Electrospinning in Liver Tissue Engineering

Subjects: Cell & Tissue Engineering

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The major goal of liver tissue engineering is to reproduce the phenotype and functions of liver cells, especially primary hepatocytes ex vivo. Several strategies have been explored in the recent past for culturing the liver cells in the most apt environment using biological scaffolds supporting hepatocyte growth and differentiation. Nanofibrous scaffolds have been widely used in the field of tissue engineering for their increased surface-to-volume ratio and increased porosity, and their close resemblance with the native tissue extracellular matrix (ECM) environment. Electrospinning is one of the most preferred techniques to produce nanofiber scaffolds. The various technical aspects of electrospinning that have been employed for scaffold development for different types of liver cells were discussed. The use of synthetic and natural electrospun polymers along with liver ECM in the fabrication of these scaffolds was highlighted. Novel strategies that include modifications, such as galactosylation, matrix protein incorporation, etc., in the electrospun scaffolds that have evolved to support the long-term growth and viability of the primary hepatocytes were also described .

Keywords: liver tissue engineering ; electrospinning ; nanofibers ; natural and synthetic polymers

1. Electrospinning

The invention of electrospinning dates back to 1980, by Yoshito Miura and group who were working on textile fibers [1], yet the use of electrospinning techniques in the field of tissue engineering to prepare nano-range scaffolds increased only in the past two decades [2]. A major advantage of this technique is that nanofibrous scaffolds with high surface area and porosity can be developed for the exchange of nutrients and oxygen and also allows infiltration of cells within the scaffold [3]. Electrospun fibers are in the nano range (from 100 nm to 50 µm) and have been observed to mimic the ECM architecture of a biological tissue. Several growth factors and drugs can be incorporated into these electrospun scaffolds through simple chemical modification of the surface [4][5][6][7][8][9][10], which can then be used as sustained drug releasing materials in vivo owing to their porous nature. Electrospun scaffolds are ideal for in vitro cultures and are also now being used for in vivo transplantation, especially in vascular reconstruction and skin tissue engineering [11][12][13]. Advancements in the field of electrospinning have given rise to its various subtypes, including coaxial electrospinning, multiple needle electrospinning, melt electrospinning, wet electrospinning and blend electrospinning. Core-sheath and hollow fibers can be produced by the coaxial spinning type, which has outer and inner spinnerets that can contain two different polymer solutions [14][15]. Blend electrospinning is different from coaxial spinning, where two polymers, or polymers with drugs or growth factors, can be blended and electrospun. Due to the toxicity of the nonpolar solvents used in a conventional electrospinning process, blending may result in the degradation of growth factors and active metabolites in drugs. To overcome this difficulty, two-phase electrospinning, where two different electrospinning methods are combined, is preferred, which allows the stability of growth factors and drugs to be maintained [16][17]. Melt electrospinning does not involve the use of toxic solvents, and, thus, is favorable for both in vitro cultures and in vivo conditions [18][19]. This process, however, requires very high temperatures to melt the polymer and only very few polymers are stable at high temperatures (e.g.: polycaprolactone and polyethylene). Wet electrospinning is another widely used electrospinning method which produces highly porous scaffolds with better cellular infiltration [20]. Conventional electrospinning systems have also been modified with multiple needles and multiple spinneret systems to fabricate scaffolds on a large scale.

A basic electrospinning set-up comprises of a syringe pump, syringe with blunt needle containing polymer solution, a collector and a high voltage current source. A high-intensity electric field (15 to 30 kV) is applied between two oppositely charged electrodes to set up electrospinning for scaffold production. One electrode is connected to the collector and the other is attached to the needle of the syringe containing the polymer solution. The flow rate at which the polymer solution is ejected out of the syringe pump is optimized according to the user’s experiment. The polymer is electrically charged as soon as it comes out of the nozzle as a spherical droplet. A charge–charge repulsion within the droplet creates a surface tension over the droplet, which is overcome by the high intensity electric field drawing the spherical droplet into a cone towards the collector [21] This is called Taylor cone formation, which is then followed by jet propagation. During jet

