Biofunctionalization and Applications of Polymeric Nanofibers

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The limited ability of most human tissues to regenerate has necessitated the interventions namely autograft and allograft, both of which carry the limitations of its own. An alternative to such interventions could be the capability to regenerate the tissue in vivo.Regeneration of tissue using the innate capacity of the cells to regenerate is studied under the discipline of tissue engineering and regenerative medicine (TERM). Besides the cells and growth-controlling bioactives, scaffolds play the central role in TERM which is analogous to the role performed by extracellular matrix (ECM) in the vivo. Mimicking the structure of ECM at the nanoscale is one of the critical attributes demonstrated by nanofibers. This unique feature and its customizable structure to befit different types of tissues make nanofibers a competent candidate for tissue engineering.

Keywords: nanofiber ; scaffold ; electrospinning ; tissue engineering

1. Biofunctionalization of Polymers

As discussed in previous paragraphs, most of the natural polymers used to construct scaffolds retain some form of similarity with the ECM found in tissues, but these polymers lack the required attributes such as mechanical strength, adequate stability in vivo and elasticity for its application in TE. Thus, investigators have incorporated synthetic polymers for their favorable mechanical qualities such as strength and elasticity, along with other desirable features of hydrophobicity and slow degradation rate. But synthetic polymers are also ridden with many drawbacks such as inadequate cellular interaction and nonresponse toward tissue integration. These challenges linked with synthetic polymers are due to the structural differences at molecular level which leads to lack of cell surface recognition sites.

One of the way to get around this barrier is surface alteration with biomolecules, where the bulk properties of the polymer especially elasticity and its ability to withstand stress remain unaffected, although alterations in the surface confer necessary characteristics. Such superficial modifications favor an enhanced cellular adherence, causing a drastic improvement in cellular proliferation and supports faster integration of the implant in vivo [1].

Surface modification using biomolecules has remained one of the preferred methods for the advantages it provides in tissue regeneration. Such biofunctionalization involves immobilization of biomolecules on the polymer matrix surfaces to promote cell adhesion and proliferation. Preferred biosignal molecules used for immobilization are cell-growth-factor proteins, therapeutic proteins and cell-adhesion-factor protein [2][3]. Such biomolecules for immobilization includes growth factors, peptide sequences (RGD), natural ECM proteins (fibronectin, laminin, collagen), heparin, heparin sulfate binding peptides among others [4]. Besides providing structural backbone, the scaffolds modified with ECM components initiate cellular interactions which are decisive for cell attachment, growth and differentiation [5].

Numerous techniques have been worked out for physical or chemical immobilization of such protein molecules. These are grafting, polymer blending and chemically modifying the polymers. To comprehend about the biofunctionalization of polymers, is it necessary to be aware about the composition of the ECM.

ECM is a complex network comprised of a cluster of macromolecules organized according to tissue type. It is composed of two prime families of macromolecules: fibrous proteins and proteoglycans (PGs) [6]. Collagens, elastins, fibronectins and laminins are the fundamental fibrous ECM proteins [7]. Collagen is the principal structural element of the ECM and the most extensive fibrous protein forming the ECM. It makes up about 30% of the total protein weight in animals and perform an array of functions such as providing resistance to breaking under tension, controlling cell adhesion, assisting chemotaxis and directing development of tissues [8]. Collagen is accompanied by elastin, which is another essential ECM fibrous protein. Elastin confers recoiling property to those tissues which undergoes frequent stretching. Fibronectins are engaged in guiding the arrangement of ECM with an essential role in facilitating cell attachment. These proteins are associated together by proteoglycans and makes up the thin fibers of the ECM [9]. Proteoglycans (PGs) are constituted of

glycosaminoglycan (GAG) chains linked to a core protein with covalent bonding. Proteoglycans perform an important function of signal transduction by binding various signal molecules and regulate many cellular processes, in addition to being a structural protein [10]. GAGs are highly water loving and adopt immensely extended conformations that lead to development of hydrogels. The matrices formed by GAGs are capable to withstand high compressive forces [7].

Collagen (type I) is the most copious extracellular protein and it exists in a nanorange fibrillar structure. Such fibrillar morphology has been demonstrated to be crucial for attachment of cells, their growth and differentiation [11]. Collagen is one of the most favored bioactive molecules used for coating, as it provides the biomimetic environment for cell life cycle. Duan et al. constructed PCL nanofibers using electrospinning and layered it with collagen to merge the desirable attributes of collagen and PCL. PCL possess superior mechanical characteristics, yet its hydrophobicity and poor cell affinity results into poor cell attachment and proliferation. Collagen was immobilized on PCL nanofibers with the aim to improve the cell affinity of nanofibers after surface modification using remote plasma treatment.

The inclusion of Gelatin in scaffolds enhances the characteristics such as cell attachment, cell growth and biomineralization. Coating of the polymer matrices using gelatin resulted into enhanced biocompatibility and mechanical performance [12]. Such coating with gelatin also suppresses the activation of the complement system and opsonization, thus reduces immunogenicity of other polymers in matrix [13]. The presence of gelatin improved cellular proliferation of mouse embryonic fibroblasts (MEF) in electrospun PCL nanofibers blended with gelatin and those coated with gelatin, but the highest improvement was observed for nanofibrous scaffolds prepared using blend of PCL and gelatin [14]. Safaeijavan et al. altered the surface of PCL nanofibers by gelatin grafting to enhance their compatibility with living medium. For grafting, PCL scaffolds were initially given air plasma treatment which adds carboxyl groups on polymer surface. Gelatin molecules were then covalently grafted on nanofiber, which inserted amine functional groups on the surface. Such grafting not only increased the hydrophilicity of the scaffold but also enabled the scaffold to hold fibroblast cells and support their survival and functioning [15].

