Chapter 20
Electrospinning and Three-Dimensional (3D) Printing for Biofabrication

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Abstract Biofabrication of engineered cell-laden constructs and scaffolds is essential for tissue engineering and tissue modeling. Electrospinning is a highly scalable technology to fabricate porous scaffolds with micro or nano-fibrous structure. Three-dimensional (3D) bioprinting has been recently developed for tissue engineering by providing control over cell location and multicellular structure. With the availability of electrospinning techniques, it is possible to combine nano- and microfiber-based

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structures with 3D bioprinted constructs, to obtain composite structures that have biomimetic or functional features and improved mechanical stability. In addition, electrospun fibers can add various functions such as drug release properties to the developed 3D bioprinted constructs. In this chapter, we will discuss the techniques for electrospinning, 3D bioprinting, and the approach of combining electrospinning and 3D bioprinting for biofabrication. We also highlight current challenges and future research directions.

Keywords 3D bioprinting · Electrospinning · Regeneration · Tissue engineering · Tissue models

20.1 Introduction

Tissue loss results in the loss of function and integrity of tissues and organs and therefore, it requires treatment [1]. The gold standard of treatment is the use of autografts [2]. However, because of limited supply and donor site morbidity, their use is not widely spread [3]. Allografts or even xenografts can also be used for the treatment of tissue defects [4]. Although these are available without limitations, their use is associated with the risk of infective agent transmission [5] and other concerns [6]. Therefore, the use of biomaterials for tissue repair has been explored [7–11]. For the purpose of tissue replacement, reconstruction and repair, various biostable or biodegradable biomaterials were developed [7, 12]. Unfortunately, these are not living and can lead to unwanted inflammatory response [13, 14], fibrous tissue encapsulation [15, 16], infection [17] and failure [18]. The use of bioabsorbable membranes for in situ tissue formation by guiding tissue regeneration has been explored [19–22]. However, this is limited to the treatment of small tissue defects. The advent of tissue engineering led to the development of tissue-like constructs produced by seeding cells into biodegradable scaffolds that have been usually made from polymers [23] with or without ceramics [24, 25] and additives such as growth factors [26–28]. Inability to ensure homogenous cell distribution in these scaffolds often led to failure of engineered tissues in vivo [29, 30]. The development of three-dimensional (3D) printing [31] and its adoption to 3D bioprinting led to development of a more advanced generation of engineered tissue constructs [32–35]. In 3D bioprinting, cells are combined with biomaterial and together printed into constructs. This way, more homogeneous and controlled deposition of cells, pore size or the spatial distribution of pores in the constructs can be achieved [36]. Furthermore, different types of materials or cells and additives as well as gradients can be built into these constructs [37, 38] to develop more complex structures and interface tissues such as muscle/tendon, tendon/bone or bone/cartilage interface tissues [39–43].

Today, 3D bioprinting represents a powerful approach for the fabrication of potentially successful engineered tissue constructs that can be used for the repair or regeneration of human tissues and organs [44–46]. Despite major advances that have been made in 3D bioprinting [3, 47–49], this method is still associated with many

limitations that hinder its translation to the clinic [50, 51]. Among the important challenges is the relatively low mechanical properties of 3D bioprinted constructs [52], which limits their use in mechanically demanding applications such as hard tissue engineering [33, 53, 54]. To tackle this problem, various approaches have been investigated, and they included so far the use of ceramic particles [55–57] or fibers [58–60] in the bioinks and external support frames [61, 62] or sacrificial materials that provide temporary support [63, 64]. The latter helps to build larger sized of constructs but does not provide permanent support to printed constructs and thus, it does not contribute to the improvement of their mechanical properties. In addition, pore size in constructs created by 3D bioprinting promote the deposition of cells in the bottom of scaffolds [65]. Constructs made using 3D bioprinting also lack the nano-/microfibrous structure the native extracellular matrix (ECM) has [66], which beside other functions is suggested to enhance initial cell attachment and proliferation [67]. Native ECM can also store and release biomolecules [68], which can similarly be mimicked by using drug releasing nanofibers to develop novel biomimetic 3D bioprinted constructs.

Therefore, the development of methods to enhance mechanical properties of the 3D bioprinted scaffolds and improve their biomimetic properties are necessary to overcome the current limitations of 3D bioprinted tissue constructs and enhance their applications in the clinic [69, 70]. Among important strategies to achieve this is the combination of the 3D bioprinting technique with other fabrication methods to create the 3D constructs that have controlled pore structures and nano-scale features [65] as well as improved mechanical properties [71, 72]. In this respect, the use of nanofibers is an attractive and useful technique in many aspects, that include providing mechanical reinforcement to the constructs and improving their ECM biomimetic properties. There are different methods that can be used for the production of nanofibers, among which electrospinning [73] is an attractive technique because it is characterized by its simplicity, versatility and scalability [73–75]. Recently, methods to combine electrospinning and 3D bioprinting have been developed [76–79], and are expected to have an impact on the development of new generation of engineered tissue constructs, that can have enhanced cell attachment and function. Therefore, in this chapter, we go over electrospinning and 3D bioprinting methods and their combination towards developing better engineered constructs and look at challenges and future directions to provide important information for readers and stimulate the development of new ideas to advance the field of tissue engineering.

20.2 Electrofabrication

Electrofabrication techniques are electrohydrodynamic methods that use electrostatic forces to generate polymeric nano-microstructures. Electrospinning is the most widely investigated electrofabrication technique for fabricating nano-microfiber based membranes for tissue engineering and wound healing applications [80, 81]. Other related approaches such as electro-spraying and melt electrospinning are also

explored in biomedical applications. In electrospinning, an electric field is applied to polymer solution so that polymer droplets can move towards an oppositely charged collector as fibers. Unlike electrospinning, electrospraying uses less viscous polymer solutions and generates nano-microparticles that can be used in drug delivery applications. Melt electrospinning uses molten polymer which is taken in a heated syringe or comes from an extruder, followed by charging the polymer melt with an electric field to produce fibers. Electrospun nanofibrous materials have a high surface-to-volume porosity ratio and can be produced in a broad range of sizes and forms [82]. These specific characteristics of nanofibrous materials provide flexibility to use them in a wide variety of biomedical and tissue engineering applications [83, 84].

Electrospinning is one of the well-established methods for generating nano/submicron fibrous materials having a soldering-like attachment at intersections formed by entanglement of polymer fibers [85]. Nanofibrous membranes are well accepted in bioengineering applications due to their high processing flexibility that helps to optimize their physical parameters like fiber diameter, thickness, porosity and pattern formation. One of the most significant advantages of electrospinning technique is that the fibers produced by it can mimic the fiber-like architecture of extracellular matrix (ECM). Electrospun fibers can be used in various tissue engineering applications, including vascular grafts [86], skin tissue engineering, and bone regeneration [87, 88]. Electrospun membranes are also being used as wound dressings and wound healing patches [89, 90]. The advantage of electrospinning in the context of biomedical applications is that fibers based made of a wide range of polymers, and their blends with other polymers, their composites with non-polymeric fillers can be produced with varying physicochemical as well as biological performance. Moreover, electrospun polymeric meshes can be loaded with suitable bioactive molecules or drugs [91–95] to provide tissue regeneration capacity as well as to prevent problems at the implantation site [96].

20.2.1 Electrofabrication Methods: Principles, Techniques and Conditions

20.2.1.1 Electrospinning

Electrospinning employs a direct current (DC) electric voltage in the range of 5–30 kV to generate highly porous mesh like structures composed of nano-micron fibers with high surface area to volume ratio [97]. Both synthetic polymers such as poly(vinyl alcohol) (PVA) [98], poly-L/D-lactide (PLDLA) [99, 100], poly(lactide-*co*-glycolide) (PLGA) [92], polycaprolactone (PCL) [100, 101] and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)] and natural polymers such as chitosan, elastin, collagen, gelatin, fibrinogen and fibrin [102, 103] hyaluronic acid (HA) [104], and silk fibroin) or the combination of both classes can be used to make electrospun scaffolds. Generally, polymers which are dissolved in an appropriate

solvent or molten polymer are constantly expelled from a syringe at slow flow rate (Usually, 0.5–3 mL/h). When a high DC voltage in the range of 5–30 kV is applied on the polymer droplet coming out, the polymer solution in the syringe gets charged, and the droplet tends to move towards the grounded collector as submicron fibers. The solvent evaporates and relatively dried polymer fibers will be deposited on the collecting platform as a membrane or a mesh. Typical electrospun scaffolds used for biomedical applications show 70–90% porosity and pores with 5–150 μm of average diameters [105]. The scaffolds have the potential to produce functional tissues with nano-size fibrous structures that replicate native ECMs and have connected pores [106–108].