propagation, solvent evaporation occurs and the charge within the jet increases with time and voltage. This causes instability of the jet and the fibers become patterned in the nanoscale range, which are then drawn towards the collector. The orientation of the patterned fibers formed depends on the collectors used. For example, the rotating drum collectors lead to the formation of aligned fibers, while static collectors would form random fibers [22] (**Figure 1**). In case of the wet electrospinning method, the fibers are collected in the water bath and rest of the set-up remains the same. Cell behavior varies drastically according to the surface topography of the electrospun scaffolds. Studies have reported that primary hepatocytes and cells of elongated phenotype-like myocytes and neuronal cells show improved cellular attachment and proliferation when cultured on aligned fibers, while non-elongated cell phenotypes are more proliferative on random fiber mats [23][24][25][26][27]. The seeded cells sense the surface changes through integrin receptor signaling (present on the surface of the cells) and different levels of receptor activation by different scaffolds cause a variability in cell adhesion and attachment.

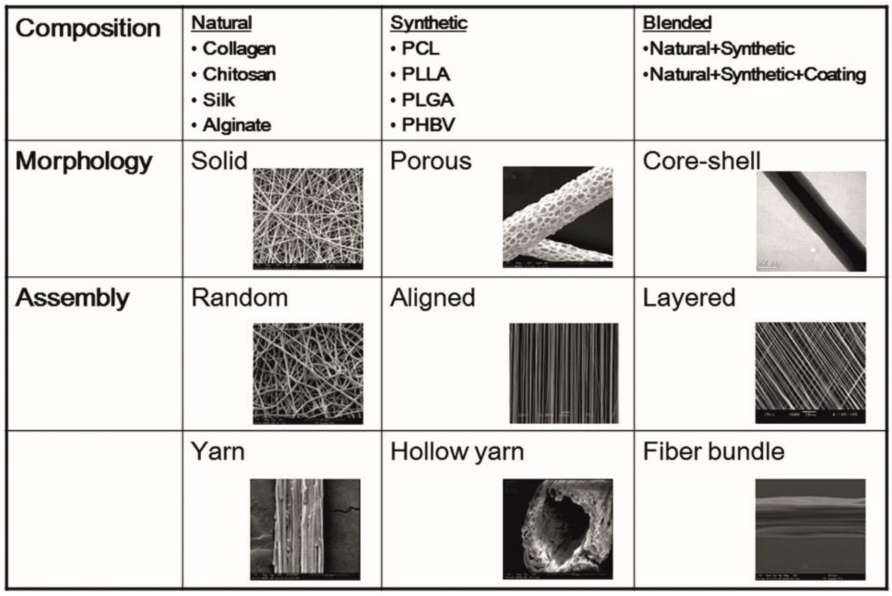

Figure 1. Patterns of the electrospun nanofibers categorized. Nanofibers can be electrospun in different formats such as random, aligned and layered depending on the instrumentation. Figure formatted with permission (NUSNNI, NUS).

Every component of the electrospinning setup can affect the formation of fibers and can also change the range/size at which the fibers are formed [28]. Rahmati et al. categorized various factors affecting fiber formation into three major groups, where the first group revolves around intrinsic properties of the materials used, including majorly the molecular weight of the polymer, viscosity and solvent nature. The molecular weight of the polymer plays a major role in determining the fiber formation and diameter. Increased molecular weight of the polymer increases the viscosity of the electrospinning solution. Highly viscous polymers tend to surpass the bending instability and form fibers with large diameters. The second group involves the processing parameters related to the equipment such as flow rate, distance between the needle and collector and voltage. Improper fiber formation may occur due to inadequate solvent evaporation when the flow rate and the distance are not adjusted. With increased flow rates and decreased distance between the collector and the needle, the solution in which the polymer is dissolved does not get the requisite time to evaporate, due to which a thick fiber mesh with inadequate pore size is formed. Jet propagation and solvent evaporation are thus two crucial factors that determine the fiber formation, which is governed by appropriate flow rates for developing several patterns of the fibers. The third category of factors affecting the electrospinning of fibers accounts for environmental factors such as humidity and temperature. Low temperature and high humidity in the air affect solvent evaporation and lead to improper fiber formation [29][30].