Fibronectin is large adhesive glycoprotein of the ECM essential for cell functions such as adhesion, spreading and motility. In a study, the functionalization of PCL electrospun fibers with fibronectin was achieved using three different approaches —protein surface entrapment, chemical functionalization and coaxial electrospinning. Improved cell adhesion and proliferation of bone murine stromal cells was obtained for scaffolds functionalized using all the three approaches. But sample with the surface entrapment of fibronectin demonstrated better performance in terms of cell response, which indicated that surface entrapment was the best approach to attain efficient functionalization for electrospun fibers [16]. Xie et al. fabricated scaffolds of PCL nanofibers with radial alignment. Influence of fiber alignment and fibronectin surface layering on cell motility of fibroblasts was studied. It indicated that fibronectin coating was able to boost the effect of topographic cues offered by the fiber alignment on cell morphology. Even in the case of randomly aligned nanofibers coated withfibronectin, cell adherence and distribution were enhancedcompared to the unlayered sample [17].

One of the most frequently employed peptides is RGD (arginine-glycine-aspartic acid) which originates from fibronectin. RGD is the leading integrin-binding domain situated inside various ECM proteins such as fibrinogen, fibronectin, vitronectin, osteopontin, bone sialoprotein as well as in some laminins and collagens [18]. It not only regulates the endothelial cells adhesion, migration and proliferation but also can be utilized to preferentially focus on certain cell lines and bring out specific cell responses. The grafting of short peptide sequences like RGD has some benefits when compared to entire protein molecules, such as greater stability under sterilization processes, storage, heat application, pH alterations and against enzymatic degradation. Short peptides also has lower space requirement, which leads to a higher density packaging of the peptides [19]. But RGD is recognized by numerous integrins, thus acts as a nonspecific peptide [20]. Choi et al. developed electrospun nanofibrous matrix of polyurethane over which RGD peptides were immobilized to enhance affinity of endothelial cells. RGD-immobilized matrix exhibited improved viability of human umbilical vein endothelial cells in comparison with an unaltered surface, proving that immobilization of RGD peptide has benefitted cell proliferation [21]. Besides RGD, several other cell adhesion motifs have been recognized namely DGEA peptide from collagen, GREDVY, KQAGDV peptide from fibronectin, PHSRN, etc. [19]. Thus, the RGD sequence can not be considered as the “universal cell recognition motif”, nevertheless it is one-of-a-kind given its broad distribution and usage.

Laminin (LM) is heterotrimeric glycoprotein having high molecular weight. It is an essential constituent of basement membrane lining many tissues. This glycoprotein is necessary for activities like cell attachment, survival, growth, mobility and specialization [22]. Junka et al. developed electrospun nanofibers for tissue regeneration in large-gap peripheral nerve injury. Nanofibrous scaffolds employed blends of PCL and chitosan. Functionalization of the scaffold surface with laminin was done by crosslinking and by using conventional adsorption method. Schwann cell attachment and proliferation rates were found to be significantly greater on laminin crosslinked to PCL-chitosan scaffolds in comparison to scaffolds

adsorbed with laminin or scaffolds without laminin [23]. Incorporation of Laminin in scaffolds has been tried for the regeneration of many diverse tissues including intervertebral fibrocartilage, muscles, neurons and blood vessels [22].

The natural adhesion between the ECM and cells generally depends on the creation of integrin-interceded bonds between integrins in the cell membrane and adhesion proteins or motifs in ECM. Here, the presence of cell membrane integrin controls the efficiency of cell adhesion. However, avidin-biotin linkage is an extrinsic, integrin-independent, high affinity receptor-ligand complex. This avidin-biotin system can be utilized for improved seeding of the cells into scaffolds. Given approach is founded on the existence of multiple binding sites on avidin for biotin and the strong non-covalent interaction between them. In TERM applications, biopolymer matrices are conjugated with avidin and cell membranes are attached with biotin to enhance cell interaction with matrices. Pan et.al evaluated avidin-biotin technology with poly(caprolactone-co-lactide)/Pluronic (PLCL/Pluronic) nanofiber based scaffolds for improving cell adhesion. Nanofiber surface is coated with avidin, whereas cellular membrane is attached with biotin.

After in vivo exposure of scaffold, fibronectin and vitronectin gets adsorbed on the surface of scaffold non-specifically. By virtue of such adhered ECM proteins, the cell-scaffold interaction improves. Such interactions are controlled by integrins, which are cell surface receptors principally involved in attachment of cells to ECM [3].

Another commonly exercised approach in TE to bring out cellular differentiation is the utilization of growth factors. Yet, the constraints linked with the use of growth factors, such as rapid blood clearance, large dose requirement and heavy price, have aroused the exploration of growth factor substitutes, including mimicking molecules. Insulin has been examined as a biochemical signal due to its structural alikeness with Insulin Growth Factor-1 (IGF-1) and similarity between their receptors [24]. Ramos et al. developed insulin functionalized scaffolds where insulin was immobilized on polycaprolactone—cellulose acetate electrospun fiber matrices. The cells incubated on insulin conjugated scaffolds presented a rise in tendon markers, indicating potential of its use for tendon repair and regeneration [25]. In another study, a significantly increase in collagen I and III was observed postsurgery where bioactive insulin-immobilized electrospun nanofiber matrices cultured with mesenchymal stem cells were sutured to transected Achilles tendons in animal model. Furthermore, these matrices promoted alignment of collagen fibrils in regenerated tendons [26].

