Near-field electrospinning polycaprolactone microfibers to mimic arteriole-capillary-venule structure

Imtiaz Qavi¹ · George Z. Tan¹

Received: 19 July 2021 / Accepted: 10 September 2021 / Published online: 22 September 2021
© Islamic Azad University 2021

Abstract

The ability to create three-dimensional (3D) cell-incorporated constructs for tissue engineering has progressed tremendously. One of the major challenges that limit the clinical applications of tissue engineering is the inability to form sufficient vascularization of capillary vessels in the 3D constructs. The lack of a functional capillary network for supplying nutrients and oxygen leads to poor cell viability. This paper presents the near-field electrospinning (ES) technique to fabricate a branched microfiber structure that mimics the morphology of capillaries. Polycaprolactone solution was electrospun onto a sloped collector that resulted in morphological and geometric variation of the fibers. With proper control over the solution viscosity and the electrospinning voltage, a single fiber was scattered into a branched fiber network and then converged back to a single fiber on the collector. The obtained fibers have a diameter of less than 100 microns at the two ends with coiled and branched fibers of less than 10 microns that mimics the arteriole-capillary-venule structure. The formation of such a structure in the near-field ES strongly depends on the solution viscosity. Low viscosity solutions form beads and discontinuous lines thus cannot be used to achieve the desired structure. The branching of PCL fiber occurs due to an electrohydrodynamic instability. The transition from the straight large fiber to smaller coiled/branched fibers is not instantaneous and stretches over a horizontal region of 1.5 cm. The current work shows the feasibility of electrospinning the stem-branch-stem fibrous structure by adopting a valley-shaped collector with potentials for tissue engineering applications.

Keywords Electrospinning · Microvasculature · Arteriole · Venule · Capillary

Introduction

Tissue engineering is an interdisciplinary research field that aims to develop therapeutic products integrated with living cells and biomaterials to repair or replace damaged tissues and organs (Mandrycky et al. 2017; Rustad et al. 2010; Shieh and Vacanti 2005). Most engineered tissues rely on a three-dimensional (3D) structure in which living cells are seeded and grown (Javaid and Haleem 2020; Marga et al. 2012; Zadpoor and Malda 2017). One of the biggest impediments towards complex tissue-engineered organ synthesis is the lack of a proper micro-circulatory network. The micro-circulatory system comprises the microvascular blood vessels like the arteriole, venule, and capillary network (Paula et al. 2010). The capillary network consists of a single-layered

endothelial wall and is responsible for the supply of vital nutrients, oxygen, and the removal of waste materials from different parts of the organ or tissue (Taylor and Moore 1999). A single capillary branch can exchange fluid within a few hundred micrometers (about 200–300 μm) in its proximity (Sukmana 2012). In absence of a proper distribution of capillary network, a necrotic core is formed which compromises the functionality of the engineered tissue or organ (Chang and Niklason 2017; Sharma et al. 2019; Vajda et al. 2021). Because of the challenge of vascularization, the successful clinical application of engineered tissues is currently limited to thin or a vascular tissues, such as skin or cartilage. To extend the application of tissue engineering, proper capillary vessel networks must be incorporated into the 3D biological constructs.

Recently different methods are under investigation for the synthesis of organs and tissues perfused with a microvascular network. Vasculature techniques include both scaffold cellularization and growth factor-induced cell culture techniques. The most common processes are scaffold functionalization

✉ George Z. Tan
george.z.tan@ttu.edu

¹ Department of Industrial, Manufacturing and Systems Engineering, Texas Tech University, Lubbock, USA

using Vascular Endothelial Growth Factor (VEGF), multicellular spheroid cultures, microfluidics, modular assembly, lithography-based patterned perfusion, Arteriovenous (AV) loop, Flap technique, angiogenic ingrowth, etc. (Laschke and Menger 2016; Lovett et al. 2009; Neumann et al. 2003; Tien 2011). Scaffold-based microvasculature has the advantages of rapid prototyping, customization, and forming complex mimicking structures compared to the randomly formed cell-based techniques. Different microfabrication techniques are currently being investigated for synthesizing the ideal biomimicking scaffolding structure for the microvasculature of artificial organs. Some of the notable processes such as photolithography, stereolithography, inkjet printing, laser-assisted bioprinting, casting, etc. have shown remarkable progress in fabricating both large and small vascular scaffolds for the synthesis of artificial vascular grafts (Vyas et al. 2017). But these fabrication methods are difficult to create channels with diameter structures less than 10 μm , which is the average size of human capillary vessels (Britanica). Self-guided assembly of cells on hydrogels using bio-laser-printing methods has shown potentials in capillary network fabrication but is still in its early development stage (Wu and Ringeisen 2010).

