Review Article International Journal of Dental Materials 2020; 2(2) ISSN:2582-2209

A comprehensive review on electrospinning design, parameters and potential use of electrospun nanofibers in regenerative endodontics

Sai Lakshmi I [1,*], Madhu Varma K[2], Girija S Sajjan[2], Kalyan Satish R[2], Sindhu D[1], Sowmya M[1]

[1]Postgraduate Student, [2]Professor, Department of Conservative Dentistry and Endodontics, Vishnu Dental College, Bhimavaram -534202, West Godavari, Andhra Pradesh, India.

INFORMATION

Article History

Received *3rd April 2020*
Accepted *5th May 2020*
Available online
31st May 2020

KEYWORDS

Electrospinning

Regenerative endodontics

Stem cells

Toxicity

Triple antibiotic paste

Nanofibres

ABSTRACT

Electrospinning is a versatile technique that has gathered interest due to its ability to fabricate nano and microscale fibres with unique properties of high surface area and fibrous porosity. This technique has been widely used in the late 20th (1990) and early 21st (2000) centuries. Since the beginning of its use, significant improvements have been made in the design, materials used, and fibres produced. The electrospinning technique is used to fabricate a material with therapeutic properties as it allows the researchers to incorporate various anti-microbial agents to different polymers without altering the chemical characteristics of polymers.

The production of nanofibres through electrospinning is affected by many operating parameters. It is, therefore, essential to know various parameters and processes that aid in fabricating the desired fibre assemblies. The nanofibres remain an essential division of biomaterials due to a wide range of biomedical applications. Nanofibres have unique properties such as protein absorption, binding sites to cell receptors, can provide maximum volume fraction by controlling fibres' alignment and orientation hence improving the material properties like surface morphology, porosity, and geometry.

Recent trends in endodontics, encourage regenerative therapy for the treatment of necrotic immature permanent teeth for root development and maturation. In this context, efficient disinfection of the root canal system is a crucial step. Existing chemical irrigating solutions (for eg., NaOCl) and antibiotic pastes (for eg., Triple antibiotic paste) usage at higher doses showed toxic results on the pulpal stem cells. Therefore, it was found to be beneficial to use a nanofibre-based intracanal drug delivery construct to release antibiotics at lower, yet anti-microbially effective concentrations.

This review aims to discuss the basic concepts of electrospinning and its potential application in regenerative endodontics along with various parameters, which affect the fibre morphology and properties of produced nanofibres.

1. Introduction

Electrospinning is initially known as electrostatic spinning because it makes use of electrostatic force for the process of spinning. This spinning was first

Correspondence: *Corresponding author Email Address: lakshmiindukuri111@gmail.com

How to cite this article: Sai Lakshmi I, Madhu Varma K, Girija S Sajjan, Kalyan Satish R, Sindhu D, Sowmya M. A comprehensive review on electrospinning design, parameters and potential use of electrospun nanofibers in regenerative endodontics.. Int J Dent Mater 2020;2(2): 37-44.
DOI: http://dx.doi.org/10.37983/IJDM.2020.2202

investigated by Zeleny in 1914 [1]. This electrostatic force stretches the Visco-elastic solution as it solidifies to fabricate electrospun fibres [2]. Electrospinning is simple in its basic set-up, thereby making this accessible to almost every laboratory. It is a versatile technology that is efficient in producing nanofibres suitable for various Bio-medical applications [3].

The technology of withdrawing ultra-thin fibres from visco-elastic fluid under a strong electric field was discovered about a century ago [4]. Electrodynamics led to the development of electrospinning to produce fibres, which was invented in 1902 by Cooley and Morton [5,6]. The term electrospinning was taken into records in 1993 by Darrell H. Renker [7,8]. Most of the time, materials for dental applications were found to be smaller in size and volume due to the size restriction of the oral cavity. The ability of electrospinning technology to produce fibres in sub-micron to nano-meter dimension and its flexibility in material selection led to the production of materials suitable for dental applications [2].