Mussle inspired peptides have attracted significant attention to functionalize material surfaces because it caters a simple and flexible approach and eliminate the requirement of expensive or complex instruments and procedures. Mussle inspired chemistry is founded on catechol-effectuated molecular adhesion [27]. Polydopamine (PDA) is one of the mussle inspired molecule. Chen et al. successfully used PDA to mediate bromelain immobilization on electrospun PCL fibers. Purpose of such immobilization was to apply antibacterial, anti-inflammatory, anti-edematous activities of bromelein and its capability to hydrolyze necrotic tissues to augment rates of wound healing. Bromelain–polydopamine–polycaprolactone (BrPDA-PCL) fibers exhibited superior biocompatibility given the hydrophilicity of the PDA coating which provides a suitable surface for cell adhesion. BrPDA-PCL fibrous membrane was observed to be highly effective wound dressing. It exhibited antibacterial activity, in addition to assist both cellular adhesion and proliferation [28]. In another study, mussel-inspired polynorepinephrine (pNE) was used to coat PCL fibers to improve hydrophilic nature and cellular interaction of hydrophobic surfaces. pNE functionalization created suitable environment both in vitro and in vivo for skeletal muscle cell adhesion and proliferation [29]. pNE coating has been also been utilized to create bio-interface by applying smooth coating of pNE on electrospun Poly(lactic acid-co-caprolactone) fibers. Here, the catechol groups from pNE assisted in collagen anchoring to improve cell adhesion and to immobilize nerve growth factor to advance differentiation to neurons [30].Polyphenol is another biomolecule whose addition in nanofibrous scaffolds increases cell adhesion, proliferation and differentiation, along with exhibiting their antioxidant and antimicrobial activity. Many polyphenols such as curcumin, naringin, apigenin, icarrin have been studied for bone tissue regeneration, which indicates their prospective for use in TE [31].

Along with the improvement in cell adhesion and proliferation with adoption of biofunctionalization using different approaches as seen in earlier paragraphs, further improvement in tissue regeneration can be achieved with the use of various growth factors. The simultaneous deliverance of angiogenesis-related factors and other biomolecules by nanofibrous matrices has demonstrated to boost tissue repair and regeneration [32]. Angiogenesis is of a pivotal occurrence in tissue regeneration which is essential to carry out the functions such as delivery of oxygen, nutrients, growth factors, ligands and disposal of metabolic byproducts. Therefore, numerous bioactive molecules have been incorporated in biomaterials to impart angiogenic activity. Scaffold-based transfer of vascular endothelial growth factor (VEGF) and bone morphogenetic protein 2 (BMP2) is the commonly investigated combination to promote angiogenesis and osteogenesis owing to their respective pro-angiogenic and osteoinductive activities [33][34]. Kai et al. fabricated PCL-gelatin (PG) nanofibers in which VEGF was incorporated using two individual methods namely blending and co-axial electrospinning to induce the cardiac differentiation of cells. The VEGF incorporated nanofibers improved the cell growth

and division of mesenchymal stem cells (MSCs), promoted cardiac differentiation of MSCs and helped in enhancing the translation of cardiac-specific proteins [35].

VEGF has reported to be angiogenic and promoted formation of natural bypasses in cases of myocardium infarction by promoting generation of neovasculature and dissolution of existing vasculature [36]. Many recent findings in TERM offer proof that surface immobilization of growth factors helps in induction of activity for prolonged duration. Guex et al. used electrospinning for fabricating PCL nanofibrous constructs and VEGF was covalently bound to it. On evaluation of its effect on cell division of endothelial cells in vitro, it was observed that number of endothelial cells were noticeably increased on VEGF-immobilized scaffolds in comparison to non-functionalized PCL scaffolds, suggesting that biological activity of immobilized VEGF was maintained [37].

Epidermal growth factor (EGF) induces growth, proliferation, differentiation as well as cell survival by binding with its membrane receptor [38] and is considered the frontrunner in advancement of wound healing [39]. EGF facilitate wound healing by improving epidermal and mesenchymal restoration, cell migration, proliferation and ECM regeneration [39]. PVA electrospun nanofibers were fabricated to act as biological wound dressing scaffolds by Asiri et al. EGF and fibroblast growth factor (FGF) were incorporated in the PVA nanofibers which resulted in the improvement in wettability and surface roughness. Growth factor release from the PVA nanofibers resulted in stimulation of cell adhesion, proliferation and improvement in cell viability. In vivo evaluation showed that GFs added PVA nanofibers expedited the healing process in burn wound by boosting epithelialization and proliferation of dermal fibroblasts [40].

2. Fabrication Techniques of Nanofibers

Diverse ways has been explored to fabricate nanofibers, some of which are template synthesis, phase separation, self assembly, interfacial polymerization and electrospinning [41]. Apart from the selection of the material from the broad range of polymers for fabricating nanofibers, the management of nanofiber diameter is extremely decisive in biomedical applications, as it decides the surface area for cellular interactions. Alongside fiber diameter, other attributes namely fiber morphology, architecture and alignment are also the significant variables instrumental in deciding the cell-fiber interactions for biomedical applications [42][43]. Above mentioned are some of the parameters used in selection of nanofiber fabrication technique. Scalability to the commercial scale is another crucial factor to consider while selecting fabrication technique. Among the mentioned techniques, electrospinning is the extensively experimented nanofiber fabrication technique and it has offered the most encouraging outcomes for TERM applications. Nanofiber synthesis using other techniques for TE application has been studies on relatively limited basis.

In Phase separation technique, nano-fibrous matrices are prepared following a process that involves polymer dissolution, thermally induced gelation, exchange of solvent, freezing and freeze-drying [44]. Gelation is the decisive stage in this technique for the creation of fibrillar matrix. Gelation of polymer solution depends upon solvents used, polymer concentration and gelation temperature. Gelling temperature is another critical element influencing the porous structure of the fibrous matrices. Porosity up to 98.5% had been achieved using this technique [45]. Some of the strengths of phase separation technique are minimum requirement of sophisticated equipments, simplified procedure, ability of the method to produce fibers in nanorange and capability to construct fibrous scaffold matrix in the anatomical shape of the body part using mold.

