Recent studies on electrospinning preparation of patterned, core–shell, and aligned scaffolds

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ABSTRACT: Electrospinning is one of the most important ways to prepare continuous, high porosity, large specific surface area, and uniform diameter micro- and nanoscale fibers. So, it has been widely used in the preparation of micro/nano-sized polymer scaffolds for tissue engineering in recent years. In addition to the versatility in material selection and the processing variables, electrospinning also provides a lot of methods to regulate fiber structure and scaffolds morphology. For example, the near-field electrospinning can provide a method to solve the problem of uncontrollable fiber path; the melt electrospinning eliminates the risk of solvent residue in the construct; the addition of different auxiliary electrodes can make the fiber patterned. This review introduces the underlying principle and characteristics of above electrospinning applied in biomedicine. Herein, we highlight a comprehensive understanding of the technical aspect of this technology for versatile fibers with patterned, core–shell and aligned morphology.

INTRODUCTION

Biomaterial designs attempt to harness the regenerative capacity of the body by merging principles of materials engineering and biological science to repair damaged tissue. An ideal tissue engineering scaffold needs to meet the following requirements: (1) the scaffold material should have good biocompatibility and no cytotoxicity, obvious inflammatory reaction, and immune rejection; (2) the material should have the appropriate biodegradability; (3) the scaffold should have appropriate pore size, and high porosity (>90); (4) the scaffold requires the three-dimensional (3D) shape of a specific tissue or organ shape; (5) the scaffold should have the high surface area and appropriate surface physicochemical properties to facilitate cell adhesion, proliferation, and differentiation; (6) the scaffold should be matched with the mechanical properties of implanted sites in order to maintain structural stability and integrity in the biomechanical microenvironment, and it should provide suitable micro stress environment for implanted cells.

Over the last few decades, several multimodal biomimetic strategies have emerged to alleviate damaged tissue using a wide range of fabrication methods. Of these, electrospinning gained a rapid recognition to open a new horizon in tissue engineering methodologies owing to its simple yet precise methods to fabricate scaffolds with nano/macro scale topography. The diameter and aperture of the prepared fibers are very uneven because of the jet whipping during the traditional electrospinning process. Near-field electrospinning is a relatively new technique that utilizes electrically charged polymer solution to deposit continuous fibrous meshes. These meshes are porous, biocompatible, have a high surface, and can be fabricated with advanced features such as drug elution of an orientation from a range of polymers. Such scaffolds ultimately resemble the structure and size of the extracellular matrix natural tissues. This has made electrospinning an attractive strategy to produce surgical constructs for regenerative medicine. Although, near-field electrospinning, as a concept was proposed in 2006, direct-write electrospinning research has experienced exponential growth in the past decade. This is attributed to the accuracy of the printing technique in recapitulating the composite micro/nanoscale structure required to meet the needs of individual patients.

The three elements of tissue engineering include cells, scaffold, and growth factors. One key area of tissue engineering research is the fabrication of scaffolds to deliver a 3D biocompatible support for cell migration, proliferation, and differentiation. There are numerous routes to scaffold fabrication, each
with advantages and disadvantages in their processing and cellular response. Many traditional methods of preparation of tissue engineering scaffolds include thermally induced phase separation method, particle leaching method, and freeze-drying method. Electrospinning can make the fiber diameter reach 500–800 nm by adjusting the spinning parameters and the properties of the solution. The structure similar to the extracellular matrix is prepared. However, there are two main problems limiting electrospinning fibrous scaffold applications. One is a fibrous scaffold fabricated with an