Electrospinning (ES) is a manufacturing process that can generate tubular fibers on a nano/micro-scale (Teo and Ramakrishna 2006). This process has the potentialities for fabricating tubular scaffolds for the smallest blood vessels in the human body i.e., the capillary blood vessels. Process parameters like voltage, polymer solution viscosity, needle traversing speed, spinning distance, etc. allow wide control over fiber morphology in the ES process (Beachley and Wen 2009; Haider et al. 2018; Li and Wang 2013b). An important parameter is the spinning distance which results in fiber morphology and diameter variations. This study hypothesizes that continuous and interconnected fibers of varying diameters will form on a collector with a graduate change in spinneret-to-collector distance. The resultant fibrous structure should mimic the morphology of the microvascular network. To test this hypothesis, a linearly traversing spinneret was used to electrospun fibers over a contoured collecting surface. Results show that this technique can be used to form a continuously branched fiber structure similar to the arteriole-capillary-venule structure (Fig. 1).

Materials and methods

Fiber fabrication process

The ES system used in the fiber fabrication process is shown in Fig. 2. The fiber fabrication process was carried out using the TL-Pro-BM Robotic Electrospinning Platform (Tongli@Tech, China). The robotic ES system can provide as high

Fig. 1 A simplified representative image for the human arteriole-capillary-venule network

as 50 kV voltage to both positive and negative terminals. Linear actuation was controlled using the ES system's built-in traversing system with digital speed inputs. A syringe pump was used to hydraulically provide a feed of the liquid polymer solution. The syringe pump system has two syringes interconnected via flexible tubing. Needles with a gauge value of 23 (0.337 mm internal diameter) were used as spinneret for the high voltage static charge nozzle for the electrospinning process. A poly(lactic) acid (PLLA)-based 3D printed collecting substrate was built using the Ultimaker 3D printer. The dimensions for the 3D model are shown in Fig. 3. The top surface of the 3D printed substrate was smoothly covered with 3.175 mm aluminum foil paper to act as the conducting surface for the negative terminal. The two ends of the 3D printed collecting surface had an elevation of 10 mm, which maintained a fixed gap of 5 mm with the needle. The middle surface, with an elevation of 5 mm maintained a gap of 10 mm with the spinneret. Following this design, observation of the change of fiber morphology over different spinneret-collector distances was made as the spinneret horizontally moved from one end to the other. A horizontal space of 35 mm was maintained at the initial and final planes and 50 mm in the middle plane. Slightly larger space was allocated for the middle region as the random, coiled fibers were expected to show greater variability than the continuous fibers as seen from the preliminary experiments.

Thus, this allowed collecting of greater number of images for obtaining more data points in the regions of high variability. The inclination angle was kept less than 11° as higher inclination could potentially disrupt the continuous collection of the jet when the spinneret to collector distance would change more rapidly. The spinneret was connected to a 2 kV positive terminal and the aluminum surface was connected to a -1 kV terminal. The

Fig. 2 The ES experimental setup **Fig. 3** The geometry of the contoured collector

actuation speed was set to 5 mm/s based on the results obtained from preliminary results. The solution flow rate in each case was set to 1.0 mL/h. Four different solution viscosities were tested to observe the continuation and fiber boundary regularity of the collected fibers using the same spinneret traversing speed and solution flow rate.

Polymer solution preparation

Polycaprolactone (PCL) pellets having an average molecular weight of 80,000 (Sigma-Aldrich®, St. Louis, MO) were used as the solute. The volatile solvent component used for the experiment was dichloromethane (DCM, $\geq 99.5\%$ stabilized ACS, VWR Chemicals BDH®). The solution concentrations prepared for the experiment were 12, 13, 14, and 15% (w/v). Solutions were used immediately after 4 h of mixing by magnetic stirrer. The properties of the PCL and DCM materials used for the experiment are shown in Tables 1 and 2.