Biomolecular self-assembly presents an easy way to manufacture functional nanomaterials. The self-assembly mechanisms of biomolecules are based on varied internal interactions, such as hydrogen bonding, electrostatic interactions, hydrophobic interactions, π–π stacking, ligand–receptor binding and DNA base pairing. In addition, self-assembly can be induced using external stimulations like making alterations in solution attributes such as pH, ion concentration and temperature, by addition of organic solvents or enzymes, and with the help of light [46]. Self assembly is a bottom up technique to manufacture nanofibers in which molecules tend to align themselves in specific patterns to generate nanofibers. The structure of the individual molecules taking part in self-assembly and the intermolecular forces involved in molecular interactions decides the morphology of the nanofibers. This technique is capable of producing fibres in nano range. Yet the drawbacks such as low productivity, arduous handling of the fiber dimensions, constricted choices of materials which can self-assemble and being a cumbersome process, this method is of least preference [41].

Template synthesis of nanofibers involves extrusion of polymer precursor solution from the nanoporous membrane into the solidifying solution under pressure. As soon as polymer solution touches solidifying solution, nanofibers are created. Nanofibrous membrane containing cylindrical pores is used as as a template/mold. Aligned nanofibers with different diameters can be fabricated using templates with different pore diameters [47]. Limitation of this technique is the formation of discontinuous fibers having variable diameters.

Interfacial polymerization is another method of generating nanofibers, which is mainly a polycondensation reaction happening at the interface between two kinds of monomers solubilised in two non-miscible solvents. On mixing two distinct phases containing monomers, polymerization happens at the interface of the dispersed phase and dispersion medium of emulsion. Homogeneous nucleated growth is the key determinant in this technique.

2.1. Electrospinning Method

Electrospinning has come out as one of the most rewarding techniques, considering its capability to manufacture fibers in the nanometer range, having morphology which is comparable to the ECM fibrous structure. It also provides control over the fiber diameter, its composition and the porosity of nanofiber meshes [44]. Furthermore, this technique is remarkably simple, robust, and versatile, thus making it the preferred choice for preparing nanofibers. Polymer can be selected from a wide range of materials amenable to electrospinning and this technique has been employed by now to manufacture nanofibers of more than 100 different kinds of polymers [48].This method is adept for scaling up to make production at commercial scale [49]. Since the attributes of electrospun nanofibers are highly manageable and can be customized to befit various tissues or to encapsulate different drugs, such fabricated nanofibers are highly resilient for use in different biomedical applications.

The electrostatic repulsion between polymer molecules help in overcoming surface tension of the polymer solution, drawing a jet from polymer solution drop and further stretching of the jet is the principle on which electrospinning is based. Such electrostatic repulsion between polymer molecules develops with the help of electrical potential difference applied between the electrodes. A symbolic electrospinning setup. On the induction of voltage between the needle and the collector, charges begin to develop on the polymer molecules in the needle. The magnitude of charge in the polymer molecule determines the extent of electrostatic repulsion experienced by the molecules of polymer in polymer solution. Surface area of the polymer solution increases as the electrostatic repulsion between polymer molecules increase [42]. But to produce fibers in nanometer range, charges on the polymer molecules should be dense enough and at the same time it should not that high to cause the solution jet to split into droplets [50]. The electrostatic repulsion developed between polymer molecules opposes the cohesive forces between polymer molecules and led to the formation of Taylor cone. This Taylor cone then turns into charged jet, which stretches, thins out and finally collects on the metal surface. All along the travel of polymer solution from Taylor cone to the collector, solvent evaporation provides rigidity to the fiber. The solvent evaporation mechanism also influences the porosity of the fibers [42].

The parameters which influences the characteristics of the nanofibers obtained can be broadly divided into parameters related to electrospinning solution, parameters related to processand environmental parameters. Polymer solution concentration controls viscosity, surface tension as well as charge density and is thus the prime parameter deciding fiber diameter [51]. Other solution related parameters includes polymer molecular weight and distribution and solvent or mixture of solvent used. Process parameters include parameters related to equipment set up namely orifice diameter, voltage, solution feed rate, spinning distance, design of collector and motion of collectorthat affects the nanofiber attributes. Apart from these, the ambient factors like temperature and humidity, which are covered under environmental parameters affect the quality of nanofibers obtained. High humidity lengthens the solidification time required by the fibers after their ejection from the needle orifice, whereas low humidity assists in efficient removal of the solvents from the nanofibers. Humidity also influences the surface morphology of the fibers, with increase in humidity has been reported to cause an increase in number and size of the pores in the nanofibers [52][53]. Increase in ambient temperature has been reported to cause reduction in diameter of electrospun fibers [54]. Yet, the influence of the environmental factors on the properties of fibers should be studied on case by case basis.

Fridrikh et al. presented a model that predicts terminal jet diameter based on the availability of information on flow rate, applied voltage, and interfacial tension of the liquid. Fiber formation in electrospinning is a result of counterbalance between polymer adhesive forces due to surface tension and repulsive forces due similar charges on polymer molecules. On this relation, the prediction of fiber diameter has been based [55]. However, electrospinning is also linked with certain shortcomings such as wide range of electrospun fiber diameter, irregular alignment of fibers, and poor mechanical performance of the fiber matrices [43].

2.1.1. Single Nozzle Electrospinning

Single nozzle electrospinning uses a nozzle with single aperture through which polymer melt or solution outflows. Composite nanofibers can be manufactured using this mode of electrospinning. Compatible polymers can be employed for electrospinning of polymer blends. Moreover, solid nanoparticles can be embedded within electrospun fibers and liquid phase particles can be electrospun using emulsion electrospinning method. Single nozzle electrospinning method uses either blend of bioactive molecules in polymer dissolved in solvent, melt of polymers or emulsions for electrospinning.