Synthetic polymer-based scaffolds show excellent mechanical properties, stability, and controlled degradability. However, they generally lack bioactivity. Other natural materials, such as different collagen types, laminin and glycosaminoglycans may be used as coating on fibers to promote cell differentiation, growth, and adhesion [109, 110]. Electrospun scaffolds can be used for a wide range of purposes, including bone, cartilage, vascular and neural tissue engineering [111, 112]. Natural polymers generally show good biocompatibility and cell proliferation properties [113]. However, their strength and stability in the human body are not very high. Thus, blending of natural polymers with synthetic biopolymers is promising approach in biomedical research. For example, vascular scaffolds developed by blending of natural polymers like collagen or elastin and synthetic polymers like PLGA or PCL are highly promising for vascular reconstruction due to their suitable mechanical properties, degradability, cell proliferation and biocompatibility [114].

Electrospinning process is influenced by several equipment related, polymer solution related and ambient parameters (Table 20.1). These factors influence fiber morphology. Through proper control of these parameters, fibers with desirable morphologies and diameters can be produced [115]. A stable process requires a suitable range of viscosity and polymer solution concentration [116, 117]. Conductivity and polarity of the solvent is another important property of the polymer solution that influence the process of electrospinning. The increased conductivity or charge density of polymer solutions usually results in enhanced ion mobility and polymer jet whipping instability during electrospinning [118, 119]. Solvent properties, such as the boiling point, can influence solvent evaporation in electrospinning, causing either smooth surfaced fibers or those with nano-porosity on the surface. It can also affect the morphology of electrospun fibers [120]. The Taylor cone, a cone like structure formed by the deformation of polymer droplet at the tip of spinneret, shrinks and stretches towards collector as the applied voltage is increased during electrospinning, resulting in the formation of fibers with smaller diameters. A low feeding rate usually generates small droplets at the tip of needle, smaller Taylor cone and facilitates the rapid evaporation of solvents, which produces electrospun fibers with smaller diameters.

The consequences of environmental factors such as temperature and moisture on electrospinning must not be neglected. A slight temperature rise can reduce polymer solution viscosity and can result fibers with smaller diameters. Electrospinning at sufficiently low temperatures can considerably delay solvent evaporation and lead

Table 20.1 Summary of the effect of various electrospinning parameters on fiber morphology, fiber diameter, porosity and fiber spacing

Parameter	Effect on fiber morphology	Effect on fiber diameter	Effect on porosity
Solution parameters			
Polymer concentration	Low-bead generation, disappearance of beads with increase in concentration [125, 126]	Increase in fiber diameter with increased polymer-concentration [127–129]	Decrease in porosity with increased polymer concentration [130]
Viscosity	Low-bead formation, disappearance of beads with increase in viscosity [131]	Increase in fiber diameter with increase in viscosity [132]	–
Molecular weight of the polymer	Reduction in the number of beads and droplets with increase in molecular weight [133–135]	Increase in fiber diameter with increase in molecular weight [136]	Increase in molecular weight decreases porosity [137]
Solution conductivity	Higher conductivity leads to defect-free fibers [138–140]	Decrease in fiber diameter with an increase in conductivity [126, 128]	Increasing conductivity leads to increased porosity [141, 142]
Processing parameters			
Flow rate	Generation of beads with too high flow rate [143, 144]	Decrease in fiber diameter with decrease in flow rate [145]	Increase in flow rate results in increased porosity [146]
Distance between tip and collector	Generation of beads with too small and too large distance, minimum distance required for uniform fibers [147]	Decrease in fiber diameter with increase in distance [148, 149]	Increase in distance leads to decrease in porosity [150]
Applied voltage	Increase in voltage results in bead formation [139, 151]	Increase in voltage may decrease fiber diameter [115, 152, 153]	Increase in applied voltage increases porosity [154]
Collector type	Influence structural morphology of electrospun fibers. A non-conductive collector creates a porous structure with circular pores on fiber surfaces. Using patterned collectors influences fiber alignment and uniformity [155]	Fiber diameter can be tailored by modifying the collector according to requirements [156, 157]	Porosity can be tuned by using appropriate collectors [158]

(continued)

Table 20.1 (continued)

Parameter	Effect on fiber morphology	Effect on fiber diameter	Effect on porosity
Ambient parameters			
Humidity	High humidity results in circular pores on the fibers [122, 159]	Increasing humidity results in uniform fibers upto a certain limit [160]	Porosity increases with increasing humidity followed by flat beads
Temperature	Increasing temperature leads to decrease in viscosity and increase in solvent evaporation resulting in low bead formation [161]	Increase in temperature results in decrease in fiber diameter [162]	–

to solid–liquid phase separation and consequently lead to the generation of tunable porous surface morphology on the fibers [121]. The effects of increasing humidity on the fiber surface are similar for solvent evaporation and pore formation [122]. The combination of breath figures and supercritical fluids ($SCCO_2$) techniques allows the preparation of porous scaffolds with interconnected outer and inner porosity. These scaffolds with secondary pore formation and fiber interconnectivity exhibit good cell adhesion as well as cell proliferation indicating their huge potential in tissue engineering and wound healing applications [123, 124].

20.2.1.2 Melt Electrospinning

Melt electrospinning, an advanced technique in electrospinning, can produce precisely deposited fibrous scaffolds having a porous architecture and has been inspired by additive manufacturing techniques. A molten polymer jet is ejected or extruded from a reservoir and driven towards the collector with the help of a high-voltage as in solution electrospinning. In recent years, this technique has become increasingly popular because it permits the direct production of scaffolds with defined structures as in 3D printing [163]. Melt electrospinning creates 3D fibers by programmed stacking and addresses issues such as difficulty in the formation of thick and interconnected pores with a defined pattern of fibers. The range of suitable materials that can be used for melt electrospinning is however limited as most of the bioactive agents are susceptible to damage at high-temperature required in melt electrospinning [164].

Melt spinning is the most cost-effective spinning method because no solvent is recovered or evaporated, as in solution spinning, and the spinning rate is relatively high. Sutures are the most common biomaterials that can be produced with the help of melt-electrospinning. However, the use of melt electrospun matrices as tissue

engineering scaffolds is increasingly being investigated [163]. Melt electrospun scaffolds with multifilament yarns are also being investigated as vascular grafts. Melt electro writing (MEW), which incorporates 3D printing technology to melt electrospinning, makes it possible to print porous scaffolds with well-defined geometrical features of ultrafine fibers for tissue engineering applications [165, 166]. No solvent is needed in the spinning process, so spun polymers are highly pure. In another study, double-layered tubular scaffolds containing both melt-spun macrofibers (<200 μm in diameter) and electrospun submicron fibers (>400 nm in diameter) were developed [167]. Thus, melt electrospinning can be considered as a promising electrofabrication method in tissue engineering and wound healing applications due to the advantages such as solvent free fabrication process and the ability to control the fiber architecture using recent approaches like MEW [69].

20.2.1.3 Electrospraying

Electrospraying is a process that uses the same processes as in electrospinning, but which produces nano or microcapsules. Fluid from a capillary nozzle is exposed to electrical forces, and due to this, droplets move towards the oppositely charged collecting platform as nano or microparticles. First, electric forces deform the natural spherical meniscus and result in the formation of Taylor cones, just as in electrospinning process. Electrically charged jet is accelerated through an electric field by electric repulsion of charges on the surface and breaks into droplets at the free-end. Droplet production process can be pulsating or continuous, depending on the physical properties of the solution, fluid flow rate, magnitude and the polarity of the potential imposed on the nozzle [168, 169]. No mechanical energy other than the electric field supplied is used for electrohydrodynamic atomization. Droplets formed by electrohydrodynamic atomization have an electric charge that is typically half that of the Rayleigh limit, which is the maximum amount of power on a drop that produces a repulsive force equal to the strength of the surface tension. Electrospraying has several advantages over traditional mechanical induction-based droplet spraying systems such as the ability to develop equally sized droplets, droplet sizes can be less than 1 μm in diameter, less agglomeration and coagulation of droplets, and the ability to control spatial deposition of charged droplets. Electrospraying has been extensively used to develop drug delivery systems for diverse medical applications [170–172].

20.2.2 Developments and Generations of Electrofabrication Methods

Early in the twentieth century, the first patent on electrospinning was granted. Zeleny published a paper in 1914, in which he tried to mathematically model the behavior of

fluids in an electrostatically charged field [173]. In the early 1960s, Taylor was a trailblazer in the field of jet-forming mechanisms [174]. In subsequent literature, the conical shape of the jet was referred to as "Taylor cone" by scholars. To offset the surface with electrostatic forces, Taylor discovered that a 49.3° angle is required. In the following years, researchers concentrated on researching the structural morphology of nanofibers and gaining a better understanding of how nanofiber properties are affected by fiber morphology and process parameters. In 1987, Hayati et al. investigated the effects of an electrical field, laboratory circumstances, and all other factors that influence fiber stability, morphology, and atomization [175]. They found that coupling a high-conducting fluid with a high electrostatic field resulted in an extremely unstable current that looped and whipped around in different directions. Their findings also showed that this unstable jet generated fibers with much wider diameter distributions. Due to expanded awareness of the possible applications of electrospun nanofibers in different industries, electrospinning activity has increased significantly after a two-decade hiatus [176].