Polymers used for electrospinning of fibers can be natural (such as alginate, chitosan, silk, etc.) or synthetic PLA (polylactic acid), PLGA (polylactic co glycolide), PCL (polycaprolactone) or even both (hybrid polymers) [31][32]. The degradation products of PLA and PLGA polymers (lactic acid) are biocompatible. PCL is known for its non-toxicity and lower immunogenicity and cases where slower degradation of polymer is needed, such as in cases of nerve regeneration through tissue engineering [33]. The degradation products of PCL (caproic acid, succinic acid, valeric acid and butyric acid) have shown to be toxic for cell culture systems, but, surprisingly, PCL implants have been reported to perform well inside the host body [34]. The slow degradation rate of this polymer is the main reason for it to be used widely in drug delivery systems. PCL scaffolds are also porous, which allows improved growth of cells in the in vitro systems and also in vivo. When transplanted in vivo, endothelial cells can infiltrate and form new blood vessels to support angiogenesis and cell viability on PCL scaffolds. It has been reported that electrospinning of natural polymers (collagen, silk fibroin) alone results

in a bead on string fiber formation and, hence, natural polymers are often mixed with a synthetic polymer to improve the mechanical properties of the formed fibers [35][36]. Overall, the high surface area of the electrospun scaffolds provide a suitable environment for cellular attachment and the nano size of the fibers that mimics the cellular protein size present in the natural tissue matrix improves the focal adhesion.

2. Hepatic Cell Types on Electrospun Nanofiber Scaffolds

Hepatocytes account for 80% of the hepatic volume and perform all the major functions of liver. The other cell types present in the liver are grouped as non-parenchymal cells (NPC), which mainly includes hepatic stellate cells, kupffer cells, sinusoidal endothelial cells and other cell types such as pit cells and cholangiocytes. NPCs hold for about 6.5% of the total hepatic volume. Sinusoidal endothelial cells are found on the lining of the space of Disse. They are different from the conventional endothelial cells due to the presence of fenestrae that facilitates the improved exchange of nutrients and oxygen. Kupffer cells are the major phagocytic cell type in the liver. Hepatic stellate cells are the reservoir of vitamin A and pit cells are the natural killer cells of the liver. Cholangiocytes are the cells lining the bile ducts [37]. The cellular architecture of the liver is supported by the extracellular matrix (ECM) with an array of several different macromolecules that together comprise the scaffolding of the liver. In a healthy liver, it forms only about 3% of its total area. The most abundant proteins of liver ECM are isotypes of collagen (I, III, IV and V), with different isotypes localized to different areas.

