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Heparin coating of poly(ethylene terephthalate) decreases hydrophobicity, monocyte/leukocyte interaction and tissue interaction

Paul H.J. van Bilsen[a,b,*], Guido Krenning[b], Didier Billy[a], Jean-Luc Duval[c], Judith Huurdeman-Vincent[a], Marja J.A. van Luyn[b]

[a] Medtronic Bakken Research Center BV, PO Box 1220, 6201 MP Maastricht, The Netherlands
[b] Stem Cell & Tissue Engineering Research Group, Department of Pathology & Medical Biology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands
[c] UMR CNRS 6600 – Domaine Biomatériaux-Biocompatibilité, Université de Technologie de Compiègne, BP 20529, 60205 Compiègne, France

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ABSTRACT

Woven poly(ethylene terephthalate) (PET) is widely used in implantable medical devices. Upon implantation, fibrinogen interacts with the PET and changes conformation, such that the fibrinogen P2 epitope may become exposed. This allows inflammatory cells to interact with the material. In this study we have coated PET with heparin and show that this decreases PET hydrophobicity and the presence of the fibrinogen P2 epitope on the material surface. In addition, we show that heparin-induced reduction of PET hydrophobicity correlates with decreased exposure of the fibrinogen P2 epitope and reduced adhesion of monocytes. Reduction of PET hydrophobicity was furthermore associated with reduced PMN elastase production and decreased interaction between PET and embryonic chicken tissue. We conclude that the heparin coating-induced decrease in PET hydrophobicity is associated with decreased interaction between PET and inflammatory cells. Independent of this interaction, the hydrophobic nature of the heparin coating is related to tissue interaction as demonstrated by a reduction in adhesion, growth and spreading of tissue on PET. The combination of these properties makes heparin coating a candidate for improving biocompatibility of PET.

1. Introduction

Implantable medical devices are used for a wide range of applications to support damaged tissues, repair tissue integrity or enhance the function of diseased organs. Many of these devices, which include vascular grafts, artificial heart valves and mesh materials for intraperitoneal applications, contain non-degradable polymeric materials. The foreign body reaction (FBR) that is triggered by such materials can potentially cause device-related complications [1]. Woven or knitted poly(ethylene terephthalate) (PET) is used as a medical implantable material. The FBR to woven PET is characterized by a chronic inflammation and subsequent formation of a fibrous capsule [2]. The nature of this FBR is initially determined by the protein interface that forms on the biomaterial surface upon implantation. Tang et al. showed that adsorption and denaturation of fibrinogen allows Mac-1 positive cells such as neutrophils and monocytes to interact with the material [3]. Subsequent activation of neutrophils and monocytes results in secretion of inflammatory proteins, causing a FBR. The physicochemical and biological properties of a biomaterial are important factors that influence the conformation of the proteins that accumulate on it. As such, the protein interface on hydrophobic materials differs from the protein interface on hydrophilic materials [4]. Consequently, the nature of the developing FBR is different as well [5]. To alter the physicochemical properties, it has been proposed to chemically modify the material surface. Such modifications aim to improve the materials' performance. Such a strategy involves providing a material with particular biological functionality so as to improve the outcome.

Heparin is a naturally occurring glycosaminoglycan that is known to enhance the function of heparin-binding proteins [6], including antithrombin III [7,8], extracellular matrix (ECM) proteins, growth factors and cytokines [9,10]. This property makes heparin potentially useful as an anti-inflammatory compound. In this respect, heparin prevents leukocyte chemotaxis [11], inhibits

* Corresponding author at: Stem Cell & Tissue Engineering Group, Department of Pathology & Medical Biology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel.: +31 50 3615181.
E-mail addresses: paul.van.bilsen@home.nl (P.H.J. van Bilsen), m.j.a.van.luyn@med.umcg.nl (M.J.A. van Luyn).

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leukocyte adhesion [12] and slows ECM breakdown by inhibiting the expression of neutrophil elastase [13]. In extracorporeal circulation systems, heparin surface modification is used to reduce complement activation and inflammation during cardiopulmonary bypass procedures [14].

Besides enhancing the function of heparin-binding proteins, immobilized heparin also potentially reduces PET hydrophobicity. This change in surface chemistry might contribute to alter the proteins present at the interface, thereby influencing biocompatibility in a manner that is independent of the biological action of heparin. We hypothesized that coating of PET with heparin improves its performance as an implantable medical material [2]. More precisely, we hypothesize that immobilized heparin changes the physicochemical and biological properties of PET, altering the protein interface and improving the cellular response, as compared to uncoated PET. In order to assess the influence that heparin coating has on PET biocompatibility we have used a number of different in vitro techniques. First we characterized hydrophobicity of heparin-coated PET. Subsequently, we used an immunochemical method to measure exposure of the fibrinogen P2 epitope on each of the materials. The anti-inflammatory potential of our heparin coating was examined by measuring adhesion of freshly isolated monocytes. Next, leukocyte activation in the presence of PET was determined in a blood-contacting model. Lastly, tissue response to heparin coated PET was evaluated in an embryonic chicken tissue-based organotypic culture method.