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<th>Experimental materials</th>
<th>Conclusion</th>
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<td>Alginate, chitosan, collagen, and hydroxyapatite</td>
<td>Reduce the disintegration by 35% for 10 days in collagenase solution</td>
<td>75</td>
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<td>A series of herbal extract incorporated into poly(lactic acid) (PLA), Equisetum arvense extract (EE), and nano-hydroxyapatite (nHA)</td>
<td>Surface hydrophobicity was reduced while the tensile strength and Young's modulus were increased satisfactorily. Regarding samples containing EE and nHA, cellular adhesion was observed with flattened normal morphology</td>
<td>76</td>
</tr>
<tr>
<td>Poly(β,γ-lactic-co-glycolic acid) (PLGA), collagen</td>
<td>The diameter and orientation of electrospinning nanofibers can affect the extracellular matrix of bone marrow stem cells and mimic the structure of periosteum, so as to achieve the purpose of bone defect repair and reconstruction</td>
<td>77</td>
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inhomogeneous pore size. The other is a fibrous scaffold with a smaller pore size. These two problems impede cell immersion, vascularization, and the integration of the scaffold with the host cell. Electrospinning scaffolds have been widely used in vascular, bone, skin, neutron, and ligament tissue regeneration. The effect of process variables and material diversity on fiber has been well documented. In this review, we provide an in-depth focus on electrospinning underlining techniques to produce fibers for biological morphology and application. Herein, we discuss the basic working principles and compare several additional features electrostatic lens auxiliary electrode, core–shell, the core structure of the spray nozzle and a needle core induction receiver.

PREPARATION OF SCAFFOLDS WITH DIFFERENT CELL CULTURES

Different scaffolds were ready, depending on the materials needed to culture the cells, such as skin cells, bone cells, periodontal ligament cells, blood vessel. This section reviews recent advances in the use of these scaffolds in recent years. Atypical cells require different mechanical properties of scaffolds, so the scaffold materials are different. Most researchers use electrospinning to study scaffolds for bone cells and skin cells.

Table II. Four Examples of the Soft Scaffold Prepared by Electrospinning

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<th>Experimental materials</th>
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<tr>
<td>Dexamethasone-loaded PGS-PCL/Gt</td>
<td>Revealed lower elastic modulus, ultimate tensile, and ultimate elongation than those of PGS-PCL scaffold and close to mechanical properties of natural tissue; the lower contact angle of PGS-PCL/Gt than that of PGS-PCL demonstrated improved surface hydrophilicity of scaffold</td>
<td>86</td>
</tr>
<tr>
<td>Poly(1,8-octanediol citrate) (POC)</td>
<td>Much simpler to obtain by melt polycondensation of 1,8-octanediol and citric acid under relatively mild conditions without any toxic catalysts or crosslinking regents</td>
<td>87</td>
</tr>
<tr>
<td>POC elastomer, PLLA</td>
<td>Formed so long as the POC prepolymer’s content was no more than 50 wt %; good biocompatibility for potential applications in soft tissue engineering</td>
<td>88</td>
</tr>
<tr>
<td>PCL/gelatin</td>
<td>Have good biocompatibility and can be used as potential scaffolds for the construction of subcutaneous soft tissue of the foot</td>
<td>89</td>
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Bone Tissue Engineering Based Electrospinning Scaffolds

Electrospinning composite nanofibers for culturing natural bone, which is porous and hierarchical, can promote cell growth and invasion by improving the performance of scaffolds. The extracellular matrix environment of bones is mainly formed by collagen and hydroxyapatite, therefore, these two materials are often used as bone scaffolds. There are many materials that can be applied to prepare the bone scaffolds based electrospinning as shown in Table I. It can be found from the Table I that poly(lactic acid) (PLA), chitosan, and other polymer materials with good performance is still the first choice for preparing scaffolds. The drug or protein is loaded in nanofiber scaffolds at the same time, and then the drug or protein can be released slowly. It can promote the formation of new bone. Scaffolds materials with 3D structure have attracted more and more attention in bone tissue engineering recently. They are seen as ideal bone substitutes. Preparation of ideal biomaterials, including bone induction, vascularization, biocompatibility, and biodegradability, is a difficult problem for bone tissue engineering.

