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Comprehensive assessment of electrospun scaffolds hemocompatibility

Jana Horakova [a,*], Petr Mikes [a], Ales Saman [a], Tereza Svarcova [a], Vera Jencova [a], Tomas Suchy [b], Bohdana Heczkova [c], Sarka Jakubkova [d], Jaroslava Jirousova [d], Renata Prochazkova [d,e]

[a] Technical University of Liberec, Faculty of Textile, Department of Nonwovens and Nanofibrous Materials, Studentska 2, 461 17 Liberec, Czech Republic
[b] The Czech Academy of Sciences, Institute of Rock Structure and Mechanics, V Holesovickach 94/41, 182 09 Prague, Czech Republic
[c] Liberec Regional Hospital, Department of Clinical Hematology, Baarova 15, 460 01 Liberec, Czech Republic
[d] Liberec Regional Hospital, Department of Blood Transfusion, Baarova 15, 460 01 Liberec, Czech Republic
[e] Technical University of Liberec, Faculty of Health Studies, Studentska 2, 461 17 Liberec, Czech Republic

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ABSTRACT

Biodegradable polyesters, namely polycaprolactone (PCL) and copolymer of polylactide and polycaprolactone (PLCL) were electrospun into various fibrous structures and their hemocompatibility was evaluated *in vitro*. Firstly, hemolytic effect was evaluated upon incubation with diluted whole blood. The results showed that the degree of hemolysis depended on chemical composition and fibrous morphology. Electrospun polycaprolactone induced slight degree of hemolysis depending on its molecular weight and fibrous morphology; copolymer PLCL did not cause detectable hemolysis. The influence of coagulation pathways was examined by measurement of coagulation times. It was showed that intrinsic coagulation pathway assessed by activated partial thromboplastin time (APTT) was moderately accelerated after incubation with PCL and prolonged after incubation with copolymer PLCL. Extrinsic activation of coagulation tested by prothrombin time (PT) was slightly accelerated after incubation with all tested electrospun samples. Thrombogenicity assessment of fibrous samples revealed high thrombogenic properties of fibrous materials that was comparable to high degree of collagen thrombogenicity. The level of platelet activation was dependent on chemical composition and surface morphology of tested materials.

1. Introduction

Tissue engineering scaffolds are designed as structural and functional analogues of extracellular matrix assuming that cells recognize their natural environment and undergo the regeneration of the damaged tissue. Extracellular matrix is a natural cell environment composed of complicated nano- and micro-architecture. Most body tissues are hierarchal fibrillar or tubular structures with various size, organization and composition that affect the tissue mechanical and biophysical properties [1]. Mimicking this 3D web by nanofibers is a challenge in the modern tissue engineering [2]. Electrospinning is the most well-known method for production of nanofibrous materials. The structure of resulting fibers is very similar to the native extracellular matrix therefore it facilitates cell adhesion and spreading [3].

Synthetic biodegradable polyesters such as polycaprolactone (PCL), polylactic acid (PLA) and their copolymers have been widely used in various applications of tissue engineering such as bone replacement [4,5], vascular tissue engineering [6–8], nerve repair [9,10] *etc.* Implantable devices have to be hemocompatible besides other important properties. There are several studies reporting cell adhesion and proliferation on various morphologies of electrospun fibers [11,12]. On the other hand, the effect of electrospun materials on hemolysis, coagulation and platelet activation has not been comprehensively studied. Interactions of materials with blood are divided in various categories evaluating potential influence of thrombosis, coagulation or hemolysis [13]. Impact of various structure of polycaprolactone has been tested by Leszczak et al. In their study, polycaprolactone in the form of flat film, nanowires and nanofibers were compared. They concluded that nanofibers promote platelet adhesion and clustering compared to flat films and nanowires surfaces [14]. Other studies were focused on nanofibrous surface functionalization with heparin leading to hemocompatibility improvement as described by Xiong et al. and Cestari et al. [15,16].

