Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 1416–1424
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Peptide modification of polyethersulfone surfaces to improve adipose-derived stem cell adhesion

Yen-Chih Lin [a], Candace A. Brayfield [b], Joerg C. Gerlach [b,c], J. Peter Rubin [a,c], Kacey G. Marra [a,b,c,*]

[a] *Division of Plastic Surgery, Department of Surgery, University of Pittsburgh, 1655E Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15261, USA*
[b] *Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA*
[c] *McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA*

Received 23 July 2008; received in revised form 24 November 2008; accepted 25 November 2008
Available online 24 December 2008

Abstract

Polyethersulfone (PES) is a nondegradable, biocompatible, synthetic polymer that is commonly utilized as a membrane material for applications such as hemodialysis, ultrafiltration and bioreactor technology. Various studies have shown surface modification to be a valuable tool in the development of nondegradable materials which promote cell adhesion. Cells of interest include adipose-derived stem cells (ASCs). ASCs are multipotent mesenchymal stem cells that are useful for various regenerative medicine applications. In this study, we hypothesized that PES surfaces modified with a peptide sequence based from fibronectin, such as Arg-Gly-Asp (RGD), Arg-Gly-Asp-Ser and Gly-Arg-Gly-Asp-Ser, would increase ASC adhesion compared to unmodified PES surfaces. The synthetic peptides were covalently bonded to amine-modified PES surfaces using 1-ethyl-3-(dimethylaminopropyl) carbodiimide. The surfaces were characterized using a ninhydrin assay and contact angle measurements. The ninhydrin assay confirmed the presence of amine groups on the surface of peptide-treated PES disks. Advancing water contact angles were analyzed to detect changes in the hydrophilicity of the polymer surfaces, and results indicated our PES membranes had excellent hydrophilicity. The attachment and proliferation of human ASCs was assessed and RGD-treated surfaces resulted in a higher number of attached ASCs after 6 and 48 h, as compared to unmodified PES surfaces. Additionally, varying concentrations of the RGD peptide sequence concentration were examined. These results indicate that PES membranes modified with the RGD peptide sequence can be utilized for enhanced ASC attachment in biomedical applications.

Keywords: Cell adhesion; Polyethersulfone; Adipose stem cells; Surface modification

1. Introduction

Biomaterials of varying compositions, both natural and synthetic, are being investigated for use in tissue engineering applications. Synthetic polymers are appealing as they can easily be mass-produced and modified for specific applications [1]. Polyethersulfone (PES) is a nondegradable, biocompatible polymer utilized as a membrane material in such applications as hemodialysis, ultrafiltration, filtration and bioreactor technology. PES is highly hydrophobic, has excellent thermal stability and is resistant to chemicals. There are a variety of cell types that have been reported to adhere to PES, such as endothelial cells, fibroblasts, osteoblasts, epithelial cells and keratinocytes [1–3]. For example, Unger et al. [4] observed that human endothelial cells grown on fibronectin-coated PES fibers retain important endothelial-cell specific morphological features. However, there are fewer studies available that report the behavior of stem cells on PES surfaces.

* Corresponding author. Address: Division of Plastic Surgery, Department of Surgery, University of Pittsburgh, 1655E Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15261, USA. Tel.: +1 412 383 8924; fax: +1 412 648 2821.
E-mail address: marrak@upmc.edu (K.G. Marra).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.11.031

One strategy to modify hydrophobic surfaces involves coating with polymers, proteins or peptides. For example, Liu et al. [5] reported that grafting of polyvinyl alcohol, polyethylene glycol or chitosan onto PES membranes increased hydrophilicity and lowered protein adsorption on the surface. To promote cell adhesion, fibronectin or fibronectin-derived peptides can be grafted onto polymer surfaces. Fibronectin-derived peptide sequences, such as Arg-Gly-Asp (RGD), represent cell adhesion ligands present in the extracellular matrix. The fibronectin-derived sequences represent an attractive alternative to fibronectin, as human-derived fibronectin is difficult to isolate in significant quantities, may be immunogenic and lacks large-scale reproducibility. Pierschbacher and Ruoslahti [6] reported that fibronectin-derived peptides promote cell attachment to synthetic surfaces. Loredana et al. [7] demonstrated that the immobilization of RGD on a membrane surface improved not only cell adhesion, but also the function of human hepatocytes. Therefore, we aimed to evaluate a PES surface modified with synthetic peptide sequences derived from fibronectin to promote adult mesenchymal stem cell adhesion.