Electrospinning also aids in the incorporation of additives like medicaments to get desired properties in the final materials [9,10]. This technique has been widely employed for the fabrication of nanofibrous scaffolds with fibre diameter alignment tailorability and diversity in raw-material [11]. The tailorability of fibre diameter and pore size provides optimal conditions for differentiation and proliferation of cells [12]. Electrospun materials have the benefits of improved cellular interactions, enhanced protein absorption, which facilitates binding sites for cell receptors and high surface area to volume ratio [13].

2. Basic Set-up of electrospinning

Electrospinning set-up typically consists of four major components. which includes high voltage power supply, syringe with pump, metal tip needle (spinneret) and collector [14,15].

2.1 Principle

The basic principle of electrospinning involves potential voltage difference between the polymer solution flowing through a spinneret into the collector. When the potential difference overcomes the surface tension of the solution, then fluid jet splits to form fibres that are solidified with the evaporation of the solvent [16].

A detailed explanation of the conversion of visco-elastic polymer solution into nanofibres was shown in Figure 1.

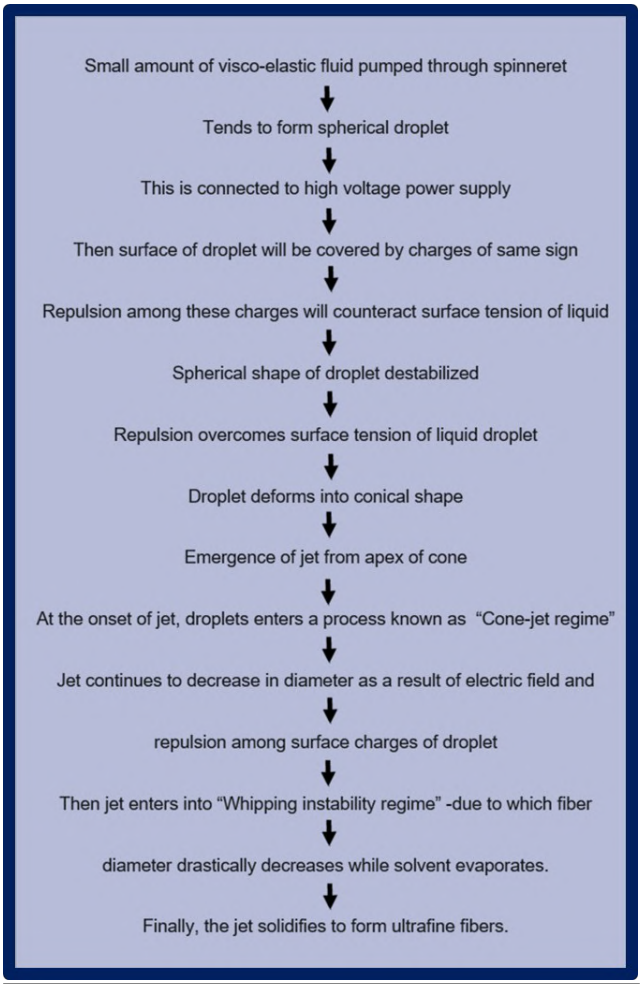

Figure 1. Steps involved in conversion of visco-elastic polymer solution into nanofibres.

2.2 Factors affecting fibre morphology and properties of electrospun fibres

The process of electrospinning produces continuous nanofibres through uniaxial stretching of visco-elastic solution [2]. To appreciate the process of nanofibre formation through electrospinning, different parameters that affect the process have to be considered. The key parameters affecting the properties of electrospun fibres and fibre morphology were described into four types. These parameters include processing, systemic, solution and physical parameters. The list of factors in each parameter affecting the characteristics of electrospun fibres was shown in Table 1.

2.2.1 Processing parameters

2.2.1.1 Voltage

Increase in voltage would discharge the polymer jet with a stronger repulsion, accelerates more volume of electrospinning solution, resulting in more stretching and decreased diameter of the fibres. However, an optimal voltage is necessary to initiate the polymer jet from the Taylor cone apex [17]. The applied voltage also

Table 1. Factors affecting the characteristics of electrospun fibres in each parameter.