Researchers are also trying to improve the productivity of electrospinning as it is very important for commercialization. By replacing the needle with a wide-open bath of polymer solution and a copper spiral coil (electrode) inside the polymer solution, needleless electrospinning solves the issue of low efficiency and nonuniform fiber orientation [177, 178]. The copper coil acts as a fiber generator when an electrostatic force is applied. When the copper coil and polymer solution are charged, several jets are launched from the copper coil. More and more jets are launched from the coil and deposited on the spinning drum's surface as the voltage is increased. This method has the benefit of increased production as well as improved fiber orientation and alignment. Electrospinning with rotating electrodes is another approach for dealing with problems like poor efficiency and fiber orientation uniformity [179]. A collector screen is suspended over strings of electrodes rotating in a bath of polymer solution. The electrodes are usually attached to the positive terminal of the power supply, while the collector screen is either grounded or connected to the negative terminal of the power supply. Electrode strings, rather than using several spinnerets, execute the same operation with more control and ease of fabrication [180]. Compared to conventional electrospinning, this process is less costly and quicker. In addition to these, several other experiments were carried out to develop electrospinning method with large scale production potential.

Stable (non-porous) nanofibers with a smooth or nano-porous surface are generated by conventional electrospinning with a polymer solution (due to gradual evaporation of solvent from the polymer solution) (Fig. 20.1a). Electrospinning using an emulsion is known as emulsion electrospinning, where fibers with core–shell structure can be obtained. Cores of nanofibers made by emulsion electrospinning is ideal reservoirs for the encapsulation and protection of bioactive molecules [181]. As a result, emulsion electrospinning has been thoroughly investigated for the development of nanofibrous delivery vehicles for the controlled release of medications, growth factors, genes, and other biomolecules. During emulsion electrospinning, two immiscible solutions are spun out through two spinnerets and extended by electrostatic force. The driving liquid is a solution with a high conductivity that passes the

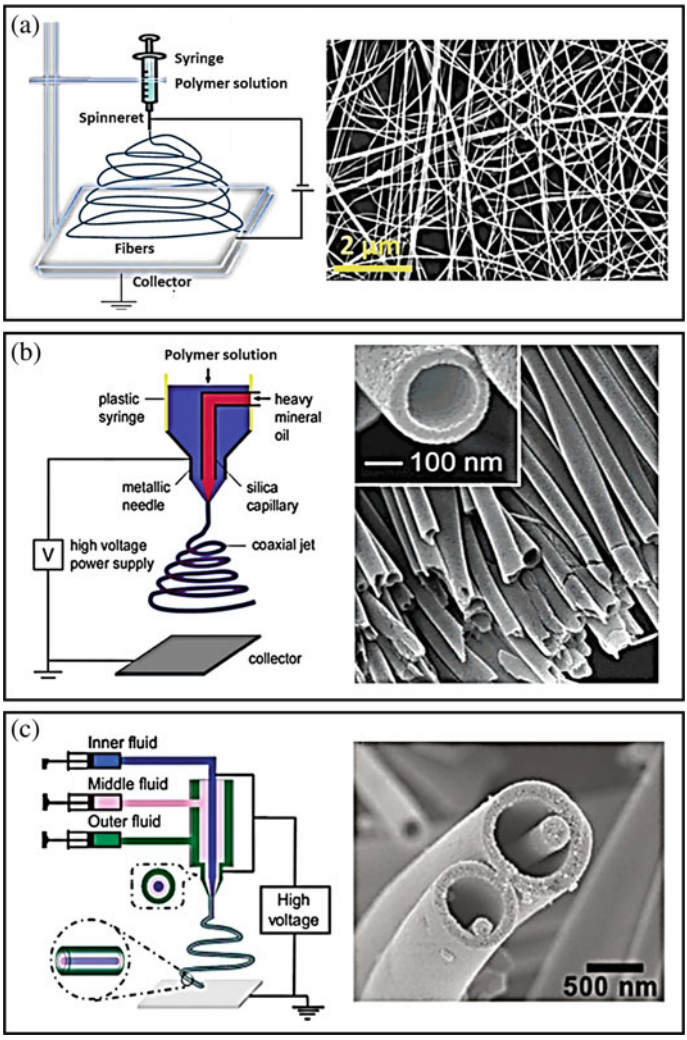

Fig. 20.1 Major types of electrospinning based on spinnerets. **a** Single needle approach. **b** Coaxial electrospinning. **c** Triaxial electrospinning. Figure a is reproduced from [209], with permission from Elsevier. Figure b is reproduced from [210], with the permission of American Chemical Society. Figure c is reproduced from [211], with permission from the American Chemical Society

tangential electrostatic force by viscosity on the liquid interface and deforms fused droplets to form small jets with an aqueous interior, gradually resulting in core–shell shaped nanofibers when stretched enough [182, 183]. If bioactive factors can be directly dissolved or evenly blended in the polymer solution before electrospinning, this can facilitate high loading and uniform distribution of the active agents in the resulting fibers [184, 185]. When this method is used alone, a large burst release of

active agents is observed [186]. It has been shown that altering the polymer structure of the fibers changes the release profile and, in some cases, greatly increases release time. It has been shown that coating fibers with a polymer slows burst release and decreases the severity of burst release [187]. Sometimes, phase separation can occur between protein and polymer solution, decreasing protein encapsulation efficiency and resulting in the formation of thin, brittle fibers. In such situations, surfactant or compatibilizer may be used to resist phase separation.

When initial burst release of drugs must be stopped or their functioning is harmed by the solvents used for dissolving the polymer, the use of coaxial electrospinning is highly recommended. In this procedure, two or three solutions are applied to the concentric needle independently from various tubes, resulting in the production of core shell layered fibers. Typically, drug-containing solution is loaded in the middle core component (Fig. 20.1b). Drug release profile can be fully or partially regulated by controlling the thickness, presence of pores and biodegradation rate of the shell polymer. Cell adhesion and proliferation profiles on scaffolds and their nanofibrous surfaces can also be fine-tuned using this approach. Coaxial electrospinning has been used in several reports to build scaffolds with better cell and skin-friendly surface properties, such as optimal hydrophobicity and cell affinity [188, 189]. Chen et al. suggested a new nanowire-in-microtube structure for fabricating core/shell ultrathin fibers (Fig. 20.1c).

Fiber orientation in scaffolds influence cell morphology and differentiation. For instance, the ECM of certain human tissues, such as cardiac tissue and ligament/tendon, provides topographical cues for guiding cell elongation and cytoskeleton growth on electrospun scaffolds with aligned nanofibers [190, 191]. By modifying the type of collector used for electrospinning, it is possible to control the morphology of the collected fibers. Spinning discs, drums, and mandrels are examples of specialized collectors in which the rate of fiber deposition is influenced by the rotation speed. A basic collector plate can generate fiber mat or mesh composed of randomly oriented fibers, while a drum or a rod collector can generate meshes with oriented fibers at fast rotation (more than 1000 rpm). At lower rotation speeds, drum-based collectors generate non-aligned cylinder like scaffolds. These hollow tubes could be used in a number of tissue engineering applications, including vascular scaffolds and nerve lead conduits [192]. Scaffolds with well aligned nanofibers can be generated by electrospinning with a point electrode and a ring electrode as collector, which can be useful for wound closure because these scaffolds can facilitate skin cell migration towards the center of the scaffold [193]. It is possible to control both the alignment and architecture of electrospun nanofibers by varying collector design. Micropatterned collectors have been used to make scaffolds that can better support cell adhesion, cell proliferation, and differentiation [194, 195]. Microtopography features of the collector such as shape and depth of patterns affect fiber conformity and alignment. In comparison to fibers collected on a flat surface, those collected on patterned surfaces show an increase in pore size and pore size distribution [156, 196]. Patterned fibrous scaffolds with defined ridges and grooves can be obtained by electrospinning on the top of metallic strips [195]. Interestingly, cell spreading was much higher in ridges than in the grooves. In another study, micropatterned

polydimethylsiloxane (PDMS) templates were used as collectors to obtain patterned scaffolds [157]. Cell morphology, cellular organization and cell migration were influenced by the 3D scaffold structure formed by the patterns on collector. Zhu et al. created a bilayer nanofibrous nerve conduit with longitudinally aligned nanofibers in the luminal layer to facilitate nerve regeneration and randomly arranged nanofibers in the outer layer for mechanical support [197].

Unlike electrospun microfibrous scaffolds, small pores are common in electrospun nanofibrous scaffolds, limiting cell penetration and vascularization. Increased fiber diameter, on the other hand, often leads to poor cell adhesion and migration due to the lack of ECM-like nanofibrous architecture [198]. Particulate-leaching electrospinning, wet electrospinning and selective removal of sacrificial electrospun fibers or electrosprayed microparticles have been investigated to increase fiber spacing in electrospun scaffolds [199, 200]. For instance, as water-soluble gelatin fibers are removed from gelatin and PLGA bicomponent scaffolds generated through dual-electrospinning, it was found that micropores were greatly expanded and the nanofibrous features were well maintained [201]. Another method is salt leaching, which involves mixing salt crystals with polymer solution and then leaching them in water after spinning [202]. While these methods are effective in increasing scaffold pore sizes and overall porosity, they had unintended consequence such as decreased structural integrity and poor mechanical properties of resulting scaffolds. Because maintain the bioactivity or functionality of drugs that can be damaged by strong electrical field or solvent(s) used during electrospinning, more complex scaffold surface functionalization chemistries may be employed [121, 203, 204]. To boost physical adsorption, controlling electrostatic or van der Waals interactions between the drug and the scaffolds may be needed (For example, by introducing surface functional groups on the surface of fibers) [205].

