JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 1–4

Surface modification of a biocompatible polymer based on polyurethane for artificial blood vessels*

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An OH-functionalized poly(carbonate urethane) (PCU) is used for developing a new antithrombogenic surface. Biomolecules like glycine or the fibronectin fragment Gly-Arg-Gly-Asp-Ser (GRGDS) are covalently bound to the PCU surface by succinyl dichloride coupling. The modification steps are controlled by infrared spectroscopy and amino acid analysis. Successfully modified films are tested under stationary cell culture conditions.

1. Introduction

Due to the continued high incidence of arteriosclerosis there is a great interest in polymers for artificial vessels [1]. Homo- as well as graft polymers are being investigated for their compatibility with blood [2]. Commercial polyester prostheses (Dacron^R) are only applicable up to 6 mm diameter. Smaller diameters cause rapid thrombus formation and vessel obstruction. In the late 1970s Eskin and Union Carbide, India, had the idea for endothelial cell seeding on polypropylene microfilaments, but this solution has not been tested in clinical use [3, 4].

Other groups prefer polyurethanes for the development of a synthetic vessel. The good mechanical properties and the biocompatibility favour this material. Biomer^R is a well-known example of this polymer group.

The best protection against thrombus formation, however, is an intact endothelium. The concept of our work is to build up a synthetic extracellular matrix covalently bound to a carrier polymer consisting of an OH-functionalized poly(carbonate urethane). The artificial matrix consists of the fibronectin fragment Gly-Arg-Gly-Asp-Ser (GRGDS), which is bound via succinyl dichloride as coupling reagent onto the polymer surface. In contrast to the model published by Breuers *et al.* [5] this polymer is non-porous and chemically functionalized. The performance of an OH-functionalized polyurethane consists of PCU/poly (4-hydroxybutyl acrylate) (HBA) and its characterization by means of ^{13}C-NMR, FTIR and X-ray photoelectron spectroscopy was carried out as described by Anderheiden *et al.* [6].

The protein-coated polymer film allows endothelial cell seeding at the surface. Thus the polymer–blood contact can be prevented. The development of a surface for endothelial cell seeding and the characterization of the modification steps is described in the present article.

2. Materials and methods

2.1. Activation reaction

The activation of the OH-functionalized PCU was performed with succinyl dichloride. The reaction was carried out in dried toluene with polymer film pieces (9 cm^2). 2 mmol of succinyl dichloride was added and stirred for 20 min at room temperature. Subsequently, 9 mmol dried pyridine and then 1 ml 12 M hydrochloric acid solution with water were added. The film was then washed in $NaHCO_3$ buffer (0.1 mol; pH 8.5) and distilled water.

2.2. Immobilization reaction

The activated film was added to 30 ml of an acetate buffer (3 mol; pH 3). 1 mmol 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as coupling reagent was dissolved in the buffer. Subsequently, the amino acid glycine (5 mmol) or the pentapeptide GRGDS (300 mg) were added and stirred for 16 h at room temperature. The GRGDS was synthesized chemically by using the Merrified solid-phase procedure as described by Breuers *et al.* [5]. The immobilization reaction was terminated in 300 ml acetate buffer (1 mol; pH 9.25). Three washing steps with 200 ml acetate buffer (0.1 mol; pH 4.5), $NaHCO_3$ (0.1 mol; pH 8.5) and distilled water followed. The treated film was dried in a vacuum-drying oven for 24 h.

* This paper was presented at the BIOMAT 90 Conference.

0022–2461/92 $03.00 + .12

2.3. Analytics

Fourier transform infrared spectroscopy (FTIR) measurements were done in attenuated total reflexion (ATR) with a Nicolet 60 SXR spectrophotometer (Offenbach, Germany). By using a germanium crystal for scanning a FTIR spectrum, the penetration depth of the IR beam into the polymer surface can be reduced compared with investigations with a KRS-5 crystal.

