Special Issue: Positioning of Tissue Engineering in Regenerative Medicine

Mini Review

Fibrous scaffolds for tissue engineering

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Scaffold, along with seed cells and microenvironment, is an essential component of tissue engineering, and plays an important role in engineered tissue regeneration. Fibrous scaffold is an important part of scaffold materials due to its ability to mimic the structure of native extracellular matrix. With the advancement of materials science and related techniques, fibers can be modified either chemically or physically to gain special physicochemical and biological properties that can provide an artificial niche environment for cell adhesion, proliferation and differentiation and ultimately lead to tissue regeneration. This paper reviews recent advances in the preparation and the application of fibrous scaffold for engineered tissue regeneration.

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Introduction

In the past decades, significant progress has been made in the area of tissue engineering, which reveals a promising future for engineered tissue/organ regeneration. The general principle of tissue engineering is to isolate seed cells from a patient or a donor, to expand the cells *in vitro* and then seed them onto a scaffold material followed by *in vivo* implantation for tissue reconstruction and functional restoration. Thus, scaffold is an essential player for engineered tissue regeneration. In a native tissue, the extracellular matrix (ECM) substances contain a specific topography with a porous structure and unique three dimensional (3D) structure, which composes an important part of extracellular niche environment. With the development of material science technology, mimicking structural characters of native ECM should become an important strategy in order to provide a suitable microenvironment with porous structure, special topography and proper mechanical properties for seed cells to adhere, migrate, proliferate and differentiate as well as for tissue regeneration[1)].

With the development of materials science, scaffolds can be prepared in a variety of physical formats, such as fiber,

mesh, membrane or solid. Among them, fibrous scaffolds have a tremendous potential in tissue engineering as the material can be shaped into a significantly long and narrow shape. Macroscopically, fiber shape scaffolds can accommodate seed cells and guide their growth into fibrous or tubular-like structured tissues as natural tissue ECMs are likely to do. Microscopically, the nanofibrous scaffold can mimic the native ECM alignment (such as collagens) topographically. Furthermore, with the techniques of braiding, knitting and membrane lamination, fibers can be post-processed into mat, tube, or other 3D shaped scaffolds.

Materials

The selection of a proper chemical substance for scaffold preparation is essential for two major characters, biocompatibility and biodegradability. A good scaffold candidate should be able to accommodate itself to the regenerated tissue and do not stimulate undesired tissue response that could lead to inflammation, cell apoptosis, or tissue necrosis. A controllable degradation with a proper degradation time period resulting in nontoxic products should also be an essential character.

Among various chemical substances, synthetic polymer is one of the promising materials for generating fibrous scaffolds. Natural polymers including collagens, chitin and silks, can best simulate the cellular microenvironment chemically, but they are usually difficult to isolate and purify, and the output is usually limited and thus have a high cost. Additionally, they usually exhibit week mechanical properties such as tensile strength and compressive strength. By contrast, synthetic polymers including polylactide (PLA) and polyglycolide (PGA) have enough physical strength, and with proper fabrication and modification, desired fiber properties such as diameter, strength, pore size and porosity can be generated in manufactured scaffolds to meet the tissue engineering requirements.

Fabrication techniques for fibrous scaffolds

With the development of manufacture techniques, numerous methods become available for fabricating fibrous scaffolds, and the major techniques are briefly described in the followings.

1)Phase separation

The essence of phase separation is to separate a polymer solution into two phases with different materials. A polymer is dissolved in a solution and followed by phase separation induced either thermally or via the addition of a non-solvent agent to the polymer solution to create a gel. Then water is used to extract the solvent from the gel. Therefore the pore structure of the scaffold can be generated accordingly (reported porosities as high as 98.5%). Phase separation has been used to produce porous polymer micro-fibers scaffolds and membranes[2-4]. Compared to self-assembly, phase separation is a simple technique without the need of specialized equipment and is easy to achieve batch-to-batch consistency. However it also has its own disadvantage because only a selected number of polymers can be used and the output of the fiber scaffolds is strictly limited to a laboratory scale[5].