Near-field electrospinning (NFES) has been proposed and developed in recent years to improve controllable deposition of electrospun fibers [206]. NFES greatly extends the spectrum of fiber-fabrication uses, including electronic parts, energy harvesting, lightweight sensors, and tissue engineering, thanks to its position-controlled deposition characteristics. Overall, NFES technique demonstrated that it has the ability to be used in tissue engineering and bioprinting [207]. Melt electro writing (MEW) is a layer-by-layer additive manufacturing technique for fabricating highly ordered 3D tissue engineering scaffolds from micron-diameter fibers [208]. A voltage is applied to create a steady fluid jet with a predictable direction that is continuously deposited on a collector. The diameter of the fibers varies in the process, and it works with polymers that have been previously used in clinical settings.

20.2.3 Advantages and Limitations

Advantages: Electrospinning has gained a lot of attention in the past several years due to it potential use for producing highly porous tissue engineering scaffolds. Loosely connected 3D mats having high porosity and high surface area, which

can easily mimic native ECM, can be generated through this method [212–214]. Nano-submicron fibrous wound coverage matrices show more promising ability to support wound healing as compared to traditional bandages, due to their ability to partially recapitulate native ECM structure (Fig. 20.2). This encourages fibroblast/keratinocyte attachment and migration, which facilitates skin tissue regeneration in the wound. Electrospun membranes can act as barrier to invading microbes and prevent [215]. Electrospinning can be used to generate fibrous scaffolds consisting of a wide variety of materials with tunable properties, composition, shape, and fiber dimension, as compared to other nanofiber generating techniques. These benefits allow researchers to use electrospinning to produce wound dressing and tissue engineering scaffolds that recapitulate the native tissue microenvironment and use them to treat wounds more effectively. Moreover, electrospun meshes possess strongly intertwined porous structure that allows oxygen permeation, absorbs wound exudate, exchanges fluids, and prevents the wound dehydration [216]. Long et al. recently created a battery-operated, compact electrospinning system with a total circuit length of just 10 cm to directly electrospin fibers over the wound [217]. This advance could make electrospinning more widely accessible in hospitals and surgical centers, allowing for more personalized individualized wound treatment. Advanced bioactive wound dressings also require regulated, on-demand release of therapeutic agents to

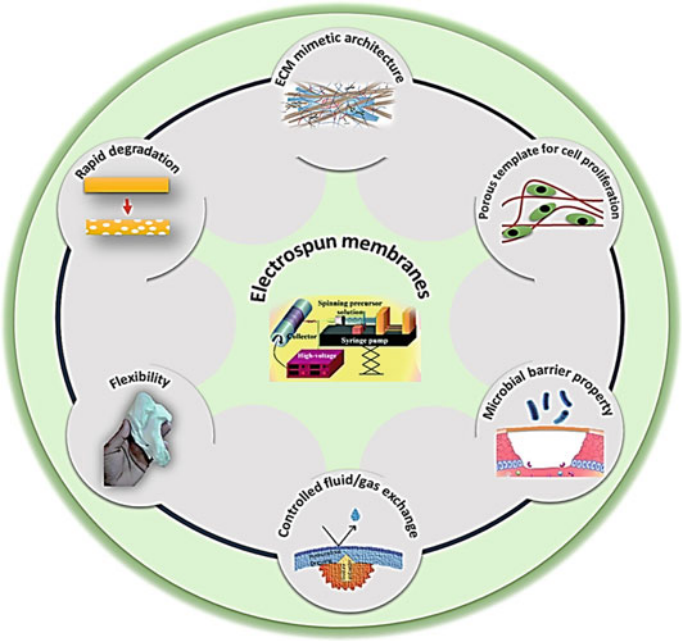

Fig. 20.2 Schematic showing the major advantages of using electrospun membranes for in tissue engineering and wound healing applications

facilitate wound healing without producing unwanted side effects [218]. Electrospinning holds great promise in this respect, as it allows loading of therapeutic agents and other active agents/biomolecules that can support tissue regeneration. Unlike bulk form, electrospun scaffolds may also have customized physiochemical properties, such as tunable degradation and drug release profiles. Bioactive agents such as growth factors, peptides and chemokines may be added after electrospinning or integrated into the scaffolds during the fabrication process.

Nano-fibrous membranes have been shown to adsorb more serum proteins than macro-fibrous membranes that are less porous [219]. In addition, compared to macroporous scaffolds, nano-fibrous scaffolds adsorbed more fibronectin from serum, according to other absorption study reports [220]. These findings suggest that nanofibrous scaffolds have a greater chance of mimicking natural ECM with improved tissue regeneration, as well as avoiding potentially negative immune response and pathogen transmission risks associated with naturally derived ECM-based constructs. Owing to the ECM mimicking features, electrospun nanofibers-based structures have shown excellent cell proliferation and differentiation capabilities both in vitro and in vivo [221, 222].

Limitations: Many of the parameters that influence electrospinning process are highly related and thus it is difficult to individually study the effects of such parameters. For instance, changing the concentration of a polymer solution, may also change its viscosity. Due to the high speed of the ejected liquid and the dynamic bending motion, it is difficult to control the deposition of fibers. It is also difficult to fabricate scaffolds in the shape of specific organs. Controlling fiber spacing is another challenge yet to be tackled. The complex process of fiber formation is still not fully understood. While electrospinning is ideal for producing thin membranes with large surface area and small pore size, it is not ideal for producing thicker sheets as this requires long production times. Melt electrospinning can only be used with polymers that have high decomposition temperatures and low melt viscosities. As a result of denaturation or decomposition of sensitive materials, the range of biopolymers that can be used in melt electrospinning is also limited.

Challenges: Even though electrospinning is a commonly used method for fabricating nanofibers, there are still many challenges to tackle. The use of toxic solvents in the polymeric solution, for example, causes health and safety concerns, and it limits industrial scalability. Electrospun scaffolds have several intrinsic limitations, one of which being their slow production rate. A typical laboratory experimental setup spins at a rate of a few mL/h (usually about 0.25–2 mL/h), which implies that if a solution containing 10% w/v polymer is spun for 10 h, the maximum amount of nanofibers formed is 1 g. To address this issue, multi-needle spinnerets have been proposed for industrial applications as a modification to the existing lab scale electrospinning setup. Multi-needle spinnerets, on the other hand, pose certain difficulties including polymer clogging on the spinneret, difficulty to clean the spinneret etc. Because of the effect of the electric field of adjacent needles, interference between the jets will affect the homogeneity of generated membrane/scaffold [223].

Another major challenge in electrospinning is the selection of appropriate solvent and ensuring that the solvent is fully removed from the finished product [224]. Electrospinning a polymer in its liquid state without the use of a solvent is better for polymers that are not soluble in common solvents. However, due to the high viscosity of polymers in the liquid state and the high melting temperature of most polymers, this process can only be used with a small number of natural and synthetic polymers. Because of the low solubility and high viscosity of high molecular weight natural polymers, the high density of intramolecular and intermolecular hydrogen bonding, the polyelectrolyte nature of aqueous solution, and the lack of appropriate organic solvent, electrospinning of natural polymers such as alginate and pectin is difficult [225]. While recent attempts to fabricate chitosan-based nanofibers are promising, crosslinking them under non-toxic conditions remains a challenge [90]. While it is highly desirable to combine natural and synthetic polymers to create natural synthetic hybrid scaffolds with superior physio-chemical properties of synthetic polymers while retaining favorable biological properties of natural polymers, finding a common solvent for both material types poses a challenge.

Total cell penetration through the pores is another problem for electrospun fibrous mats. Therefore, a combination of nano- and microfibers was introduced for cartilage tissue engineering [226]. Ideally, a scaffold should have a hierarchical geometry, adequate compressive power, and densely interconnected pores. While incorporating salt particles into electrospun scaffolds and their removal to generate pores has shown promising cell infiltration effects, uniform particle distribution within the structure remains a challenge [227, 228]. Using electrospun scaffolds to have most of the topographic and biochemical properties of native tissues is currently a difficult to achieve task and has not been realized yet. Another challenge when performing electrospinning is maintaining the bioactivity of drugs during and after spinning [229]. To summarize, the use of electrospun scaffolds for tissue engineering poses several challenges that must be carefully addressed and tackled by coupling with other recent approaches such as 3D printing/bioprinting so that electrospun scaffolds can be used for clinical applications.

20.3 3D Bioprinting

The main 3D bioprinting techniques include microextrusion, inkjet, and laser-assisted bioprinting (LAB). Also, the use of stereolithography (SLA) has recently been extended into bioprinting applications [230]. The efficacy of bioprinting techniques for fabricating biomimetic tissue constructs can be evaluated based on critical printing parameters, such as printing resolution, cell viability, and printing speed (Table 20.2). The deposition of bioinks in bioprinters is controlled by a digital system, which results in the fabrication of customized and complex constructs (Fig. 20.3) [231].