Parameters	Individual factors
Processing parameters	Voltage
	Feed rate
	Distance of Collector
	Volumetric Flow rate
	Needle diameter
	Motion
Systemic parameters	Molecular weight
	Solvent
	Polymer type
Solution parameters	Viscosity
	Concentration
	Conductivity
	Surface Tension
	Dielectric constant
Physical parameters	Relative Humidity
	Temperature

had an effect on droplet shape prior to jet formation. Higher voltage results in an increased flow rate of solution and faster electrospinning [18].

2.2.1.2 Feed rate

The feed rate mainly determines the amount of available solution between the tip of the needle and electrospinning target. Increased feed rate causes the fusion of fibers due to improper evaporation of the solvent before reaching the collection point [18].

2.2.1.3 Distance of Collector

The reduction in the distance causes shorter flight time for the jet. So, it may not have sufficient time to solidify and results in the fusion of fibers. Increasing the distance drops the surface charge density, decreases the magnitude of the electric field, forming fewer charged ions [19]. This increase in distance results in elongation and decreases the diameter of the polymer jet.

2.2.1.4 Volumetric Flow Rate

Faster flow may stagnate the solution at the tip of the needle. As the rate of flow increases, the surface charge density decreases. The flow rate of the solution affects various features of nanofibres, such as diameter, porosity, and geometry [18]. A constant flow-rate is required to minimize the bead formation in electrospun materials [20]. Slow flow-rate reduces the diameter of the electrospun nanofibres [21]. In addition, the slow flow rate resulted in a smaller number of beads compared to a faster flow rate [22]. Therefore, in order to fabricate nanofibre continuously, the flow rate needs to be optimized [23].

2.2.1.5 Needle diameter

Fibre diameter was reported to increase with a greater needle tip diameter [24,25]. Smaller internal diameter reduces the clogging due to less exposure time of the jet to the environment. Reduction in inner needle diameter increases the solution surface tension corresponding to a smaller droplet that causes the jet to decrease its acceleration. So, jet gets more flight time before deposition and has more stretching and elongation; this results in smaller diameter fibres [23].

2.2.1.6 Motion

Regular electrospinning yields randomly aligned nanofibres [26]. Control on the geometry of the deposition of fibre or getting other desired fibre patterns can be achieved with a change in the design of the collector. One of them includes parallel bars with a gap in-between the two that leads to aligned nanofibres [27].

2.2.2 Systemic parameters

2.2.2.1 Molecular weight

Molecular weight represents the length of the polymer chain that, in turn, influences the entanglements. These will prevent the jet from premature splitting during the process. Higher molecular weight results in a viscous solution when compared to lower molecular weight [26]. Increasing molecular weight can result in decreased beading [28].

2.2.2.2 Solvent

The solubility and boiling point of the solvent are two essential factors to choose the desired solvent before electrospinning. Volatile solvents are considered to be an ideal option due to rapid evaporation and dehydration of the nanofibres [29]. A very low boiling point favours rapid evaporation, so this should be avoided to prevent the obstruction of needle orifice before electrospinning.

It was found that high boiling point solvents may not dehydrate completely before reaching the target resulting in a flat ribbon-shaped fibre instead of round fibre [30,31]. The volatility of the solvent might affect the features of electrospun nanofibres, including shape, porosity, and size. Hence, particular attention must be taken during the evaluation and selection of electrospinning solvents [30].

2.2.3 Solution-related Parameters

The solution properties are important to attain uniform fibres. It should have optimal low surface tension and high enough charge density and viscosity so that the collapse of the jet into droplets can be prevented before the solvent evaporates [32]. Polymer characteristics such as solution viscosity, concentration, surface tension, and solution conductivity influence the nanofibre morphology and properties.

2.2.3.1 Viscosity

Viscous solutions (optimum) enhance chain entanglements and result in uniform fibres without any beads. Less viscous polymer solution breaks up into small droplets or creates beaded fibres [33]. However, if the viscosity of the solution is too high, then it will be difficult to force the solution through capillary, and the solution at the tip may dry up [34].