2)Melt spinning

Usually a polymer is heated above the glass-transition temperature first, then the fibers are produced with a piston melt-spinning machine by melt extrusion and hot drawing process. The compound is extruded through a round shape spinneret with a micrometer diameter under an inert gas flow. The heated spun fibers were collected under an optimized rate around several godets with different temperatures, then onto a final winder. The diameter of generated fibers is determined by the pore size of spinneret[6]. To generate pore size contained fibers, polymer can also be mixed with other materials such as gelatin, heated, extruded and collected similarly, and the pore size of fibers can be directly controlled by the microsphere diameters of mixed materials such as gelatin particle, and the general porosity changes with the polymer/gelatin ratio[6].

3)Self-assembly

Using natural processes as a guide, self-assembly is a bottom-up fabricating approach for biomaterials, in which materials are assembled molecule by molecule. In this process, a disordered system of pre-existing bioactive molecular components forms an organized structure or patterns as a specific consequence. During the procedure, the assembling process is precise and restricted. To make the self-assembled structure hold together, two key elements in molecular fabrication are chemical complementarity and structural compatibility, both of which confer the weak and non-covalent interactions that bind building blocks together during self-assembly. The non-covalent

bonds, including hydrogen bonds, ionic bonds, hydrophobic interactions, van der Waals interactions, seem to be insignificant separately, when combined together as a whole, they control the structure conformation and effect the interaction among molecules[7, 8]. Therefore self-assembly technique can provide nanofibers with diameters of approximately 10 nm from small molecules. As reported by Davis et al., their experiments demonstrated that the self-assembling peptides could create the micro-environment which is able to promote cell recruitment once implanted *in vivo*, and thus this progress enables the application of injectable scaffolds to tissue regeneration[9]. Collectively, self-assembly presents us another useful tool for domesticating the structure and function of bioactive molecules, though the complexity of the process and required facilities might be the limitation to its wide applications.

4)Electrospinning

In electrospinning, a syringe with a needle, a syringe pump, a high voltage power supply and a collecting plate are the main facilities. The selected polymer solution or melt is loaded into a syringe with a capillary needle and placed in the syringe pump, which is capable of deploying the polymer solution at a controlled rate[10]. With the sufficient high voltage added between the needle and the collecting board, the liquid droplet below the needle gets charged, and electrostatic repulsion counteracts the surface tension of the liquid surface which holds the polymer to the needle tip. Therefore a fluid jet is ejected from the needle and the polymer fibers are deposited on the collector in the form of a non-woven fabric. Electrospun scaffolds are porous materials composed of micro- or nano-scale polymer fibers, which readily mimic structural elements of the natural extracellular matrix[11]. Electrospun scaffolds provide an ideal substrate for cellular adhesion and in-growth[12].

The diameter of the generated fibers and their other properties can be manipulated by a variety of parameters, including polymer solution viscosity/concentration, conductivity/solution charge density, surface tension, polymer molecular weight, dipole moment and dielectric constant, flow rate, field strength/voltage, distance between needle tip and collector, needle tip design and placement, collector composition and ambient parameters. Instead of a flat plate, a rotating drum collector can provide the nanofibers with a directed orientation. The simplicity and efficiency of the process, the economical facilities and the controllability of the fiber properties are all the advantages of electrospinning technique. The limitation is the limited control of the pore structure[13, 14].

Based on electrospinning technique, coaxial electrospinning has two separated coaxial containers to load two different solutions (usually polymer and biomolecule solution). In fabrication, these isolated solutions are simultaneously pumped through capillary channels and a core-shell structure. The different solubility of the two components prevents the two phases from mixing to each other during the fabrication process. The hydrophilic core serves as a reservoir of bioactive molecules; meanwhile the hydrophobic shell presents an output protection and provides a relatively steady release and interaction of biomolecules. In Ji et al. research, the coaxial scaffolds revealed more sustained release profiles than the comparable blend scaffolds, though both methods decreased the biological activity of the core molecules[15]. Accordingly, coaxial electrospinning presents a promising process to manufacture nanofibers with bioactive properties and steady physical and chemical properties fitting for tissue engineering applications.