Table 20.2 Comparison of different types of three-dimensional (3D) bioprinting techniques and their characteristics

Property	3D Bioprinting type				
	Inkjet	Microextrusion	Laser-assisted	Stereolithography	Electrospinning
Material viscosity	3.5–12 mPa/s	30 mPa/s to >6 × 10^7 mPa/s	1–300 mPa/s	Not required	High, usually >300 mPa/s
Gelation methods	Chemical, photo-crosslinking	Chemical, photo-crosslinking, sheer-thinning, temperature	Chemical, photo-crosslinking	Light	Chemical, photocrosslinking
Preparation time	Short	Short to medium	Medium to long	Short	Short to medium
Printing speed	Fast (1–10,000 droplets per second)	Slow (10–50 μm/s)	Medium-fast (200–1600 mm/s)	Fast	Slow
Resolution or droplet size	<1 pL to >300 pL droplets, 50 μm wide	5 μm to millimeters wide	Microscale resolution	Micrometers	Nanometers to micrometers
Cell viability	>85%	40–80%	>95%	>85%	N/A
Cell density	Low, <10^6 cells/mL	High, cell spheroids	Medium, 10^8 cells/mL	Medium, 10^8 cells/mL	N/A
Printer cost	Low	Medium	High	High	Low

Adapted from Murphy and Atala [232], with permission from Nature Publishing Group and from Ashammakhi et al. [233], with permission from Wiley

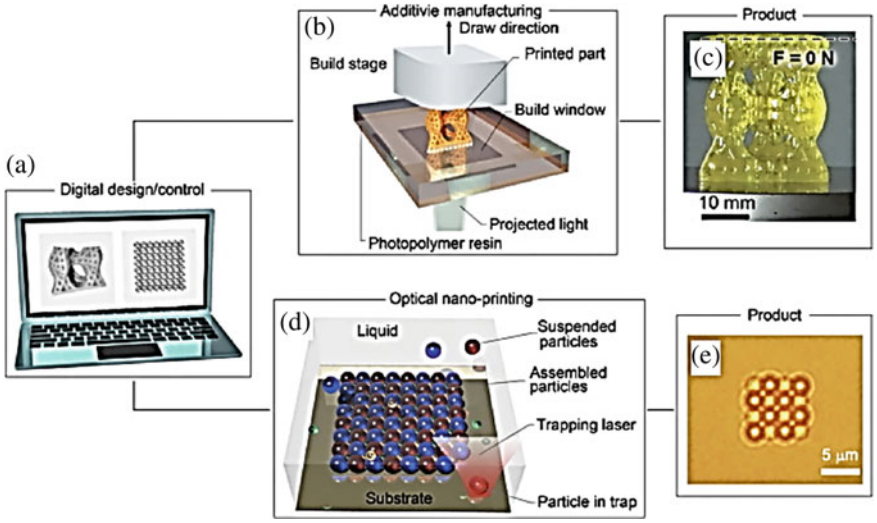

Fig. 20.3 Schematic illustration of digital manufacturing technique. **a** Computer-aided design for three-dimensional (3D) printing and the digital control of the printing process. **b** and **c** Printing of Kagome tower using the stereolithography (SLA) technique. **d** and **e** Nano-printing of a hybrid structure composed of particles at different sizes. Reproduced from Lin et al. [231], with permission from Elsevier

20.3.1 Microextrusion 3D Bioprinting

Microextrusion 3D bioprinting technique involves extruding printing bioinks into continuous filaments, which can be patterned based using a CAD program. Microextrusion technique enables simultaneous deposition of cells and matrix materials through multiple printheads to generate geometrically complex and hybrid biological structures. This technique can provide higher structural integrity due to its ability to deposit continuous filaments of printed material [234].

20.3.1.1 Principle

In microextrusion, printing material is pushed through printer nozzles by using a pressure-assisted system, which can either be pneumatic or mechanical (Fig. 20.4a, b). The extrusion of the ink can also be driven by a solenoid-based system, as shown in Fig. 20.4c [235]. Microextrusion printing would generate continuous streams of materials, which can be patterned to desired 3D structures by using a printing software connected to the 3D printer. Most of the extrusion-based 3D printers provide single nozzle dispensing systems. However, recent advances in multi-head deposition systems enable simultaneous deposition of multiple materials.

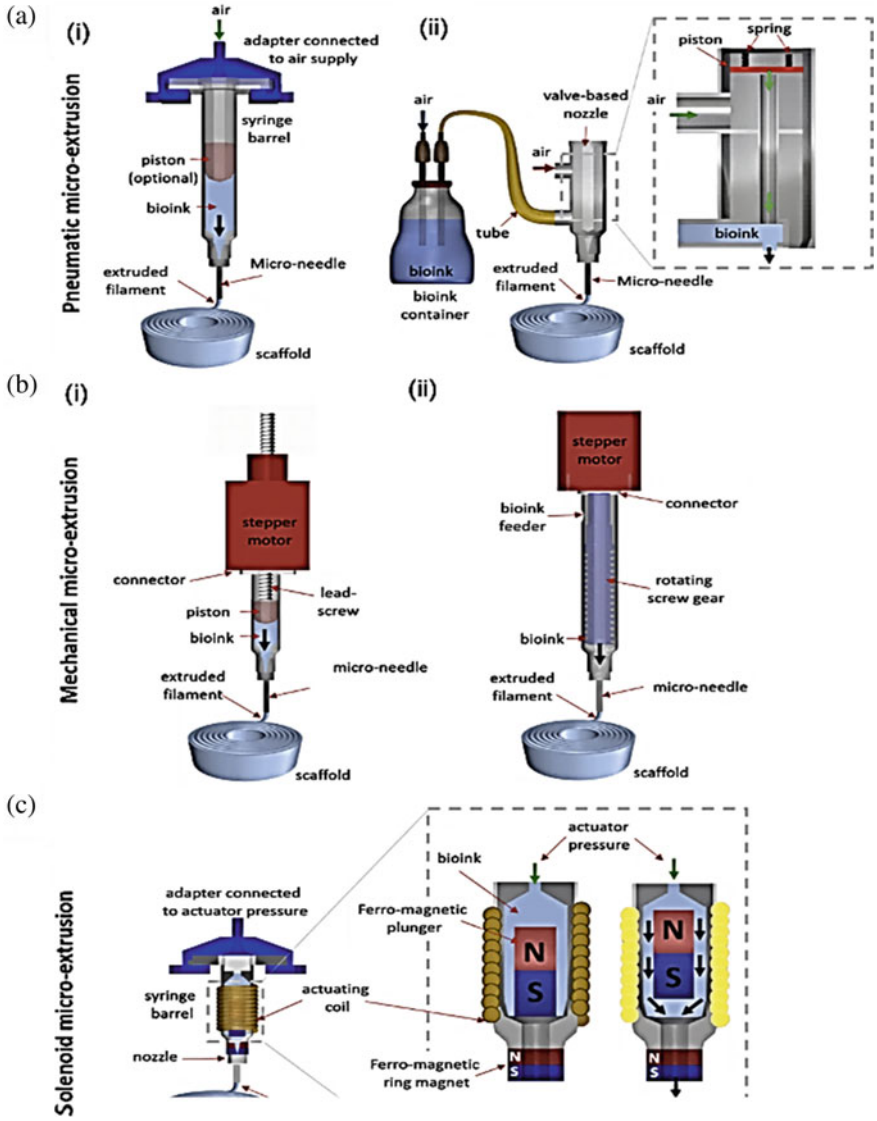

Fig. 20.4 Schematic illustration of different types of microextrusion three-dimensional (3D) printing techniques. **a** Pneumatic microextrusion can be either (i) valve-free or (ii) valve-based system. **b** Mechanical microextrusion that includes (i) piston- or (ii) screw-based system. **c** Solenoid microextrusion technique. Reproduced from Ozbolat et al. [235], with permission from Elsevier

Pneumatic Systems

Pneumatic 3D microextrusion systems are based on the use of a simple extrusion mechanism, where force needed to dispense ink is generated by using compressed air and a valve-free or valve-based configuration. Valve-free systems have been used more commonly due to their simple structure; however, valve-based systems have the advantage of better control over the printing pressure for high-resolution printing applications [235]. Overall, pneumatic systems are generally characterized by slow material deposition process due to the use of compressed gas.

Mechanical Systems

Mechanical extrusion systems usually have a more complex structure, resulting in a more precise deposition of materials or cells compared to the pneumatic-based microextrusion systems. However, this would reduce the maximum force that the instrument can apply to dispense the printing ink [236]. Piston-based microextrusion systems have demonstrated a high capability to control the flow of ink [235] while the screw-based systems can dispense highly viscous inks [237]. However, the high pressure needed for the screw-based systems to dispense viscous materials can adversely affect cell viability and function. To mitigate this problem, the use of shelf screws should be avoided. For example, Visser et al. employed a screw-driven system to melt and deposit polycaprolactone (PCL) while a piston-driven syringe was used to print a hydrogel [238].

