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Biomaterials 27 (2006) 4315–4324
Biomaterials
www.elsevier.com/locate/biomaterials

Hemocompatibility evaluation of poly(glycerol-sebacate) in vitro for vascular tissue engineering

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Received 8 February 2006; accepted 1 April 2006

Abstract

Poly(glycerol-sebacate) (PGS) is an elastomeric biodegradable polyester that could potentially be used to engineer blood vessels in vivo. However, its blood-material interactions are unknown. The objectives of this study were to: (a) fabricate PGS-based biphasic tubular scaffolds and (b) assess the blood compatibility of PGS in vitro in order to get some insight into its potential use in vivo. PGS was incorporated into biphasic scaffolds by dip-coating glass rods with PGS pre-polymer. The thrombogenicity (platelet adhesion and aggregation) and inflammatory potential (IL-1β and TNFα expression) of PGS were evaluated using fresh human blood and a human monocyte cell line (THP-1). The activation of the clotting system was assessed via measurement of tissue factor expression on THP-1 cells, plasma recalcification times, and whole blood clotting times. Glass, tissue culture plastic (TCP), poly(l-lactide-*co*-glycolide) (PLGA), and expanded polytetrafluorethylene (ePTFE) were used as reference materials. Biphasic scaffolds with PGS as the blood-contacting surface were successfully fabricated. Relative to glass (100%), platelet attachment on ePTFE, PLGA and PGS was 61%, 100%, and 28%, respectively. PGS elicited a significantly lower release of IL-1β and TNFα from THP-1 cells than ePTFE and PLGA. Similarly, relative to all reference materials, tissue factor expression by THP-1 cells was decreased when exposed to PGS. Plasma recalcification and whole blood clotting profiles of PGS were comparable to or better than those of the reference polymers tested.

Keywords: Hemocompatibility; Blood compatibility; Coagulation; Thrombosis; Vascular grafts; Tissue engineering

1. Introduction

It is estimated that 1.2 million Americans will suffer a heart attack this year, resulting in nearly 500,000 deaths, of which 50% are due to coronary heart disease [1]. In treating this disease, approximately 500,000 bypasses are performed every year with autologous blood vessels as the first option for revascularization. Nevertheless, up to 30% of the patients who require arterial bypass surgery do not posses suitable or sufficient autologous blood vessels, necessitating the use of synthetic grafts [2]. Polyethylene-terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE) are currently the standard biomaterials used for prosthetic vascular grafts [3]. Satisfactory results have been obtained with PET or ePTFE vascular grafts used to replace or bypass, large-diameter blood vessels; however, results reported when used in small-diameter blood vessels (<6 mm) are poor when compared to those of autologous vein grafts [4]. For example, when used for femoropopliteal bypass grafting, PET and ePTFE are only 36% and 47% functional after two years, respectively [5].

To address this problem, small-diameter vascular grafts have become a major area of interest, spanning multiple disciplines. A variety of biodegradable polymers, scaffolds, and matrices have been evaluated for the development of a tissue engineered vascular graft [6,7]. The tissue engineering approach would rely on either the in vitro or in vivo cellular remodeling of a polymeric scaffold. In order for in vivo cellular remodeling to be successful, the biocompatibility, degradation rate and mechanical characteristics of the scaffold must be well-suited to the dynamic environment of the blood vessel. The ideal scaffold for vascular tissue engineering should employ a biocompatible and degradable polymer with elastomeric properties that interact favorably with cells and blood.

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doi:10.1016/j.biomaterials.2006.04.010

Poly(glycerol-*co*-sebacate) (PGS) is an elastomeric biodegradable polymer developed recently for use in soft tissue engineering [8,9]. It has been investigated for potential uses in regenerative medical approaches including the development of an artificial microvasculature [10] and a nerve guidance material [11]. Also, due to the elastomeric and degradation properties of PGS, it could potentially be used to engineer blood vessels in vivo. However, the incorporation of PGS into a scaffold for vascular tissue engineering and the hemocompatibility of this material have not been investigated. In this study, the fabrication of PGS-based biphasic tubular scaffold and the interaction of PGS in vitro with blood and plasma were investigated in order to assess its application in vascular tissue engineering.

2. Experimental

2.1. Buffers and cells

The phosphate-buffered saline (PBS) contained 137 mM NaCl, 2.7 mM KCl, 10.1 mM sodium phosphate dibasic and 1.8 mM potassium phosphate monobasic, pH = 7.4. The platelet-suspending buffer (PSB) contained 137 mM NaCl, 2.7 mM KCl, 0.4 mM Sodium Phosphate Monobasic, 5.5 mM Dextrose, 10 mM HEPES, 2.5 mM $CaCl_2$, 0.1 U/mL Apyrase, 1.0 mM $MgCl_2$ and 4 mg/mL BSA, pH = 7.4. The Triton-PSB buffer contained 2% (v/v) Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in PSB, pH = 7.4. The glutaraldehyde buffer contained 2.5% (v/v) glutaraldehyde in PBS, pH = 7.4. One milliliter of undifferentiated THP-1 cells were purchased from American Type Culture Collection (ATCC, Rockland, MD) and grown in suspension with complete RPMI-1640 media (containing 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 10% newborn calf serum, and 0.05 mM 2-mercaptoethanol). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise specified.