Another method derived from electrospinning for generating bioactive nanofibers is to disperse biomolecules in the polymer solution and the compound can be used to fabricate a hybrid scaffold. Nevertheless, the physical characters of biomolecules make them unsteady in a solvent usually, and are easily to precipitate and polymerize, and thus may lead to an unpredictable property of the span nanofibers. As reported by Zeng et al., a significant burst release was observed when using this blending method alone, and a coating polymer by chemical vapor deposition was able to retard the releasing of enzyme[16]. Yang et al. reported an emulsion electrospinning via preparing a compound solution of biomolecules and polymers. To make the macromolecule suspending, they used ultra-sonication and homogenizer as a process of emulsification[17]. Therefore, this technique has been advanced by emulsifying the mixed solution before electrospinning and coating additional polymer after fabrication.

5)Twisting

A few numbers of fibers can be twisted in a clockwise or counter-clockwise manner to form a fiber bundle. And then the thicker bundles are continuously twisted in the same

manner to form a yarn. Finally, the yarns are twisted to form one scaffold. When needed, the procedure can be repeated for several times. The number of fibers in each twisting and the diameter of twisted fibers can be regulated during twisting and thus can exert an influence on the physical properties of fibrous scaffolds[18]. The structure of the twisted fiber scaffold is similar to the organization of a native ligament and is designed to mimic the biomechanical behavior of the anterior cruciate ligament (ACL)[19-21]. The twisted fiber scaffolds display a toe region and a linear region when placed under increasing load.

6)Braiding

In the study by Freeman et al., 3 single PLLA fibers were combined together to form 1 yarns. And then these 3 yarns were braided together to form one scaffold. With different numbers of fibers in braiding and different braiding patterns, the braiding angles also varies, which can be observed and may be used to estimate the force that is placed on the scaffold. Furthermore, the twisting and braiding angles can be determined with a stereoscope, and the angles can be optimized for required mechanical properties (such as Young's modulus) in order to simulate the physiological properties of native tissues[22].

7)Knitting

Knitting scaffold is a flat membrane-like scaffold knitted by yarns or monofilaments. With the membrane lamination technology, a 3D shape can be prepared with fibrous materials. Evenly distributed tangled loops are created in the commissures which still open enough to allow cellular ingrowth[23]. Unlike braided fabrics, knitted scaffold is shown to achieve a better internal communication spaces and favors for the deposition of collagenous connective tissue matrices, which is crucial for tissue reconstruction[24]. However, knitted scaffold usually requires a gel system for cell seeding and has nutrient transmission problems. To conquer this problem, Liu et al. used a combined silk scaffold with microporous silk sponges that are introduced into the openings of a knitted silk scaffold and thus to form a hybrid mesh[25].

Surface bioactive modification

With the increased demands of functional scaffolds for tissue regeneration, scaffolds that only provide physical support can no longer meet the requirements of tissue engineering. Bioactive scaffolds combining biomolecules, such as proteins, small molecules and nucleic acids, can better interact with the tissue and regulate adhesion, migration, proliferation and differentiation of the seeded cells[26].

In recent years, the electrospinning nanofibers become a suitable delivery of biomolecules via scaffold materials due to the great surface-volume ratio. There are three major methods of scaffold based bioactive modification.

1)Physical adsorption

In this procedure, after fabrication, nanofiber scaffolds can be simply dipped into an aqueous solution of biomolecules, and those molecules can be attached to the scaffold by electrostatic forces. Because of the weak attachment, this method is seldom used. It has been revealed that the proteins added on the poly (D,L-lactide-co-glycoside) (PLGA) scaffold had completely released in 20 days and the 95% of incorporated genes usually lasted less than 10 days[27, 28].

