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Influence of the fiber diameter and surface roughness of electrospun vascular grafts on blood activation

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ABSTRACT

Electrospun grafts have been widely investigated for vascular graft replacement due to their ease and compatibility with many natural and synthetic polymers. Here, the effect of the processing parameters on the scaffold's architecture and subsequent reactions of partially heparinized blood triggered by contacting these topographies were studied. Degrapol® (DP) and poly(lactic-co-glycolic acid) (PLGA) electrospun fibrous scaffolds were characterized with regard to fiber diameter, pore area and scaffold roughness. The study showed that electrospinning parameters greatly affect fiber diameter together with pore dimension and overall scaffold roughness. Coagulation cascade activation, early platelet adhesion and activation were analyzed after 2 h of exposure of blood to the biomaterials. While no differences were found between DP and PLGA with similar topographies, the blood reactions were observed to be dependent on the fiber diameter and scaffold roughness. Scaffolds composed of thin fibers (diameter <1 μm) triggered very low coagulation and almost no platelets adhered. On the other hand, scaffolds with a bigger fiber diameter (2–3 μm) triggered higher thrombin formation and more platelets adhered. The highest platelet adhesion and activations rates as well as coagulation cascade activation were found in blood incubated in contact with the scaffolds produced with the biggest fiber diameter (5 μm). These findings indicate that electrospun grafts with small fiber diameter (<1 μm) could perform better with reduced early thrombogenicity due to lower platelet adhesion and lower activation of platelets and coagulation cascade.

1. Introduction

In Europe, cardiovascular diseases (CVD) are responsible for about 50% of all mortality, causing about 4.3 million deaths per year [1], and in 2008 over 2.6% of the overall population in Europe was admitted to hospital for CVD [2]. The restriction of blood flow by arteriosclerosis thus represents a significant medical burden. If detected early, many obstructed blood vessels can be bypassed or replaced by vascular substitutes, including arterial autografts, polytetrafluoroethylene (ePTFE) and polyester grafts [3].

Autografts have been observed to be the most successful choice for the repair of small-diameter vascular grafts, with primary patency rates of 73%, compared to 47% for ePTFE and 54% for polyester grafts [4]. The main limitations of autologous grafts are their availability and donor site morbidity [3]. Therefore, tissue-engineered vascular grafts (TEVG) using autologous cells are promising alternatives. While large-diameter TEVG grafts regenerated successfully in humans with a five-year patency of about 90% [5], small-diameter vascular grafts (diameter <5 mm) are still a challenge [6]. Among the main reasons for graft failure of small-diameter grafts (anastomotic intimal hyperplasia, aneurysm formation, infection and progression of atherosclerotic disease), acute thrombogenicity of the graft is one of the most important [7–9].

Reduction of thrombogenicity is crucial for improving the graft success rate, and several strategies have been developed with partial success. The most studied graft surface modifications are the addition of anti-thrombotic factors, coating with cell-adhesive ligands to promote endothelialization and seeding with endothelial cells. However, the addition of soluble anti-thrombotic factors has a time-limited activity, which eventually stops when the whole drug supply is used up. Coating with cell-adhesive ligands and seeding with endothelial cells both aim at better endothelialization, which provides excellent anti-thrombogenic properties [10]. However, coating with cell-adhesive ligands is not specific to endothelial cells and also supports platelets and smooth muscle cells adhesion, leading to clotting and subsequent pseudointimal thickening [3]. Also, in vitro endothelialization cannot guarantee full

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coverage of the graft and cells are lost during transplantation, and complete endothelialization in vivo is not instantaneous and takes at least 10 days [11], thus acute thrombogenicity cannot be fully prevented by this strategy.

Electrospinning has been widely used as a processing method for the design of vascular grafts [12,13], with performances equal to or superior than the current standard, ePTFE [14]. This technique is quite popular as it allows easy production of fibrous tubular scaffolds with controllable composition, architecture, fiber diameter and mechanical properties [12,15,16]. Scaffold properties greatly affect the reaction of contacting cells and tissues; for instance, scaffold porosity affects the cell infiltration in vitro [15,16] and tissue integration in vivo [17], and fiber orientation can affect cell alignment [18]. Regarding vascular grafts, a number of studies have shown that endothelialization is improved on scaffolds with nanofibers compared to microfiber scaffolds [12,19].

Although early thrombosis has been recognized as a major cause of graft occlusion, no study has investigated the influence of electrospun graft topography on acute thrombogenicity. In the present study, two polymers – DegraPol® (DP), which has been shown to meet essential requirements for vascular grafts, such as good biocompatibility [20], mechanical properties [16] and hemocompatibility [21], and poly(lactic-co-glycolic acid) (PLGA), which has been approved by the US Food & Drug Administration and has been widely exploited for many biomedical applications [22–25], were processed into scaffolds with defined fiber diameters. We subsequently evaluated the effects of the size of the fiber diameters and of the resulting surface topography of the electrospun scaffolds on early coagulation, platelet adhesion and activation. The blood coagulation cascade can be activated by a specific stimulus, such as an injury or exposure to a thrombogenic surface. A key enzyme required for the activation of the cascade is thrombin, which triggers the polymerization of fibrinogen into fibrin fibrils [26,27]. Thrombin–antithrombin (TAT) complexes have been used as a surrogate marker for thrombin generation [28] and were used in this study as a marker for coagulation cascade activation. The aim of this contribution is to understand the effect of the scaffolds' architecture on the blood reaction in order to be able to design a scaffold with minimal acute thrombogenicity.