For amino acid analysis a Biotronic LC 6000 E (Maintal, Germany) was used.

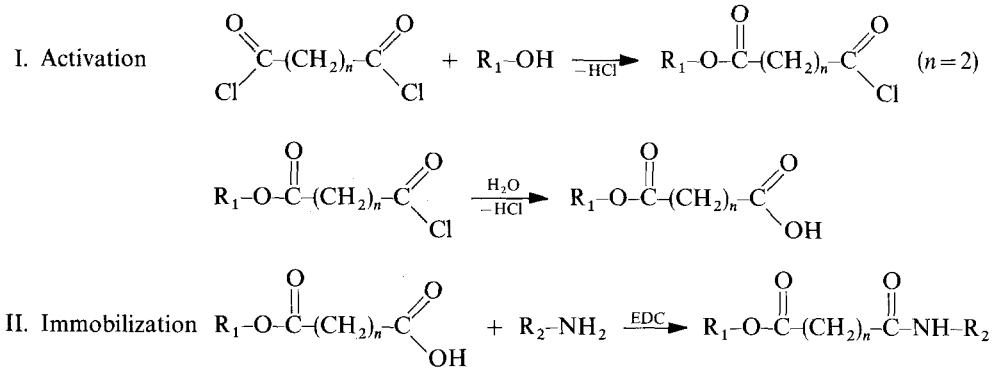
Scanning electron microscopy (SEM) was carried out with a SEM 515 electron microscope (Philips, Eindhoven, The Netherlands).

Biocompatibility was investigated with a cell culture test using epithelial cells, type $H.E_P$ 2, and human umbilical vein endothelial cells (HUVEC). Cells from the logarithmic growth phase were incubated with the materials until confluence was reached at a reference glass surface. The incubation time was 24 h for epithelial cells and six days for HUVEC. Morphological investigations were done with a haemalaune-stained specimen. In parallel, specimens for SEM were prepared by critical point drying and subsequent gold sputtering.

3. Results and discussion

3.1. Immobilization of glycine and fibronectin fragment

To activate the OH-functionalized poly(carbonate urethane) surface succinyl dichloride was used as a bifunctional spacer. The immobilization of peptides can be obtained at pH 3 with a water-soluble carbodiimide (EDC) at room temperature. Initially, a carbodiimide-carboxylic adduct was achieved [7]. The nucleophilic amine group displaced the carbodiimide group and resulted in a stable product [8]. The reaction mechanism is shown in Formula 1.



Formula 1 Mechanism of the succinyl dichloride activation and immobilization of biomolecules using EDC.