2)Plasma treatment

Plasma treatment is a convenient method to modify the surface properties of the scaffolds, which is able to induce the desired chemical groups or chains onto the polymers for enhancing surface hydrophilicity or reducing tissue inflammatory reaction. During the process, the manufactured scaffolds are placed over an electrode in an evacuated plasma chamber. After filling with a desired gas and maintaining a proper pressure value, glow discharge plasma will be created with a predetermined electrical power and treatment time. The advantage of this method is its availability to complex surface even for porous structure[29].

3)Covalent immobilization

Covalent immobilization uses chemical bond to immobilize biomolecules onto the nanofiber surface for guided cell behavior[30]. Due to the complexity of the process and the unstableness of molecule release, covalent immobilization is not a routine method to deliver biological message. However it can improve the surface properties of the fibers, such as biocompatibility and bioactivities[31].

Application of fibrous scaffolds in tissue reconstruction

Based on the characters above described, fibrous scaf-

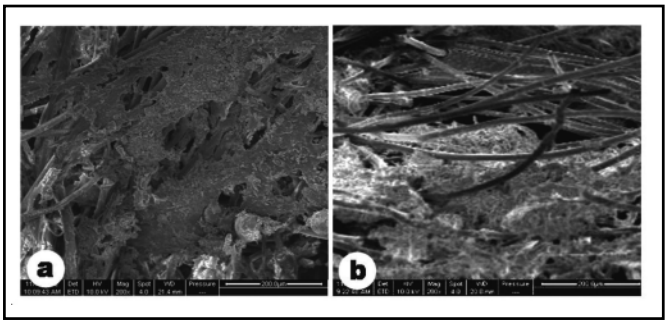

Fig.1 Attachment and proliferation of BMSCs on PLA/PGA scaffold

Scanning electron microscope (x200) demonstrates that both induced BMSCs (a) and dexamethasone treated cells (b) adhere to and spread on PGA fibers very well and produce abundant extracellular matrices. (Reprinted from[36])

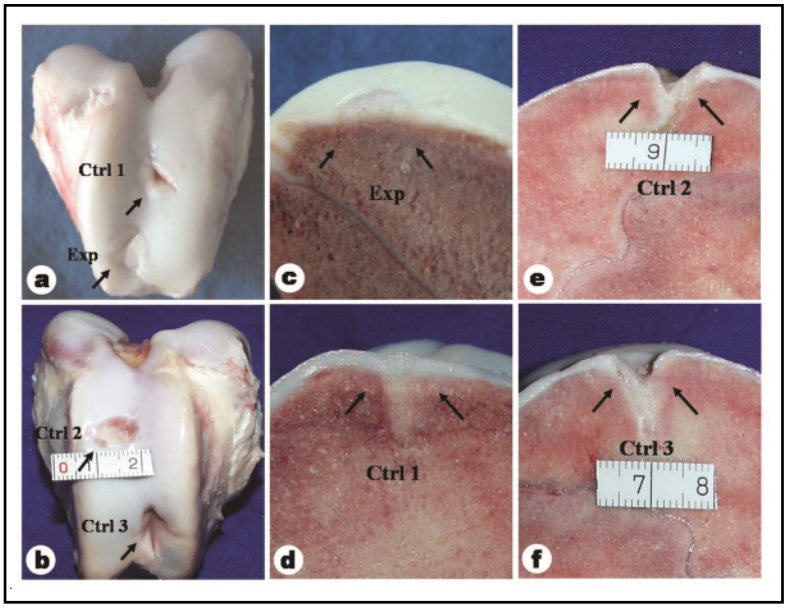

Fig.2 Gross and cross section view of repaired defects at 6 months post-repair

Arrows indicate the repaired regions. The experimental defect exhibits a relatively regular surface (Exp, a) and the osteochondral defect is completely repaired with both engineered cartilage and bone when observed at the cross section (c). The repaired surface of control 1 group remains irregular (Ctrl 1, a), but the osteochondral defect is mostly repaired at the cross section (d). The defects in control 2 group (Ctrl 2, b, e) and control 3 group (Ctrl 3, b, f) remain largely unrepaired at both cartilage and bony layers. (Reprinted from[36])

folds have the advantages to mimic the natural ECM alignment and to maintain the functional macrostructure for tissue or organ regeneration. With the advancement in cell and tissue culture technology, fibrous scaffolds become one of the popular scaffolds for tissue engineering and are widely applied for various tissue and organ regeneration and functional restoration. Several common applications are described in the followings.