Periodontal Ligament Fibroblast on the Electrospinning Scaffolds

The biocompatibility of the electrospinning PLA/poly(caprolactone) (PCL) blend fibrous scaffold with human periodontal ligament fibroblast is investigated. A healthy tooth bone and periodontal ligament can be seen in Figure 1. Bai and Chen found that the fibroblast can grow and proliferate on the nanofibrous scaffold which has good biocompatibility. Such nanofiber can be used as the candidates. After 3 days, the proliferation rate of cells is obviously accelerated. The cells are rapidly spreading over the field of vision and full of body. The cell membrane is intact, and there is no obvious cytotoxicity. The main method for the treatment of periodontal ligament injury is using polytetrafluoroethylene membrane and collagen membrane guided tissue regeneration, but the polytetrafluoroethylene membrane is difficult to degradation. It needs the reoperation for removal, while the collagen membrane has different degradation speed. So it is difficult to maintain the effect of the spatial form of treatment. Therefore, the combination...
of autologous cells and biological scaffold for periodontal tissue defect repair has become the focus of periodic nodal tissue regeneration at this stage.\textsuperscript{81–83} Dan et al.\textsuperscript{84} studied the influence of cellular source on periodontal regeneration using calcium phosphate coated PCL scaffold supported cell sheets. Cell-based therapy is considered a promising approach to achieve predictable periodontal regeneration.\textsuperscript{84,85} The osteogenic potential of the primary cultures was demonstrated \textit{in vitro}. Cell sheets are supported by a calcium phosphate coated melt electrospun PCL (CaP-PCL) scaffold were transplanted to denuded root surfaces in surgically created periodontal defects, and allowed to heal for 1 and 4 weeks. The
CaP-PCL scaffold alone was able to promote alveolar bone formation within the defect after 4 weeks. The addition of alveolar bone and periodontal ligament sheets resulted in significant periodontal attachment formation. The gingival margin-derived cells sheets did not promote periodontal regeneration on the root surface and inhibited bone formation within the CaP-PCL scaffold. In conclusion, the combination of either gingival margin-derived cells or alveolar bone sheets with a CaP-PCL scaffold could promote periodontal regeneration, but alveolar bone sheets were not as effective as periodontal ligament sheets in promoting new attachment formation.

**Scaffolds by Electrospinning for Soft Tissue Engineering**

The size of the collagen fiber in the natural extracellular matrix is 50–500 nm. So nanoscale scaffold material can imitate the characteristics of the extracellular matrix in the body to the greatest extent. At present, electrospinning is the only method that can be used to prepare polymer nanofibers directly and continuously. Small leg skin flap transfer and other methods are used to repair soft scaffold. After repairing, local soft tissue stability is poor. Electrospinning to prepare soft scaffold can solve the problem. There are many materials applied to prepare the soft scaffold based electrospinning as shown in Table II. It can be found from the Table II that the core/shell poly(glycerol sebacate)-PCL/gelatin (PGS-PCL/Gt-Dex) fibrous and poly(1,8-octanediol citrate)/poly(l-lactide) could be used as a carrier for the sustained release of drugs relevant for scaffold. Poly(1,8-octanediol citrate) and poly(l-lactide) are much simpler to obtain by melt polycondensation of 1,8-octanediol and citric acid under relatively mild conditions without any toxic catalysts or crosslinking reagents.

**BASIC PRINCIPLES OF TRADITIONAL ELECTROSPINNING, NEAR FIELD ELECTROSPINNING, AND 3D PRINTING BASED ON NEAR FIELD ELECTROSPINNING**

The polymer melt or solution, processed by conventional electrospinning, is subjected to a high-voltage electrostatic field and the excited micro/nano jet produces direct writing of a single fiber of needle-collector at an appropriate distance. Yet, 3D printing based on electrospinning is a fabrication device that combines near-field electrospinning with computer-aided design/computer-aided manufacturing system. Near-field electrospinning drives the formation of fibers in a shorter distance through stretching of melted or dissolved polymer into the Taylor cone. In 3D printing with electrospinning, single fiber produced is particularly superimposed on the dielectric plates collectors geometrically assisted by the computer-aided design/computer-aided manufacturing component. As the polymer solution is electrostatically drawn into a jet following an initially vertical stability zone, bending instabilities develop in a second zone along the flight path of the charged jet. However, the range of the needle–collector distance of the direct writing method, although in an initially straight stable zone, is limited.