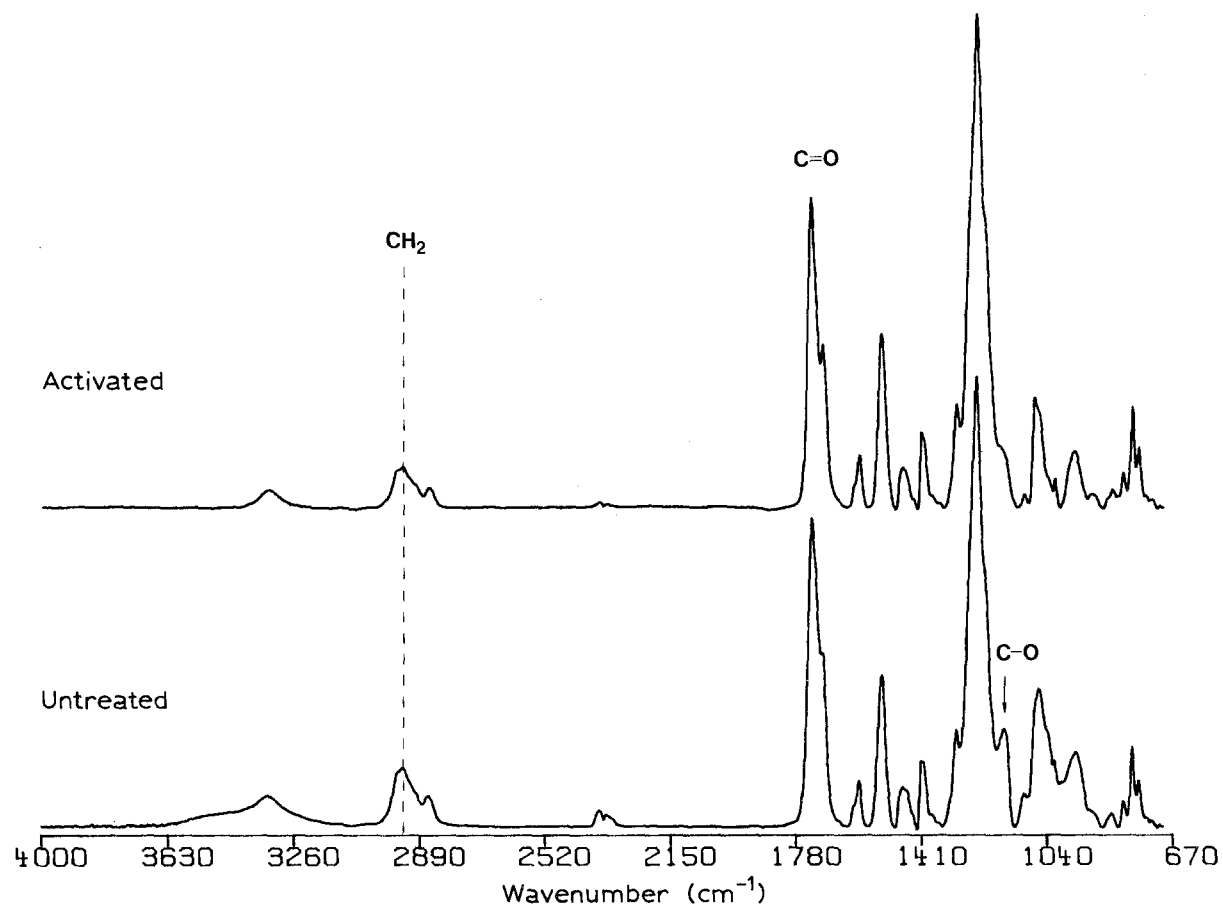


Figure 1 Superimposed FTIR–ATR spectra of the untreated and the activated polyurethane in the range of 4000–670 cm^{-1}.