Prolonged cultures of viable and functional liver cells such as primary hepatocytes and hepatoma cell lines on nanofiber scaffolds is the major goal of liver tissue engineering. The survival of primary hepatocytes ex vivo has remained a challenge for years. Adult primary hepatocytes do not survive after 3–4 days of culture as they change their phenotype and transform into mesenchymal cell lineages. The use of electrospun scaffolds for liver tissue engineering was first reported in the 21st century [30][31][32][33][34][35]. Zhang-Qi Feng et al. were some of the few researchers who pioneered the culturing of liver cells on electrospun scaffolds [34][38][39]. Among the cell lines, HepG2 shows better efficiency when cultured on electrospun fibrous scaffolds, on both natural (silk fibroin) as well as (PCL) synthetic polymer. Other than the hepatoma cell lines, cryopreserved human primary hepatocytes and rat/mice isolated primary hepatocytes have also been cultured on electrospun scaffolds, however, with limited success. It has been observed that primary hepatocytes display better viability with the natural polymers or with synthetic polymer scaffolds when they are modified with matrix proteins such as collagen, fibronectin or RGD peptides [40][41][42][43][44][45]. Another bottleneck of culturing primary hepatocytes is their limited replicative potential in vitro. An effective approach that has now emerged is to grow these cells as spheroids. Bell et al. have shown that hepatocytes can be cultured as spheroids for up to 35 days without compromising their functionality [44]. Hepatocytes have also been seen to form spheroids when cultured on galactosylated surfaces [45][46][47][48][49][50][51]. Galactose–asialoglycoprotein receptor (ASGPR) present on the hepatocytes has been demonstrated to be a key player mediating this interaction. Kian-Ngiap Chua et al. used poly (e-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) polymer for scaffold formation and surface-modified it with polyacrylic acid and -O-(60- aminohexyl)-D-galactopyranoside (AHG) for galactosylation [49]. Isolated rat hepatocytes began to form clusters after day 1 on these galactosylated scaffolds. This was not observed on the non-galactosylated substrate, where the cells took an irregular shape and topography. Functional analysis revealed that an increased secretion of albumin and urea synthesis was observed with hepatocytes cultured on galactosylated substrates after 2 days and P450 activity increased after day 5. This was a major advantage of the developed substrate as it has been reported that P450 activity of primary hepatocytes usually deteriorates with time in culture conditions otherwise [46][47]. Hong-Fang Lu et al. showed that hepatic spheroids co-cultured with non-parenchymal cells on galactosylated PVDF (Polyvinylidene fluoride) surface have enhanced P450 activity [48]. Several other studies also reported the efficiency of galactosylated surfaces in maintaining hepatic spheroid phenotype, function and preventing their trans-differentiation [49][50][51][52][53]. Another study by Kian-Ngiap Chua et al. employed a dual-functional scaffold for facilitating adhesion and enhanced functionality of the primary hepatocyte spheroids. 3-methylcholanthrene (3-Mc) is a selective inducer of P450, and this group prepared 3-Mc-loaded electrospun PCLEEP polymer scaffolds by mixing the inducer with the polymer solution before spinning them into scaffolds. The galactosylated surface helped in the prolonged culture of the hepatocytes as spheroids and the bio-molecule-loaded feature improved the functionality of hepatocytes. Galactosylated scaffolds showed 85% cell adhesion, whereas attachment was a little low with 3-Mc loaded scaffolds (76%) and very poor attachment was observed with the unmodified scaffolds (PCLEEP alone) (37%). The P450 function of the dual scaffolds increased by 1.5-fold in comparison with the galactosylated scaffolds, clearly demonstrating the usefulness of this approach [54]. Besides primary hepatocytes, bone marrow stem cells (BMSCs), human mesenchymal stem cells (hMSCs), etc., have also been used in liver tissue engineering, where these cells are seeded onto the electrospun fibrous scaffolds and are trans-differentiated into hepatocytes with appropriate growth factors. This approach has also extended the life span and functionality of the differentiated hepatocytes in vitro [55][56][57][58].

3. Recent Innovative Approaches in Electrospinning for Liver Tissue Engineering

For prolonged cultures of hepatocytes, studies have now reported the use of hepatic cells in the form of 3D spheroids. Innovative approaches have been employed by researchers to incorporate the hepatocytes as spheroids, which is discussed in the following section. Wei et al. modified the conventional electrospinning method and came up with the idea of short fibers to support culture of hepatic spheroids without the need for surface modifications. They reported that the length of the fibers can be modified according to the length of the spheroids and showed that spheroids cultured on the PSMA (Poly(styrene-co-methyl acrylate) fibers of about 50µm length have improved drug metabolism and drug clearance [59]. Carbon nanotubes (CNTs) in the form of nanofibrous mats are known to provide electrically conductive surfaces and have been used for prolonged 3D spheroid cultures [60]. Koga et al. have reported that CNTs have the ability to induce the formation of hepatocyte spheroids [61]. Wei et al. have also used multiwalled CNTs functionalized with galactose moieties on the surface for efficient hepatic spheroid cultures. They demonstrated that hepatocytes cultured on these functionalized fibrous mats showed better functions, namely, better drug clearance and increased expression of drug metabolizing genes [62].