2.2.3.2 Concentration

The concentration of the solution below the threshold value will result in the formation of droplets instead of fibres. The high concentration of the solution increases viscosity and may lead to processing problems. Increasing concentration can result in decreased beading and increased fiber diameter [35].

2.2.3.3 Effect of Conductivity

High conductivity facilitates polymer solution to carry greater charge compared to low conductivity. Hence, high conductivity yields greater tensile forces to applied voltage and reduction in nanofibre diameter [36]. Fong *et al.* examined the effect of sodium chloride on polymer for the fabrication of electrospun nanofibre and reported a higher charge density of electrospinning jet. This increased charge density results in the formation of smooth and uniform nanofibre [33]. Increasing conductivity through the addition of salt can result in defect-free, smaller diameter fibers [33,37].

2.2.3.4 Surface tension

Surface tension results in decreased surface area of the solution and aids in the formation of a spherical droplet. In case of low concentration, a high ratio of solvent molecules have an increased tendency to assemble and form a spherical or bead formation [33]. Low surface tension solvents should be used to get bead free uniform fibres.

2.2.3.5 Solvent dielectric constant

Increasing dielectric constant can result in decreased bead formation. The solvent with a higher dielectric constant has a higher density in solution [38].

2.2.4 Physical parameters

2.2.4.1 Relative humidity

Humidity causes changes in the diameter of the nanofibres by controlling the solidification process of the charged jet. An increase in humidity results in a decreased diameter of nanofibres. Further, an increase in humidity led to bead fibre for individual polymers and almost no electrospinning for the blends [39].

2.2.4.2 Temperature

Temperature causes two opposing effects to change the average diameter of the nanofibres: (i) it increases the rate of evaporation of the solvent and (ii) it decreases the viscosity of the solution. The increase in the evaporation of the solvent and the decrease in the viscosity of the solution work by two apposite mechanisms, however, both lead to a decrease in the mean fibre diameter [40].

3. Applications of electrospun nanofibres in regenerative endodontics

Pulp-dentin complex regeneration aids in extending the normal function of the natural dentition, especially in cases of traumatized permanent immature teeth, which hinders completion of root development and maturation [41,42]. The idea behind the regenerative endodontics is mainly based on stem cells' capacity to regenerate. These stem cells will be introduced into root canals through the intentional laceration of periapical tissue after a thorough disinfection protocol. The growth factors and stem cells from the apical area populate the scaffold, inducing tissue regeneration [43,44]. Therefore, both root canal disinfection and blood-clot formation have been shown to play a critical role in new tissue formation and overall root maturation and development.

The application of the antibiotic mixture in regenerative endodontic procedures was introduced in 2001 [45]. Since its emergence, this intra-canal antibiotic paste, i.e., either Triple antibiotic paste (TAP) or Double antibiotic paste (DAP), has been the most commonly used inter-appointment medicament [46]. Even though root canal irrigation with sodium hypochlorite (NaOCl) associated with antibiotic mixtures (i.e., TAP or DAP) has led to maximum bacterial elimination, but their use at high concentrations have been shown to negatively

impact dental derived stem cells survival and function [41,47]. It was shown that the widely used creamy paste (1 g/mL) of the triple antibiotic mixture is toxic to stem cells from the apical papilla (SCAPs) [48].

It was stated that Triple Antibiotic paste concentrations ranging from 0.01 to 0.1 mg/mL were not cytotoxic when applied directly onto the stem cells from apical papilla (SCAP) [48] and had no effect on viability after its removal from the root canal lumen [49]. However, antibiotics mixed with water or saline in such low concentrations result in a watery mixture that cannot be retained inside root canals. Therefore, it would be beneficial to use a biocompatible nanofibre-based intracanal drug delivery construct to release antibiotics at lower yet anti-microbially effective concentrations [50].

