Poly(L-lactide-co-ε-caprolactone) Nanofiber Morphology Control and Influence of Properties

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Abstract—Electrospinning has the advantages of large specific surface area and high porosity, and the nanofibers prepared by it can be widely used in environmental engineering, wound dressing, scaffold materials, biomedicine and other fields. Because gelatin is a biological material with biodegradable and good biocompatibility properties; while poly(L-lactide-co-ε-caprolactone) (PLCL) is a biological material with excellent mechanical properties and good biocompatibility. Therefore, the prepared PLCL/gelatin nanofiber membrane has good biocompatibility, and has minimal repellency to the body during clinical use, and is effective as a scaffold material. This article uses PLCL and gelatin as experimental materials to study the development and optimization of fiber scaffold materials. First introduce the existing tissue engineering scaffold materials, and then describe the basic principles of electrospinning technology. Used 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as solvent to prepare PLCL/GE spinning solution and prepare nanofiber membranes with different total concentrations and different ratios of PLCL to GE, then used the electrospinning process as preparation method to spin nanofiber membranes separately, and the microstructure and hydrophobic performance parameters of the nanofibers were tested and characterized.

1. Introduction
The rapid development of social economy has brought about various problems such as ecological imbalance and virus ravages, which has caused people to pay more and more attention to health, so the requirements for medical drugs have become increasingly strict. As the main medical treatment method, drugs, in addition to external oral administration, treatment methods such as in vivo targeted drug release and tissue engineering technology, have significant effects on various cancers[1-3]. For damaged tissues in the human body, scaffolds are often used for repair and replacement. Traditional scaffolds represented by metal and polymer fibers have a single material, low maneuverability and low degree of individualization, and are highly repellent to human tissues has gradually no longer adapted to clinical needs[4]. The synthesis ratio, structure and surface treatment of materials are the main research contents of stent material optimization[5, 6]. With the development of life sciences and cross-discipline, various new materials and natural biomaterials and their mixtures have gradually become hot new talents in scaffold development and optimization[7]. At present, most of the scaffold materials with strong human compatibility are natural materials, which have a large market gap, but few research results. Therefore, how to overcome the compatibility problem with the human body and further optimize the use of the scaffold material has become an urgent problem.
Electrospinning technology is based on electro-hydrodynamics, using high-voltage electrostatic field to atomize the polymer spinning solution at the spinneret into a tiny jet, forming a conical “Taylor cone”[8]. The spinning solution is ejected from the spinneret, and the formed jet overcomes its surface tension in the high-voltage electrostatic field, and is always dry during the ejection process, and maintains a certain amount of charge[9]. After running for a long receiving distance, it will finally fall into the receiving area. The board solidifies to form fibers. Electrospinning to prepare nanofibers is simple, fast, reliable, and superior in porosity, specific surface area and other properties, and can be used as medical materials such as tissue engineering and drug carriers[10]. Tissue engineering scaffold materials prepared by electrospun fibers are beneficial to the optimization of tissue scaffold materials and also provide more possibilities for the development and research of drug-controlled release systems. PLCL is composed of polylactic acid (PLA) and polycaprolactone (PCL) in a mass ratio of 50:50 through the ring-opening polymerization of monomers catalyzed by metal anion coordination to form high molecular organic polymers[11]. In addition, PLCL is a hydrophobic aliphatic polyester copolymer[12]. It is a commonly used electrospinning material. It has achieved significant scientific results in the application of tissue engineering in the medical field. Gelatin is obtained by partially hydrolyzing the collagen in animal skin, bone and ligament (acid method, alkali method, acid-base mixing method or enzymatic method) and then purified. It is a protein-like peptide molecular polymer. Gelatin has many excellent properties and properties, and is both acidic and alkaline, and is an amphoteric substance[13]. The molecular structure of gelatin has a large number of hydroxyl groups, carboxyl groups and amino groups. It is a hydrophilic colloid. Although gelatin has many advantages, its low solubility and mechanical strength limit their application. It is often mixed with other materials with good mechanical properties. Gelatin has good biocompatibility. When it is mixed with PLCL with good mechanical properties and strong hydrophobicity for electrospinning, the good mechanical properties and biocompatibility of the two are not only a simple superposition, but also have an optimized effect on the nanofibers formed by electrospinning, and the resulting nanofibers have a more uniform morphology. Electrospinning mixed with gelatin and PLCL can achieve the expected improvement in mechanical properties and biocompatibility.