Besides in vitro cultures, electrospun liver scaffolds are also being used for in vivo applications. The scaffolds can be fabricated as patches containing nano-fibrous mesh that can then be implanted at the site of injury. Kim et al. have recently shown that electrospun scaffold patches can be used to deliver healthy hepatic cells in toxin-induced liver injury mouse models. In this study, they used PCL for fabricating electrospun scaffolds/sheets and seeded them with patient-derived primary hepatocytes in a stacking manner by 3D bioprinting to mimic the native liver environment. The survival of the animals with the hepatic sheet transplant was 70% as compared to that of the control group without the scaffold. This study has opened the doors of using electrospun liver cell scaffolds for liver transplantation and in vivo therapy [63]. Another study by Salerno et al. has also proved the potential of electrospinning in mimicking the native liver tissue architecture with multiple cell types. They used the dry jet–wet electrospinning method to prepare hollow PCL fibers, and placed them in a bioreactor which contained an outer luminal segment where primary human hepatocytes were cultured and an inner luminal segment where endothelial cells were seeded in a hexagonal manner, thereby mimicking the native liver architecture [64]. The authors observed improved hepatic functions such as glucose consumption and albumin secretion for up to 18 days in the perfusion bioreactor. The recent studies on the electrospun scaffolds show the potential of them on the clinical front, an overview of the recent strategies employed for the fabrication of electrospun nanofiber scaffolds for liver cells is given schematically in **Figure 2**. Patient-specific scaffolds made out of this technique would give us the edge of replacing liver transplantation treatment with tissue engineering.

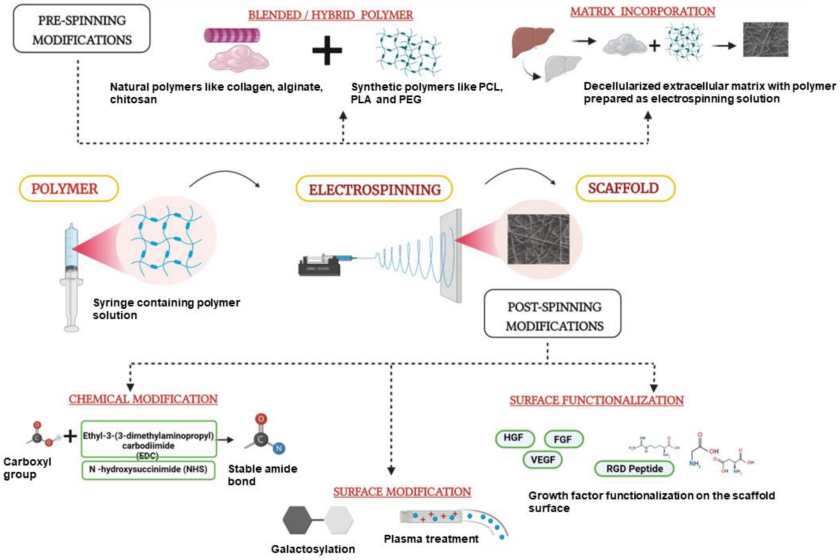

Figure 2. New Strategies employed in Electrospinning for Liver Tissue Engineering. Pre-spinning modifications including incorporation of matrix proteins, drugs or decellularized whole tissue matrix. Post-electrospinning strategies such as surface modification of the fibers with galactose, growth factors and RGD peptide conjugation, thereby improving the quality and functionality of the developed fibers for culture of liver cells. HGF—Hepatocyte Growth Factor, FGF—Fibroblast growth factor, VEGF—Vascular Endothelial Growth Factor (Unpublished original picture by authors created using Biorender.com).

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