There are considerable differences in the setup of melt electrospinning writing and solution electrospinning. Figure 2 represents a schematic overview of a 3D printing device with the solution and melts electrospinning that is widely used for manufacturing support and scaffold. Melt electrospinning writing is largely based on the fused deposition modeling combined with a near-field electrostatic field that controls the path of the fiber falling. The setup uses a heating coil along with a temperature control in order to provide enough heat to melt the polymer. The raw material commonly used in melt electrospinning writing is granular material, while the filamentous thermoplastic material is usually used to the fused deposition modeling technology (3D printing) as shown in Figure 2(c). Melt electrospinning writing has a high viscosity and low electrical conductivity requiring the needle–collector distance to be a wide range. Melt electrospinning writing bypasses medical translational challenges such as solvent toxicity and accumulation associated with solution electrospinning system. While both methods provide orderly structures, charge accumulation in solution electrospinning impacts a number of layers causing the fibers to be as one coherent structure. This largely impacts the average pore size of the scaffold and overall quality of the tissue engineered product. On the other hand, melt electrospinning writing allows larger pore size of the scaffold products that is required for optimal cell invasion and growth. So far, melt electrospinning writing has been used to create 3D bone scaffold, vascular, and bone cell implants.

**DEVELOPMENT OF DIFFERENT AUXILIARY ELECTRODE AND DIELECTRIC PLATES COLLECTORS**

The pore size and regular shape of scaffolds are important indexes to evaluate the quality of tissue engineered scaffolds. This requires that the device be more controllable to the fiber path. Traditional electrospinning process is reasonably unstable and almost uncontrollable due to the bending of a charged jet under coupled multifield forces. 3D printing device with near field electrospinning helps to overcome this hurdle and improve the fine control over the construction design. In addition, to narrow down the collector distance, it is now possible to change the deposition of fibers by increasing the auxiliary electrode, including electrospinning with an electrostatic lens system.
pin electrode, and so on. This, in turn, affects the electric field between the nozzle and the collector plate that optimally guides the fiber in a controlled fashion. The resultant scaffold has enough internal space and larger pore size that resembles the extracellular microenvironment of tissue despite a complex exterior contour.

Electrospinning Setup with Electrostatic Lens System

Electrospinning with an electrostatic lens system has a characteristic annular auxiliary electrode in form of a tube between the nozzle and the collector plate. Notably, the ring electrode comprised of a single coil conductor, as shown in Figure 3(a,b). It is subject to a lower voltage than those of nozzle. Neubert et al. developed a distinct ring auxiliary electric circle to manufacture nylon, poly(vinyl chloride) and PLA-co-PCL. To this end, they achieved complex maneuvers with an average diameter of 0.15 mm and point positioning accuracy of 3 mm. This highlights a novel method to prepare scaffolds aimed at cellular differentiation. In contrast, Uyar et al. applied two different auxiliary electrodes. While cylindrical electrode was used in the first study, the second design emphasizes the use of both cylindrical electrode and four laminate electrodes, as highlighted in Figure 3(c). Their study showed different patterns of poly(vinyl alcohol) nanofiber mats that significantly enhanced the mechanical properties of the material. Such constructs are expected to find a wide range of application in clinical grade dental composites. Researchers can consider the effects of unusual diameter ring coils and different voltage while making scaffolds.

Auxiliary electrode offers many advantages over traditional electrosprning. First, it enhances the precision of point positioning. Second, a staged electric-field used to increase the collection distance. However, this device has a high requirement for the building.

Application of Core/Shell Nozzle

The core/shell or coaxial nozzle is a concentric set of coaxial spinneret composed of an outer and inner needle as illustrated in Figure 4(a,b). Coaxial electrospinning using various solution pairs can generate fibers from core-sheath to hollow fibers, which may contain particles or bioactive agents to accelerate the growth and differentiation of cells. Both of the composite structures of the nozzle, the inner and outer shell are, respectively, connected with the controllable voltage of the same polarity. The solution of the internal and external channels, although dissimilar, convergence at the end of the nozzle. The flow rate of each concentric nozzle is independently controlled to produce construction with optimal feature. Recently, Kim and Kim proved that the coaxial microporous superfine fiber of PCL/collagen scaffold had about three times better mechanical properties than the fibers prepared by a
standard electrospinning. An additional coating of type I collagen further improved the ability of proliferation and differentiation of osteoblast cells (MC3T3-E1 cells) in vitro. Overall different physical and mechanical properties of the absorption of water and protein showed better cell activity than traditionally electrospun fibers.\textsuperscript{106}
Coaxial spinning for regeneration is based on the concept of designing optimal fiber such as hollow or drug-filled core to develop functional substitutes that restore, improve, or repair damaged tissues and/or organs. The setup can be employed on an industrial scale without compromising the structural, function, and the mechanical integrity of the material.