2. Materials and methods

2.1. Polymers

Polyester urethane (trade name DegraPol® (Mw = 70 kDa)) was produced according to the procedure described elsewhere [29,30]. Poly(lactic acid-*co*-glycolic acid) (Resomer®, PLGA, Type RG 85:15, Mw = 280 kDa) was obtained from Boehringer Ingelheim, Germany. Chloroform (stabilized with ethanol) was obtained from Emanuele Centonze SA, Switzerland and hexafluoro-propanol (HFP) was purchased by Sigma.

2.2. Scaffold production by electrospinning and solvent casting

Fibrous scaffolds were produced with an electrospinning set-up, assembled in-house, comprising a syringe pump (Racel Scientific Instruments Inc., USA), a spinning head consisting of a central stainless steel tube, a hollow cylindrical rotating aluminum mandrel for fiber collection (length: 100 mm, diameter: 80 mm, wall thickness: 5 mm) and a high-voltage DC supply (Glassman High Voltage Inc., USA).

Homogeneous DegraPol solutions were prepared by letting the desired amount of polymer dissolve in an HFP–chloroform mixture (wt.% 25:75) at room temperature (RT) overnight, while PLGA solutions were dissolved in chloroform only. The homogeneous solutions were loaded into a 2 ml syringe (B. Braun Melsungen AG, Germany) and pumped into the spinning heads. Electrospinning parameters, such as the polymer concentration, the distance between the spinning head and the collecting mandrel (referred to as working distance), the flow rate and the applied voltage between the spinning head and the collector, were set for producing electrospun scaffolds with three different desired diameters. These we called ES1, ES2 and ES3, and they are summarized in Table 1.

Table 1
The electrospinning parameters used for the production of the different DegraPol and PLGA fibrous scaffolds.

Polymer	Scaffold	Concentration (%)	Flow rate (ml h^{-1})	Working distance (cm)	Applied voltage (kV)
DegraPol	ES1	15	0.1	25	5
	ES2	25	0.4	20	10
	ES3	25	1	15	15
PLGA	ES1	15	0.4	25	10
	ES2	25	1	20	10
	ES3	25	1.5	15	10

The concentration represents the weight portion of the polymer in the solvent mixture (25% HFP and 75% chloroform were used for DegraPol and pure chloroform was used for PLGA).

Uniform smooth polymeric films were used as plain controls to the porous electrospun fibrous scaffolds. For production of the polymeric films used as controls, a 30 wt.% DegraPol solution and a 10 wt.% PLGA solution (in chloroform) were prepared. The solutions were then cast using a stencil with a 500 μm gap on a polytetrafluoroethylene-coated plate. The films were left under a fume hood until complete evaporation of the solvent. The so-produced cast scaffolds are referred as CS throughout.

Disks of 20 mm diameter were punched out from the polymeric scaffolds and used for the blood incubation experiments.

2.3. Scaffold characterization

Fiber diameters were measured as described [18] using scanning electron microscopy (SEM) micrographs: first a diagonal line was drawn from the bottom left to the top right of the image and the fiber diameter was measured, perpendicular to the fiber length, at the points where the line crossed the fiber using ImageJ (http://rsbweb.nih.gov/ij/) after calibration with the scale bar of the microscope image. The diameters of the fibers were averaged over all the images of a sample (50 fiber diameters measured per sample). The pore area was determined as described elsewhere [12] using SEM micrographs and manually approximating the surface pores (50 pores measured per sample).

Surface roughness was measured by means of stereo-SEM, whereby a surface is imaged twice with conventional SEM, eucentrically tilted (i.e. in the focus plane) by 10° [31]. From these two images of the same region of interest (ROI), three-dimensional (3-D) information of the surface topography was calculated by means of automatic image correlation with specialized software (MeX, Alicona, Graz, Austria) [32]. The resulting height profiles were evaluated using the window-roughness method, whereby values above a certain cut-off wavelength λ_c are identified as waviness and are not included in the surface roughness calculation [33]. Here, the cut-off wavelength was set at λ_c = 200 μm and the surface roughness was expressed as R_a, describing the arithmetic average of the absolute values in the *z*-direction.

The water contact angle was measured using a Ramé-Hart model 100 goniometer (Ramé Hart Inc, Moutain lakes, NJ, USA) at RT. Water drops of 5 μl were used for water contact angle measurements in air.