2. Materials and methods

2.1. Materials

Smooth PET (Goodfellow, Huntingdon, UK) was used for all experiments, except for the blood-loop study. Knitted PET (Bard, Tempe, Arizona, USA) was used for the blood-loop study. PET was first coated with plasma-polymerized ethylene gas. These surfaces were then incubated in 25% acrylamide/acrylic acid (Sigma–Aldrich, Zwijndrecht, The Netherlands) containing 1 M ceric ammonium nitrate (Merck VWR, Amsterdam, The Netherlands). The PET was then washed in DI water for 4 h at 50 °C. Poly(ethylene imine) (PEI; BASF, Arnhem, The Netherlands) was bound to the poly(acrylamide) graft by incubation in a solution of 0.1% PEI and 1% carbodiimide (Sigma–Aldrich, Zwijndrecht, The Netherlands) in borate buffer (pH 9.0; Merck VWR, Amsterdam, The Netherlands) for 45 min at room temperature. Heparin was covalently coupled to the PEI layer by incubation of the PET surfaces in a solution of 0.25 mg/ml heparin (Diosynth, Oss, The Netherlands) and 0.2 mg/ml sodium cyanoborohydride (Sigma–Aldrich, Zwijndrecht, The Netherlands) in acetate buffer (pH 4.6; Sigma–Aldrich, Zwijndrecht, The Netherlands) for 4 h at 37 °C. Lastly the surfaces were rinsed in DI water for 3×15 min, followed by rinsing in 1 M NaCl (Sigma–Aldrich, Zwijndrecht, The Netherlands) for 3×15 min. After completing the coating process, toluidine blue staining was used to confirm presence and the homogeneity of the heparin coating. In addition, the surfaces were exposed to fresh human blood, following the blood exposure protocol described below. Generation of thrombin was determined by a thrombin-antithrombin assay (Enzygnost, Dade Behring, Marburg, Germany) in order to confirm the anti-coagulant action of the coupled heparin. Disks of 1 cm^2 were punched out of uncoated, PEI coated and heparin coated PET and ethylene oxide (EtO) sterilized (Sterigenics, Verviers, Belgium). These materials are further referred to as BARE, PEI and HEP, respectively.

In addition, poly(tetrafluoro ethylene) film (PTFE; Goodfellow, Huntingdon, UK) was used as a hydrophobic control for the contact angle measurements as well as in the fibrinogen P2 measurements. Thermanox® tissue culture polystyrene film (Merck VWR, Amsterdam, The Netherlands) was used as a control in the organotypic culture model.

2.2. Contact angle measurements

In order to measure the effect of heparin coating on hydrophobicity of PET (Goodfellow, Huntingdon, UK), coupons of PET were first coated with heparin as described above. Dynamic contact angle measurements were conducted using the Wilheney plate technique (Cahn dynamic contact analyzer 332). Triplicate measurements were conducted on different areas of one coupon of the BARE, PEI and HEP. PTFE film was used as a hydrophobic control.

2.3. Fibrinogen adsorption and exposure of the P2 epitope

To assess fibrinogen adsorption and conformational change on heparin coated PET (Goodfellow, Huntingdon, UK), the presence of total fibrinogen as well as the P2 fibrinogen epitope was determined using an immunoassay. Three coupons of PTFE, BARE, PEI and HEP were incubated with human fibrinogen (15 μg/ml; Sigma–Aldrich, Zwijndrecht, The Netherlands) for 180 min, after which they were washed with phosphate buffered saline (PBS; Invitrogen, Breda, The Netherlands). Next, the surfaces were blocked with PBS containing 1% BSA (Sigma–Aldrich, Zwijndrecht, The Netherlands) at 37 °C for 1 h, after which the surfaces were incubated with goat polyclonal antibody (Ab) to human fibrinogen (1:20,000 in TBS/BSA/Tween; Accurate Chemical, Westbury, New York, USA) at 37 °C for 1 h. After incubation with the primary Ab, the surfaces were incubated with alkaline phosphatase-conjugated polyclonal rabbit Ab to goat IgG (1:4000 in TBS/BSA/Tween; Abcam, Cambridge, Massachusetts, USA) at 37 °C for 1 h. Next, the surfaces were incubated with Alkaline Phosphatase Yellow (pNPP) Liquid Substrate System for ELISA (Sigma–Aldrich, Zwijndrecht, The Netherlands) at 37 °C for 45 min. Absorbance of the substrate solutions was measured at 405 nm using an ELISA-plate reader (Molecular Devices, Sunnyvale, California, USA). Another set of three coupons of PTFE, BARE, PEI and HEP was incubated with mouse monoclonal Ab to the fibrinogen γ 392–411 and 392–406 chain (1:750 in TBS/BSA/Tween; Accurate Chemical, Westbury, New York, USA) at 37 °C for 1 h. After incubation with the primary Ab, these surfaces were incubated with HRP-conjugated polyclonal goat Ab to mouse IgG (1:12,000 in TBS/BSA/Tween; U.S. Biological, Swampscott, Massachusetts, USA) at 37 °C for 1 h. Next, the surfaces were incubated with tetramethyl benzidine (TMB; Sigma–Aldrich, Zwijndrecht, The Netherlands) at room temperature for 10 min. The substrate conversion reaction was stopped by adding 1N sulfuric acid (Sigma–Aldrich, Zwijndrecht, The Netherlands). Absorbance was measured at 450 nm.

2.4. $CD14^+$ adhesion test

$CD14^+$ Mononuclear cells (MNC) were isolated from buffy coats obtained from healthy donors (Sanquin, Groningen, The Netherlands) using density gradient centrifugation on lymphoprep (Nycomed Pharma, Zurich, Switzerland). Donors signed a donor consent form prior to the experiment. $CD14^+$ monocytes were isolated by magnetic bead separation [15]. Briefly, 1×10^7 MNC were labeled with 20 μL MACS MicroBeads (Miltenyi Biotec, Utrecht, The Netherlands) and incubated in a volume of 80 μL PBS supplemented with 0.5% fetal bovine serum (BioWhittaker, Verviers, Belgium) and 2 mM EDTA (BioWhittaker, Verviers, Belgium) on ice for 15 min. Cells were washed with buffer and resuspended in 500 μL PBS. The cell suspension was separated on a LS^+/VS^+ column placed in a strong magnetic field. $CD14^+$ cells were retained in