2.1.2. Co-Axial Electrospinning

Co-axial electrospinning produces core-sheath fibers by physically separating them using two co-axial electrospinning needles and two solutions. Co-axial electrospinning uses the concurrent flow of different solutions through two co-axial capillaries to physically separate core and shell fibers. Specific processing parameters such as solution flow rates and solution properties like viscosities and electrical conductivities are typically taken into account while attempting to apply the co-axial approach. For example, the compositions of the fiber's core and sheath may be chosen depending on their ability to provide strength and their ability to support cells respectively. Selection of polymeric material and solvent is of importance for consistent generation of coaxial fibers. Viscoelasticity of the polymer solution forming shell should be sufficient to stabilize the fluid jet to create core-shell morphology of the fibers. A study shows that the morphology of the nanofibers depends on the interaction between the core and shell solutions during co-axial electrospinning, rather than their individual effects. If two highly miscible solutions are used, then partial mixing of those solutions occurs during co-electrospinning, which significantly influences the morphology of resulting nanofibers [56].

This technique can be applied to even non-electrospinnable materials, which can form the core of the core-shell nanofibers, whereas solutions forming the shells should possess spinnability. Moreover, active compounds devoid of fibrous characteristics can also be enclosed in the fiber core. The technique also offers the benefit of building a single drug delivery system from two or more bioactive compounds with varied biological activity and solubilities. Coaxial electrospun fibers with topographical and biochemical features are utilized for TE applications. Drugs are often incorporated in the core and released by shell polymer degradation or sheath pores. A longer-lasting drug release is possible using coaxial electrospinning of drugs and polymers. The sheath barrier effect can stop an initial burst discharge of the drugs [57]. Core–shell nanofibers have also been explored for the dual discharge of growth factors, wherein growth factor incorporated in core followed a time-controlled release compared to the growth factor attached on the shell surface [58]. Dual drug loading has also been achieved using core-shell nanofibers by loading drugs each in the core and the shell. Core exhibited the long-term release, whereas shell showed short-term release of the drugs to improve the tissue regeneration efficiency of the scaffolds fabricated from such fibers [59].

Although it requires a complicated setup, coaxial electrospinning offers numerous benefits like one-step technique for encapsulating, ability to make composite nanofibers, and its applicability for a variety of materials. With all of its benefits, coaxial electrospinning has been extensively utilised in the creation of nanofibers for varied applications [60].

2.1.3. Multiple-Jet Electrospinning

The preliminary form of electrospinning uses a single-needle to efflux the polymer solution and to create fibers. Notwithstanding the range of benefits offered by this simple form of electrospinning, the major drawback of the lower production rate with conventional electrospinning restricts the utilization of the process at a commercial scale. Multiple-jet electrospinning technique has been considered to surmount this deficiency of lower rate of production, but creation of multiple jets carries with it the issues including repulsion between jets, non-uniformity of electrical fields, poor control over the process and decline in fiber quality [61]. This necessitates even further development and optimization of the process. The operating principle of multi-nozzle electrospinning is same as that of conventional single-needle electrospinning technique, with the major difference lies in use of multiple nozzles. In a single setup, multiple nozzles are arranged in various configurations to generate multiple jets [62]

2.1.4. Blend Electrospinning

In Blend electrospinning technique, bioactive materials are solubilised or suspended within polymer solution. Physicochemical characteristics of the solution and its interaction with the bioactive materials decides the disposition of bioactive molecules within fibers [63]. Blend electrospinning method is uncomplicated compared to coaxial and emulsion electrospinning, but it also has some drawbacks such as sensitive bioactive agents may get denatured due to presence of the solvents and thus suffer from loss of their bioactivity [64]. Polymers such as poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) having good water solubility, have been utilized to encapsulate bioactive proteins [65][66]. Surface accumulation of bioactive molecules is commonly observed in nanofibers, because such molecules are charged and they migrate towards the surface of the jet due to repulsion between them, during jet ejection and elongation.

2.1.5. Emulsion Electrospinning

The emulsion electrospinning involves basic set up similar to that of blend electrospinning but comprises spinning of emulsion. This is another unique and simple approach to electrospin core-shell nanofibers using a single nozzle spinneret. In comparison with coaxial electrospinning which employs coaxial needles to manufacture nanofibers with core-shell morphology, emulsion electrospinning uses single nozzle to electrospun nanofibers, therefore making it simple and more

conducive for scaling up [67]. Core-shell structure is obtainable in electrospun nanofibers using either water-in-oil (W/O) [67] or oil-in-water (O/W) emulsions [68] to load respectively hydrophilic or hydrophobic compounds into the core of nanofibers [69]. In this method, polymer is solubilised in organic or aqueous solvent to form the dispersion medium whereas bioactive substances are solubilised in organic or aqueous solvent forming dispersed phase. Formulation of emulsion eliminates the requirement for common solvent for polymer and bioactive molecules. Availability of common solvent is considered as a primary necessity of the blend electrospinning technique, which is omitted in emulsion electrospinning. After ejection of jet in emulsion electrospinning, evaporation of the solvent of dispersion medium from ejected jet increases the viscosity of that phase. As a consequence, droplets of the dispersed phase travel to the core of the jet due to viscosity gradient [70]. Mutual dielectrophoresis caused by electric field led to coalescence of the droplets at the centre of the fiber, thus giving fiber a core–shell morphology. Stability of the emulsion is a decisive consideration for emulsion electrospinning, which necessitates addition of emulsifier to prevent emulsion from breaking down. This technique has been developed to incorporate functional elements such as enzymes, bioactive proteins and drugs. This technique is a potential alternative to conventional electrospinning methods because it enables loading of lipophilic drugs using affordable hydrophilic polymers and bypass the requirement of restricted, less safe solvents [63]. Some other crucial determinants of fiber characteristics include the nature of emulsion, strength of applied electric potential difference, conductivity of dispersed phase, interfacial tension exhibited by emulsion, and cooling time among others [71].

