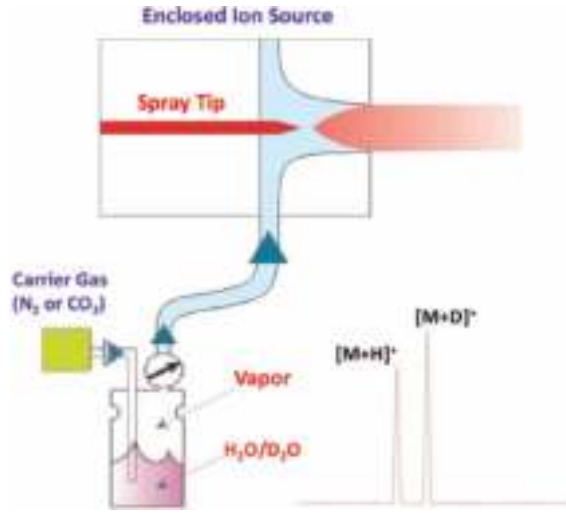


**Figure 1.**
*Schematic diagram of CEESI ion source used for protonation studies of caffeine.*

environment around the spray tip could be precisely controlled. A Thermo LTQ linear ion trap mass spectrometer was used to acquire mass spectra. The temperature of the heated capillary tube in the LTQ mass spectrometer was set at 200°C, and the syringe pump was running at 2 μl/min, unless noted otherwise. All tests were performed in the positive ion mode, with spray voltage set at 1.05× of the threshold voltage $V_0$, below which there was no detectable signal in the mass spectrometer. The threshold voltage $V_0$ varied from tip to tip and was experimentally determined in each case. Usually, $V_0$ was around 1.2–1.5 kV. Therefore, spray voltages were always below 1.6 kV, ensuring no corona discharge existed around the spray tip.

For the production of protonated small water clusters (PSWC), reverse osmosis purified water (to remove all ions) stored in water storage tank *1* was pumped through capillary array *3* using water supply pump *2* (**Figure 2**). The capillary array was connected to the positive electrode of high voltage power supply *4*, serving as the anode. The tips of the capillary array were aligned with the circular holes of cathode 5,

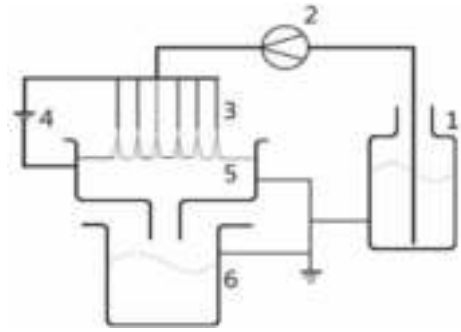


**Figure 2.**
*Schematic diagram of production equipment, where 1 – water storage tank; 2 – water supply pump; 3 – capillary arrays; 4 – power supply; 5 – circular holes aligned with capillary array tips serving as cathode; 6 – water storage tank.*

which were connected to the negative electrode of power supply *4*. The PSWC was collected in water storage tank *6*. The voltage of the home-made power supply *4* was set at 10 kV, and the flow rate of water supply pump *2* was 12 ml/min.

## 3. Results

First, a caffeine ESI signal was established when the spray chamber was open to the atmosphere through an open tubing. Then, the tubing was connected to an ultra-pure $N_2$ or $CO_2$ tank; while maintaining the same pressure in the spray chamber, the ESI signal dropped to zero. Disconnecting the tubing and releasing air into the spray chamber restored the ESI signal to the previous level. A video of the process is shown (Supplementary Video 1, https://bit.ly/3UrNDz3). These results could be obtained regardless of whether caffeine was dissolved in and sprayed out from pure water or pure acetonitrile. In the case of pure acetonitrile as a solvent and mobile phase, there was no source of $H^+$ in the solution to protonate caffeine. However, $[M+H]^+$ was still readily observed in the mass spectra. These observations indicated that protonation might be related to compounds in the air, other than $N_2$ or $CO_2$.