Table 1 Physical properties of the solute

Chemical Formula	Average molecular weight	Melting point (°C)	Density (at 25 °C)	Impurity
PCL (C ₆ H ₁₀ O ₂) _n	80,000	60 °C	1.145 g/mL	<0.5% water

Experimental results

Viscosity measurement

A dynamic shear Rheometer (SmartPave 92, Anton Paar) was used to measure the rheological properties of the prepared solutions. A flat surface disk spindle having a diameter of 25 mm was used to apply rotary shear force ranging from 0 to 100 S⁻¹ with a data collection interval of 1 S⁻¹. The measurement and analysis mode was set to viscoelastic. The viscosity measurement was limited to 100 S⁻¹ owing to the volatile nature of DCM. Prolonged

Table 2 Physical properties of the solvent

Chemical formula	Vapor density (vs air)	Vapor pressure (mmHg, at 20 °C)	Boiling point (°C)	Melting point (°C)	Density g/cm ³ (at 25 °C)	Solvability for PCL (23 °C)
DCM CH ₂ Cl ₂	2.9	353	39.8 °C	– 95 °C	1.322	100

Fig. 4 Viscosity measurement plots for 12%, 13%, 14%, and 15% PCL concentration

experimental run and higher shear rate aids evaporation of DCM and thus resulting in erroneous values. The obtained plots for the viscosity of different solutions are shown in Fig. 4. The data is presented in a log-scale for the viscosity

values. It is observed that at 100 S⁻¹ solutions at 12% and 13% have almost similar viscosity levels whereas 14 and 15% solution concentrations have higher viscosity levels. This is possible due to the polymer chain entanglement effect in higher solution concentration that results in higher viscosity (Graessley 1967).

Fiber visualization

The fabricated fibers were collected on the modified collector base as shown in Fig. 5. For simplification, the initial 35 mm plane region with a spinneret-to-collector distance (SCD) of 5 mm is termed as “initial plane” (IP). The mid-plane spanning over 50 mm with SCD of 10 mm is termed as “middle plane” (MP) and the final plane with 35 mm span and SCD of 5 mm is termed as “final plane” (FP).

The aluminum fiber was carefully pulled off from the collector base and observed under Olympus DSX 500 digital microscope using a DSX-HR specialized 40× objective lens. The obtained images were further post-processed to obtain sharper and noise-free images. The fiber morphologies for all experimented solution concentrations at IP, MP, and FP regions are shown in Fig. 6. It is observed that almost all the solution concentrations could produce capillary-like microtubules from the qualitative images. The microtubules had

Fig. 5 **a** 3D printed variable height PLLA collector base. **b** Conductive aluminum layer attached on the collector base

Fig. 6 Fiber morphology in the IP, MP and FP regions of the contoured collector for (a) 12% (b) 13% (c) 14% (d) 15% PCL concentration

an average diameter of 3–10 μm for the tested solution concentration. These values are well within the acceptable range of human blood capillary indicating the potentiality of near-field ES for fabricating scaffold structures for microvascular cell culture. For solution concentrations of 12 and 13%, it is observed that fibers with irregularly formed boundaries are observed in the IP region. In the FP region, the solutions produced broken fibers. The solutions having a concentration of 14% and 15% showed consistent uniform boundary both in the IP and FB regions.

A Phenom table top scanning electron microscope (SEM) was used to investigate the fine fiber morphology. Figure 7 illustrates electron-microscopic images of fiber

morphologies in different regions of the modified collector. The results shown in Fig. 7 are for solution viscosity of 15%. The start and the end region produced fibers characterized by thick, solid tubular shapes with a diameter value of around 100 μm . Fibers in the divergent region show gradual thinning (reduction of diameter) of the main tubular trunk followed by outgrown smaller branched tubular structures. It is evident that with increase in SCD, the whipping instability is induced in the polymer solution jet (Huang et al. 2013). It was measured that at around 0.7 cm SCD, for the current experiment, whipping instability initialization occurs. In the MP region, fine tubular and branched structures having a diameter of less than 10 μm are observed. The convergent

Fig. 7 Scanning electron microscopy (SEM) images of fiber morphology at different parts of the collector for 15% solution concentration

region has a different pattern compared to the divergent zone. Visually it is observed that instead of forming a central trunk region, finer whipped fibers start to concentrate in a narrower region. The structure can be explained as overlapped microfibers in a narrow region forming a tubular structure.

