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Biomaterials 26 (2005) 2401–2406
Biomaterials
www.elsevier.com/locate/biomaterials

Synthesis of photoreactive pullulan for surface modification

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Received 21 June 2004; accepted 27 July 2004
Available online 17 September 2004

Abstract

Photoreactive pullulan was prepared, the polymer was photoimmobilized on polymeric or organic surfaces, and its interactions with a protein and a cell type were investigated. The photoreactive pullulan was synthesized by a coupling reaction with 4-azidobenzonic acid. Surface modification was carried out in the presence or absence of a micropatterned photomask containing 100 μm transparent stripes with 150 μm gaps, making it easy to confirm the immobilization. By the micropatterning method, immobilization of the photoreactive pullulan on polystyrene, polyethylene, and silane-coupled glass was confirmed. Contact angles were measured on the unpatterned surfaces. Although the original surfaces have different contact angles, the contact angle on Az-pullulan-immobilized surface was the same on all surfaces. This result demonstrated that photoimmobilization completely covered the surface with Az-pullulan. Protein adsorption was investigated using fluorescently labeled albumin applied to the micropatterned surface: fluorescence microscopy demonstrated that adsorption was reduced on the pullulan-immobilized regions. Culture of RAW264 cells, derived from mouse leukemic monocytes, on the micropatterned surface for 22 h showed that cells did not adhere to the immobilized pullulan regions. In conclusion, photoreactive pullulan was covalently immobilized on various surfaces and tended to reduce interactions with proteins and cells.

Keywords: Photoimmobilization; Photolithography; Polysaccharide; Pullulan; Protein adsorption; Cell adhesion

1. Introduction

Many types of carbohydrate derivatives have been synthesized for biomedical applications. Some types of sugar-derivatized polystyrenes were synthesized to cultivate hepatocytes [1], and it was reported that a galactosylated surface is an attractive substrate for hepatocyte culture because of the specific interaction between the galactose ligand and the asialoglycoprotein receptor on hepatocytes [2–4]. In addition, polysaccharides such as chitin [5], chitosan [6–10], heparin [9–11], alginate [12–14], and pullulan [15,16] have been derivatized for biomedical applications.

Recently, Li et al. [17] reported photocrosslinkable polysaccharides based on chondroitin sulfate. Barbucci et al. [18] synthesized photoreactive hyaluronan and its sulfated derivative for micropattern immobilization. We have modified growth factors [19], sulfated hyaluronic acid [20], heparin [21], thermo-responsive polymers [22], and β-galactose derivatives [23], and photoimmobilized them on various surfaces using the method devised by Matsuda and Sugawara [24]. This photoimmobilization method can be applied to various materials that have organic surfaces. In addition, by using photolithography micropatterning, direct comparison between immobilized and nonimmobilized surfaces is possible, resulting in surface patterning of proteins and cells according to the properties of the micropatterned polymers.

In this study, pullulan was chosen as a new material for micropatterning, and the subsequent interactions

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doi:10.1016/j.biomaterials.2004.07.065