Adult mesenchymal stem cells (MSCs) have the capacity for self-renewal and capability of differentiation to various cell lineages [8]. MSCs can be found in various adult tissues, such as bone marrow, cartilage and adipose tissue. Adipose tissue can be harvested from a patient in a minimally invasive manner and provides a large quantity of autologous cells [9,10]. Adipose-derived stem cells (ASCs) derived from human adipose are multipotent, which renders these cells ideal for regenerative medicine applications, such as cartilage, bone and soft tissue reconstruction. Furthermore, MSCs derived from adipose tissue possess a high proliferation potential, followed by MSCs derived from bone marrow [11]. The rapid expansion of ASCs as well as the development of comprehensive three-dimensional (3-D) in vitro culture models of ASC differentiation will be of great significance in increasing the availability of ASCs for clinical use. Furthermore, 3-D in vitro models will enable researchers to evaluate the efficacy of ASCs as a multifaceted tool for biomedical applications.

In this study, we hypothesized that amine-functionalized PES membranes covalently bonded to synthetic peptide sequences derived from fibronectin would significantly enhance ASC adhesion compared to unmodified PES membranes. As such, we assessed the attachment and proliferation of human ASCs on three different fibronectin-derived, synthetic peptide sequences bound to PES membranes. Improved cell adhesion on PES surfaces will have implications in 3-D bioreactor technology, which currently uses PES hollow fibers.

2. Materials and methods

2.1. Materials

All chemicals were obtained from Sigma–Aldrich unless otherwise specified. CyQuant cell proliferation assay kit, DMEM/F12, penicillin/streptomycin and fetal bovine serum were obtained from Gibco (Invitrogen Corporation, Carlsbad, CA). Collagenase (type II, M8B10274) was obtained from Worthington Biochemical Corporation, Lakewood, NJ. The fibronectin-derived peptide sequences tested, Arg-Gly-Asp (RGD), Arg-Gly-Asp-Ser (RGDS) and Gly-Arg-Gly-Asp-Ser (GRGDS), were purchased from Sigma–Aldrich. PES membrane (Micro PES-2F, hydrophilic flat membrane) was obtained from MEMBRANA (Underling Performance, Germany); the average thickness of the PES membrane for this product was 110 μm with a mean pore size of 200 nm.

2.2. Chemical modification of PES membranes

A procedure described by Higuchi et al. [12] was followed in order to incorporate amine groups onto the surface of PES disks (Fig. 1). To attach fibronectin-derived RGD, RGDS and GRGDS peptide sequences to the amine group on the modified PES surface, we used 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC). The fibronectin-derived peptide sequences were dissolved at the desired concentration in 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffer. The peptide and EDC solution were mixed in a 1:1 peptide:EDC molar ratio. PES disks were immersed in the above solution for 3 h at room temperature, and then the solution was replaced by double distilled

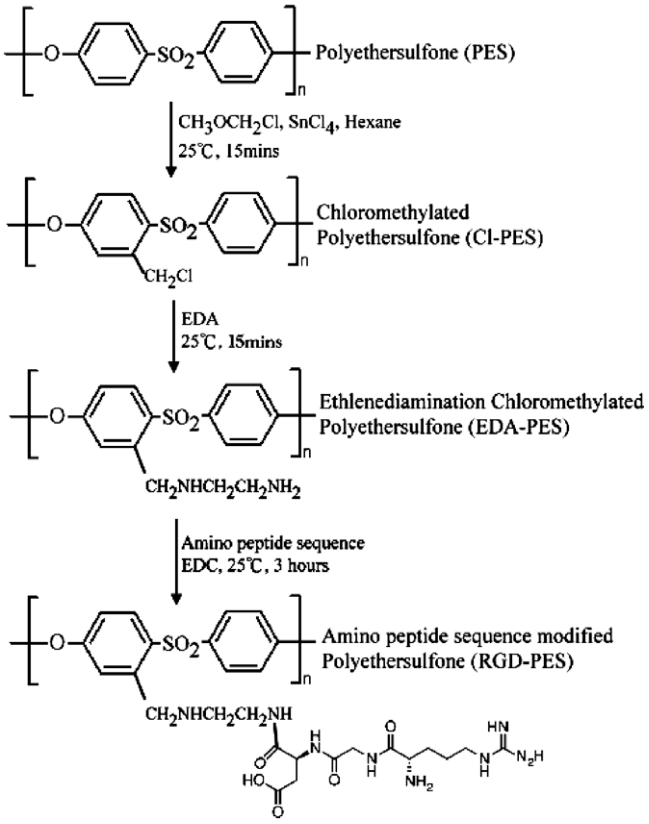

Fig. 1. Reaction scheme of modification of PES.