The transition of fibers from linear and continuous to branched and coiled has been demonstrated in Figs. 8 and 9. Initially, the linear fiber in the IP region starts forming auxiliary lateral branches when the spinneret-to-collector distance (SCD) in the divergence section of the collector is at 0.60 cm as seen in Fig. 8a. At this point, a main fiber in

the center with lateral fiber branches is observed. Eventually, when the SCD increases to 0.75 cm, the main fiber undergoes whipping instability and starts forming a coil-like pattern instead of a linear pattern. The side-wise branches are still prominent as seen in Fig. 8b. Beyond 0.90 cm, the main fiber and the side branches are hardly distinguishable from the microscopic images as the SCD is large enough for the whipping instability to stretch the main fiber into a size comparable to the side branches. This has been demonstrated in Fig. 8c. The branching of fiber still exists even in the FP region, where fully electrospun coiled fibers are observed. Figure 9 portrays a scanning electron microscopic

Fig. 8 Optical microscope images of fiber transition and branching from continuous to branched for a spinneret-to-collector distance of (a) 0.60 cm (b) 0.75 cm (c) 0.90 cm in the divergence section for 15% solution concentration

(SEM) image of a branched coil fiber in the MP region. In this region, both the main micro-fiber and the side branches are in the scale region of 3–10 μm . It is to be mentioned that the transition from large diameter continuous and linear fiber to small diameter branched and coil pattern does not occur at an instantaneous point. The transition occurs over a region spanning a horizontal distance of approximately 1.5 cm in the inclined part of the collector.

The diameter of the fibers in the IP, MP, and FP regions was measured using the SEM's built-in measurement

system. The fiber diameter values are illustrated using a violin box-plot diagram in Fig. 10.

The continuous fibers in the IP and FP regions had average fiber diameters of approximately 95 and 100 μm , respectively for the 15% solution concentration. The capillary sized fibers in the MP region has fiber diameter ranging from 2 to 8 μm with an average value of 6.5. To investigate the variance in the fiber diameter, one-way ANOVA was conducted between diameter values of fibers obtained in the IP and FP regions. The results from the ANOVA analysis are tabulated in Tables 3 and 4.

Fig. 9 SEM Image of a branched micro-fiber in the MP region for 15% solution concentration

Fig. 10 Diameter distribution for different fiber morphologies for 15% solution concentration

Table 3 Average and Variance of obtained fibers

Groups	Count	Sum	Average	Variance
Initial plane (SCD=0.5 cm)	10	950.5	95.05	22.60722
Mid plane (SCD=1.0 cm)	10	55.99	5.599	5.356321
Final plane (SCD=0.5 cm)	10	1005.9	100.59	34.19656

conditions, it is possible to produce arteriole-capillary-venule-like structures with similar-sized start and end fiber diameters representing the arteriole and venule sections. Furthermore, the whipping instability induced microfibers ($< 10 \mu\text{m}$) produced in the MP region suggests the potentialities of the near-field ES system in generating capillary-like structures.

The results from ANOVA analysis suggest that a significant difference does not exist ($P < 0.05$) between the fibers produced in the IP and FP region. It is evident that for a solution viscosity of 15%, with the current experimental

Table 4 Analyses of variance between start and end fiber morphology (ANOVA)

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	364.658	1	364.658	8.401892	0.009574	4.413873
Within groups	781.234	18	43.40189			
Total	1145.892	19				

Discussion

The electrospinning process is a complex phenomenon that involves basic fluidic properties like viscosity, solution concentration, polymer entanglement, surface tension, charge conductance, etc. (Drew et al. 2003). Stable jet and fiber formation in the electrospinning process relies on the delicate harmonies of the whipping instability and Rayleigh–Taylor-induced droplet breakup (Shin et al. 2001). The whipping instability causes fiber stretching and eventually fine micro/nanofibers (Fridrikh et al. 2003). The Rayleigh-induced instability impedes fine and homogeneous boundary fiber formation when the surface tension force exceeds the viscous force (Cengiz et al. 2010). Drew et al. explained that beyond a minimum threshold value, the morphology of the electrospun fibers is less dependent on the viscosity parameter (Drew et al. 2003). This could potentially explain the irregularly formed boundaries for lower solution concentrations of 12% and 13%, signifying inadequate viscous drag force to generate a uniform boundary. Li and Wang postulate that for low-viscosity solutions, surface tension becomes the determinant factor and often result in beaded fibers (Li and Wang 2013a). Surface tension tends to form droplets by the process of capillary breakup in the spinning jet (Fong et al. 1999). This further strengthens the proposition of irregularly formed boundaries at lower viscosity solution, where surface tension might be responsible for boundary deformation. On the other hand, increased viscosity results in higher viscoelastic forces in the jet that resists boundary deformation by surface tension forces (Nezarati et al. 2013). This explains the phenomenon of regular boundaries formed in solutions having a viscosity of 14% and 15% in Fig. 6.