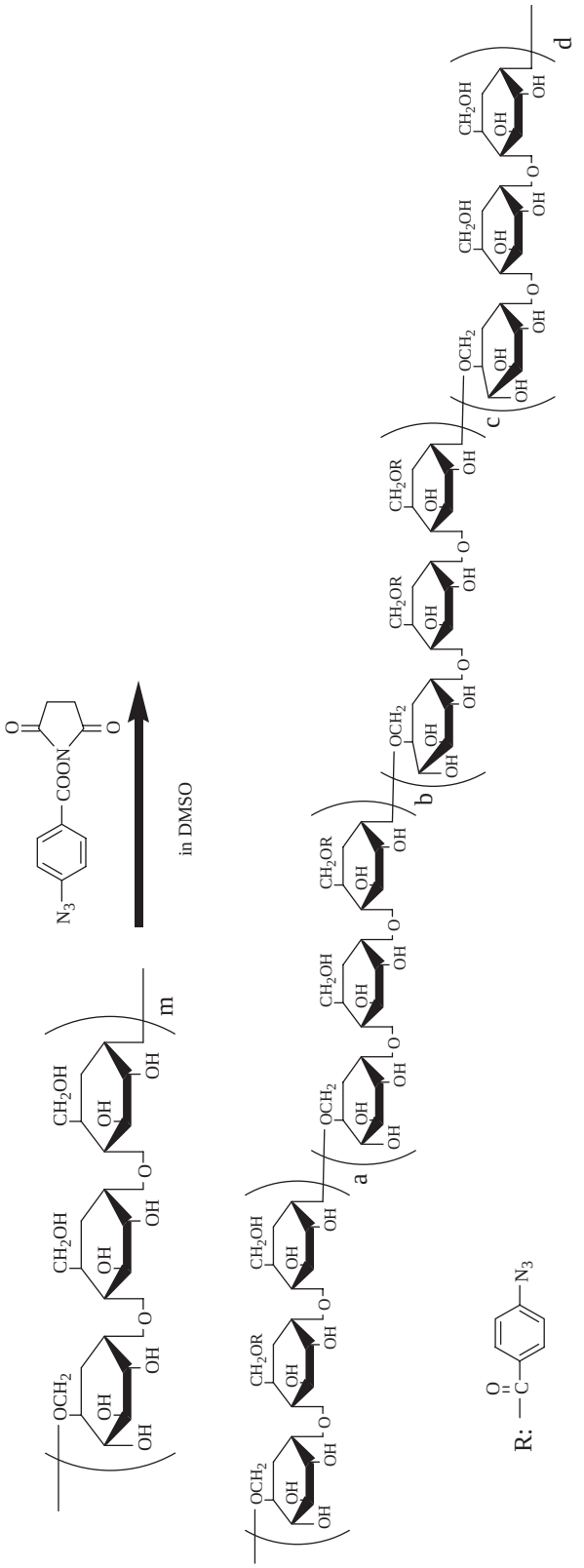

Fig. 1. Synthesis scheme for photoreactive pullulan.

with proteins and cells were investigated. Pullulan is a polysaccharide that has been used in drug delivery because of its solubility and biocompatibility [15]. In addition, although the polysaccharides have many ionic groups, both anionic and cationic, pullulan is nonionic. It was expected that the hydrated nonionic surface would reduce interaction with biocomponents Fig. 1.

2. Materials and methods

2.1. Materials

Pullulan of a molecular weight of about 200,000 was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and used without further purification. Circular plates of polyethylene, 22 mm in diameter, were purchased from Sarstedt (Newton, NC). Polystyrene dishes were purchased from Bibby Sterilin Ltd., (Stone, Staffs, UK). Aminosilane-coupled glass plate was purchased from Matsumani Glass Ind., Ltd. (Osaka, Japan). Dimethylsulfoxide (DMSO) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used without further purification. Fluorescein isothiocyanate (FITC)-labeled bovine serum albumin was purchased from Sigma (St. Louis, MO).

2.2. Synthesis of photoreactive pullulan

Modification of the pullulan was performed as follows. First *N*-(4-azidobenzoyloxy)succinimide was synthesized as previously reported [25]. Pullulan (125 mg) was mixed with the *N*-(4-azidobenzoyloxy)succinimide (200 mg) in DMSO (10 mL) and the mixture was left to stand for 40 h at 40 °C. The resultant solution was poured into toluene and the precipitate was recovered. The precipitate was again dissolved in DMSO and poured into toluene, and subsequently freeze dried. The yield was 43.3 mg. This azidophenyl derivatized pullulan is referred to as Az-pullulan.

2.3. Gel permeation chromatography and spectroscopic measurements

The pullulan and the purified Az-pullulan were measured by gel permeation chromatography (GPC) using COSMOSIL 5-Diol-300-II [Nacalai Tesque (Kyoto, Japan)] at 25 °C. Pure water (milli-Q water, pH 7.3) and a buffered solution (0.15 M Tris-HCl, pH 8.2) were used as elution solvents. Detection was carried out using the refractive index. UV and fluorescence measurements were performed using a JASCO V-550 spectrophotometer (Tokyo, Japan) and a JASCO FP-6500 fluorometer (Tokyo, Japan), respectively.