1)Bones

The skeleton provides the motor system of human body structure and provides the protection of internal organs. Congenital defects, trauma and pathological injury can all cause certain types of bone damages that are difficult to heal due to the limited regenerate ability. Traditionally, bioinert materials such as metals, alloys, ceramics and composites have been widely used, but they are barely degradable. Electrospinning scaffolds provide a resorbable scaffold material for bone regeneration[32, 33].

In the work by Liu et al., MC3T3-E1 osteoprogenitor cells were cultured in a highly porous poly(L-lactic acid) with nano-fibrous architecture (NF-PLLA) and gelatin were incorporated onto the surface. The cell number at 4 h and 24 h post-seeding and the rate of proliferation on surface-modified NF-PLLA are all higher than those on the control scaffolds. The results demonstrated that the electrostatic self-assembly can have a potential for bone tissue engineering[2].

2)Cartilages

Articular cartilage is a thin layer of avascular, low cellular, connective tissue covering synovial joint surfaces, which has the limited self-healing capacity after the damage. Cartilage can be considered as a fiber-reinforced composite material, which is both non-homogeneous and anisotropic, consisting of two major structural macromolecules

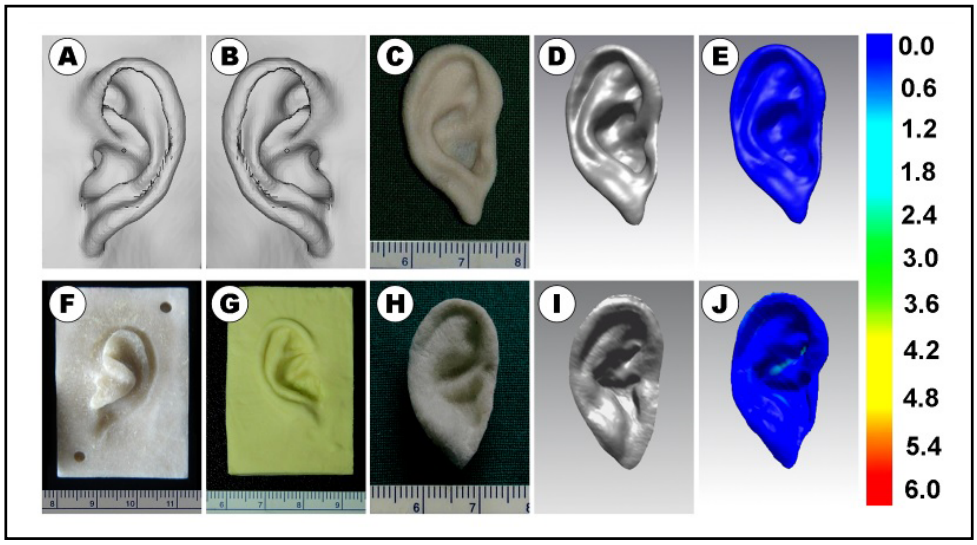

Fig.3 Preparation and shape analysis of the ear-shaped scaffolds
(A)3D image of the normal ear; (B)the mirror image of A; (C)The half-sized resin positive mold; (D)laser scan image of C; (E)color map of D; (F)inner part of the resin negative mold fabricated by 3D printing; (G)outer part of the negative mold cast from F with silicon rubber; (H)the ear-shaped PLA/PGA scaffold; (I)laser scan image of H; (J)color map of I compared to D. (Reprinted from[37])

(collagen and proteoglycans), chondrocytes, water, ions and nutrients. Due to the inability of cartilage to regenerate itself when injured, tissue engineering provides the promise for repairing large size defects.