The effect of SCD on fiber morphology in the IP, MP, and FP regions can be explained with the help of “stable” zones formed in the electrospinning jet. Šimko and Lukáš used numerical simulations to show that in the electrospinning process, a stable zone is formed between the Taylor cone and the initiation point of the whipping instability (Šimko and Lukáš 2016). For the current near-field ES experimentation, it is possible that for an SCD of 0.5 cm, only a stable zone is formed. This explains continuously deposited linear fibers in the IP and FP regions. But when the SCD exceeds 0.7 cm, the intermediate distance is long enough to cause whipped fibers to form and acquire deposited on the aluminum surface. At SCD of 1.0 cm, the whipping instability causes the maximum bending and whirling of fiber that

results in coiled micro-fibers in the MP regions. The slight increase in fiber diameter for the 15% solution concentration in the MP region may be attributed to the high volatility of DCM. DCM is a highly volatile substance with an evaporation rate of 27.5 with reference to butyl acetate = 1 (NIH). It takes approximately 27 s for the needle to come to the FP region starting from the left-most end of the IP region. DCM in the Taylor-Cone may have undergone evaporation, thus resulting in higher solute concentration. Increased solute concentration results in higher viscosity (Li and Wang 2013b). Increased viscosity results in a larger fiber diameter assuming that the other ES parameters are constant (Nayak et al. 2013). Thus it is reasonable to assume that the slight increase in continuous fiber diameter in the FP region compared to the IP region is due to higher viscosity from solvent evaporation.

The transition from continuous and linear fiber to coiled and branched fiber is a function of the SCD distance increment. As the SCD distance increases, the whipping instability induced in the jetting fiber increases and thus stretches and bends the main fiber into a shorter diameter. The existence of lateral branches can be explained by an electrohydrodynamic theory proposed by Yarin et al. (2005). A perfectly smooth cylindrical electrospinning jet may develop static undulations under the combined effects of electric Maxwell stress and surface tension (Yarin et al. 2005). This undulated surface in the jet may experience instabilities at the sites of the highest local curvatures that result in emanating lateral branches (Yarin et al. 2005). This branching phenomenon was observed in a 15% (w/v) PCL-acetone solution jet using high-speed visualization techniques (Yarin et al. 2005). The existence of lateral branches in the current experiment aligns with the theory proposed in the work by Yarin et al.

The human microvascular system has interconnected arterioles ranging up to 100 μm in diameter and capillaries less than 10 μm (Levick 2013; Tuma et al. 2011). With proper solution viscosity, the fibers formed in the IP, MP, and FP regions were continuously connected and were within the diameter range. This validates our initial hypothesis that in-process SCD variation in the near-field electrospinning process can generate fibrous structures similar to the natural microcirculatory network. We plan to extend this work through the fabrication of porous tubular fibers bearing similar morphologies and hierarchies presented here. The microtube scaffolds can be seeded with endothelial cells and

checked for cellular viability for potential applications in the microvasculature.

In the future, electrospinning can be integrated into additive manufacturing (AM) technologies (Haleem and Javaid 2020; Haq et al. 2020; Javaid and Haleem 2018a) for fabrication of micro-vessel-incorporated scaffolds and implants. Tissue-engineered scaffolds for soft tissues synthesized using AM technology coupled with advanced technologies in the Industry 4.0 framework and Internet of things (IoT) have minimal wastage, rapid production rates, and can meet patient-specific needs (Ashima et al. 2021; Fatima et al. 2021; Javaid and Haleem 2020). For hard tissues, various advanced materials, such as polyether ether ketone (PEEK) (Haleem and Javaid 2019a, 2019b), have demonstrated both high biocompatibility and excellent mechanical properties which are similar to the native hard tissues in the human body for load-bearing applications (Haleem et al. 2020; Javaid and Haleem 2018b, 2019). The cell-laden implants integrated with biomimetic microtubes will have great potential for regenerative medicine.