Next, caffeine dissolved in $D_2O$ (without adding any $H_2O$ or acid) was used for ESI while the ion source was opened to the atmosphere through a tubing. $[M+1]^+$ was observed as the predominant peak, and the relative intensity for the $[M+2]^+$ peak was about 13% (**Figure 3c**). Compared to the standard caffeine spectrum when $H_2O$ was used as a solvent (**Figure 3b**), the relative peak intensities for the $[M+2]^+$ peaks were very similar, close to the theoretical natural isotope abundance of protonated caffeine (**Figure 3a**). However, when $D_2O$ was introduced in the gas phase while pure water was used as a solvent for caffeine, then $[M+2]^+$ became the dominant peak in the mass spectrum (**Figure 3d**), presumably representing ionization through protonation by adding one $D^+$ to the caffeine molecule, forming $[M+D]^+$.

Similar results were obtained when caffeine was dissolved in pure acetonitrile and sprayed out while the chamber was filled with air or with ultra-pure nitrogen gas carrying $D_2O$ (Supplementary Video 2, https://bit.ly/3UrNDz3). When the ion source was open to the atmosphere through a tubing, $[M+1]^+$ was observed as the dominant peak, and $[M+2]^+$ was at 11%, representing natural isotope distribution. When the tubing was connected to the ultra-pure $N_2$ source carrying gas phase $D_2O$, the $[M+2]^+$ peak increased rapidly to be the dominant peak, while the $[M+1]^+$ peak decreased to <10%, probably due to a trace amount of air coming from the incomplete seal of the ion source. The $[M+3]^+$ peak was presumably due to the natural isotope distribution in caffeine.

Furthermore, a mixture of $D_2O/H_2O$ with a known molar percentage of $D_2O$ $\zeta = D_2O/(H_2O + D_2O)$ was used in the solvent bottle, and its vapor was carried by either $N_2$ or $CO_2$ gas to the spray chamber. A typical mass spectrum obtained is shown in **Figure 2b**.

The intensity of each isotope peak can be expressed as

$$M1 = A + \beta * B \tag{1}$$

$$M2 = B + \alpha * A \tag{2}$$

where $A$ is the peak intensity contributed from the monoisotopic peak of $[M+H]^+$, $B$ is the peak intensity contributed from the monoisotopic peak of $[M+D]^+$, $\alpha$ is the