In drug delivery systems, coating the electrospun fiber with a shell is considered to be effective in controlling the release kinetics of the drugs [51]. The shell coat serves as an outer protective layer. Hydrophilic drugs can be incorporated in the core phase and hydrophobic polymers in the shell phase. In core-sheath nanofibers, the core swells or dissolves, forming pores in the shell after the dissolution of hydrophilic portion in the core, thereby allowing for the sustained release of the drug [52].

In order to overcome the inherent toxicity of the agents mentioned above, the concept of a cell-friendly disinfection strategy was introduced through the successful development of novel antibiotic-containing polymer nanofibres [43,50,53]. These novel drug delivery systems designed for regenerative endodontics are predicated on the fact that controlling the antibiotic dose and release rate will lead to enhanced stem cell viability while preserving anti-microbial activity [43,50,54-57]. In electrospinning, a polymer solution containing the desired concentration of antibiotics is prepared to produce nanofibres [43,50].

The reason behind the use of antibiotic-containing nanofibres as a three-dimensional (3D) tubular drug delivery construct [53,58] is based on the fact that the addition of low antibiotic concentrations and the slow drug release provided by these nanofibrous constructs will be able to eradicate the infection and thus create a bacteria-free environment favourable to tissue regeneration [56,57,58]. Importantly, in this strategy, the anti-microbial agents are delivered directly onto the dentinal walls, where microbial biofilms have been found to be present.

Collagen or Polycaprolactone (PCL) gelatin-based nanofibrous scaffolds incorporating bioactive glass nano particles were developed for dentin-pulp regeneration and showed enhanced growth and odontogenic differentiation from human dental pulp stem cells (DPSCs) compared to collagen nanofibrous scaffold via the integrin-mediated process [58,60]. Bottino *et al.* incorporated antibiotics (metronidazole and ciprofloxacin) to polydioxanone (PDS) electrospun scaffolds and observed that these scaffolds were more effective at delivering antibiotics. They require a lower dose against pathogenic bacteria, including Porphyromonas gingivalis and Enterococcus faecalis, compared to drugs delivered via pastes [50].

It was shown that these electrospun meshes of PCL have a strong potential for promoting odontogenic growth and differentiation, as suggested by increased turnover of collagen I and other proteins when tested in vitro with human pulpal cells [61]. Taken together, the major advantage of electrospinning might be its ability to produce complex geometry of nanofibrous scaffolds for dentin-pulp complex regeneration.

Electrospinning was used to fabricate tubular 3D drug delivery constructs comprising polydioxanone and three antibiotics (metronidazole, ciprofloxacin, and minocycline) at a much lower concentration than in the triple antibiotic paste. The 3D construct was designed to smoothly fit within the individual anatomy of immature teeth, that is, a parallel and tubular thin root dentin wall. The tubular 3D triple antibiotic-eluting drug delivery constructs were found to be effective in ablating intracanal biofilm in a similar fashion to the well-established triple antibiotic paste [62].

In electrospinning, the chosen polymer solution can be incorporated with one or a combination of antibiotics, making it possible to fabricate fibres with a narrow or wide spectrum of action (e.g., ciprofloxacin (CIP), metronidazole (MET), and minocycline (MINO) that have been shown to inhibit the growth of endodontic pathogens [50,56,57]. In a study, ciprofloxacin-containing polymer nanofibres were tested against E. faecalis biofilm developed on human root fragments and found maximum bacterial biofilm elimination [55].

4. Conclusion

Nanofibres used for the seeding of regenerative cells (like dental pulp cells, mesenchymal cells, odontoblasts, growth factors, etc.) provide a porous 3D surface that promotes regeneration of teeth [50]. Apart from this, the nanofibres produced by electrospinning offers various advantages like adhesion to cells, mimicking extracellular matrix, differentiation, and proliferation

of cells. In fact, scaffolds containing antibiotics have been proven to reduce or completely eradicate infection by the controlled release of a wide variety of antibiotics [63]. Ultimately, the nanofibrous scaffold's ability to deliver intracanal, controlled amounts of antibiotics might have positive treatment outcomes conducive to tissue regeneration by rendering a bacteria-free environment while minimizing the toxic effects associated with the use of the antibiotic paste.