Subramanian et al. had evaluated an electrospun chitosan mat composed of oriented sub-micron fibers for its tensile property and biocompatibility with chondrocytes. The electrospun mats had significantly higher elastic modulus than the cast films and provided good biocompatibility with chondrocytes. The chitosan mats have the potential to be further processed into three dimensional scaffolds for cartilage tissue repair[34].

In the research of Liu et al., they used PGA fibers and premixed combination of chondrocytes and 30% Pluronic F127 as a implant to repair an 8-mm full-thickness defect of porcine articular cartilage. After 24 weeks, the surface of the repaired defects appeared smooth and histological, biomechanical examination showed an ideal interface healing and biomechanical improving. This research proved that the combination of unordered PGA fibers and Pluronic can be a candidate scaffold for articular cartilage engineering[35]. Zhou et al. used PLA-coated PGA unwoven fibers as scaffolds to create a proper niche for chondrogenically induced bone marrow stromal cells to differentiate into chondrocytes (Fig.1 and Fig.2). The research strongly indicated that the PLA/PGA scaffolds can accommodate bone marrow stromal cells to proliferation and differentiation[36].

With the similar methods, Liu et al. also use PLA/PGA scaffolds for engineering human ear-shaped cartilage. And with the assist of computer aided design and manufacturing technology, the scaffold was fabricated in an ear-shaped negative mold. The similarity level was over 84% compared to the original shape after chondrocyte seeding and *in vitro* engineering for 12 weeks. In conclusion, the scaffold was able to gain sufficient mechanical strength by coating PGA unwoven fibers with PLA in order to form a complicated 3D macro structure by CAN/CAM (Fig.3)[37].

Collagen-based nanofibrous scaffolds have also been proposed for cartilage tissue engineering, as collagen is one of the components of the native cartilage. The feasibility of collagen II electrospinning had been demonstrated by Matthews et al.. And the novel scaffolds produced are composed of the fibers with their diameter scales from nano- to micron, which have been shown to be compatible with chondrocytes[38].

3)Tendons

Tendon is a tough band of fibrous connective tissue that connects muscle to bone and is capable of withstanding tension. Similar to ligaments and fasciae, they are all avas-