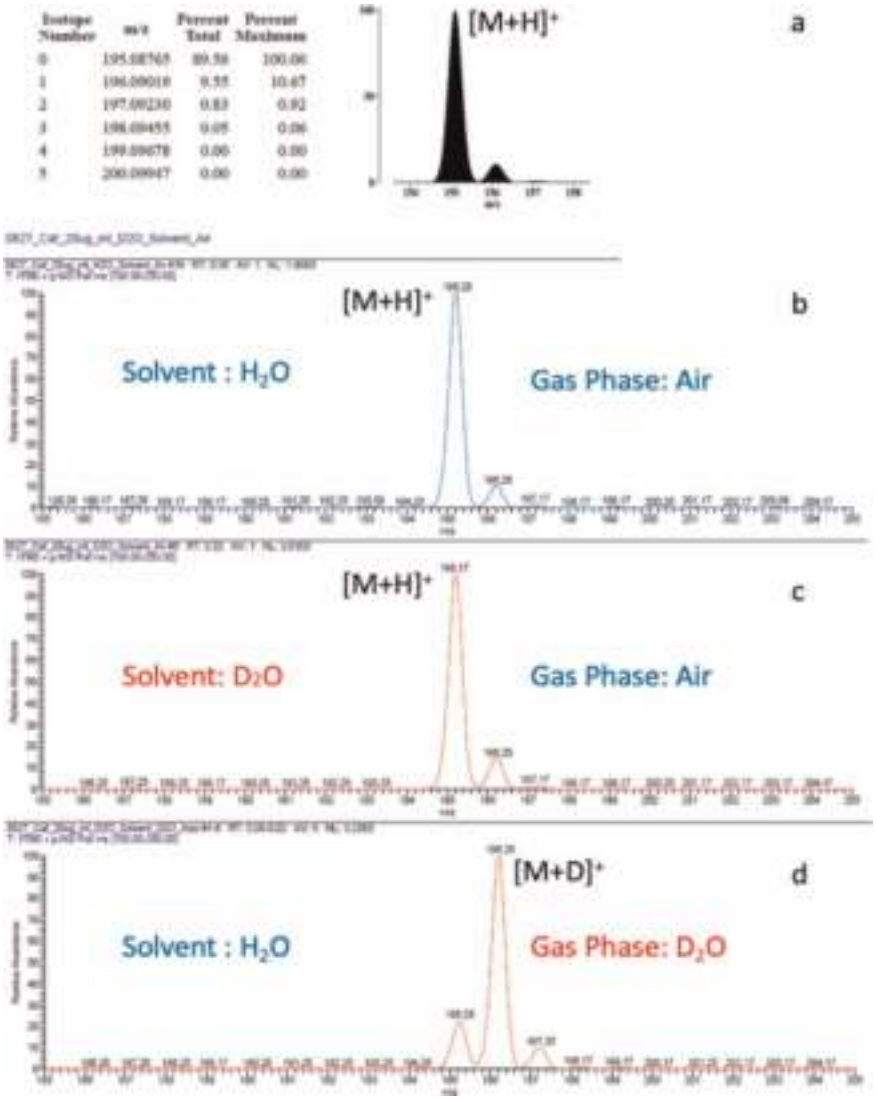


**Figure 3.**
*Isotope profile of charged caffeine molecules.* a. *Simulation of natural isotope abundance of $[M+H]^+$ using MS-Isotope. While the m/z of the dominant monoisotope is normalized as 100%, the monoisotope +1 peak is at about 11% of the maximum.* b. *Measured isotope profile of $[M+H]^+$ when caffeine was dissolved in $H_2O$ and the gas phase molecule was air.* c. *Measured isotope profile when caffeine was dissolved in $D_2O$ and the gas phase molecule was air.* d. *Measured isotope profile when caffeine was dissolved in H2O and the gas phase molecule was $D_2O$.*

percentage of the second isotope peak intensity relative to $A$ and $\beta$ is the percentage of $[M+H]^+$ peak intensity relative to $B$ when no $H_2O$ is used in the mixture ($\zeta$ = 100%, **Figure 4c**). The existence of a small $\beta$ may represent an incomplete seal of the ion source and a trace amount of $H_2O$ in the $D_2O$ sample. The value of $\alpha$ can be obtained theoretically (10.67%, **Figure 3a**) or experimentally when no $D_2O$ was used in the mixture ($\zeta$ = 0%, **Figure 4a**). The value of $\beta$ was determined experimentally to be 9.35% (**Figure 4c**).

