Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 1016–1023
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Immobilization of glycoproteins, such as VEGF, on biodegradable substrates

J.L. Sharon, D.A. Puleo *

Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506, USA

Received 14 December 2007; received in revised form 23 January 2008; accepted 19 February 2008
Available online 5 March 2008

Abstract

Attachment of growth factors to biodegradable polymers, such as poly(lactide-co-glycolide) (PLGA), may enhance and/or accelerate integration of tissue engineering scaffolds. Although proteins are commonly bound via abundant amino groups, a more selective approach may increase bioactivity of immobilized molecules. In this research, exposed carboxyl groups on acid-terminated PLGA were modified with dihydrazide spacer molecules. The number of hydrazide groups available for subsequent attachment of protein was dependent on dihydrazide length, with shorter molecules present at significantly greater surface densities. The potent angiogenic glycoprotein vascular endothelial growth factor (VEGF) was oxidized with periodate and the aldehyde moieties allowed to react with the hydrazide-derivatized PLGA. Derivatization initially affected the amount of protein bound to the surfaces, but differences were substantially reduced following overnight incubation in saline. More importantly, use of shorter dihydrazide spacers significantly enhanced accessibility of immobilized VEGF for binding neutralizing antibody and soluble VEGF receptor. Furthermore, immobilized growth factor enhanced endothelial cell proliferation, with surfaces having the shortest and longest spacers stimulating greater effects. The present work has not only demonstrated an alternative approach to immobilizing growth factors on biodegradable materials, but the scheme can be used to alter the amount of protein bound as well as its availability for subsequent biointeractions.

Keywords: Protein immobilization; Glycoprotein; PLGA; VEGF

1. Introduction

Generally when the body encounters a biomaterial surface, the result is a repair response rather than a regenerative response. The repair response aims to restore function quickly, but the tissue formed does not mimic the normal structure and properties of the original [1]. Treating a wound site with growth factors can not only accelerate healing, but it may also promote regeneration of more native tissue. Oral delivery of growth factors is not ideal because of low bioavailability resulting from the peptide/protein's enzymatic degradation and poor absorption [2], and intravenous delivery of growth factors is not effective because of the short plasma half-life [3]. Therefore, local delivery of bioactive molecules is needed.

The simplest way to deliver biomolecules to the tissue–implant interface is by dipping the material in a solution of protein before inserting it. Although some encouraging results have been reported [4–6], a major drawback with the adsorption method is that it provides little, if any, control over delivery, including retention and orientation, of molecules. Proteins are initially retained on the surface by weak physisorption forces, and then, depending on the implant microenvironment, which varies among anatomical sites and among patients, the molecules desorb from the surface in an uncontrolled manner to interact with cells.

An alternative approach is to chemically attach biomolecules to surfaces. Adhesive peptides, such as the Arg-Gly-Asp (RGD) sequence that binds to cell surface receptors of the integrin superfamily, have been the focus of much

* Corresponding author. Tel.: +1 859 257 2405; fax: +1 859 257 1856.
E-mail address: puleo@uky.edu (D.A. Puleo).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.02.017

attention (e.g. [7–9]). Immobilization of proteins, such as growth factors, on the surface may provide more control over cell–biomaterial interactions. Whereas RGD peptides primarily mediate adhesion of cells to substrates, immobilized growth factors may be able to modulate subsequent cell functions, such as proliferation, differentiation, and activity, on biomaterial surfaces.

Most schemes used for immobilizing biomolecules involve formation of covalent bonds between surface-exposed functional groups of amino acids with suitable substrates [10]. Moieties commonly used for attachment include amino, carboxyl, and thiol groups. However, most proteins contain several of each type of these reactive groups. In contrast, proteins and peptides do not naturally contain aldehyde groups. Thus, introduction of aldehyde groups, such as by oxidation of carbohydrates, offers an alternative approach to tethering biomolecules on biomaterials.