Conclusion

This study demonstrates the feasibility of near-field ES in fabricating fibers with varying morphology using modified a collector base. The shift of SCD was implemented by the actuation of the spinneret over a contoured collector. This facilitated in-process variation of one of the ES parameters which resulted in fiber diameter and orientation changes. It was observed that below a certain viscosity range, uniform fiber boundary was not obtained in either the IP or FP regions. Coiled fibers bearing a diameter less than 10 μm were common to all the solution concentrations in the MP region. Higher solution yielded uniform fibers of approximately 100 μm in the IP and FP regions with 10 μm coiled fibers in the MP region. The converging and diverging regions showed the effect of SCD variation on the whipping process. The variation of SCD causes large diameter fibers to branch off into small diameter coiled fibers starting in the diverging region ($\text{SCD} = 0.7$ cm approximately) and vice versa in the converging region. The transition from single large fiber to smaller branched/coiled fiber occurs in the inclined planes with gradual narrowing and branching of the deposited fibers owing to initiation of the whipping instability. The whipping instability stretches and shrinks the fiber diameter from an SCD of 0.6 cm–0.9 cm in the inclined portion of the collector. The lateral branching of the fiber occurs owing to an electrohydrodynamic instability proposed in prior literature. The morphologies of the obtained fibers resemble the arteriole–capillary–venule structure as seen in humans. This experiment validates the hypothesis that near-field electrospinning with gradient SCD

will generate a branched microfiber structure similar to the natural microvascular network. Future experimental scope includes replicating the structure using the core-sheath electrospinning technique to fabricate a hollow tubular structure with varying geometry and morphology for internal cell culture. The core-sheath hollow structure seeded with endothelial and smooth muscle cell culture on the fabricated scaffolds would provide valuable insights on the usability of this technique in the vascularization of synthesized tissues or organs.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Ashima R, Haleem A, Bahl S, Javaid M, Mahla SK, Singh S (2021) Automation and manufacturing of smart materials in additive manufacturing technologies using internet of things towards the adoption of industry 4.0. *Mater Today* 45:5081–5088. <https://doi.org/10.1016/j.matpr.2021.01.583>
- Britannica. Capillary. <https://www.britannica.com/science/capillary>
- Beachley V, Wen X (2009) Effect of electrospinning parameters on the nanofiber diameter and length. *Mater Sci Eng, C* 29(3):663–668. <https://doi.org/10.1016/j.msec.2008.10.037>
- Cengiz F, Dao TA, Jirsak O (2010) Influence of solution properties on the roller electrospinning of poly (vinyl alcohol). *Polym Eng Sci* 50(5):936–943. <https://doi.org/10.1002/pen.21599>
- Chang WG, Niklason LE (2017) A short discourse on vascular tissue engineering. *NPJ Regenerative Med* 2(1):1–8. <https://doi.org/10.1038/s41536-017-0011-6>
- Drew C, Wang X, Samuelson LA, Kumar J (2003) The effect of viscosity and filler on electrospun fiber morphology. *J Macromol Sci Part A* 40(12):1415–1422. <https://doi.org/10.1081/MA-120025320>
- Fatima S, Haleem A, Bahl S, Javaid M, Mahla SK, Singh S (2021) Exploring the significant applications of internet of things (IoT) with 3D printing using advanced materials in medical field. *Materials Today* <https://doi.org/10.1016/j.matpr.2021.01.305>
- Fong H, Chun I, Reneker DH (1999) Beaded nanofibers formed during electrospinning. *Polymer* 40(16):4585–4592. [https://doi.org/10.1016/S0032-3861\(99\)00068-3](https://doi.org/10.1016/S0032-3861(99)00068-3)
- Fridrikh SV, Jian HY, Brenner MP, Rutledge GC (2003) Controlling the fiber diameter during electrospinning. *Phys Rev Lett* 90(14):144502. <https://doi.org/10.1103/PhysRevLett.90.144502>
- Graessley WW (1967) Viscosity of entangling polydisperse polymers. *J Chem Phys* 47(6):1942–1953. <https://doi.org/10.1063/1.1712222>
- Haider A, Haider S, Kang I-K (2018) A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arab J Chem* 11(8):1165–1188. <https://doi.org/10.1016/j.arabjc.2015.11.015>