Conflicts of interest: Authors declared no conflicts of interest.

Financial support: None

References

1. Zeleny J. The electrical discharge from liquid points and a hydrostatic method of measuring the electric intensity at their surfaces. J. Phy.1914; 69–91.
2. Teo WE, Ramakrishna S. A review on electrospinning design and nano- assemblies. Nanotechnology. 2006; 17(14): 89-106.
3. Xue J, Wu T, Dai Y, Xia Y. Electrospinning and electrospun nanofibres: Methods, materials, and applications. Chemical reviews. 2019;119(8):5298-415.
4. Xue J, Xie J, Liu W, Xia Y. Electrospun nanofibres: new concepts, materials, and applications. Accounts of chemical research. 2017;50(8):1976-87.
5. Cooley JF, Charles S Farquhar, Ambrose Eastman, assignee. Apparatus for electrically dispersing fluids. United States patent US. 1902; 692-631.
6. Morton WJ. Method of dispersing fluids US Patent Specification.1902; 705691.
7. Yadav TC, Srivastava AK, Mishra P, Singh D, Raghuwanshi N, Singh NK, Singh AK, Tiwari SK, Prasad R, Pruthi V. Electrospinning: An Efficient Biopolymer-Based Micro-and Nanofibres Fabrication Technique. In Next Generation Biomanufacturing Technologies, American Chemical Society. 2019 ; 209-241.
8. Doshi J, Reneker DH. Electrospinning process and applications of electrospun fibres. In Conference Record of the 1993 IEEE Industry Applications Conference Twenty-Eighth IAS Annual Meeting. 1993;1698-1703.
9. Greiner A, Wendorff JH. Electrospinning: A fascinating method for the preparation of ultrathin fibres. Angew. Chem. Int. Ed. 2007; 46: 5670–5703.
10. Vaishali A, Varma KM, Bhupathi PA, Bharath TS, Ramesh MV, Varma PV. In vitro evaluation of antimicrobial efficacy of 2% chlorhexidine loaded electrospun nanofibres. J Pierre Fauchard Acad (India Section). 2017;31(2-4):105-8.
11. Shin SH, Purevdorj O, Castano O, Planell JA, Kim HW. A short review: Recent advances in electrospinning for bone tissue regeneration. J Tissue Eng. 2012;3(1):1–11.
12. Jozsef Bako, Farkas Kerenyi, Lajos Daroczi, Csaba Hegedus. Biodegradable polymer based electrospun nanofibres for dental applications. J Biotechnol Biomater. 2018; 8: 87-88.
13. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science. 2005;310 (5751):1135-8.
14. Zhang X, Reagan MR, Kaplan, DL. Electrospun silk biomaterial scaffolds for regenerative medicine. Adv. Drug Deliv. Rev. 2009;61: 988–1006.
15. Reneker DH, Yarin AL. Electrospinning Jets and Polymer Nanofibres. Polymer 2008; 49: 2387−2425.
16. Subbiah T, Bhat GS, Tock RW, Parameswaran S, Ramkumar SS. Electrospinning of nanofibres. J App Polymer Sci. 2005;96(2):557-569.
17. Taylor G. Electrically Driven Jets. Proc. R. Soc. Lond. A Math. Phys. Sci. 1969; 313: 453–475.
18. Deitzel JM, Kleinmeyer J, Harris D, Beck Tan NC. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 2001; 42:261–272.
19. Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Electrospinning of collagen nanofibers. Biomacromolecules. 2002; 3: 232–238.
20. Zeleny J. The role of surface instability in electrical discharges from drops of alcohol and water in air at atmospheric pressure. J Frankl Inst 1935; 219: 659–675.
21. Garg K, Bowlin GL. Electrospinning jets and nanofibrous structures. Biomicrofluidics 2011; 5: 013403-1- 013403-18.
22. Megelski S, Stephens JS, Chase D.B, Rabolt JF. Micro-and nanostructured surface morphology on electrospun polymer fibers. Macromolecules 2002; 35: 8456–8466
23. Seo SJ, Kim HW, Lee JH. Electrospun nanofibres applications in dentistry. J Nanomate. 2016; 1-7.
24. Tong HW, Wang M. Effects of Processing Parameters on the Morphology and Size of Electrospun PHBV Micro-and Nano-Fibres. Key Eng. Mater. 2007; 334:1233–1236.
25. Jeun J, Kim Y, Lim Y, Choi J, Jung C, Kang P, Nho Y. Electrospinning of Poly(L-lactide-co-D,L-lactide). J Ind Eng Chem. 2007; 13: 592–596.
26. Zafar M, Najeeb S, Khurshid Z, Vazirzadeh M,