In this work, hemocompatibility of electrospun biodegradable polyesters with various fiber morphology as promising candidates of novel implantable biomaterials was evaluated since information about electrospun scaffold morphology and chemical composition effect on blood component interactions are missing. Various structures of polycaprolactone and copolymer polylactide and polycaprolactone

* Corresponding author.
E-mail addresses: jana.horakova@tul.cz (J. Horakova), petr.mikes@tul.cz (P. Mikes), ales.saman@tul.cz (A. Saman), tereza.svarcova@tul.cz (T. Svarcova), vera.jencova@tul.cz (V. Jencova), suchyt@irsm.cas.cz (T. Suchy), bohdana.heczkova@nemlib.cz (B. Heczkova), sarka.marikova@nemlib.cz (S. Jakubkova), jaroslava.jirousova@nemlib.cz (J. Jirousova), renata.prochazkova@nemlib.cz (R. Prochazkova).

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were tested for hemolysis degree, influence of coagulation and platelet activation. Moreover, thrombogenic potential of fibrous mats was compared to naturally thrombogenic collagen.

2. Materials and methods

2.1. Preparation of foils and fibrous materials

Biodegradable polyesters, namely poly(ε-caprolactone) (PCL) and copolymer PURASORB PLC 7015 composed of L-lactide and ε-caprolactone in a 70/30 molar ratio (PLCL) were electrospun into planar fibrous mats for further hemocompatibility assessment. Polycaprolactone with different molecular weights (average M_n 45,000 and 80,000) were obtained from Sigma Aldrich and copolymer PLCL was purchased from Corbion. Polymers were dissolved in solvent system composed of chloroform and ethanol or chloroform, ethanol and acetic acid (Penta, Czech Republic) in different weight ratios leading to 4 various fibrous structures as listed in Table 1. Final concentration of electrospinning solutions was 16 wt% and 22 wt% of polycaprolactone with lower molecular weight of 45,000 (marked as PCL45 16% and PCL45 22%, respectively). Polycaprolactone with molecular weight of 80,000 (PCL80) and copolymer PLCL were electrospun from 10 wt% solution.

Prepared solutions were electrospun using Nanospider™ 1WS500U. The forming fibers were collected on spun bond layer that was rolled by speed of 12 mm/min. The distance between electrode and collector was kept at 18 cm. The potential difference between spinning electrode and collector was 45 kV. The temperature was kept in 23 °C and relative humidity between 35 and 42%.

Morphology of electrospun layers was assessed by scanning electron microscopy (SEM) and image analysis software to characterize fiber diameter distribution. Samples for SEM analyses were sputter coated with gold and analyzed using TESCAN Vega 3SB Easy probe (Czech Republic). Further image analysis was used to characterize fiber distribution of electrospun samples from SEM pictures employing software NIS Elements (LIM s.r.o., Czech Republic). Fiber diameter was evaluated from 200 measurements of fiber thickness from 5 SEM photos.

For thrombogenicity testing, foils were prepared from the same polymers as electrospun mats resulting in 3 types of materials marked as PCL45 F, PCL80 F, PLCL F. Polymers were dissolved in chloroform in 2 wt% and let evaporated in the fume hood. After complete solvent evaporation the polymer formed a smooth foil that was used for thrombogenicity assessment.

Collagen samples were prepared as positive controls for thrombogenicity testing since collagen is considered to be one of the most thrombogenic material [17]. Control collagen samples were prepared from type I collagen isolated from calf skin (VUP Medical, Czech Republic) in the form of foils as well as in the form of electrospun mats. Collagen foils (COL F) were prepared from 8 wt% solution in phosphate buffer saline (PBS, pH 7.4, Sigma-Aldrich) and ethanol (1/1, v/v), the detailed preparation is published elsewhere [18]. The fibrous mats (COL) were electrospun from 8 wt% collagen solution in PBS/ethanol (1/1, v/v) with addition of polyethylene oxide (Sigma-Aldrich) in amount of 0.5 wt%. Both collagen materials were cross-linked employing the *N*-(3 dimethylaminopropyl)-*N*-ethylcarbodiimidehydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) in ethanol solution (EDC: 4.08 mg ml^{-1} and NHS: 1.02 mg ml^{-1}). EDC and NHS (Sigma Aldrich) were used as received. Following a reaction period of 24 h at 37 °C, all the scaffolds were washed in the 0.1 M Na_2HPO_4 (Penta, Czech Republic) for 20 min followed by rinsing using deionized water (2 × 20 min).