A Pole Nozzle
The key feature of a pole nozzle, illustrated in Figure 4(c), is the solid core that serves as a conductive tip. The remainder of the device resembles a traditional 3D printer with electrospinning. The needle core is robust and allows flexibility in the elongation length. As the auxiliary electric field is formed at the needle core it decreases the average voltage of the jet. This forms the underlying principle of the device since the decrease in voltage plays a critical role in jet stabilization.

Sun et al. have demonstrated the application of a core nozzle to pattern direct writing of poly(ethylene oxide). Their research showed that the swing amplitude is considerably smaller than noncore nozzle direct writing used in a traditional method. Secondly, the voltage required as well as the jet oscillation amplitude is significantly lower. In a parallel study, they showed an enhancement in the overall stability of single fiber produced through core nozzle and the induced type device. Owing to the stability of the jet, the fibers obtained are uniform and accurate in their size. The fibers were of micro/nanoscale magnitude with a spiral structure fiber that may be applied as a biological imaging aid of artificial organs. This is attributed to the tunability of the fibers according to the desired scale of application. In order to create successful biological substituent, it is desirable that the scaffold mimics the biomechanics of the tissue microenvironment. Patterned and controlled deposition of fibers is one such commonly used method for 3D scaffolds that enable biomechanical tunability by optimizing fiber layer forms. It addresses the impediment posed by the dense arrangement of fibers in the traditional electrospinning process. Yuan et al. directly printed the patterned 3D PLA fibrous scaffolds by stable jet electrospinning to promote cell in-growth. Stable jet allows ultra-fine PLA fibers form a patterned scaffold, which facilitated vascular smooth muscle cells to penetrate and grow within the internal space of the scaffold.

Figure 6 depicts the preparation of tubular fibers by electrospinning. Although traditional methods of patterning are associated with lack of orderly fibers, the direct writing method eliminates most of such organizational problems. Jana et al. demonstrated the production of these tubular constructs with one natural and two synthetic polymers (chitosan, PCL, and polyvinylpyrrolidone). They further demonstrated in a model bioapplication that muscle cells cultured on the inner surface of an aligned nanofibrous tubular scaffold enabled the formation of aligned and densely populated myotubes organized as in native muscle tissues.

Porous fibers prepared by electrospinning has more obvious advantages over traditional fibers such as (1) orderly and controlled fibers with large fiber diameter, (2) superior biocompatibility, and (3) superior cell permeability within the scaffold. This imparts biomimetic character to the scaffold which resembles the extracellular matrix comprising of cells within the structural compartments. In nature, the extracellular matrix is composed of 3D structural components in micro/nanoscale that form a supportive meshwork around cells. In order to produce successful tissue engineered constructs, it is desirable to have a 90% porous environment which can mitigate cell adhesion as well as the infiltration of nutrients and metabolic products. The control over the microstructure offered by electrospinning recapitulates the natural form and function of the tissue microenvironment through the high surface area to volume ratio and porosity.

CONCLUSIONS
Novel methods for scaffold fabrication are based on complex external geometry, which offers improved permeability of cells in the internal space and increases the overall quality of the surgical construct. The recent advances in this field have helped to overcome the limitations of traditional electrospinning, especially in terms of control of fiber deposition. Although in its infancy, pioneering research with electrospinning shows great promise in the field of tissue engineering and regenerative medicine. This review focuses on different techniques for control of dimensions and morphology, especially the preparation technology of patterned tissue engineering scaffold. The patterned scaffold has obvious advantages in regulating cell morphology, cell distribution, and multicellular coculture. With such technology in place, it is now possible to deliver personalize surgical construct for treating tissue effects, that mimic tissue
microenvironment and release bioactive agents, to the desired site. Nanofiber scaffolds should be combined and matched with different materials according to different needs, using scaffold materials with good mechanical properties, high porosity, good compatibility, and good degradation performance. This is probably one of the important future directions.

Currently, there are limited equipment options on the market that require researchers to assemble their setup manually. Overall, a significant and promising path lies ahead for multidisciplinary research combining materials engineering, computational modeling, medical imaging, and cell biology to fully achieve the potential of electrospinning in regenerative medicine.

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