From Eqs (1) and (2), $A$ and $B$ can be written as

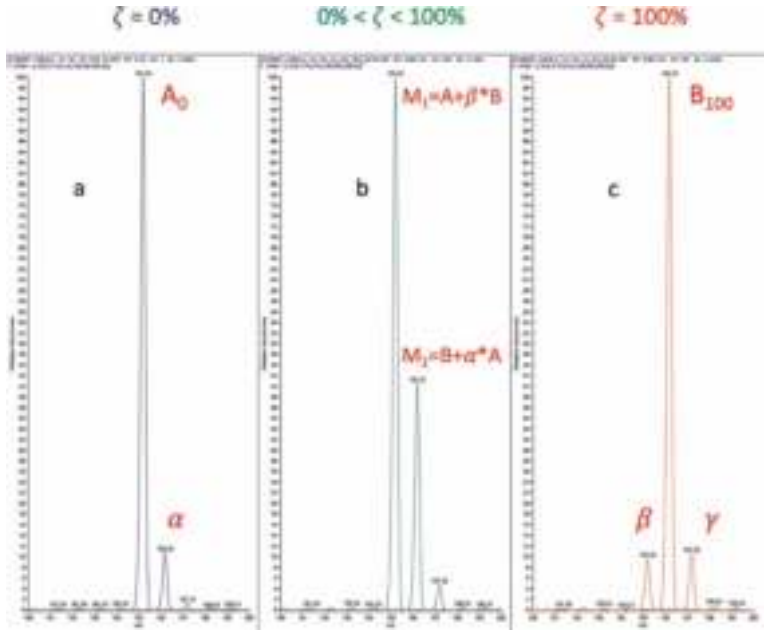


**Figure 4.**
*Isotope profiles of charged caffeine molecules dissolved in pure acetonitrile when mixtures of $H_2O$ and $D_2O$ with different molar ratios were used in the gas phase.* a. *Isotope profile when only $H_2O$ was used. α represents the percentage of the second isotope peak intensity relative to the monoisotope peak $A_o$.* c. *Isotope profile when only $D_2O$ was used. β represents the percentage of the remnant $[M+H]^+$ compared to the $[M+D]^+$.* b. *Isotope profile when a mixture containing ζ molar percentage of $D_2O$ was used. $M_1$ and $M_2$ are the measured intensities of the first and second isotope peaks, respectively, and A is the peak intensity contributed from the monoisotopic peak of $[M+H]^+$ and B is the peak intensity contributed from the monoisotopic peak of $[M+D]^+$.*

$$A = \frac{M1 - \beta M2}{1 - \alpha\beta} \tag{3}$$

$$B = \frac{M2 - \alpha M1}{1 - \alpha\beta} \tag{4}$$

The percentages of $D_2O$ in the mixture can be calculated from the mass spectra as

$$\zeta_{Exp} = \frac{B}{A + B} = \frac{M2 - \alpha M1}{(1 - \alpha)M1 + (1 - \beta)M2} \tag{5}$$

We tested molar percentage of 25%, 50% and 75% $D_2O$ in the gas phase mixture using either $N_2$ or $CO_2$ as carrier gas. The experimental values of $\zeta_{Exp}$ were obtained by using Eq. (5) and listed in **Table 1**. The results showed that they faithfully reflected the molar percentage of $D_2O$ in the original solution.

When caffeine was dissolved in pure acetonitrile, there were no $H_2O$ molecules in the liquid phase, nor did there exist any liquid phase molecules that could provide $H^+$. These results strongly indicated that the gas phase water molecules around the spray tip provided the only source of protonation in ESI, at least in the current experiment setup.

Using the same experimental setup, when pure water (after reverse osmosis to remove all ions) was used for ESI, the protons from the surrounding air would protonate water molecules. Normal water molecules exist as clusters $[H_2O]_n$, where n

| | $N_2$ | | | | | $CO_2$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ζ | **0%** | **25%** | **50%** | **75%** | **100%** | **0%** | **25%** | **50%** | **75%** | **100%** |
| M1 | 100% | 100% | 100% | 42.57% | 9.35% | 100% | 100% | 97.86% | 38.66% | 6.43% |
| M2 | 10.56% | 42.50% | 100% | 100% | 100% | 10.35% | 42.75% | 100% | 100% | 100% |
| α | 10.56% | | | | | 10.35% | | | | |
| β | 9.35% | | | | | 6.43% | | | | |
| $\zeta_{Exp}$ | 0% | 24.96% | 49.66% | 74.19% | 100% | 0% | 24.99% | 49.57% | 74.87% | 100% |

**Table 1.**
*$D_2O$ molar percentages calculated from the mass spectra ($\zeta_{Exp}$) using Eq. (5) when mixtures of $D_2O/H_2O$ with different $D_2O$ molar percentages (ζ) were used in the gas phase.*