- Haleem A, Javaid M (2019a) Polyether ether ketone (PEEK) and its 3D printed implants applications in medical field: an overview. *Clinical Epidemiology and Global Health* 7(4):571–577. <https://doi.org/10.1016/j.cegh.2019.01.003>
- Haleem A, Javaid M (2019b) Polyether ether ketone (PEEK) and its manufacturing of customised 3D printed dentistry parts using additive manufacturing. *Clin Epidemiol Global Health* 7(4):654–660. <https://doi.org/10.1016/j.cegh.2019.03.001>
- Haleem A, Javaid M (2020) 3D printed medical parts with different materials using additive manufacturing. *Clin Epidemiol Global Health* 8(1):215–223. <https://doi.org/10.1016/j.cegh.2019.08.002>
- Haleem A, Javaid M, Khan RH, Suman R (2020) 3D printing applications in bone tissue engineering. *J Clin Orthopaedics Trauma* 11:118–124. <https://doi.org/10.1016/j.jcot.2019.12.002>
- Haq MIU, Khuroo S, Raina A, Khajuria S, Javaid M, Haq MFU, Haleem A. (2020). 3D printing for development of medical equipment amidst coronavirus (COVID-19) pandemic—review and advancements. *Research on Biomedical Engineering*, 1–11. <https://doi.org/10.1007/s42600-020-00098-0>
- Huang Y, Bu N, Duan Y, Pan Y, Liu H, Yin Z, Xiong Y. (2013). Electrohydrodynamic direct-writing. *Nanoscale*, 5(24):12007–12017. <https://doi.org/10.1039/C3NR04329K>
- Javaid M, Haleem A (2018a) Additive manufacturing applications in medical cases: a literature based review. *Alexandria J Med* 54(4):411–422. <https://doi.org/10.1016/j.ajme.2017.09.003>
- Javaid M, Haleem A (2018b) Additive manufacturing applications in orthopaedics: a review. *J Clin Orthopaedics Trauma* 9(3):202–206. <https://doi.org/10.1016/j.jcot.2018.04.008>
- Javaid M, Haleem A (2019) Current status and applications of additive manufacturing in dentistry: a literature-based review. *J Oral Biol Craniofacial Res* 9(3):179–185. <https://doi.org/10.1016/j.jobcr.2019.04.004>
- Javaid M, Haleem A (2020) 3D printed tissue and organ using additive manufacturing: an overview. *Clin Epidemiol Global Health* 8(2):586–594. <https://doi.org/10.1016/j.cegh.2019.12.008>
- Laschke MW, Menger MD (2016) Prevascularization in tissue engineering: current concepts and future directions. *Biotechnol Adv* 34(2):112–121. <https://doi.org/10.1016/j.biotechadv.2015.12.004>
- Levick JR. (2013). *An introduction to cardiovascular physiology*: Butterworth-Heinemann.
- Li Z, Wang C. (2013a). Effects of working parameters on electrospinning. In *One-dimensional nanostructures* (pp. 15–28): Springer. https://doi.org/10.1007/978-3-642-36427-3_2
- Li Z, Wang C (2013b) One-dimensional nanostructures: electrospinning technique and unique nanofibers: Springer-Verlag. Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-36427-3>
- Lovett M, Lee K, Edwards A, Kaplan DL (2009) Vascularization strategies for tissue engineering. *Tissue Eng Part B Rev* 15(3):353–370. <https://doi.org/10.1089/ten.teb.2009.0085>
- Mandrycky C, Phong K, Zheng Y (2017) Tissue engineering toward organ-specific regeneration and disease modeling. *MRS Commun* 7(3):332. <https://doi.org/10.1557/mrc.2017.58>
- Marga F, Jakab K, Khatiwala C, Shepherd B, Dorfman S, Hubbard B, Colbert S, Forgacs G. (2012). Toward engineering functional organ modules by additive manufacturing. *Biofabrication*, 4(2):022001.
- Nayak R, Padhye R, Kyratzis IL, Truong YB, Arnold L (2013) Effect of viscosity and electrical conductivity on the morphology and fiber diameter in melt electrospinning of polypropylene. *Text Res J* 83(6):606–617. <https://doi.org/10.1177/0040517512458347>