2.1.6. Cell-Electrospinning

On account of aforementioned benefits, electrospinning has earned noteworthy attention for applications in biomedical field. Nevertheless, it carries some constraints with it, such as utilization of toxic solvents, low and uncertain cell penetration and non-uniform cell distribution [72]. In addition, to seed cells in scaffold, it needs to be kept in bioreactor for long durations. Even then, there remains the uncertainty about the distribution of the cells within scaffold. To subdue such limitations, a unique approach, termed cell-electrospinning (C-ES), was invented. Cell electrospinning was discovered in around 2005–2006 [73][74].

In this technique, one of the approach is to encapsulate the biosuspension containing living cells in the core of a fiber, using a coaxial needle, within a shell fabricated out of a biocompatible polymer [74]. Electrical conductivity of biosuspension flowing through the inner needle and polymer solution flowing through the outer needle make an important consideration for the electrospinning. Viscosity, flow rate of both the liquids and the strength of the applied electric field are critical variables to analyze in this technique. Needle with different configuration such as single as well as tri-needle can also be used. The type of needle used decides the core arrangement, which can vary from single to tri-core morphology. Another consideration is that the ground electrode in cell electrospinning is significantly different compared to those used in conventional electrospinning technique. This modification is to avoid dehydration of the encapsulated cells to avert cellular damage or death [73]. Due to the negative effect of the electric field on the viability of the cells in biosuspension, magnitude of the electric field can not be raised above threshold. Dehydration and shear developed during stretching of the fibers are the probable reasons besides the applied electric field for the low viability of the cells during cell electrospinning process.

A study developed active biological microthreads using coaxial electrospinning method. A concentrated living biosuspension was used to form the core and a medical grade poly(dimethylsiloxane) was used to form the shell of the microthreads. Along the length of the microthreads, cell aggregates generated the capsules. Cell viability assay showed that the viability of the cells passed through the electric field to be around 67%, which was not statistically much different from the viability of the control cells. No indications of any harm to encapsulated cells were observed while generating microthreads through cell electrospinning using co-axial needles [74].

Guo et al. developed an electrospinning approach to enclose cellular aggregates into fibrin/polyethylene oxide microfibers. Encapsulated cellular aggregates within fibrinogen microfibers were suspended into a rotating bath containing thrombin to produce fibrin fibrils by thrombin induced polymerization of fibrin. Researcher established that loading cellular aggregates less than 100 µm in size and adjusting process parameters in electrospinning led to improved cell survival [75]. Considering the great interest developed in the area of cell electrospinning owing to the benefits provided, more of the studies are expected in future.

3. Applications of Polymeric Nanofibers

3.1. Neural Tissue Regeneration

The peripheral nerve injury creates a major problem in their repair and restoration. Autografting, allografting and xenografting offers recourse to overcome this difficulty. However, donor site morbidity, the lack of donors and low

proficiency in grafting techniques turn up to be limitations of these alternatives [76]. In contrast, the electrospun nanofibers offer multiple benefits, including controlled alignment which provides spatial assistance for neurite outgrowth, axon lengthening [77] and mechanical cues for differentiation of stem cells [78]. Apart from this, aligned nanofibers were noticed for supporting Schwann cells migration and thus assist in reestablishing a growth cone at the tip [79].

Afrash et al. developed a nerve growth factor (NGF) functionalized aligned nanofibrous scaffold based on polycaprolactone/chitosan (PCL/CS) polymers for tissue regeneration of neural cells. NGF was used as a neurotrophin and it was attached to PCL/CS nanofibers with the use of dopamine coating. Polydopamine coating reduced the hydrophilicity of the nanofibers, whereas immobilization of NGF on the nanofibers improved the hydrophilic nature. It was observed that, aligned fibers were more hydrophilic compared to randomly aligned fibers. It established that topography and morphology can control interfacial tension. It also demonstrated that regular alignment of PCL/CS nanofibers could provide desirable conditions for neural cell growth [80]. In another study by Xieet al., the characterization of embryonic stem (ES) cell culture on electrospun PCL nanofibers with regular and irregular alignment, manifested the significance of material topography in cell differentiation. PCL nanofibers seeded with ES cells showed that stem cells specialized to oligodendrocytes and astrocytes along with many other neural lineage cells.

3.2. Vascular Tissue Regeneration

An impediment of in vitro fabrication of vascular tissues to fulfill the clinical necessity of tissue grafts is lingering since the dawn of TERM [81].The 1950s saw the development of synthetic tissue-engineered vascular grafts (TEVGs) to restore blocked arteries after surgical complications. TEVGs were used as a remedy to the regular scarcity of allogenic tissue grafting and to mitigate the problem of immunological rejections after transplantation. But these synthetic TEVGs were found to be unable to noticeably reduce overall mortality and morbidity [82]. To solve this issue, the researchers have employed various in vitro strategies to prepare vascular tissue having ability to interact with cells to develop new blood vessels [83].

Shin et al. fabricated PLGA nanofibers with co-functionalization of RGD peptide and graphene oxide (GO) for vascular TE using the electrospinning technique. Surface functionalization with RGD and GO on PLGA nanofiber improved hydrophilicity and facilitated interaction between nanofiber and cells. RGD peptide functionalization greatly increased initial attachment and growth of vascular smooth muscle cells (VSMCs). In addition, GO also supported enhanced proliferation of VSMCs. The study shows the promising potential of RGD-GO-PLGA nanofiber matrices for vascular tissue regeneration [84]. Marelli et al.electrospun SF into fibers with tubular morphology for small diameter vessel grafting. These electrospun tubes were able to resist pressure up to 575 ± 17 mm Hg, which is more than fourfold of normal systolic pressure i.e., 120 mm Hg and more than twice that of pathological upper pressure of 220 mm Hg. SF tubes displayed good cytological compatibility in in vitro analysis.