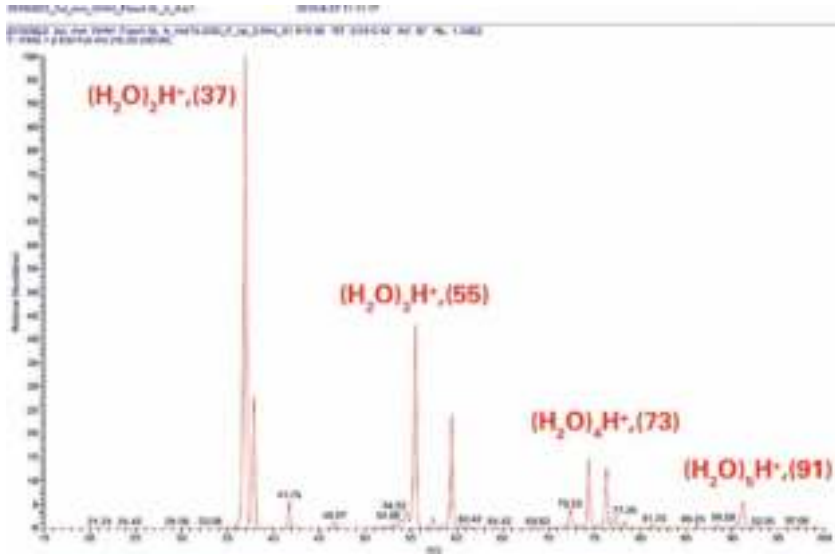


**Figure 5.**
*Observed ESI mass spectra of pure water, indicating the presence of protonated dimer, trimer, tetramer and pentamer water clusters.*

ranges from 2 to ~100. In order to minimize the influence of high temperature on the integrity of clusters, the temperature of the heated capillary tube in the LTQ mass spectrometer was set at 30°C. The mass spectrum is shown in **Figure 5**. Protonated small water clusters $[H_2O]_n$, where n = 2, 3, 4 and 5, were observed. No protonated clusters where $n \geq 6$ were found.

Due to the protonated nature of the small water clusters, they could serve as a carrier for protons to neutralize negative reactive oxygen species. In order to further test their utility, a high-throughput production device was built according to **Figure 2**. The collected protonated small water clusters were then tested with a pH paper and a pH meter. The measured pH values were 7 and 4.67, respectively. This indicated that the protonated small water clusters were chemically neutral but had an $H^+$ concentration of $10^{-4.67}$ mol/L. As a control test, the reverse osmosis-purified water before going through the ESI process was also tested with the same pH paper and pH meter. The measured pH values were 6.5 and 5.94, respectively. When the collected

protonated small water clusters underwent multiple rounds of ESI, the pH value measured by the pH meter could go as low as 2.39, while the pH strip still gave a pH of 6.

The stability of the protonated small water clusters was also tested by boiling and maintaining the boiling temperature for 5, 10, 15, 20, 25 and 30 min. After cooling down to room temperature, their pH values were again tested with the same pH meter. The obtained values were 4.64, 4.62, 4.62, 4.62, 4.61 and 4.61, respectively. The protonated small water clusters were stored in plastic water bottles and tested repeatedly during a 2-year period. No significant change in pH values was ever found. These indicated that the protons were well-protected in the protonated small water clusters and stable under heating and over a long period of time.

## 4. Discussions

In many previous publications, pure $N_2$ was used as carrier gas or curtain gas in the ESI ion source. However, no signal quenching was ever reported. We attribute this to the atmosphere-open ESI ion source used. With an open source, there was no way to guarantee that there were no $H_2O$ molecules from the air still existed around the spray tip. In our setup, as the CEESI source was an enclosed ion source, we could effectively remove any remaining water molecules inside the ion source by using inert gases. Therefore, the quenching of the ESI signal was observed when ultra-pure $N_2$ or $CO_2$ gas was introduced.

We used the lowest spray voltage possible inside the CEESI source, which was 500–1000 V lower than that normally used for an atmosphere-open ESI source. This ensured that corona discharge did not occur in the CEESI source.