Glycoproteins, such as vascular endothelial growth factor (VEGF), possess oligosaccharide chains that can be used for immobilization. VEGF is a key regulator of angiogenesis [11]. This 42 kDa glycoprotein is involved in all phases of neovascularization, including enzymatic degradation of the extracellular matrix, migration and proliferation of endothelial cells, and organization of the cells into tubules. Several groups have investigated use of VEGF for controlling tissue repair and tissue–biomaterial interactions in applications such as arterial graft endothelialization [12], bone grafting [13], in-stent intimal formation [14], and limb [15] and myocardial ischemia [16].

The objective of this study was to explore an alternative approach to immobilize growth factors on biodegradable surfaces. In particular, a method for binding glycoproteins to polymers was used. The amount, accessibility, and bioactivity of VEGF bound to poly(lactic-co-glycolic acid) (PLGA) were determined.

2. Experimental procedures

2.1. Surface preparation

Non-end-capped (acid-terminated) PLGA (5050 DL 2A; Alkermes, Wilmington, OH) was used in these studies. Although porous scaffolds are also being examined, results for PLGA-coated coverslips are presented because their more uniform surfaces enabled more accurate comparison between treatments. Coverslips (12 mm diameter) were coated with approximately 30 μl of 10% w/v PLGA solution in methylene chloride and allowed to dry overnight in vacuum; all samples had comparable surface area.

2.2. Surface modification

Four dihydrazides of varying length were investigated as spacer molecules: oxalic (Aldrich, Milwaukee, WI), succinic (Aldrich), adipic (Sigma, St. Louis, MO), and sebacic dihydrazide (TCI America, Portland, OR). Each of these has the same basic backbone, with a carbon chain of different length at its center. The spacer arms were 2 (oxalic; C2), 4 (succinic; C4), 6 (adipic; C6), and 10 (sebacic; C10) carbon atoms long (Fig. 1). Based on results from a pilot study, a dihydrazide concentration of 0.057 mM dissolved in 0.1 M 2-(*N*-morpholino)ethanesulfonic acid (MES; Sigma) at a pH of 6.0 was used. Samples were placed in wells of a 24-well plate along with 0.5 ml of one of the dihydrazide solutions. To activate the surfaces, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC; Sigma) and *N*-hydroxysuccinimide (NHS; Fluka, Buchs, Germany) were added. This solution was prepared to contain EDAC and NHS at a molar ratio of 5:2 in 0.1 M MES, pH 6.0 [17,18]. After adding 0.5 ml of the solution, plates were gently shaken at room temperature for 2 h. All liquid was then aspirated from the plate, and the samples were rinsed three times with deionized water. The derivatization reactions are shown in Fig. 2.

2.3. Quantification of hydrazide groups

To measure the number of hydrazide groups available on each PLGA sample, surfaces were reacted with 0.1%

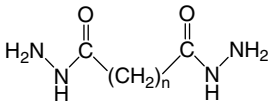

Fig. 1. Structure of dihydrazide spacer molecules having increasing length.

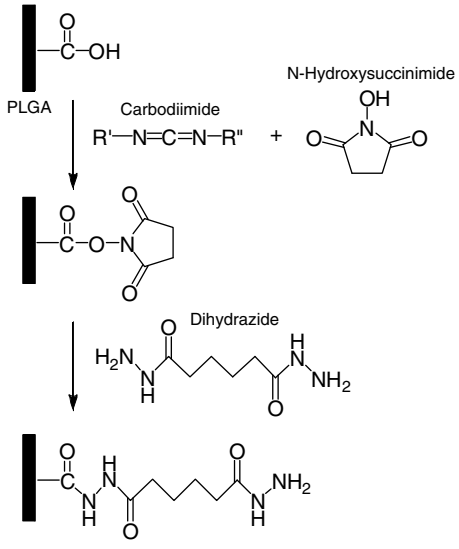

Fig. 2. Idealized scheme for dihydrazide attachment to PLGA.

ID	Title	Pages
2114	Immobilization of glycoproteins, such as VEGF, on biodegradable substrates	8