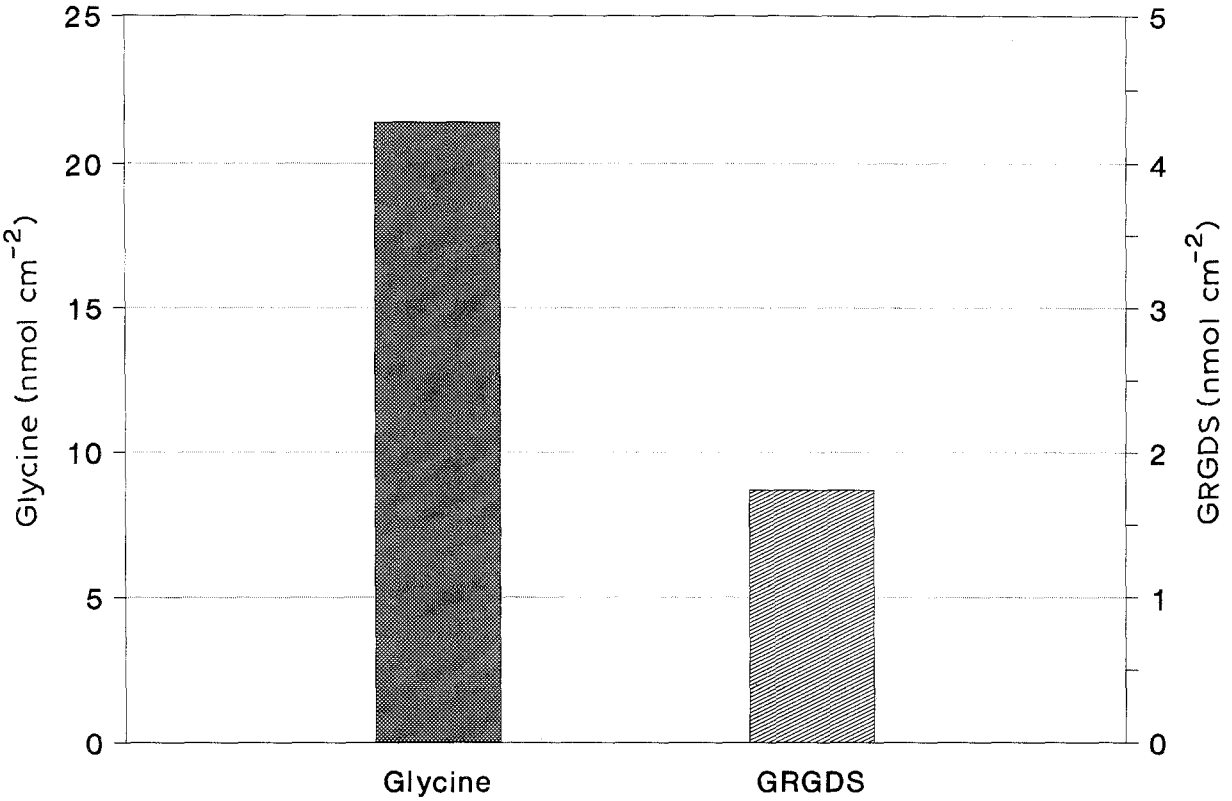


Figure 2 Immobilization reactions of glycine/GRGDS at succinyl dichloride activated polyurethane surfaces.

After activation and hydrolysis of the spacer molecule carboxylic acid groups were obtained. Steric hindrance as a cause of failed coupling could be excluded with glycine. To improve cell adhesion the fibronectin fragment GRGDS was immobilized in a second step. The differences between the FTIR spectra of the untreated film and the surface after succinyl dichloride activation can be seen in Fig. 1.

The absorption band in the range of 3650–3200 cm^{-1} for the untreated PCU is broadened with respect to that of the activated PCU due to the hydrogen bonds of the OH-groups. The resolution of the bands between 1740 and 1700 cm^{-1} is less pronounced in the untreated polymer than in the activated PCU. A decrease in the C–O peak intensity of the OH-function in the poly(HBA) can be observed at 1168 cm^{-1} in the activated polymer surface.

The yield of covalently bound glycine and GRGDS was obtained after total hydrolysis of the modified films by amino acid analysis. A high quantity of fixed molecules was achieved for both reactions at pH 3 and is shown in Fig. 2. Biomolecule fixation could not be proved by FTIR because the negligible layer thickness limited the applicability of this method.

3.2. Cell culture investigations

Untreated and modified polyurethane surfaces with $H.E_P$ 2 cells are morphologically characterized by regular nucleus structure and normal cell spreading behaviour. The photomicrographs in Figs 3 and 4 show that chemical modification of the basic material to the GRGDS coated surface had no adverse effect on cell proliferation.

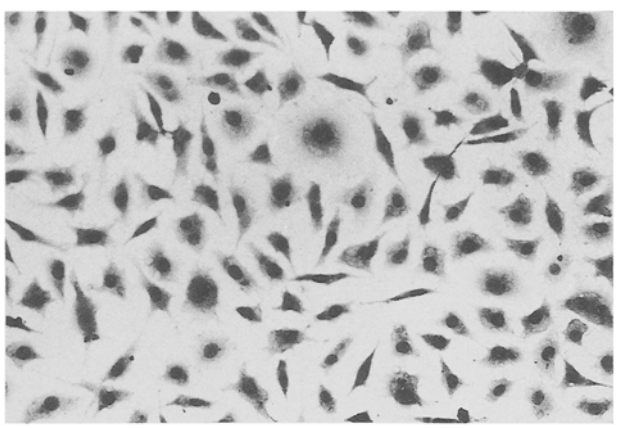


Figure 3 Photomicrograph of the untreated polyurethane surface with HEP 2 cells, after haemalaune staining.