Zohaib S, Najeeb B, Sefat F. Potential of electrospun nanofibres for biomedical and dental applications. Materials. 2016;9(2):73.
27. Li D, Wang Y, Xia Y. Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays. Nano Lett. 2003; 3: 1167–1171.
28. Koski A, Yim K, Shivkumar S. Effect of molecular weight on fibrous PVA produced by electrospinning. Mater Lett. 2004; 58 (3-4);493-7.
29. Pillay V, Dott C, Choonara YE, Tyagi C, Tomar L, Kumar P, du Toit LC. Ndesendo VM. A review of the effect of processing variables on the fabrication of electrospun nanofibers for drug delivery applications. J. Nanomater. 2013; 1–22.
30. Sill TJ, von Recum HA. Electrospinning: Applications in drug delivery and tissue engineering. Biomater. 2008; 29: 1989–2006.
31. Lannutti J, Reneker D, Ma T, Tomasko D, Farson D. Electrospinning for tissue engineering scaffolds. Mater. Sci. Eng. C 2007; 27: 504–509.
32. Ioannis, S.C. Novel nanocomposites and nanoceramics based on polymer nanofibers using electrospinning process—A review. J. Mater. Process. Technol. 2005; 167: 283–293.
33. Fong H, Chun I, Reneker, D.H. Beaded nanofibers formed during electrospinning. Polymer.1999; 40: 4585–4592.
34. Ramakrishna S, Fujihara K, Teo W, Lim T, Ma Z. Electrospinning process. In An Introduction to Electrospinning and Nanofibers; World Scientific, Singapore. 2005;135–137.
35. Gupta P, Elkins C, Long TE, Wilkes GL. Electrospinning of linear homopolymers of poly(methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. Polymer 2005; 46: 4799-4810.
36. Huang L, Nagapudi K, Apkarian RP, Chaikof EL. Engineered collagen–PEO nanofibers and fabrics. J. Biomater. Sci. Polym. Ed. 2001; 12: 979–993.
37. Mit-uppatham C, Nithitanakul M, Supaphol P. Ultrafine electrospun polyamide-6 fibers: effect of solution conditions on morphology and average fiber diameter. Macromol Chem Phys. 2004; 205: 2327-2338.
38. Son WK, Youk JH, Lee TS, Park WH. The effects of solution properties and polyelectrolyte on electrospinning of ultrafine poly (ethylene oxide) fibres. polymer. 2004 ;45(9):2959-66.
39. Pelipenko J, Kristl J, Jankovic´ B, Baumgartner S, Kocbek P. The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibres. Int. J. Pharm. 2013; 456 (1):125–134.
40. De Vrieze S, Van Camp T, Nelvig A, Hagström B, Westbroek P, De Clerck K. The effect of temperature and humidity on electrospinning. J. Mater. Sci. 2004; 44 (5):1357–1362.
41. Galler KM. Clinical procedures for revitalization: current knowledge and considerations. Int Endod J. 2016;49(10):926-36.
42. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: a way forward. J Am Dent Assoc. 2016;147(5):372-80.
43. Albuquerque MT, Valera MC, Nakashima M, Nor JE, Bottino MC. Tissue-engineering based strategies for regenerative endodontics. J Dent Res 2014;93 (12):1222–31.
44. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Top 2013;28(1):2–23.
45. Iwaya SI, IkawaM, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol. 2001;17:185–7.
46. Kontakiotis EG, Filippatos CG, Agrafioti A. Levels of evidence for the outcome of regenerative endodontic therapy. J Endod. 2014;40:1045–53.
47. Galler KM, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S, et al. Dentin conditioning codetermines cell fate in regenerative endodontics. J Endod. 2011; 37:1536–41.
48. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod. 2012;38:1372–5.
49. Althumairy RI, Teixeira FB, Diogenes A. Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. J Endod. 2014;40:521–5.
50. Bottino MC, Kamocki K, Yassen GH, Platt JA, Vail MM, Ehrlich Y. Bioactive nano fibrous scaffolds for regenerative endodontics. J Dent Res. 2013;92:963–9.
51. Wang J, Windbergs M. Controlled dual drug release by coaxial electrospun fibers—Impact of the core fluid on drug encapsulation and release. Int J Pharm. 2019; 556: 363–371.
52. Pant B, Park M, Park SJ. Drug delivery applications of core-sheath nanofibres prepared by coaxial electrospinning: a review. Pharmaceutics. 2019;11(7):305.
53. Porter ML, Munchow EA, Albuquerque MT, Spolnik KJ, Hara AT, Bottino MC. Effects of novel 3-dimensional antibiotic-containing electrospun scaffolds on dentin discoloration. J Endod. 2016; 42:106–12.