2. Experimental section

2.1. Material & Equipment
Poly (l-lactide-co-ε-caprolactone) (PLCL) polymer (75:25) was purchased from Jinan Daigang Biomaterial Inc, China. gelatin (GE), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and deionized water were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd, China.

2.2. Preparation of Fibrous Membranes.
The data for electrospinning was shown in Table 1. Shell solutions were prepared with different concentration and ratio of PLCL and GE in HFIP. The solution was injected with independent syringe pumps (LSP01-1A, Shanghai Precision Instruments Co., Ltd, China). The solution was injected at 1ml/h Electrostatic discharge generator (ESD-203A, Shanghai Lingshi Electromagnetic Technology Co., Ltd, China) were used to apply a high pressure of 20 kV kilovolts between the collector and the needle. The distance between the needle and the collector was set at 20 cm. The needle used had diameter of 1.1 mm. All electrospinning experiments were performed at room temperature 20 ℃ and a relative humidity of 23% (Fig. 1). Fig. 1 demonstrates the electrospinning apparatus. As they emerge from the needle, the high voltage power was applied in the needle to cause polymer elongation and jetting towards the grounded collector thus forming nanofibers.

Table 1 Spinning parameters

| Parameter | Operation value |
|-----------|-----------------|

2
2.3. Characterization
A scanning electron microscope (SEM) (S-3400N, Teskin trading (Shanghai) Co., Ltd, China) was used for morphological characterization. Analyze SEM micrographs with software Image-J (National Institutes of Health). ImageJ was used to analyze the outer diameter or radius (r) of electrospun fibers at random locations, and at least three images were measured for each sample. The calibration of the image tool software is achieved by using the scale bar on each image.

Observe the spun fiber morphology after the electrospinning machine works normally. After 5 minutes, place the clean glass slide on the insulating rod and move it to the spinning receiving range to receive the fiber for 3 seconds. Then take the downloaded slides, place them under a microscope, observe the fiber morphology at 50x, 100x, 200x, and 500x magnifications, and select uniform and regular fiber morphology to take pictures. Save at least 3 samples for each parameter.

Water contact angles (WCA) were used to quantify the hydrophilic-hydrophobic properties of the fibers. Deionized water was used for drop formation. All apparent contact angles of droplets (5 ul) were measured by contact angles tester (Beijing Jinshengxin Testing Instrument Co, Ltd., China).

