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Composite vascular grafts with high cell infiltration by co-electrospinning

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ABSTRACT

There is an increasing demand for functional small-diameter vascular grafts (diameter < 6 mm) to be used in clinical arterial replacement. An ideal vascular graft should have appropriate biomechanical properties and be biocompatible. Electrospinning has become a popular polymer processing technique for vascular tissue engineering, but the grafts fabricated by electrospinning often have relatively small pores and low porosity, which limit cell infiltration into scaffolds and hinder the regeneration and remodeling of grafts. In the present study, we aimed to develop an efficient method to prepare electrospun composite vascular grafts comprising natural and synthetic materials. We fabricated grafts made of polycaprolactone, gelatin, and polyvinyl alcohol (PVA) by co-electrospinning, and the scaffolds were further functionalized by immobilizing heparin on them. The PVA fibers degraded rapidly in vivo and generated electrospun scaffolds with high porosity, which significantly enhanced cell proliferation and infiltration. The mechanical properties of the grafts are suitable for use in artery replacement. Heparin functionalization of the grafts yielded a good antithrombogenic effect, which was demonstrated in platelet adhesion tests. Moreover, in vitro and in vivo results demonstrated that the heparin release from the grafts enhanced the growth of endothelial cells, which is important for the endothelium of implanted grafts. The results of this study indicate that our method is effective and controllable for the fabrication of vascular grafts that meet the clinical requirements for blood vessel transplantation.

1. Introduction

At present, many patients suffer from vascular diseases, which severely damage human health. Vascular transplantation is an effective clinical strategy, but its application is often limited by a shortage of donors. Therefore, there is an urgent demand for artificial grafts as substitutes. Some artificial vascular grafts (such as allografts and xenografts) may offer long-term patency, but their clinical utility is limited by the potential for an immunogenic response [1,2]. Synthetic grafts, such as those made of polytetrafluoroethylene, have been widely used for peripheral vascular reconstructions, although they readily fail when applied to small-diameter vessels, such as the coronary artery, because of acute thrombosis and subsequent occlusion [3]. Therefore, there is an increasing demand for functional artificial vascular grafts with small diameter (<6 mm) to be used in clinical arterial replacements [4]. An ideal vascular graft should have appropriate biomechanical properties and should be biocompatible [5,6].

Recently, composite vascular grafts made of natural and synthetic materials have attracted increasing attention [7,8]. The key advantages of synthetic polymer scaffolds are their superior mechanical properties, whereas natural polymers have a high degree of biocompatibility and biodegradability. Polycaprolactone (PCL) is an aliphatic polyester that degrades slowly and possesses high tensile and elongation properties [9,10]. Evaluations have shown that PCL vascular grafts can provide sufficient mechanical strength in rat aorta implantation for up to 18 months, but they are hydrophobic and lack reactive sites for further biofunctionalization [2,3,11]. Gelatin (GT), derived from collagen denaturation, is an attractive natural polymer for tissue engineering applications because it is non-immunogenic, bioresorbable, non-cytotoxic, and low cost, wherein it can provide cells with a high degree of biocompatibility and biodegradability [12,13]. GT scaffolds have been evaluated as vectors for load growth factor [14,15]. However, being a water-soluble protein, gelatin needs to be treated in an appropriate manner to ensure its mechanical performance and temporal stability. Therefore, hybrid materials that combine both synthetic PCL and natural GT can be used in a composite vascular scaffolding system [6]. PCL polymers can be used to reinforce the mechanical properties of matrix materials, while GT can be used to improve the biocompatibility of scaffolds [16,17].

Electrospinning has become a popular polymer processing technique for fabricating vascular grafts due to its unique capacity for producing scaffolds with micro- to nano-scale topography, high surface area-to-volume ratios, and highly interconnected pores [18,19]. During this process, a high voltage is applied between a nozzle and a collector, where the potential difference between the nozzle and collector leads to stretching of the solution, thereby creating a thin jet from the solution

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toward the collector, after which the solvent evaporates and size-controlled fibers can be gathered on the collector [20,21]. However, a major problem with this technique is that the electrospun scaffolds have inherently small pore sizes, which often result in insufficient cell infiltration, and thus cell growth is restricted only to the surface [22,23]. To address this problem, various techniques have been tested to increase the pore sizes and overall porosity [24,25]. Eichhorn and Sampson showed that it is possible to control the pore sizes by controlling the average fiber diameters based on various electrospinning parameters, where greater fiber diameters yield larger pore sizes [26]. Some studies have suggested that porogens can also be used to increase the pore sizes in spun fiber mats. Thus, Baker et al. used water-soluble polyethylene oxide as a sacrificial polymer with PCL [27]. Wright et al. also attempted to incorporate salt crystals by dropping them on a rotating mandrel during the electrospinning of polymers, where the scaffolds had a reduced elastic modulus and lower yield strain after leaching out the salt crystals in water the [28]. Another method uses a modified hollow electrospinning mandrel with well-defined pores on the surface, where pressurized air is expelled from these pores during the spinning process to disrupt fiber deposition, thereby increasing the cellular infiltration of scaffolds [29]. However, these methods may affect the morphology of fibers and reduce the overall mechanical properties, which can lead to the collapse of the porous scaffolds. In this study, we aimed to develop a method for preparing porous scaffolds using co-electrospinning techniques by achieving a balance between biocompatibility and the physical properties. Thus, PCL was electrospun into vascular scaffolds with good mechanical properties, and GT and heparin were introduced to improve the biocompatibility and reduce thrombosis. Sacrificial polyvinyl alcohol (PVA) was used to create large pores within the hybrid electrospun PCL and GT scaffolds, thereby enhancing cell infiltration and ingrowth while also maintaining sufficient mechanical strength for arterial replacement.