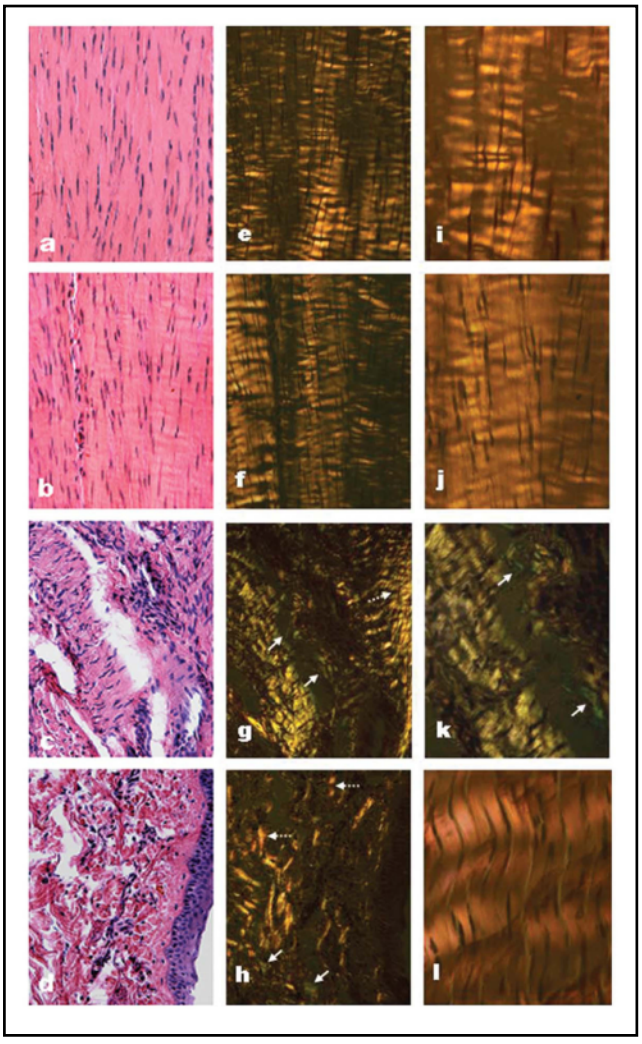

Fig.4 Histological finding of formed tissue at 26 weeks
Hematoxylin and eosin (H&E) staining shows histological structures of (A) fibroblast, (B) tenocyte-engineered tendons, (C) a control tissue in control group 2, and (D) normal pig skin. Collagen III (delicate collagen fibers with a light-green color) is detected only in the polarized images of control tissues (G and K) and in normal pig skin (H), as indicated by white arrows. In addition, collagen I (golden color, indicated by white dotted arrows) is also detected in these tissues. In the polarized images of fibroblast (E and I) and tenocyte (F and J) engineered tendons and natural tendons (L), collagen I (golden color) is the predominant collagen type. (Original magnification x400 (I-K) x200, all others.) (Reprinted from[39])

cular, low cellular and collagen as a major component. When tenocytes might be difficult for their use in tendon engineering, dermal fibroblasts can serve as seed cells in tendon engineering when combined with PGA fibrous scaffolds (Fig.4)[39].

Sahoo et al. used porcine bone marrow stromal cells as seed cells, and planted them onto the PLGA nanofibers which were electrospun onto knitted PLGA scaffold. In the study, the novel scaffold facilitated cell seeding and promoted cell proliferation, and differentiation, demonstrating that such advanced fabrication of fibrous scaffold can be applied to tendon tissue engineering[40].

In the study by Yin et al., they aimed to determine the effects of nano-topography on the differentiation of human tendon stem cells and used both aligned and randomly-oriented PLLA nanofibers. And the result showed that the aligned electrospun nanofiber structure provides an instructive micro-environment for seed cells differentiation[41].

Chen et al. used the PGA fiber scaffold (15-20 μm in diameter) and muscle derived cells for *in vitro* tendon engineering. They arranged those fibers into a cord shape with 6cm in length and 1cm in diameter, and then secured on a custom-made spring formed with stainless steel frame to maintain shape and stress of the scaffold. After being seeded with muscle derived cells and in vitro culture, neotendon tissue could be generated and became further matured after *in vivo* implantation[42].

4)Nerves

Despite the fact that the peripheral nervous system has a significant ability to regenerate after trauma or other injuries, a nerve graft is usually needed when the nerve defect is longer than 5 mm. Tissue engineered nerves provide an alternative graft for nerve transplantation and regeneration, and thus avoid the function loss and other side effects at the donor site. Due to the directional growth of neuron and axon, cell orientation is crucial for engineered nerve regeneration[43].

In the research of Yang et al., they used aligned PPLA nano/micro fibers as the scaffolds and neural stem cells (NSCs) as the seed cells. The direction of NSC elongation and its neurite outgrowth are parallel to the direction of PLLA fibers of aligned scaffolds. No significant changes were observed on the cell orientation with respect to the fiber diameters. However the rate of NSC differentiation efficiency was higher on PLLA nanofibers than that on micro-fibers and it was independent of the fiber alignment[44].

With the technique of suspension and subsequent freeze-drying, Madaghiele et al. found that the cylindrical collagen-based scaffolds with axially oriented pore channels had the potential to improve the regeneration of peripheral nerves and spinal cord. And they defined an orientation index (OI) as a means to quantify the orientation of the

pore channels inside the scaffolds[45].