Solenoid-Based Systems

Solenoid-based microextrusion systems utilize electrical pulses to control ferromagnetically or piezoelectrically actuated valve systems [239] (Fig. 20.4c). For instance, upon applying voltage, solenoid coil on the top of the printhead becomes magnetic and applies a magnetic force that can pull up the plunger to open the orifice and extrude the ink [240].

20.3.1.2 Development and Generations

Microextrusion-based 3D printing method has long been used for some time for applications that include the shape forming of metals and plastics [235]. However, this technique was first used in tissue engineering back in 2002, when fused deposition modeling (FDM) technique was employed to print melted biomaterials into porous scaffolds to support cell proliferation and growth [241]. Until about a decade ago (2005), 3D printers were generally expensive, and their high cost limited their use. Later, low-cost multimaterial extrusion-based 3D printers became accessible to the public [242]. Since then, various types of extrusion-based 3D printers have

been developed and commercialized by different laboratories and companies worldwide. These products range from large-size printers to small desktop printers that can easily fit within bio-hoods for cell-involving printing applications. Most of these printers are based on a syringe extrusion technique that allows controlling the extruded ink diameter by adjusting the needle size and properties of printing material [243]. Some new microextrusion printers have also demonstrated the capability to deposit and assemble tissue spheroids into 3D structures, which are on their way to commercialization [244].

20.3.1.3 Advantages and Limitations

Microextrusion 3D printing enables the printing of high-viscosity materials [230, 233]. Also, it allows printing of high-density bioinks to achieve physiological cell densities in the printed constructs [245, 246]. Microextrusion can easily be equipped with multiple printheads to print multimaterial constructs, mimicking the heterogeneous composition of native tissues [243].

20.3.2 Inkjet 3D Bioprinting

Inkjet bioprinting has been the first 3D bioprinting technology developed based on conventional 2D ink printers, where the ink was replaced with a biomaterial and paper with a moving stage that enabled the printing of 3D constructs by its controlled move along the x-y-z axis [243].

20.3.2.1 Principle

In inkjet 3D bioprinting, small bioink droplets are generated using thermal or piezoelectric actuation and subsequently deposited onto predefined locations in a layer-by-layer fashion to build a 3D structure. Thermal inkjet printers include electrical elements that are heated to create air bubbles that push the bioink down to the nozzle and dispense it. Alternatively, many inkjet printers are based on piezoelectric waves that can break the bioink into droplets of different sizes, thus resulting in a higher printing resolution. This technique reduces the risk of cells being exposed to excessive heat or pressure. Moreover, the nozzle-free configuration of inkjet printers can help to mitigate nozzle clogging issues [247].

20.3.2.2 Development and Generations

Inkjet 3D printing technique was invented by Thomas Boland in 2003 [243]. Later in 2015, an inkjet 3D printer, with a capability to simultaneously deposit multiple materials, was developed [248]. More recently, inkjet 3D bioprinting has been combined with a spray crosslinking, which enabled the fabrication of multimaterial large constructs [249]. Also, a high-resolution inkjet bioprinter was commercialized, with the capability to deposit low- and high-viscosity materials and even cell culture media [250].

20.3.2.3 Advantages and Limitations

Inkjet is a desirable 3D printing technique due to its low-cost and ability to print different types of biomaterials. Moreover, using this technique, it is possible to achieve printing resolutions as low as 50 μm with the capability for the precise deposition of biomaterials and cells [251]. Inkjet bioprinting is also characterized by high speed, with a deposition rate ranging from 1 to 10,000 droplet/s [252]. Inkjet printers can include multiple printheads enabling the deposition of multiple materials at the same time [248]. Another advantage is their ability to induce gradients in terms of cells or materials concentrations throughout the 3D bioprinted constructs by changing droplet size or density [237]. However, heat and mechanical stresses generated during this process may damage the cells [253]. However, no significant impact on cell activity was observed as the cooling of the printed constructs occurs within a few seconds. One major drawback of inkjet 3D bioprinting is the limitation related to the viscosity of inks. Excessive force is required to break and eject solutions of high viscosities. Bioink must be in liquid form to be inkjet-printed, and subsequently, it should have the ability to form a rigid structure after printing by using one of the crosslinking mechanisms [254]. Such processes may drastically alter the properties of the bioink or induce serious cytotoxicity effects. Difficulty with printing bioinks having high cell density (>10^6 cells/mL) is another limitation associated with the use of inkjet 3D bioprinting [232, 233].

20.3.3 Laser-Assisted 3D Bioprinting

Laser-assisted 3D bioprinting (LAB) is a less commonly used printing technique compared to inkjet and extrusion-based 3D bioprinting. However, there has been an increasing demand for the use of LAB for biomedical applications due to its high printing resolutions.

20.3.3.1 Principle

In LAB, laser is used to volatilize a donor layer (usually made of a glass coated with an energy-absorbing metallic layer) under which a cell-laden liquid bioink is suspended. This would generate a high-energy bubble that throw droplets of bioink onto the receiving plate placed below the bioink. This process continues until the 3D construct fabrication is completed (nozzle-free 3D printing) [230, 255].

20.3.3.2 Development and Generations

Laser 3D printing was initially used for high-resolution printing of metallic compounds, such as computer chips. In 2007, the use of laser 3D printing was extended to biological applications by patterning an array of micro-sized droplets [256]. LAB technique has been commercialized for 3D printing and was used for printing cosmetic tissue products, such as artificial human skin. However, widespread use of LAB for tissue engineering applications is not anticipated in near future [244].

20.3.3.3 Advantages and Limitations

LAB has a high printing resolution, and it can be used for printing bioinks at high cell densities (up to 10^8 cells/mL). This nozzle-free technique has enabled printing materials of diverse properties with viscosities ranging from 1 to 300 mPa/s. The nozzle-free system also reduces shear stresses exerted on cells during printing, thus increasing cell viability up to >95% [257, 258]. However, LAB is limited by its high production and maintenance costs, which are related mainly to the high-resolution laser diodes used in this technique [257, 258].

20.3.4 Stereolithography 3D Bioprinting

SLA 3D printing technique is based on the photocrosslinking process, where UV or visible light is used to solidify liquid ink into a desired 3D pattern. This technique is particularly interesting for printing photocurable materials, such as acrylics and epoxies [237].

20.3.4.1 Principle

In SLA, cell-laden photopolymers are deposited layer by layer, and selectively photo-crosslinked on each layer based on 2D digital models (Fig. 20.2d) [243]. After the 2D pattern is printed onto the printing bed, the printing stage is moved up along the z-axis and the photo-crosslinking process continues until 3D structure is fabricated.

20.3.4.2 Development and Generations

SLA 3D printing was first patented in 1986 [259]. This technique was subsequently introduced into the field of bioengineering in 1993 by fabricating detailed models for reconstructive head surgery [260]. More recent studies have shown the ability of SLA techniques to incorporate cells into the printed structures [261]. In this regard, high-speed and high-resolution SLA 3D bioprinters have been developed for fabricating ultra-fine structures, such as human capillaries and fine ECMs [262]. The printing process was shown to be non-toxic, and it could be used for printing cell-laden bioinks [262]. Also, recent reports have demonstrated the development of a digital micromirror device (DMD)-based SLA bioprinting. This technique includes millions of micron-sized mirrors that can be controlled individually for precise projection of light onto the polymerization stage to create high-resolution 3D structures [263].

20.3.4.3 Advantages and Limitations

SLA is a high-speed and high-resolution technique that can be used for printing complex structures at resolutions as high as <5 μm [264]. Similar to the LAB technique, SLA is a nozzle-free technique; therefore, no shear stress will be generated during printing, and high cell viability (>85%) will be achieved using this technique [243, 265]. However, in SLA, there is a need to use transparent bioinks to achieve uniform bioink photo-crosslinking, and this has limited the cell density of bioinks to $<10^8$ cells/mL [243]. Also, the use of UV light in this technique can induce cell toxicity, which has been partially resolved using visible light-assisted SLA [266].

20.3.5 Electrospinning-Based 3D Printing

Electrospinning is a high-resolution fabrication technique that can generate a small diameter of randomly oriented fibers for constructing mechanically strong structures.

20.3.5.1 Principle

Electrospinning-based printing includes charged filaments of solutions or melts, which are pulled out and deposited by electrical forces and subsequently dried to form a fibrous mat [80, 243]. This technique can produce fibers with diameters as small as 2 nm, resulting in the formation of high-resolution constructs that can be used for a variety of tissue engineering applications [73, 209, 267]. In this method, a digitally controllable stage with the capability to move spatially is combined with the conventional electrospinning machine, where a solution is extruded by a syringe or pneumatic force, making it possible to pattern a 3D construct in a customized manner. The main characteristic of electrospinning-based printers is their shorter

collecting distance (0.5–3 mm) as compared to tens of centimeters for conventional electrospinning technique. This enables the deposition of electrospun fibers in a more controllable manner. Also, the working voltage in electrospinning-based printers is usually lower than classical electrospinning, but this depends on the conductivity of biomaterial to be 3D printed [243].