- Neumann T, Nicholson BS, Sanders JE (2003) Tissue engineering of perfused microvessels. *Microvasc Res* 66(1):59–67. [https://doi.org/10.1016/S0026-2862\(03\)00040-2](https://doi.org/10.1016/S0026-2862(03)00040-2)
- Nezarati RM, Eifert MB, Cosgriff-Hernandez E (2013) Effects of humidity and solution viscosity on electrospun fiber morphology. *Tissue Eng Part C Methods* 19(10):810–819. <https://doi.org/10.1089/ten.tec.2012.0671>
- NIH. Dichloromethane (2021) https://www.euro.who.int/__data/assets/pdf_file/0013/123061/AQG2ndEd_5_7Dichloromethane.pdf
- Paula KY, Balaratnasingam C, Cringle SJ, McAllister IL, Provis J, Yu D-Y (2010) Microstructure and network organization of the microvasculature in the human macula. *Invest Ophthalmol vis Sci* 51(12):6735–6743. <https://doi.org/10.1167/iovs.10-5415>
- Rustad KC, Sorkin M, Levi B, Longaker MT, Gurtner GC (2010) Strategies for organ level tissue engineering. *Organogenesis* 6(3):151–157. <https://doi.org/10.4161/org.6.3.12139>
- Sharma D, Ross D, Wang G, Jia W, Kirkpatrick SJ, Zhao F (2019) Upgrading prevascularization in tissue engineering: a review of strategies for promoting highly organized microvascular network formation. *Acta Biomater* 95:112–130. <https://doi.org/10.1016/j.actbio.2019.03.016>
- Shieh S-J, Vacanti JP (2005) State-of-the-art tissue engineering: from tissue engineering to organ building. *Surgery* 137(1):1–7. <https://doi.org/10.1016/j.surg.2004.04.002>
- Shin Y, Hohman M, Brenner M, Rutledge G (2001) Experimental characterization of electrospinning: the electrically forced jet and instabilities. *Polymer* 42(25):09955–09967. [https://doi.org/10.1016/S0032-3861\(01\)00540-7](https://doi.org/10.1016/S0032-3861(01)00540-7)
- Šimko M, Lukáš D (2016) Mathematical modeling of a whipping instability of an electrically charged liquid jet. *Appl Math Model* 40(21–22):9565–9583. <https://doi.org/10.1016/j.apm.2016.06.018>
- Sukmana I (2012) Microvascular guidance: a challenge to support the development of vascularised tissue engineering construct. *Sci World J* 2012:201352. <https://doi.org/10.1100/2012/201352>
- Taylor AE, Moore TM (1999) Capillary fluid exchange. *Adv Physiol Educ* 27(6):S203
- Teo WE, Ramakrishna S (2006) A review on electrospinning design and nanofibre assemblies. *Nanotechnology* 17(14):R89. <https://doi.org/10.1088/0957-4484/17/14/R01>
- Tien J (2011) Tissue engineering of the microvasculature. *Compr Physiol* 9(3):1155–1212. <https://doi.org/10.1002/cphy.c180037>
- Tuma RF, Durán WN, Ley K. (2011). *Microcirculation* (Vol. 4): Academic Press.
- Vajda J, Milojević M, Maver U, Vihar B (2021) Microvascular tissue engineering—a review. *Biomedicines* 9(6):589. <https://doi.org/10.3390/biomedicines9060589>
- Vyas C, Pereira R, Huang B, Liu F, Wang W, Bartolo P (2017) Engineering the vasculature with additive manufacturing. *Curr Opin Biomed Eng* 2:1–13. <https://doi.org/10.1016/j.cobme.2017.05.008>
- Wu P, Ringeisen B (2010) Development of human umbilical vein endothelial cell (HUVEC) and human umbilical vein smooth muscle cell (HUVSMC) branch/stem structures on hydrogel layers via biological laser printing (BioLP). *Biofabrication* 2(1):014111. <https://doi.org/10.1088/1758-5082/2/1/014111>
- Yarin A, Kataphinan W, Reneker DH (2005) Branching in electrospinning of nanofibers. *J Appl Phys* 98(6):064501. <https://doi.org/10.1063/1.2060928>
- Zadpoor AA, Malda J. (2017). Additive manufacturing of biomaterials, tissues, and organs. *Ann Biomed Eng* volume 45:1–11 (2017). <https://doi.org/10.1007/s10439-016-1719-y>