3. Results and Discussions
3.1. PLCL/GE Fibrous Membranes
According to the literature, a general solvent (HFIP), the weight ratio of PLCL to GE (1:1-3:1) and the spinning solution concentration of PLCL/GE (20%-30% w v⁻¹), such as Figure 2 shows. In the spinning process, when the applied voltage is less than 18 kV, the formed fibers are uneven and twisted between the spinneret and the collector plate, and the voltage is increased to 18 kV to form uniform fibers. When the voltage is 20 kV Unstable jetting was observed, therefore, the applied voltage of the spinning experiment was maintained at 18 kV; the collector plate distance was varied between 10 and 30 cm, and the fiber produced at a spinning distance of 20 cm appeared more uniform, with fewer beads and There was no jet, so the current collector plate distance for spinning experiments was performed at a distance of 20 cm. According to preliminary experiments, it has been found that the prepared nanofiber mat has a spider web-like multi-scale structure, which promotes cell proliferation and increased water swelling capacity. When the concentration of the spinning solution is constant, the smaller the ratio of gelatin, the more uniform the fiber diameter and the smoother the fiber surface. It can be seen from Figure 2 that the increase in spinning concentration, the smaller the effect of gelatin on fiber diameter. When the spinning concentration is 20% (w v⁻¹), the ratio of gelatin has the greatest influence on fiber uniformity. The ratio of gelatin to PLCL is 1:1, and the fiber is the most uneven. 20% (w v⁻¹) PLCL/GE (1:1), 20% (w v⁻¹) PLCL/GE (2:1) and 20% (w v⁻¹) PLCL/GE (3:1) The nanofiber scaffolds are 791.47 ± 715.75 nm, 533.16 ± 310.34 nm and 508.69 ± 224.53 nm, respectively. When the spinning concentration is 25% (w v⁻¹), the diameter of 25% (w v⁻¹) PLCL/GE (1:1), 25% (w v⁻¹) PLCL/GE (2:1) And 25% (w v⁻¹) PLCL/GE (3:1) nanofiber scaffolds are 822.87 ± 771.48 nm, 561.96 ± 426.239 nm and 684.821 ± 483.292 nm, respectively, and 25% (w v⁻¹) PLCL/GE can be found (1:1) The diameter range of nanofiber scaffold is greater than 2:1 and 3:1. When the spinning concentration is 30% (w v⁻¹), the three ratios of PLCL and GE are 30% (w v⁻¹) PLCL/GE nanofibers have similar diameter distributions. The diameters are 30% (w v⁻¹) PLCL/GE (1:1), 30% (w v⁻¹) PLCL/GE (2:1) and 30% (w v⁻¹) PLCL/GE (3:1) The nanofiber scaffolds are 593.99 ± 481.32 nm, 651.43 ± 542.06 nm and 553.07 ± 545.46 nm. Since the 20% (w v⁻¹) PLCL/GE (3:1) nanofiber mat has the most stable fiber diameter, it is selected to act on the shell layer of the spinning coaxial drug-carrying fiber. Under the same ratio PLCL/GE, as the concentration of the spinning solution increases, the diameter of the nanofibers also increases significantly. This is because the greater the concentration of the spinning solution, the greater the solid content of the solution, the greater the molecular weight, the greater the degree of entanglement of the polymer molecular weight and the greater the internal polymer between molecular chains, thereby increasing the polymer spinning The viscosity of the liquid, so that the diameter of the spun nanofibers increases under the same spinning conditions. The fiber morphology in the electrospinning process is affected by the viscosity and conductivity of the spinning solution and the surface tension during the stretching process. The decrease in surface tension and the increase in electrical conductivity are both beneficial to the stretching of the fiber, and the increase in viscosity will lead to greater molecular entanglement, thereby increasing the surface tension during stretching, and therefore worsening the stretching of the fiber. During the process of spinning solution configuration, the solution ions formed by PLCL in HFIP interact with the surface of gelatin, and the entanglement of gelatin molecules increases, and the stretch ability becomes worse, thus increasing the filament diameter.
3.2. Surface hydrophilic properties

The surface wettability mainly depends on the intrinsic hydrophobicity of the material and the maximum diameter (d_{max}) of the fiber membrane. The contact angle is the angle between the liquid side and the solid-liquid boundary line when the solid material is in contact with the liquid. It is a measure of the degree of wettability and is the main parameter for measuring the hydrophilicity and hydrophobicity of the tested material. According to Young's formula, when the solid material is hydrophilic, the contact angle is less than 90°; conversely, when the material is hydrophobic, the contact angle is greater than 90°, and as the contact angle increases, the material becomes more hydrophobic. The different roughness and different surface area of the film material will produce different surface tension, which has an impact on the contact angle. When the contact angle is less than 90°, the surface tension liquid is larger and the solution wets more due to the larger action area of the rough surface. Conversely, when the contact angle is greater than 90°, the greater the roughness, the worse the wettability. Polymer copolymers with good hydrophilicity are more widely used in the field of biomedicine. In order to study the hydrophilicity of PLCL and gelatin solution, the experimental samples were tested by a contact angle/water drop angle measuring instrument (KRÜSS-DSA30, Krusch, Germany), which can intuitively reflect the properties of PLCL/GE spinning solution for electrospinning Influence of membrane affinity and hydrophobicity (Figure 3). When the ratio of PLCL to gelatin is constant, the contact angle increases as the total concentration of the spinning solution increases. When PLCL/GE is 1:1, the increase in the total concentration of the spinning solution increases. When PLCL/GE is 1:1, the increase in the total concentration of the spinning solution makes the hydrophilic properties of the electrospinning membrane further increase. The contact angle is always lower than 90°, and the nanofiber membrane always exhibits hydrophilicity, but as the concentration increases, the hydrophilicity gradually decreases. When the PLCL/GE is 3:1, the increase in the total concentration of the spinning solution still further increases the hydrophobic properties of the electrospun membrane. And the contact angle is always higher than 90°, the nanofiber membrane always exhibits hydrophobicity, and as the concentration increases, the hydrophobicity gradually increases. But when PLCL:GE is 2:1, the contact angle of the fiber drops from 25% of 135.2° to 30% of 118.2°, showing a downward trend. This is because: The PLCL: Gelatin ratio under the same ratio is 2:1, and the fiber diameter of 30% is slightly lower than that of 25%. SEM image of the combined fiber: the fiber diameter decreases, the specific surface area
increases, the pores between the fibers increase, the moisture infiltration is convenient, and the contact angle decreases. And the contact angle is always greater than 90°, showing the hydrophobicity of the fiber. When the PLCL:GE is 3:1, the contact angle of nanofibers prepared with high-quality fractions increases slowly, from 141° at 25% to 145.2° at 30%, which is a small increase compared to others. The contact angle is always greater than 90°, still showing the hydrophobicity of the fiber. In the same proportion, the fiber diameter increases with the increase of the mass fraction; the increase of the fiber diameter causes the pores to gradually decrease. The increase in the pores of the nanofiber membrane will facilitate the penetration of water and the infiltration of gelatin under the permeation theory and capillary effect. Therefore, the hydrophilicity of nanofibers gradually decreases as the concentration of the spinning solution increases, so the fiber contact angle increases as the concentration increases. Because gelatin has strong hydrophilicity, PLCL has good biocompatibility and degradability because of its raw material PLA, but it has strong hydrophobicity. Due to the hydrophilic nature of gelatin and the large specific surface area of nanofibers, the combination of gelatin and PLCL can greatly improve the hydrophilicity of the scaffold, thereby improving the biocompatibility of the scaffold.