Based on our results, we believe the evidence is strong pointing toward that the source of protonation in ESI can originate from the gas phase molecules (e.g., $H_2O$) surrounding the ESI tip. More work is needed to determine how the gas phase $H_2O$ could provide the $H^+$ for protonation in ESI.

Here, we propose one possible model in which the $H^+$ ions in the spray chamber come from the gas phase molecules (e.g., $H_2O$) surrounding the ESI tip due to field-induced cleavage of the O-H bond, while ESI droplets leaving the Taylor cone were neutral but polarized. The $H^+$ ions near the Taylor cone attach to the negative poles of the polarized droplets and eventually provide the source of protonation to the analyte molecules.

Is it reasonable to assume that the electrical field around the Taylor cone is strong enough to break the O-H bond in $H_2O$? Considering the capillary with high voltage in front of the mass spectrometer inlet, the required voltage for the onset of electrospray is given in [19]:

$$V_{on} \approx 2 \times 10^5 (\gamma R_c)^{\frac{1}{2}} \ln\left(\frac{4d}{R_c}\right) \tag{6}$$

where $\gamma$ is the surface tension of the solvent, $R_c$ is the outer radius of the spray capillary and d is the distance between the spray tip and the counter-electrode. In our setup, $\gamma$ = 0.073 N/m for $H_2O$, $R_c = 12.5\ \mu m$ and d = 1.0 mm; therefore, $V_{on}$ = 1.1 kV, which matches well with our experimental value.

The electric field at the tip of the Taylor cone is [20, 21]:

$$E_c \approx \frac{2V_c}{r_c \ln(4d/r_c)} \tag{7}$$

Here, $r_c$ is the radius at the tip of the Taylor cone, not the $R_c$ of the capillary. It is known that $r_c$ can be 100 nm [22] or even less [23]. In our case, we estimate $r_c$ is on the order of 100–600 nm. Therefore, $E_c = 2.1 \times 10^9$ V/m to $4.16 \times 10^8$ V/m. And, the electric field energy density is:

$$W_e = \frac{1}{2}\,\varepsilon_0 E^2 \tag{8}$$

Here, $\varepsilon_0 = 8.85 \times 10^{-12}$ $C^2/Nm^2$. Therefore, $W_e = 1.9 \times 10^7$ J/m$^3$ to $7.7 \times 10^5$ J/m$^3$.

On the other hand, if we assume the gas phase $H_2O$ in the spray chamber is ideal gas, we have:

$$PV = nRT \tag{9}$$

where P is the vapor pressure, V is the volume of gas, n is the mole number, R is the ideal gas constant (8.314 Pa m$^3$ mol$^{-1}$ K$^{-1}$) and T is temperature. At room temperature of 298K (25°C), the vapor pressure of $H_2O$ is 3173 Pa. Therefore, its concentration can be written as:

$$\frac{n}{V} = \frac{P}{RT} = 1.28\ mol/m^3 \tag{10}$$

The O-H bond energy is 464 kJ/mol. So, the energy density to break the O-H bond must be larger than

$$4.64 \times 10^5 \frac{J}{mol} \times 1.28\ \frac{mol}{m^3} = 5.94 \times 10^5\ J/m^3. \tag{11}$$

Therefore, the electric field energy $W_e$ is more than enough to break the O-H bond and produce $H^+$.

If this model of ionization is correct, we may be able to find other molecules containing the X-H bond which has lower bond energy than the O-H bond. This would facilitate the release of $H^+$ and increase protonation efficiency in ESI.

This mode of ionization is also compatible with other experimental phenomena observed in ESI, such as ion suppression caused by charge competition and the lack of dependency on mobile phase pH.

Our results here do not rule out the possibility that electrochemical reactions at the electrodes provide the protons in an ESI setup. In reality, multiple ionization pathways may co-exist in a given set of experimental conditions. More studies are certainly needed to determine which pathway is more dominant under normal ESI conditions. Nevertheless, realizing the source of protonation could come from the gas phase is particularly important not only for ESI mechanism studies but also for finding new ways to improve the analytical detection sensitivity of ESI by introducing novel gas phase molecules to enhance protonation efficiency.