Chew et al. cultured human Schwann cells on aligned electrospun poly(ε-caprolactone) (PCL) fibers for 7 days. Decrease in the expression of neurotrophin and neurotrophic receptors and up-regulation of the myelin-specific gene, PO, were observed in the cells on electrospun fibers, suggesting that a more mature phenotype was adopted by the human Schwann cells and the propensity of aligned fibers promoted Schwann cell maturation[46].

On the contrary, instead of trying to promote the adhesion, proliferation and differentiation of the neural cells, McKenzie et al. used carbon nanofibers to decrease the growth of astrocytes (glial scar tissue-forming cells). They prepared several types of carbon nanofibers with different diameters, surface energy, and weight percentages, then astrocytes were seeded, and it was found that astrocytes preferred larger diameter and the lower surface energy. It demonstrated that formulation of carbon containing fibers in the nanometer regime might limit astrocyte functions which led to decreased glial scar tissue formation[47].

5)Blood vessels

When dealing with vascular graft, the capability of electrospinning to mimic a cell niche microenvironment is especially important. The blood flow in the vessel creates a serious of forces against the inner lumen, which requires the ECMs or the scaffolds to be able to withstand these forces with minimal matrix loss. Electrospun nanofibers seem to be a potential material that not only can simulate the native ECM, but also achieve mechanical properties required by the tissue[32].

Xu et al. had suggested the use of aligned nanofibrous poly(L-lactide-co-ε-caprolactone) scaffolds. In their study, the smooth muscle cells (SMCs) attached and migrated along the axis of the aligned nanofibers and expressed a spindle-like contractile phenotype; the distribution and organization of the cytoskeleton proteins inside SMCs were parallel to the direction of the nanofiber; the adhesion and proliferation rate were significantly improved than those on the flat polymer films, suggesting that the nanometer-scale scaffold can mimic the natural ECM and provide a suitable architecture for replicating *in vivo*-like vascular structure[48].

Combining electrospinning and cell micro-integration in vessel tissue engineering, Stankus et al. used an electrospinning process to produce elastomeric tubular conduits from biodegradable poly(ester urethane) urea while vascular SMCs were micro-integrated into the conduits using an electrospraying process that runs parallel to the electrospinning. As a result, they obtained homogenous SMC integration into the electrospun tubular conduits. This method represents another achievement as a tissue engineering approach for blood vessel replacement[49].

Xu et al. seeded SMCs on a PGA fiber mesh, which was then wrapped around a silicone tube placed in a vessel bioreactor with pulsatile stimuli, and secured by biodegradable sutures. After 8 weeks of dynamic engineering, an elastic blood vessel wall was grossly formed and the phenotype of SMCs was confirmed by positive staining of smooth muscle markers[50].

Mechanical property and compatibility remain to be the key limitations for the application. As above described, fibrous scaffolds based on extracellular matrix molecules such as collagens are usually weak and the cross-linking using a chemical compound like gluteraldehyde usually leads to poor compatibility to cells and tissue. By contrast, polymer based fibrous scaffold can be made strong with physical modifications including knitting, twisting and braiding. Nevertheless, the acidic degradation products will also lead to poor compatibility upon *in vivo* implantation. In the future, the generation of neutralizing polymer-based fibrous scaffolds with the incorporation of basic molecules such as tripolyphosphate nanoparticles[51] or the generation of hybrid scaffolds by combining polymer fibers and extracellular molecules will be the potential directions.

Conclusion

Fibrous scaffolds are important components in tissue engineering scaffolds. With the development of material science related technologies, fibers scaffolds can be manufactured at the size levels of macro, micro, even nano magnitudes to mimic the topographical structure of cell niche environment, and the 3D morphology can be shaped precisely with CAD/CAM techniques. Furthermore, physical and chemical modifications of fibrous scaffold enable the generation of mechanically strong and bioactive fibrous scaffolds that are able to guide cell proliferation, differentiation and tissue formation, and will have great potential in their applications of tissue and organ regeneration.

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Conflict of Interests

None

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