2.2. Preparation of samples

Equimolar amounts of glycerol and sebacic acid were added to a 250 ml three-neck round-bottom flask fitted with an inlet and outlet adapter. Sebacic acid and glycerol were added in a 1:1 molar ratio and melted at 150 °C under a flow of nitrogen gas while stirring to create a prepolymer. For the fabrication of flat sheets, the pre-polymer was stirred for an additional 4 h under a vacuum of 50 mmHg and then cast into a glass Petri dish pre-heated to 120 °C and immediately placed into a 120 °C vacuum oven. The co-polymer was kept at 0.015 mmHg and 120 °C for 72 h to obtain a PGS sheet. For biphasic scaffold fabrication, the pre-polymer was mixed at 120 °C for an additional 8 h under 50 mmHg vacuum to create a viscous solution that would not phase separate once removed from the heat. To test the feasibility of incorporating PGS into a hybrid biphasic scaffold design, PGS solid tubes (inner layer) were first fabricated using a solvent coating and evaporation technique. Briefly, PGS pre-polymer was dissolved in acetone to a concentration of 35% (w/v). Glass rods were repeatedly dipped into this solution to increase the tube wall thickness and the solvent allowed to evaporate. After evaporation, the glass rods were transferred to a vacuum oven for post-polymerization at 120 °C for 72 h under 0.015 mmHg. A porous poly(1,8-octanediol citrate) (POC) [12,13] (outer layer) was added to the PGS tube by placing the PGS-coated glass rod concentrically into a Teflon tube mold that contained a salt-POC prepolymer slurry with 90% salt particles (100–200 μm). The assembly was then post-polymerized at 80 °C for 4 days. This method for the fabrication of biphasic scaffolds has been previously described [13,14]. Expanded PTFE samples were donated by the Division of Vascular Surgery, Feinberg Medical School, Northwestern University. A transparent PLGA 85/15 (Sigma-Aldrich, St. Louis, MO) film was prepared by dissolving Poly(lactide-*co*-glycolide) (PLGA) 85/15 in dichloromethane, which was cast into a poly(tetrafluoroethylene) mold. The solvent was evaporated at room temperature, producing the film. The PLGA film was removed from the mold and dried under vacuum at room temperature for 48 h. The thickness of all polymer films was 0.8 mm. All samples were cut into 10 mm diameter disks using a cork borer with a surface area of 78.5 mm^2.

2.3. Preparation of platelet-rich plasma

Blood was drawn from healthy adult volunteers by venipuncture into acid citrate dextrose anticoagulant (ACD, Solution A; BD Franklin Lakes, NJ). The methods used to collect and prepare the platelets used in this study were approved by the Institutional Review Board and the Office for the Protection on Research Subjects at Northwestern University. Platelet-rich plasma (PRP) was prepared as previously described [15]. Briefly, whole blood was centrifuged at 250*g* for 15 min and the platelet-rich supernatant was removed. Plasma proteins were separated from the platelet fraction utilizing size exclusion chromatography. The columns (Bio-Rad, Hercules, CA, #732-1010) were packed with sepharose 2B (Sigma-Aldrich, St. Louis, MO, #2B-300) and equilibrated with PSB. The PRP was run through the column and the elution volumes containing the platelets were collected and the platelet concentration determined prior to incubation with samples.

2.4. Quantification of platelet adhesion

Glass, PGS, PLGA, and ePTFE samples were incubated with the PRP for 1 h at 37 °C under static conditions. The suspension was aspirated and each well was rinsed carefully three times with PBS. The number of adherent platelets was determined by detecting the amount of lactate dehydrogenase (LDH) present after cell lysis as previously described [15,16]. Briefly, adherent platelets were lysed by incubation with 2% Triton-PSB buffer for 30 min at 37 °C. A colorimetric substrate for LDH (Roche Diagnostics Corporation, Indianapolis, IN, 1644793) was added and incubated for 20 min at 37 °C. The reaction was stopped with the addition of 1 N HCl. The optical density was measured at 490 nm with reference wavelength of 650 nm. A calibration curve was generated from a series of serial dilutions of a known platelet concentration and used to determine the number of adhered platelets. The morphology of adhered platelets was assessed via scanning electron microscopy (SEM). Briefly, adherent platelets were fixed using 2.5% glutaraldehyde in PBS for at least 2 h, dehydrated in a graded series of ethanol, and freeze-dried. The samples were then sputter coated with a 7 nm layer of gold and observed using scanning electron microscopy (SEM 3500N, Electron Probe Instrumentation Center, Northwestern University).

2.5. Quantification of platelet activation via detection of soluble P-selectin (sP-selectin)

Glass coverslips, PLGA, PGS and ePTFE samples were incubated with 200 μl of whole blood for 1 h at 37 °C under static conditions. The blood was transferred to a 1.5 ml tube and EDTA added to a final concentration of 10 mM. The sample was subsequently centrifuged at 2000*g* for 10 min to obtain the platelet poor plasma (PPP) [17]. The concentration of sP-selectin levels in the plasma was determined using ELISA kit (Human soluble P-selectin Immunoassay, R&D Systems, Minneapolis, MN, #BBE 6).

2.6. Quantification of whole blood clotting time

The thrombogenicity of PGS was evaluated using a whole blood kinetic clotting time method, as previously described [18,19]. Blood was drawn from healthy adult volunteers by venipuncture into ACD anticoagulant vacutainer tubes. The first 3 ml of blood drawn was discarded to prevent contamination by tissue thromboplastin caused by needle puncture. Four