20.3.5.2 Development and Generations

In recent years, electrospinning-based printing has captured special attention due to the increasing interest in nanotechnology and the fabrication of ultra-fine 3D structures in a controlled manner. In 2011, Brown et al. combined melt electrospinning with a digitally controlled collector and developed a new class of 3D printers called melt electrospinning writing (MEW), which enabled the deposition of well-defined filaments [268]. Since then, several laboratories across the world have developed custom-built devices to combine electrospinning with inkjet or extrusion-based 3D printing techniques [269–278]. Also, companies such as Spraybase® (Ireland) [279], NovaSpider (Spain) [280], and RegenHU Inc. (Switzerland) [281] have attempted to commercialize some MEW-based printers for various tissue engineering applications.

20.3.5.3 Advantages and Limitations

Melt electrospinning-based printing is an emerging printing technique that can print fibers with diameter in the range of a few nanometers to micron (0.650–1 μm), providing a high degree of resolution, porosity, and pore interconnectivity [271, 282, 283]. Also, a high surface area of the electrospun fibers relative to their diameters can provide highly bioactive structures. However, one major limitation is the rapid whipping of the charged fibers which results in a spatially unstable 3D structure [243]. Also, the encapsulation of cells in the electrospun biomaterials is still a significant challenge due to the high temperatures and voltages involved in the electrospinning process [243]. Melt electrospinning-based printing; however, allows for the fabrication of well-defined and highly porous structures with the possibility to seed the cells in the scaffolds after the printing process to provide a high degree of cell activity and morphogenesis in the printed structure [284–287].

20.4 Combining Electrospinning and 3D Bioprinting

20.4.1 Need, Approaches and Conditions

Advances in the field of biomaterials in the last few decades have contributed to significant improvements in tissue engineering and regenerative medicine [69, 288–291]. Specifically, biomaterials are used in 3D bioprinting to formulate bioinks to encapsulate the cells and produce highly organized structures [292]. In most cases, these bioinks are developed as hydrogels useful for bioprinting. They can provide a hydrated environment, like native tissue, due to their high water absorption and retention capability [293]. Moreover, hydrogels have favorable biocompatibility, and biodegradation profiles, as well as porous structure important for the transportation of nutrients, oxygen, and cell metabolic waste [294, 295]. Moreover, hydrogels can ensure cell viability over time. However, there are some drawbacks related to hydrogels. Most importantly, the lack of sufficient mechanical properties, which is considered a significant disadvantage. Hydrogels cannot keep their 3D structure and they deform due to gravitational forces, which reduces 3D bioprinted structure resolution and fidelity [296]. Moreover, after bioprinting, the construct is usually fragile, which makes its handling very difficult. This drawback is problematic for in vivo application of such scaffolds and it significantly hinders load-bearing applications [293, 297, 298].

To tackle this problem, most researchers try to increase the crosslinking density by increasing the crosslinking time and/or use of a second crosslinker [299–301]. Consequently, binding sites increase, and scaffold mechanical integrity improves. Other options include combining several biomaterials in the bioink or increasing the concentration of the biomaterial in the bioink. However, these solutions can reduce cell viability due to decreased diffusion rate of nutrients and oxygen. In other words, there is a trade-off between the mechanical properties of bioprinted scaffolds and the bioactivity of the scaffolds [293, 296]. As an alternative, it is possible to combine 3D bioprinting with other advanced manufacturing techniques to obtain a hybrid construct. This combination is advantageous since it can combine the benefits of two or several different fabrication techniques to compensate for each other's shortcomings.

Electrospinning is considered a simple and powerful tool that can be used to produce nanofibrous mats prepared for regenerative medicine purposes due to high porosity, high surface-to-volume ratio, and, more importantly, structural resemblance to the native extracellular matrix (ECM) [302, 303]. These structures can also enhance cell attachment and accelerate cell proliferation [304–306]. Besides, this technique can produce thin sheets from synthetic and natural polymers with great mechanical flexibility [293, 307]. Consequently, the combination of electrospinning and 3D bioprinting could be considered a promising approach to fabricate scaffolds suitable for cell survival and proliferation and mechanically stable for handling and in vivo applications. Ideally, these hybrid structures can have more complex hierarchical structures ranging from nanometer-size up to micrometer scale.

20.4.2 Application in Tissue Engineering

Xu et al. combined electrospun PCL/Pluronic F-127 nanofibers with inkjet bioprinting of fibrinogen/collagen/chondrocytes bioink for cartilage tissue engineering [297]. The two fabrication techniques were combined in one device. The addition of Pluronic F-127 into the electrospinning solution resulted in enhanced hydrophilicity of the nanofibers. In a layer-by-layer assembly, the scaffold was prepared as a five-layer structure with two layers of bioprinted hydrogels embedded within three layers of nanofibers (thickness of 1 mm). This was beneficial since extremely thin layers of nanofibers (average diameter of 422 ± 62 nm) were electrospun which is challenging to handle if they were electrospun separately. The hybrid scaffold showed better mechanical integrity with higher ultimate tensile strength than pure alginate hydrogels, pure PCL, and collagen/fibrin gels. Moreover, live/dead assay showed high cell viability (~82%) of chondrocytes within these hybrid structures, which confirmed structures' biocompatibility. Besides, after four weeks of in vitro culture, the encapsulated cells produced native ECM components, including collagen and glycosaminoglycans. The in vivo evaluations showed that hybrid scaffolds were capable of producing collagen and glycosaminoglycans after eight weeks. Deposited ECM compounds were observed to be well-organized and to resemble the native ECM. Encapsulated chondrocytes also retained normal phenotype as observed in native cartilage, demonstrating this approach's high potential for cartilage tissue engineering.

Paul et al. used this approach for vaginal wall repair [304]. PCL nanofibers were fabricated using melt electro-writing technique (a combination of melt electrospinning and 3D printing). The structure possessed suitable biocompatibility and appropriate surface topography for cell attachment, migration, and proliferation. Alginate/aloe vera hydrogels containing endometrial mesenchymal stem/stromal cells (EMSCs) were printed on top of the nanofibers using an extrusion bioprinting method. It was observed that alginate/aloe vera bioink with a 1:1 mass ratio performs the best biocompatibility and cell viability. Besides, the results revealed a homogenous cell distribution after bioprinting over PCL mesh (Fig. 20.5a). In vivo mice experiments showed that cell encapsulated hybrid scaffolds recruited lower inflammatory cells as compared to cell-free hybrid scaffolds and PCL nanofibers. More interestingly, histological analysis showed that after implantation, EMSC-loaded hybrid scaffolds resulted in infiltration of more M2 macrophages. This was attributed to the anti-inflammatory properties of EMSCs (Fig. 20.5a). Besides, cell-encapsulated hybrid structures showed better integration with host tissue.

As an alternative to the layer-by-layer stacking of nanofibers and bioprinted hydrogels perspective, Ko et al. combined the electrospinning and 3D bioprinting by dispersing nanofibers within the formulated bioink [296]. To this aim, fragmented poly(lactic-*co*-glycolic acid) (PLGA) nanofibers were dispersed in the bioink (gelatin methacryloyl, GelMA solution) through a mechanical homogenizer (Fig. 20.5b, c). Nanofibers were homogeneously distributed in the bioink and addition of PLGA nanofiber fragments (PLGA-NF) resulted in a significant increase in the compressive

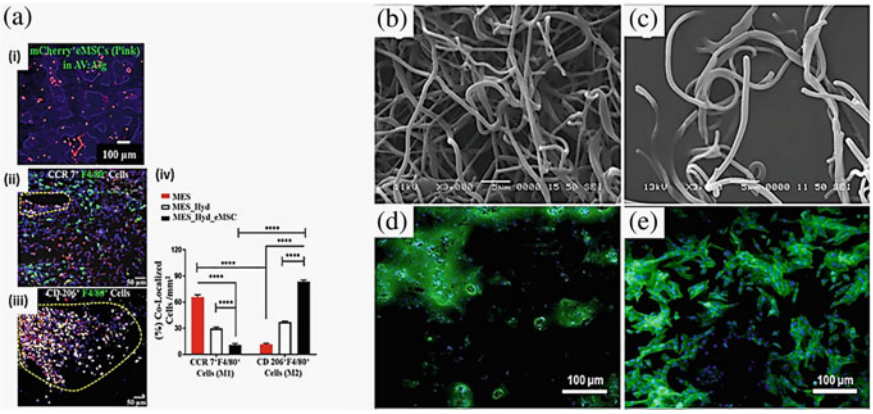

Fig. 20.5 Combination of electrospinning and three-dimensional (3D) bioprinting. **a** Combination of melt electro writing and 3D bioprinting. (i) distribution of mCherry^{+} endometrial mesenchymal stem cells (EMSCs) over the 3D printed structures, (ii, iii) M1 and M2 macrophages in tissue explant after one week, and (iv) quantification of obtained results. Reproduced from Paul et al. [304], with permission from Elsevier. **b** Scanning electron microscopy (SEM) images of poly(lactide-*co*-glycolide) (PLGA) nanofibers and grinded PLGA nanofibers (**c**). Fluorescence microscopy of fibroblast cells encapsulated in pure gelatin methacryloyl (GelMA) hydrogel (**d**), and poly(lactic-*co*-glycolic acid) nanofiber fragments (PLGA-NF) loaded (GelMA) hydrogel (**e**) after 3 days. **d–g** Reproduced from Ko et al. [296], with permission from Elsevier

strength. However, at high concentrations of PLGA-NF (10%), clogging occurred, and non-uniform strands were printed. Biocompatibility assessment revealed that nanofibers did not adversely affect cell viability. Moreover, since nanofibers could provide cells with binding sites, cells start to retain their phenotype faster than pure GelMA bioinks (Fig. 20.5d, e). A summary of the previous works that combined 3D bioprinting and electrospinning is presented in Table 20.3.