3.3. Cartilage Tissue Regeneration

Articular cartilage is a functional connective tissue which covers the ends of bones at the site of junction of two or more moving bones. The ECM in cartilage tissue is dense, while chondrocytes are thinly distributed within matrix. Such entrapment of chondrocytes within dense microenvironment prevents its mobility to adjoining regions within cartilage. Though chondrocytes responds to variety of stimuli, it rarely form cell –to-cell contacts for direct cell transduction. Moreover, limited ability of chondrocytes to replicate is responsible for poor regenerative capability of cartilage in case of an injury [85]. In addition, articular cartilage lacks blood vessels, lymphatics and nerves, limiting its ability to regenerate tissue after injury. Damage in the cartilages necessitates replacement most of the times. To settle this problem, researchers have experimented with many TE strategies, including sponges, hydrogel scaffolds, gelatin microsphere, and collagen sponges. These approaches showed limited improvement in the cartilage healing process. Conversely, the nanofibers synthesized from synthetic, natural, and composite polymers provide good results due to its resemblance with the ECM. Such Nanofibers promote the cell-ECM interaction and chondrogenic differentiation. Very high surface area compared to total volume of the aligned nanofibers manifests the potential of engineering articular cartilage using approaches of TE [43][83].

Semitela et al. synthesized the bioactive polycaprolactone-gelatin nanofibers scaffolds (PCL + GEL) with enhanced pore size and interconnectivity for cartilage tissue repair. Polyethylene glycol (PEG) was incorporated during the electrospinning process and subsequently eliminated to get enlarged pore size. This innovative method was used to subdue two weaknesses of PCL electrospun fibers which are small pore size and lack of bio-inductive property. The scaffolds with improved pore diameter and interconnectivity enabled enhanced cell infiltration and homogeneous cell distribution, thus creating the potential to generate functional tissue [86].

An electrospun composite containing uniformly distributed but distinct fibers of PCL and PEO was developed. In this composite scaffold, fibers of polyethylene oxide formed the removable sacrificial fiber fraction. Both polymers were chosen based on their stability in hydrated environment, such as PCL is slowly degrading, whereas PEO dissolves immediately upon hydration. Although, removal of sacrificial fiber content resulted in reduced mechanical properties, it increased the size of the pores within the scaffold. Increase in sacrificial fiber fraction in the construct augmented cellular infiltration within construct. Construct with 60% PEO fraction, was observed to be fully colonized with seeded cells and was able to direct cell morphology and consequent matrix formation [87].

3.4. Bone Tissue Regeneration

Bone is one of the highly vascularized tissues in the human body. It is categorized into cortical bone and trabecular bone. Cortical bone is a dense, solid bone, extends mechanical support to human body and protection to bone marrow whereas trabecular bone is biologically active, enables joint and limb movement. The bone structure is made up of 69% of inorganic component containing hydroxyapatite and calcium phosphate complex contributing to bone its compactness and stiffness, while organic component composed of collagen and other structural proteins accounts for about 22% [88].

The regeneration of bones is a complex process involving a series of osteoinductive processes. Therefore bone TE demands the utilization of scaffolding, cells, chemical signaling and mechanical forces to create customized tissues. Biomimetic scaffolding for bone repair can include features such as high porosity to aid cell attachment, migration, proliferation and differentiation and biomechanical stress tolerance ability to endure stress generated within body during tissue regeneration [83]. A growing number of bone illnesses including infections, cancer and bone loss, necessitate bone regeneration. The vigorous course of bone TE begins with movement and recruitment of osteoprogenitor cells, and continues with cellular growth, differentiation, matrix production, and bone remodelling. Mechanical characteristics of bones are due to unique structural design of bone that extends from nano range to macro range dimensions, along with specific interconnections. Bone TE focuses on developing three-dimensional scaffolds that can replicate the ECM, offer structural support as well as aid in regeneration of bone. To increase the attachment, viability and mobility of osteogenic cells, scaffolds should have osteo-conductive, osteo-inductive, and osteogenic characteristics. To impart these characteristics, scaffolds are manufactured to provide mechanical and chemical cues that induce osteoblastic lineage formation [89].

Several scientists have attempted to alter the mechanical properties of scaffolds namely stiffness, strength and toughness using various methods and to create nanostructures to imitate bone's natural architecture [90]. Despite many studies focusing on bone TE, much-needed advancements in scaffolds with conceivably superior clinical outcomes are still required. Electrospinning has long been considered an appropriate manufacturing technique for scaffolds by virtue of its multidimensional capacity of making nano- and micro-range fibrous frameworks with configurable fiber features [91].

Using an electrospinning process, PLA fibers encapsulating Fe_3O_4 nanoparticles at concentration of 2 and 5 percent were formed. Bone deformities transplanted with Fe_3O_4/PLA nanofibers displayed a significantly greater rate of bone healing compared to deformities transplanted with plain PLA nanofibers. Furthermore, CT scan demonstrated that the bone defects grafted with Fe_3O_4/PLA nanofibers encapsulating 2 and 5 percent Fe_3O_4 nanoparticles presented 1.9- and 2.3-fold enhancement, respectively, in volume of bone in comparison to the control sample [92]. Miszuket al. fabricated a composite nanofiber based scaffold using polycaprolactone/hydroxyapatite for regeneration of bone using an new thermally induced self-agglomeration (TISA) technique based on electrospinning. High elasticity, porosity even after coating with minerals and easy alteration with the applied pressure to fit to different defect shapes are the reported features making it desirable for application in bone TE. In addition, biomimetic mineral coating on fabricated scaffolds allows simultaneously encapsulation of different types of proteins, small molecules and drugs, under physiologically mild conditions.