In terms of growth rate, the growth rate of high proportion is obviously slower than that of land proportion. The spinning dope with a total concentration of 25% exhibits this characteristic most prominently. The ratio of 141° at 3:1 and 135.2° at 2:1 is significantly slower than the ratio of 76.6° at 1:1. And when the ratio of PLCL to gelatin is 1:1, the total concentration of spinning solution is 20% and 25%, both showing hydrophilicity. The nanofiber membrane prepared with high concentration and high proportion of spinning solution is always hydrophobic. When the total concentration of the spinning solution is 20%, the ratio of PLCL to gelatin increases, and the hydrophobic properties of the electrospun membrane further increase. The nanofiber membrane was initially hydrophilic, and with the increase of PLCL: gelatin, it became obviously hydrophobic. PLCL: The change of gelatin in the spinning solution, the ratio increases from 1:1 to 3:1, the PLCL ratio increases and the gelatin ratio decreases. The solvent curing process in the electrospinning process is from the outside to the inside, so the hydrophobic group tends to face outward, resulting in an increase in the proportion of hydrophobic groups towards the surface. The total concentration of the spinning solution is 20%. When the PLCL/GE is 1:1, the PLCL and gelatin in the electrospinning solution are equal in mass, each accounting for 50%, and the contact angle is 69.5°. The fiber mainly shows the hydrophilicity of gelatin. Sex. When the ratio of PLCL to gelatin is greater than 1:1, the amount of PLCL in the electrospinning solution is greater than that of gelatin, so the spun electrospun fiber shows obvious hydrophobicity of PLCL. And with the increase of the ratio, the difference between the content of PLCL and gelatin further increases, so the hydrophobicity of the nanofiber membrane further increases.

![Figure 3. Water contact angle (WCA) of PLCL/GE nanofiber membranes.](image)

4 Conclusion
In summary, using the electrospinning process, gelatin and poly-lactide-caprolactone (PLCL) are used to prepare PLCL/GE nanofiber scaffolds. The scaffold materials successfully spun from the spinning solutions with different PLCL/GE mass fractions and PLCL/GE ratios form a network structure, which facilitates the material exchange during the repair process and achieves the ideal use effect. The
Experimental results show that the increase of poly-lactide-caprolactone (PLCL) greatly improves the hydrophobic properties of PLCL/GE nanofibers. The increase in the ratio of different PLCL/GE reduces the diameter of the nanofibers, and at the same time makes the fibers more hydrophobic. Different concentrations have a great impact on the formation and structure of the nanofiber, and the increase in the total concentration of different spinning solutions can increase the diameter of the nanofiber. When PLCL/GE is 1:1, the total PLCL/GE concentration reaches 20% and 25%, and the spun nanofibers appear obvious adhesion. The distribution of fiber diameter is more uniform, and the hydrophobicity of the fiber is further increased.

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