2. Materials and methods

2.1. Materials

PCL (Mn = 120.000) was purchased from Daigang Biomaterial (Jinan, China). Polymers of GT type B obtained from bovine skin and thiazolyl blue tetrazolium blue (MTT) in powder form were both obtained from Sigma. PVA, heparin sodium, Coumarin 6, DiI, dimethyl sulfoxide (DMSO), and trifluoroethanol (TFE) were all purchased from Aladdin Chemicals (Shanghai, China).

2.2. Preparation of electrospinning solutions

Solutions of PCL/GT were prepared by mixing 10% *w*/*v* PCL/TFE and 10% *w*/*v* GT/TFE at a volume ratio of 50:50. The mixed solutions were stirred for ~24 h prior to processing to ensure thorough mixing. Visible inspection indicated that the mixed solutions exhibited a high degree of cloudiness. A tiny amount of 2% (*v*/v) acetic acid was dropped into the solutions described above to prepare a miscible and transparent GT/PCL/TFE solution. PVA powder was dissolved in deionized water at a concentration of 10% (*w*/*v*).

2.3. Electrospinning of hybrid mats and tubular grafts

Electrospinning was performed according to a previously reported method [30,31]. The instrument (Fig. 1) included a positive high-voltage DC power supplier and a negative supplier, two syringe pumps (SPLab01, Easypump, China), two 10-mL syringes, and a grounded collector. One of the syringes was fitted with blunt-ended 27G needles (internal diameter = 0.21 mm, outer diameter = 0.41 mm) and the other was fitted with blunt-ended 22G needles (internal diameter = 0.41 mm, outer diameter = 0.72 mm). The PCL/GT solution for electrospinning was fed into the first syringe (27G) and the PVA solution was fed into the other syringe (22G), and both syringes were then loaded on the pumps. The needles were connected to emitting electrodes with positive polarity at a voltage of + 12 kV and the negative electrode was connected to the collector at a voltage of − 1.5 kV. The distance between the needle tip and the collector was 12 cm for PCL and 15 cm for PVA.

Two types of electrospinning scaffolds were fabricated using different collectors under similar conditions. Mats were collected using a rotating mandrel collector with a diameter of 60 mm and tubular grafts were prepared by a rotating collector with a diameter of 2 mm. Both collectors ran at a rotation speed of 1000 rpm. The PCL/GT solutions were electrospun at a constant flow rate of 0.6 mL/h and the PVA solutions were electrospun at flow rates of 0 and 0.2 mL/h. The prepared electrospun scaffolds were designated as Type A (PCL/GT) and Type B (PCL/GT/PVA). Finally, the electrospun membranes and tubular grafts were dried under vacuum for one day to completely remove the residual solvent.

2.4. Morphological characterization of the scaffolds

PVA was removed from the composites by rinsing with phosphate-buffered saline (PBS) solution for 24 h at 37 °C. Before and after the removal of PVA, the PCL/GT and PVA composite scaffolds were processed using the same method. The surface morphology of the electrospun mats and tubular grafts were observed using a field emission scanning electron microscope (SEM; JSM-6700F, JEOL, Japan). Before imaging by SEM, the samples were sputter-coated with gold for 50 s to increase their conductivity. Based on the SEM images, the diameters of the fibers were analyzed using Image-Pro Plus software. For each sample, five SEM images were analyzed and at least 70 fibers were measured manually in each image, where the results were expressed as the mean and standard deviation. The porosity of the scaffolds was calculated using the liquid intrusion method [5]. First, the scaffolds were dried and weighed, and then immersed in 100% ethanol for 2 h. Next, the scaffolds were wiped gently to remove any excess ethanol and weighed again. The porosity was calculated using the following equations:

$$V_{eth} = (W_{wet} - W_{dry})/D_{eth};$$
$$V_s = W_{dry}/D_s;$$
$$\text{Scaffold porosity} = V_{eth}/(V_s + V_{eth}) \times 100\%;$$

where V_{eth} is the volume of ethanol trapped in the graft, V_s is the volume of the scaffold, W_{dry} and W_{wet} are the dry and wet weights of the scaffold, respectively, D_s is the density of the scaffold, and D_{eth} is the density of ethanol.

To observe the relative distributions of PCL/GT and PVA fibers, we separately labeled PCL/GT and PVA with Coumarin 6 (green) and DiI (red) by adding trace amounts of the two fluorescent dyes to the spinning solutions. The fabricated electrospun mats were then analyzed using confocal laser scanning microscopy (PerkinElmer, USA).

2.5. Mechanical testing of tubular scaffolds

Uniaxial tensile testing was performed using a tensile tester (HY-0230, Shanghai Hengyi, China) equipped with a 100 N loaded cell. Tubular specimens ($n = 5$) measuring 2 mm in diameter, 15 mm in length, and about 230 μm in thickness were immersed into PBS for 12 h at room temperature before the test. The tubular scaffolds were then clamped and pulled longitudinally at a rate of 6 mm/min until rupture, where the gauge length was 1.5 cm. The stress–strain curve was recorded by the machine. The tensile strength was defined as the maximum stress until rupture during the tensile test. Young's modulus was determined by measuring the slope of the stress–strain curve in the elastic region.