3.5. Dermis Tissue Regeneration

Skin lesions usually heal by forming epithelialized scar tissue rather than full skin regeneration. The epidermis has a poor ability to heal compared to the dermis; thus in case of substantial damage to the epidermis, biological regeneration process is insufficient. On the other hand, the dermis has a tremendous ability to rejuvenate. After a skin injury, the scar tissue develops with deficiency of dermis, thus loses the flexibility, elasticity, and toughness of natural dermis [93]. The fibrous structure in native ECM always shows a more intricate design than just straightforward unidirectional alignment. Skin tissue contains collagen fibrils that have a pattern like a basketweave or mesh. As a result, scaffolds with crossed nanofibers performed better than those with random or unidirectionally aligned nanofibers in terms of keratinocyte and fibroblast migration rates.

Collagen in its indigenous form acts as a natural foundation for cell adhesion, division, growth and specialization. Collagen exhibits significant strength in its biological form [94]. In addition, its' biological origin make it the most biomimetic skin substitute created and thus the most preferred material to fabricate nanofibrous scaffold.

Powell and Boyce prepared electrospun submicron fibers using PCL and collagen to design a scaffold. Mechanical performance of nanofibers improved noticeably with mixing of little amount of PCL to collagen without negotiating on the biocompatibility of nanofibers. Keratinocytes and dermal fibroblasts cultured on collagen/PCL nanofiber scaffolds promoted the regeneration of the layered epidermis, dermis and uninterrupted basal layers [95]. Another study intended to evaluate in vivo performance of the PGA/collagen nanofiber on granulation histology and its capability of stimulating new vasculature was conducted out by Sekiya et al. This group of researchers developed PGA/collagen nanofibers using electrospinning technique. When compared to commercially available collagen matrix, histology revealed that fabricated nanofibers demonstrated considerably higher cell density with greater number of migrating cells. These observations indicated the superior ability of the developed nanofibers in relation to cell migration and neovascularization compared to collagen matrix product. This desirable outcome was attributed to the nano-range diameter of fibers and inclusion of PGA [96]. In another study, a highly porous scaffold created out of PCL/chitosan fibers with core–shell morphology were developed using emulsion electrospinning. Presence of high porosity and interconnectivity assisted penetration and proliferation of cells. The scaffold also supported ECM protein translation and in vitro layered epithelialization. Successful incorporation of the scaffold with margins of wound in animal model and rapid healing in around 20 days established the effectiveness of the scaffold as skin graft [97].

3.6. Cardiac Tissue Regeneration

Cardiac tissue has very restricted regenerative capacity, thus cardiac tissue regeneration using the principles of TE is a necessary and appropriate alternative. Some of the challenges associated with cardiac tissue engineering are the choice of polymers for fabricating scaffolds and achieving the required alignment of the microfibrils for guiding the growth of cells and contraction of cardiac muscle cells. Another clinical challenge is the regeneration of heart valves (HVs) because of their complex anatomical structure with leaflets and numerous supporting structures along with having complex, striated ECM [98]. Earlier many attempts to engineer the valves met the failure with disordered ECM and inability to function due to use of isotropic and homogeneous scaffolds [99]. Biomimetic scaffold with heterogeneous and anisotropic characteristics which approach that of inherent heart valve tissue are applicable for regenerating HV tissue. Tissue engineered HVs are contemplated to be capable of adapting to such complexities, indicating its potential as a alternative to existing treatments.

Ahmadi et al. manufactured polyurethane/chitosan/carbon nanotubes (PU/Cs/CNT) composite nanofibrous scaffolds using two techniques. PU/Cs/CNT electrospun scaffolds were manufactured by blending CNT and electrospinning this blend of polyurathane, chitosan and CNT. In other technique, polyurethane/chitosan solution electrospun into nanofibers and CNT were electrosprayed onto nanofibers from the opposite side. The nanofibers were also collected with random and aligned orientation. Addition of CNT substantiallyameliorated the mechanical characteristicsand hydrophilicity of the nanofibers. Improvement of surface properties by hydrophilic chitosan and carboxylated CNTs led to proliferation enhancement of Human umbilical vein endothelial cells in PU/Cs/CNT scaffold compared to PU scaffold. Cardiac rat myoblast cells (H9C2 cells) proliferation on fibrous matrix with electrosprayed CNT was more notable than cell proliferation on PU scaffold. Alamar blue assays demonstrated that number of H9C2 cells on scaffold with electrosprayed CNT, in both aligned and random scaffolds, enhanced notably higher than other scaffolds and control group [100].

To fabricate fibrous scaffold for replicating the anisotropic nature of native heart valves, Xue et al. utilised ring-shaped copper collector for collecting electrospun fibers. This group of researchers fabricated anisotropic fibrous matrices manufactured with (poly(1,3-diamino-2-hydroxypropane-co-glycerol sebacate)-co-poly (ethylene glycol) (APS-co-PEG) and PCL polymer blends that hadadjustable and controllable fiber morphologies and mechanical features. The polymer formulationswere electrospun onto flat aluminum foil and ring-shaped copper wire, producing isotropic and anisotropic fibers, respectively. The scaffolds gathered on flat aluminum foil demonstratedalike mechanical properties in the two perpendicular directions, revealing an isotropic behavior, whereas the scaffolds collected on the ring-shaped collector acted differently in their fiber and cross-fiber directions, indicating mechanical anisotropy. The anisotropic scaffold also showed to possess a Degree of anisotropy (DA) close to that of a porcine aortic valve, indicating its prospectives to be used to regenerate the heart valves [101].

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