Realizing this new method of protonation mechanism, we have also discovered protonated small water clusters. The possible structures of these protonated small water clusters are shown in **Figure 6**. These protonated small water clusters are thought to comprise one single proton surrounded by 2, 3, 4 or 5 water molecules.

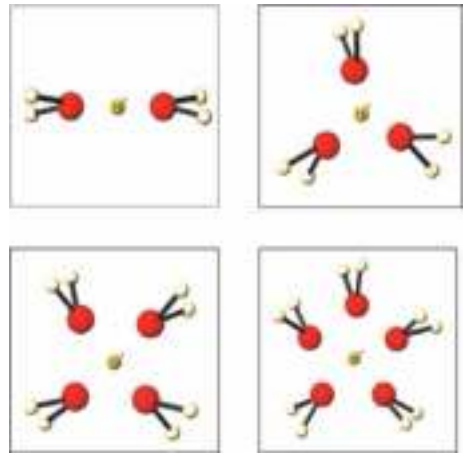

**Figure 6.**
*Proposed structures of the protonated small water clusters.*

This may explain the observed stability of the protonated small water clusters, as the protons are well-protected at the center of the clusters.

The protonated small water clusters were found to have an anti-inflammation effect in a series of animal model tests [24] and may have other biological functions.

## Acknowledgements

The authors would like to express their appreciation to Prof. Evan Williams who suggested us to try pure organic solvent for ESI in our setup and to Dr. Rong Wang, Prof. Yukui Zhang and Prof. Richard Zare for stimulating discussions. This work was partially funded by the National Major Scientific Instrument Development Project (2012YQ120044) from the Ministry of Science and Technology of China. Part of this work was presented at the 67th ASMS Conference in Atlanta, GA.

## Conflict of interest

YZ is the owner and president of Hangzhou Shanshangshui Co. Ltd. (Hz3S) and an inventor of several patents related to the enclosed electrospray ion source. KT is a consultant to Hz3S.

*DOI: http://dx.doi.org/10.5772/intechopen.1005265*

## Author details

Yixin Zhu* and Kai Tang*
Hangzhou Shanshangshui Co. Ltd., Hangzhou, China

*Address all correspondence to: zyx@hprotonwater.com
and ktang@hprotonwater.com

IntechOpen

## References

[1] Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science. 1989;**246**(4926): 64-71

[2] Fenn J, Rosell J, Nohmi T, Shen S, Banks F. Electrospray ion formation: Desorption vesus desertion. In: Snyder AP, editor. Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry. Washington, DC: American Chemical Society; 1996

[3] Cech NB, Enke CG. Practical implications of some recent studies in electrospray ionization fundamentals. Mass Spectrometry Reviews. 2001; **20**(6):362-387

[4] Kebarle P, Tang L. From ions in solution to ions in gas phase. Analytical Chemistry. 1993;**65**(22): 972A-986A

[5] Wilm MS, Mann M. Electrospray and Taylor-Cone theory, Dole's beam of macromolecules at last? International Journal of Mass Spectrometry and Ion Processes. 1994;**136**:167-180

[6] Dole M, Mack LL, Hines RL, Mobley RC, Ferguson LD, Alice MB. Molecular beams of macroions. The Journal of Chemical Physics. 1968;**49**(5): 2240-2249

[7] Iribarne JV, Thomson BA. On the evaporation of small ions from charged droplets. The Journal of Chemical Physics. 1976;**64**(6):2287-2294

[8] Konermann L, Rodriguez AD, Liu J. On the formation of highly charged gaseous ions from unfolded proteins by electrospray ionization. Analytical Chemistry. 2012;**84**(15):6798-6804

[9] Konermann L, Ahadi E, Rodriguez AD, Vahidi S. Unraveling the mechanism of electrospray ionization. Analytical Chemistry. 2013;**85**(1):2-9

