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ScienceDirect
Acta Biomaterialia 5 (2009) 3411–3422
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A novel nanostructured poly(lactic-*co*-glycolic-acid)–multi-walled carbon nanotube composite for blood-contacting applications: Thrombogenicity studies

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Received 17 December 2008; received in revised form 23 April 2009; accepted 1 June 2009
Available online 6 June 2009

Abstract

Composite films of poly(lactic-*co*-glycolic-acid) with multi-walled carbon nanotubes (PLGA–MWCNT) having two different nanotube orientations, namely random and vertically aligned, have been fabricated and characterized. The effect of these nanostructured surfaces on platelet adhesion is evaluated. In particular, the thrombogenicity of the nanostructured composite films is compared with that of pristine graphite (a low thrombogenic material) and PLGA film, in order to determine the influence of surface chemistry and topography on platelet adhesion. The results in this study show that the PLGA–MWCNT composite with vertically aligned nanotubes exhibits very low levels of fibrinogen adsorption and platelet adhesion, which can be attributed to both chemical and topographical effects. Platelet adhesion shows a good correlation with the presence of —COOH groups and appears to be sensitive to the topographic features of the composite films. The results in this study suggest that in addition to chemistry, nanotopographical surface modifications could be an effective strategy in the development of low thrombogenic and hemocompatible materials.

Keywords: Anti-thrombotic materials; Platelet adhesion; Nanotopography; Carbon nanotube composites; Poly (lactide-*co*-glycolic-acid)

1. Introduction

Blood-contacting medical devices such as stents and artificial heart valves usually fail due to thromobotic occlusion over time, caused primarily by platelet adherence to the material surface. The control of platelet–surface interactions is, therefore, particularly important in the development of blood-compatible biomaterials, in order to minimize or eliminate the need for anti-coagulation therapy. Thrombus formation occurs when a biomaterial surface comes into contact with blood, prompted by rapid adsorption of plasma proteins onto the surface within the first few seconds of contact. This adsorption is regarded as the first major event in the coagulation process. Among the plasma proteins, fibrinogen is regarded as the key protein that triggers platelet adhesion, activation and aggregation. Subsequently, coagulation factors are released, initiating the coagulation cascade and the eventual formation of a thrombus [1]. Conformational change of the adsorbed fibrinogen layer plays an important role in mediating the platelet response to an artificial surface and determines the formation of a thrombus and thrombo-embolism in patients with implanted prostheses. Current control of thrombus formation by systemic anticoagulant therapy is clearly not ideal, due to the number of associated side-effects, such as thrombocytopenia, neutropenia and hemorrhage. Therefore, the design of a suitable biomaterial that offers improved blood biocompatibility is highly desirable for blood-contacting devices.

The relatively passive nature of carbon in tissues is well known and documented, and many forms of carbon are

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doi:10.1016/j.actbio.2009.06.003

used in biomedical implant applications. Pyrolytic carbon is widely used in heart valves prostheses [2] and in biomedical implants and coatings [3] due to its resistance to thrombus formation with minimum adherence and activation of platelets. Numerous approaches have been employed in fabricating blood-compatible devices by either surface modification (by chemical and physical methods) [4,5] or topography [6,7] modifications. The coating of intracoronary stents with diamond-like carbon has been demonstrated to significantly reduce the rate of acute thrombosis [8]. Based on this premise, carbon nanotubes (CNTs) were evaluated [9–11] for their potential applications in a variety of biomedical systems and devices due to their unique structure and properties. The most widespread application has been the use of CNTs in polymer nanocomposites [12–15].

In a previous study [16] we demonstrated a significant reduction in platelet adhesion and absence of platelet activation on a poly(lactic-*co*-glycolic-acid)–carbon nanotube (PLGA–CNT) composite fabricated by the electrostatic layer by layer (LbL) technique. Enhanced platelet adhesion was observed on the surface of pristine PLGA as compared with surfaces with adsorbed CNT. The reduction in platelet adhesion to the PLGA–CNT composite was attributed to the effect of the surface chemistry and topography of the CNT, but no attempt was made to establish the relative importance of each factor. In view of these previous results, in this work we investigate the influence of surface chemistry and topography, attempting to establish the contribution of each effect.