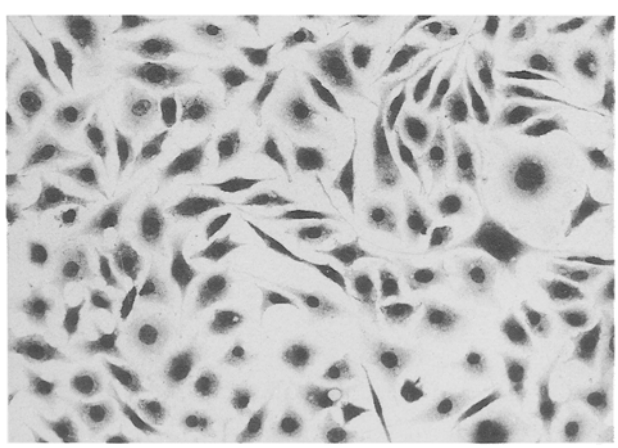


Figure 4 Photomicrograph of the GRGDS-coated polyurethane surface with HEP 2 cells, after haemalaune staining.

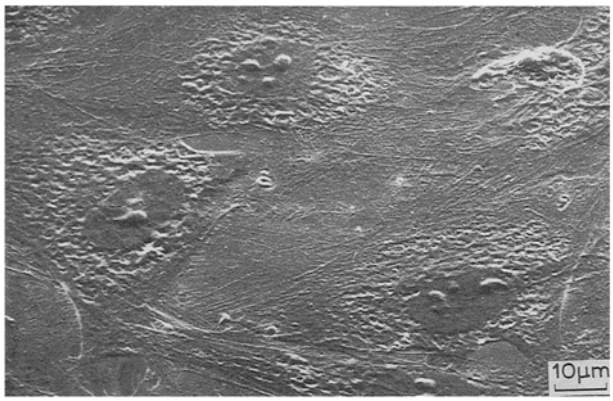


Figure 5 SEM micrograph of the glass control surface with human umbilical vein endothelial cells (HUVEC), magnification (× 1010), voltage 20.1 kV.

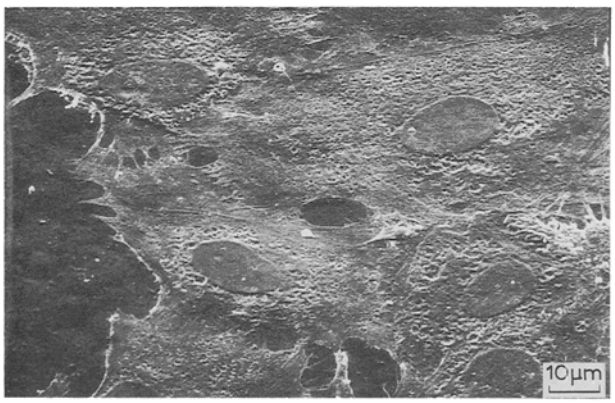


Figure 6 SEM micrograph of the polyurethane surface after succinyl dichloride activation with HUVEC, magnification (× 1010), voltage 20.0 kV.

SEM investigations of the untreated polymer and the succinyl dichloride activated surface using HUVEC show well-spread endothelial cells. As can be seen in the centre of Fig. 6 the bifunctional spacer itself induces monolayer-like cell growth.

It must be the aim of further experiments to show whether the fibronectin fragment is actually indispensable for endothelial cell immobilization.

Acknowledgement

The authors thank the European Community for financial support (BRITE/EURAM-project no. p-2217-6).

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Received 6 January
and accepted 8 April 1991