[10] Kebarle P, Verkerk UH. Electrospray: From ions in solution to ions in the gas phase, what we know now. Mass Spectrometry Reviews. 2009; **28**(6):898-917

[11] Ogorzalek Loo RR, Lakshmanan R, Loo JA. What protein charging (and supercharging) reveal about the mechanism of electrospray ionization. Journal of the American Society for Mass Spectrometry. 2014;**25**(10):1675-1693

[12] Konermann L, Metwally H, Duez Q, Peters I. Charging and supercharging of proteins for mass spectrometry: recent insights into the mechanisms of electrospray ionization. Analyst. 2019; **144**:6167-6171

[13] Marginean I, Parvin L, Heffernan L, Vertes A. Flexing the electrified meniscus: the birth of a jet in electrosprays. Analytical Chemistry. 2004;**76**(14):4202-4207

[14] Fernandez de la Mora J. The fluid dynamics of Taylor Cones. The Annual Review of Fluid Mechanics. 2007;**39**: 217-243

[15] Blades AT, Ikonomou MG, Kebarle P. Mechanism of electrospray mass spectrometry. Electrospray as an electrolysis cell. Analytical Chemistry. 1991;**63**(19):2109-2114

[16] Van Berkel GJ, Kertesz V. Using the electrochemistry of the electrospray ion source. Analytical Chemistry. 2007; **79**(15):5510-5520

[17] Fernandez de la Mora J, Van Berkel GJ, Enke CG, Cole RB,

Martinez-Sanchez M, Fenn JB. Electrochemical processes in electrospray ionization mass spectrometry. Journal of Mass Spectrometry. 2000;**35**(8):939-952

[18] Kelly MA, Vestling MM, Fenselau CC, Smith PB. Electrospray analysis of proteins: A comparison of positive-ion and negative-ion mass spectra at high and low pH. Organic Mass Spectrometry. 1992;**27**:1143-1147

[19] Taylor GI, McEwan AD. The stability of horizontal fluid interface in a vertical electric field. Journal of Fluid Mechanics. 1965;**22**(1):1-15

[20] Loeb L, Kip AF, Hudson GG, Bennet WH. Pulses in negative point-to-plane Corona. Physical Review. 1941; **60**(10):714-722

[21] Pfeifer RJ, Hendricks CD. Parametric studies of electrohydrodynamic spraying. AIAA Journal. 1968;**6**(3): 496-502

[22] Fernandez de la Mora J, Navascues J, Fernandez F, Rosell-Llompart J. Generation of submicron monodisperse aerosols in electrosprays. Journal of Aerosol Science. 1990;**21**:s673-s676

[23] Fernandez de la Mora J. The effect of charge emission from electrified liquid cones. Journal of Fluid Mechanics. 1992; **243**:561-574

[24] Zhu Y. Production, characteristics, and biological effects of protonated small water clusters. In: Proc. SPIE 12959, AOPC 2023: Laser Technology and Applications; and Optoelectronic Devices and Integration, 129590L (18 December 2023). Bellingham, WA, USA: SPIE; 2023. DOI: 10.1117/12.3005480

*Edited by Weronika Smok, Pawel Jarka and Tomasz Arkadiusz Tański*

*New Topics in Electrospraying* is a captivating exploration of two groundbreaking technologies—electrospraying and electrospinning—that are transforming industries. This book explores their innovative applications in the food industry, tissue regeneration, and drug delivery, offering fresh insights into how these methods are revolutionizing food processing, packaging, preservation, and medicine. From producing ultra-fine coatings to designing functional materials with tailored properties, the book unpacks these techniques' science and practical implications. Each chapter provides a deep dive into the principles behind the processes while showcasing their potential to address real-world challenges. Whether you're a researcher, a professional, or an enthusiast of cutting-edge technology, this book equips you with the knowledge to appreciate the versatility and the power of electrospraying and electrospinning.

Published in London, UK


IntechOpen

ISBN 978-1-83769-650-5
9 781837 696505