Platelet interaction studies in polymeric biomaterials, involving the effect of surface chemistry [17–22] and of surface topography [23–25], are still scarce and studies on both surface chemistry and topography effects in relation to platelet adhesion and hemocompatibility are still very preliminary [26]. This is comprehensible, as the evaluation of blood interactions on biomaterial surfaces becomes more complex because topographical variations are usually accompanied by the presence of chemical heterogeneities on the surfaces. In the light of this, in this study fibrinogen adsorption and platelet interactions were studied on poly (lactic-*co*-glycolic-acid)–multi-walled carbon nanotube (PLGA–MWCNT) composites with randomly dispersed and vertically aligned MWCNT orientations and compared with that of pristine films to investigate whether the different surface topography provided by the MWCNT can modulate platelet interaction behaviour. To the best of our knowledge this is the first hemocompatibility study of carbon nanotube composites with different carbon nanotube orientations and aspect ratios.

2. Materials and methods

2.1. Materials

Multi-walled carbon nanotubes (MWCNT) were obtained from Shenzhen Nanotech Port Co. Ltd. (China) and vertically aligned MWCNT from Nanoloab Inc. (Newton, MA). The chemicals used were 70% nitric acid (HNO_3), 95 wt.% sulphuric acid (H_2SO_4), hydrogen chloride (HCl), sodium hydroxide (NaOH), dichloromethane (DCM) and Tween 20. 80/20 poly(lactic-*co*-glycolic-acid) i.v. 5.01 (PLGA) was purchased from PURAC Biochem. Phosphate-buffered saline (PBS), consisting of sodium chloride, monobasic potassium phosphate, dibasic sodium phosphate (Na_2HPO_4) and potassium chloride (KCl), was obtained from Sigma–Aldrich. Fibrinogen from human plasma (Hfg) was purchased from Sigma–Aldrich (Hfg ~35–65% protein, ⩾95% of protein clottable). Platelet-rich plasma (PRP), monoclonal antibody [horseradish peroxidise (HRP) conjugated with human fibrinogen goat antibody] and 3,3′,5,5′-tetramethylbenzidine were purchased from US Biological. Graphite film [highly ordered pyrolytic graphite (HOPG)] was supplied by SPI Supplies and Structure Probe Inc. The graphite and pristine PLGA films were used as controls in this study.

2.2. Fabrication of randomly dispersed PLGA–MWCNT composite

Carboxylic acid groups were introduced into the MWCNT by acid treatment [27] to improve dispersability and to reduce their tendency to agglomerate. MWCNT were added to a 3:1 mixture of H_2SO_4 and 70% HNO_3. The mixture was sonicated for 2 h, followed by addition of HCl for another 2 h to allow the formation of carboxylic groups. The mixture was diluted with deionized (DI) water and subsequently filtered using a vacuum pump and washed with NaOH solution and DI water until pH 7 was obtained. Finally, the functionalized MWCNT were dried at 70 °C overnight. The functionalized MWCNTs (~0.059 g) were sonicated in 18 ml dichloromethane (DCM) in an ice bath to achieve a homogeneous dispersion before adding PLGA pellets to the sonicated mixture. PLGA pellets (1 g) was dissolved in 18 ml DCM to obtain a solution with low viscosity, as the subsequent addition of MWCNT increases the viscosity substantially. The solutions were mixed and cast in aluminium pans and left to dry at room temperature for a minimum of 3 h before placing in a vacuum oven at 55 °C for 4 days for solvent evaporation. Oxygen plasma treatment was performed at 250 mTorr, 40 W, 12 sccm O_2 to expose a partial layer of the embedded MWCNT in the composites.

2.3. Fabrication of vertically aligned PLGA–MWCNT composite

A polymer solution of PLGA pellets to DCM (1 g per 20 ml) was infiltrated into vertically aligned arrays of MWCNT grown on a silicon substrate by chemical vapour deposition (CVD). Prior to PLGA infiltration the vertically aligned arrays of MWCNTs were exposed to O_2 plasma treatment at 250 mTorr, 40 W, 12 sccm O_2 for 2 min to enhance PLGA infiltration between the interstitial sites of

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