54. Albuquerque MT, Ryan SJ, Munchow EA, Kamocka MM, Gregory RL, Valera MC, et al. Antimicrobial effects of novel triple antibiotic paste-mimic scaffolds on Actinomyces naeslundii biofilm. J Endod. 2015; 41:1337–43.
55. Albuquerque MT, Valera MC, Moreira CS, Bresciani E, de Melo RM, Bottino MC. Effects of ciprofloxacin-containing scaffolds on enterococcus faecalis biofilms. J Endod. 2015; 41:710–4.
56. Kamocki K, Nör JE, Bottino MC. Effects of ciprofloxacin containing antimicrobial scaffolds on dental pulp stem cell viability-In vitro studies. Arch Oral Biol. 2015; 60:1131–7.
57. Kamocki K, Nör JE, Bottino MC. Dental pulp stem cell responses to novel antibiotic-containing scaffolds for regenerative endodontics. Int Endod J. 2015; 48:1147–56.
58. Bottino MC, Yassen GH, Platt JA, Labban N, Windsor LJ, Spolnik KJ, Bressiani AH. A novel three□ dimensional scaffold for regenerative endodontics: materials and biological characterizations. J Tissue Eng Regen Med. 2015; 9(11):116-123.
59. Kim GH, Park YD, Leeetal SY. Odontogenic stimulation of human dental pulp cells with bioactive nano -composite fibre. J Biomater Appl. 2015; 29(6):854–866.
60. Bae WJ, Min KS, Kim JJ, Kim JJ, Kim HW, Kim EC, Odontogenic responses of human dental pulp cells to collagen/nano-bioactive glass nanocomposites. Dent Mater. 2012;28(12):1271–1279.
61. Kim JJ, Bae WJ, Kim JM, Kim JJ, Lee EJ, Kim HW, Kim EC. Mineralized polycaprolactone nanofibrous matrix for odontogenesis of human dental pulp cells. J Biomater Appl 2014; 28: 1069–1078.
62. Bottino MC, Albuquerque MT, Azabi A, Münchow EA, Spolnik KJ, Nör JE, Edwards PC. A novel patient□ specific three□ dimensional drug delivery construct for regenerative endodontics. J Biomed Mater Res Part B: Applied Biomater. 2019;107(5):1576-86.
63. Kim K, Luu YK, Chang C, Fang D, Hsiao BS, Chu B. Incorporation and controlled release of a hydrophilic antibiotic using poly(lactide-co-glycolide)-based electrospun nanofibrous scaffolds. J Control Release 2004; 98:47-56.