20.4.3 Advantages and Limitations

The idea of combining 3D bioprinting with other manufacturing methods is a fascinating phenomenon that can result in the formulation of hybrid structures with exclusive capabilities and features [296]. This combination can help to develop more complex structures that resemble native ECM more effectively [298]. Using 3D bioprinting, it is possible to construct exceptionally well-defined 3D structures with several cells encapsulated within the structure [310]. On the other hand, electrospinning can produce highly porous fibrous structures similar to native ECM [311]. This hybrid approach provides excellent opportunities to use and combine synthetic and natural polymers in the structure. For example, collagen, fibrin, and gelatin can be utilized in hybrid structures with significantly higher mechanical and biological

Table 20.3 Summary of works combining electrospinning and three-dimensional (3D) bioprinting

Application	Material for electrospinning	Material for 3D bioprinting	Cell types	Results	Refs.
Soft tissue engineering	PLGA	GelMA	NIH3T3 fibroblast	• Enhanced viscosity and compressive modulus of the bioink by incorporation of nanofibers • Increased cell proliferation rate in bioprinted structures by the addition of nanofibers • Cells retained their stretched phenotype faster in fiber-loaded structures	[308]
Cell/surface interaction analysis	Polyurethane	Cell spheroids	Primary human fibroblast	• Visualization of cell-fiber interfaces and cell membrane protrusions • Quantification of several important morphological and cell-scaffold interfaces	[309]
Vaginal wall repair	PCL	Alginate/aloe vera	Endometrial mesenchymal stem/stromal cells (EMSCs)	• Stimulated tissue integration in NSG mice • Improved EMSCs retention • Improved collagen deposition in EMSCs bioprinted scaffolds	[304]
Soft tissue engineering	PCL	Alginate	NIH3T3 fibroblast	• Improved mechanical properties • Decreased shrinkage and enhanced fidelity and resolution • Enhanced cell proliferation	[293]

(continued)

Table 20.3 (continued)

Application	Material for electrospinning	Material for 3D bioprinting	Cell types	Results	Refs.
NA	PLLA	GelMA	Pre-osteoblast MC3T3 cells	• Enhanced printability • Enhanced viscosity • Enhanced printing fidelity • Slightly higher cell viability compared to pure GelMA	[295]
Neural tissue engineering	PCL	Alginate	PC12 neural cells	• Improved mechanical properties • High cell viability and cell proliferation	[298]
Cartilage tissue engineering	PCL/Pluronic F-127	Collagen/fibrinogen	Rabbit chondrocytes	• Control of construct organization • Production of thin layers for layer-by-layer assembly • Improved mechanical properties • High (>80%) cell viability • Maintaining the phenotype and ECM production of cells in vivo	[297]

properties than the scaffolds made using a single soft hydrogel. These hybrid structures showed promising properties in mimicking the ECM of native tissues that might ultimately lead to clinical evaluations.

However, despite numerous advantages, there are some shortcomings with this approach. Generally, electrospinning is a time-consuming technique to fabricate thick structures. Hence, when both techniques are used simultaneously, it could take a considerable amount of time to fabricate scaffolds. Besides, any residual solvent within the electrospun fibers can negatively affect the viability of cells in the construct cell viability and final functionality. Moreover, nanofiber layers between each layer of the 3D bioprinted construct can alter nutrients and oxygen diffusion rate with some potential adverse effects on cellular behavior [297]. Besides these disadvantages, this combination is still promising and can create several new opportunities to solve critical issues in tissue engineering, regenerative medicine, and healthcare.

20.5 Challenges and Future Directions

Sterility of the fabricated constructs is a critical consideration for 3D bioprinting. Since cells are directly incorporated into the scaffold upon fabrication, any contamination could cause severe problems for further use. This phenomenon could become even more important when different fabrication techniques are combined. Especially, if electrospinning and 3D bioprinting are conducted separately, careful consideration should be held to make sure that both scaffolds (i.e., fibers and hydrogels) are prepared in a sterile environment and are kept sterile upon combination. Direct integration of electrospinning and bioprinting may pose additional challenges due to the presence of organic solvents used in electrospinning. Sufficient time should be provided between electrospinning and bioprinting processes to facilitate the evaporation of hazardous solvents from electrospun fibers before coming in contact with the bioink. Providing a higher temperature in the spinning hood may help in rapid solvent removal. A washing step can also be introduced between electrospinning and bioprinting processes to minimize the presence of residual solvent. Material level incompatibility of electrospun fibers, particularly in the case of hydrophobic electrospun fibers, in bioprinted hydrogel construct can also be a challenge. Surface modification of electrospun fibers can be a prerequisite before incorporating electrospun fibers in bioprinted constructs.

One specific consideration for future examinations could be the modulation of degradation behavior. Generally, nanofibers are added to 3D bioprinted hydrogels to enhance the mechanical properties [312–314]. For this purpose, synthetic polymers were used which are known to have a much slower degradation profile than the investigated hydrogels [315, 316]. This degradation variation could alter the remodeling of the structures and subsequently the integration of the scaffold. Hence, in future works, attempts should be made to balance the degradation rates of both hydrogels and fibers to match the specific requirements of the targeted tissue.

Vascularization is known to be a bottleneck limiting the engineering of large-scale tissues [317–319]. Consequently, in future studies, it is possible to exploit smart materials and encapsulate angiogenesis factors and biomolecules into engineered constructs to formulate stimuli-responsive scaffolds that can release the molecules upon need and accelerate vascularization. Incorporating growth factors in electrospun fibers and into 3D bioprinted constructs can be a useful approach to improve vascularization. Electrospun tubular constructs [320, 321] can be integrated in bioprinted constructs to act as vascular channels for media transport during in vitro construct maturation and as vascular network after in vivo implantation.

The widespread use of the natural polymer-based hydrogels is limited because of their low mechanical strength. Combining mechanically less stable natural polymers with electrospun synthetic polymers may solve such challenges to some extent. However, precisely mimicking the mechanical properties of ECM of various native tissues in engineered constructs is still challenging. Future research may focus on this aspect, where tissue specific ECM mimetic scaffolds with matching tensile strength, modulus and elasticity will be developed by combining electrospun fibers within 3D bioprinted constructs. Furthermore, structures with high degree of resemblance to native ECM and native tissues could be developed by using a mixture of specifically cell-laden hydrogel along with organized distribution of nanofibers. In addition, biofouling is another main challenge that limits wider clinical use of both nanofibrous membranes and 3D bioprinted constructs. To tackle infection problems, loading of antibiotics or antimicrobial nanoparticles in electrospun nanofibers has been reported [322–326]. Incorporating chopped electrospun membranes loaded with antibiotics or nanoparticles in bioprinted constructs is an interesting area for further investigation.

In addition to the integration of electrospinning and 3D bioprinting methods, combination of multiple techniques such as the use of microfluidic techniques [37], may be a promising approach for developing smart multifunctional and stimuli responsive constructs for regenerative medicine, drug delivery and personalized therapy [327–329]. In addition, other beneficial techniques and different methods could be used to promote cell movement through non-woven structures, such as the application of electrical fields, the production of gradient properties (i.e., stiffness, pore diameter, chemical gradient, directional porosity, etc.) within scaffolds.

To develop a robust construct by combining electrospinning and 3D bioprinting, it is important to completely understand all the factors related to electrospinning and 3D bioprinting processes, and to find an effective way to quantify the interaction between factors/parameters affecting these techniques and their combination. To achieve this, morphological and chemical effects need to be studied and influence of these parameters on the relationships between each variable of electrospinning and 3D bioprinting should be addressed. After this has been determined for several similar materials, a mathematical models can be drawn up to estimate conditions needed to perform electrospinning and 3D bioprinting simultaneously or one after the other in compliance with the requirements for desired application.

20.6 Conclusions

Although there has been increasing developments in 3D bioprinting toward the development of functional tissue constructs, the production of mechanically robust and durable constructs using 3D bioprinting has been a challenging task. Various strategies have been explored to overcome this limitation that recently included the integration of nanofibers that have been produced by using electrospinning to 3D bioprinted constructs. This resulted in the production of reinforced constructs with better properties. The use of nanofiber-based structures adds native ECM-mimicking properties to engineered tissue constructs. In addition, this approach enables the integration of drug release properties and building gradients into the engineered tissue constructs and widening the applications of 3D bioprinting for interface tissues. It is expected that such a combinatorial approach will be further explored in future and its applications increased.

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