Table 1
List of biodegradable polyesters used for fibrous scaffolds fabrication.

	Polymer concentration [wt%]	Solvent system [v/v/v]
PCL45 16%	16	Chloroform/ethanol 9/1
PCL45 22%	22	Chloroform/ethanol 9/1
PCL80	10	Chloroform/ethanol/acetic acid 8/1/1
PLCL	10	Chloroform/ethanol/acetic acid 8/1/1

2.2. Preparation of whole blood, platelet poor plasma and thrombocyte rich solution

Four milliliters of blood from a healthy donor was collected in BD Vacutainer containing 0.129 M sodium citrate (BD Diagnostics). Anticoagulated blood was subsequently diluted with 5 ml of PBS providing the source of hemoglobin for hemolysis assay.

Twelve milliliters of blood from a healthy donor was collected in BD Vacutainer containing 0.129 M sodium citrate (BD Diagnostics) and centrifuged for 15 min at 1500 × *g* in order to obtain platelet poor plasma (PPP) for measurement of coagulation times.

Thrombocyte rich solution (TRS) was prepared from mixed buffy coats obtained

from 4 blood donors. After centrifugation using a deleucotization filter (CompoStop® Flex 3F T&B, Fresenius Kabi), thrombocyte rich solution containing 1046 × 10^6 thrombocytes/ml was obtained. The solution containing thrombocytes was gently agitated in 20–24 °C for 48 h prior to the thrombogenicity assessment.

2.3. Hemolysis testing

The rate of hemolysis was evaluated by determining the relative amounts of hemoglobin released into solution phase from erythrocytes in diluted whole blood exposed to the test materials. Electrospun samples (PCL45 16%, PCL45 22%, PCL80 and PLCL) were cut into 1 × 1 cm squares and put individually into the test tubes. The test samples were soaked in 10 ml PBS, negative controls contained 10 ml PBS only and positive controls were comprised from 10 ml distilled water in order to induce maximal lysis of erythrocytes ($n = 5$ for each testing group). All test tubes were preheated in a 37 °C for 30 min followed by addition of 200 μl diluted anticoagulated fresh whole blood. The test tubes were kept for 60 min in 37 °C, after which the tubes were centrifuged at 100 × *g* for 5 min. The supernatant containing the solubilized hemoglobin was removed and its absorbance was measured at a wavelength of 570 nm (two measurements from each test tube).

2.4. Coagulation times

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined by measurement of clotting times of platelet poor plasma (PPP) after incubation with tested electrospun biodegradable polyesters (PCL45 16%, PCL45 22%, PCL80 and PLCL). PT is an indicator of extrinsic and common coagulation path of activation. APTT measures activity of intrinsic and common pathways of coagulation. Prolonged time of PT or APTT signifies an enhanced anticoagulant activity of tested materials.

Tested materials were cut into squares of 1 × 1 cm size and placed in a test tube followed by addition of 300 μl PPP. As controls, 300 μl of PPP was incubated in test tubes without any material present. After 45 min of incubation in 37 °C, the material was removed from test tube and clotting time was measured using automatic analyzer BCS XP (Siemens) according to the manufacturer's instructions. Ten samples were measured for evaluation of clotting times by PT and APTT.

2.5. Thrombogenicity

Thrombogenic potential was tested *in vitro* after incubation of biodegradable polyesters and collagen samples in the form of electrospun layers and foils with thrombocyte rich solution. Materials used for