

펩타이드가 고정된 표면을 이용한 혈소판 활성화 평가

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Evaluation of Activated Platelet Using Peptide-Immobilized Surface

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ABSTARCTS

RGDF immobilized micro-patterned surface was developed to detect the functional state of platelets. Using photolithographic technology, an RGDF micro-patterned surface was prepared on silicon wafer. Platelet adhesion to this surface was observed by fluorescence microscopy after staining platelets with mepacrine. Nonactivated platelets pretreated with PGE₁ interacted incompletely with the RGDF micro-patterned surface, whereas activated platelets treated with ADP interacted with the surface extensively. These results show that the distinct selectivity of an RGDF-immobilized micro-patterned surface can be used to detect the functional state of platelets.

Key Words: Platelet, RGDF, Immobilization, Photolithography

INTRODUCTION

The adhesion of normal and activated platelets to fibrinogen requires the receptor-binding sites of platelet membrane GP IIb/IIIa; these are at the A α (RGDS at 572-575 and RGDF at 95-98) and carboxy-terminal γ chain (HHLGGAKQAGDV at 400-411) of fibrinogen.¹ Maximal adhesion of nonactivated platelets is supported only by intact fibrinogen; even "early" fragment X (lacking the carboxyl-terminal segment of the A α chain with the putative adhesion sequence Arg-Gly-Asp-Ser (RGDS) at A α ⁵⁷²⁻⁵⁷⁵, and containing a large β_3 chain) is less effective in this respect. Fibrinogen-derived fragment E, which contains the Arg-Gly-Asp-Phe (RGDF) sequence at A α ⁹⁵⁻⁹⁸, fails to support adhesion

of nonactivated platelets, whereas fragment D, which contains the dedecapeptide adhesion recognition sequence at γ ⁴⁰⁰⁻⁴¹¹, supports adhesion only partially.² In the present study, GGGRGDF-immobilized micro-patterned surface was prepared using a photolithographic technology.³ The final goal of this study is to develop a micro-patterned RGDF peptide chip, which specifically interacts with activated platelets.

MATERIALS AND METHODS

Preparation of micro-patterned peptide chip

Silicon wafer was spin-coated with a solution of 5 % polystyrene (PS ; MW 250,000, Aldrich, Milwaukee, WI) in xylene (Aldrich) at 1,000 rpm for 1 min to produce a PS film. A solution of 0.5 wt % N-hydroxysuccinimidyl perfluorophenyl azide (NHS-PFPA; Ikanos Co., Portland, OR) in nitromethane (Aldrich) was spin-coated on the top of the PS film. A photomask was then placed on the surface, which was UV-irradiated with a 254 nm UV lamp at a distance of 30 cm (intensity, 3.2 mW/cm²). Subsequently, the surface was washed with nitromethane to remove unreacted NHS-PFPA. A solution of 50 μ M GGGRGDF in 0.1 M NaHCO₃ buffer (pH 8.2) was spread and incubated at 25 $^{\circ}$ C for 3 hrs. The GGGRGDF-immobilized micro-patterned surface was washed thoroughly with phosphate buffered saline (PBS, pH 7.4).

Platelet adhesion to micro-patterned RGDF array

For fluorescence labeling of platelets, PRP was treated with the mepacrine (quinacrine dihydrochloride, Sigma) at a final concentration of 10 μ M for 30 min at 37 $^{\circ}$ C

Mepacrine labeled platelets were activated with ADP or PMA. For adhesion of nonactivated platelets to the surface, platelets were inactivated with PGE₁. 50 μ l of PRP was applied to the RGDF array to which it adhered for 45 min at room temperature. Following incubation for 50 min at 22~25 $^{\circ}$ C, nonadhered platelets were removed and washed three times with PBS (pH 7.4). Adhered platelets on the surface were fixed with 2% (w/v) paraformaldehyde (Sigma) in PBS for 10 min. After replacing the paraformaldehyde solution with PBS, the fixed platelets were mounted with gel-mount (Biomedica Corp., Foster City, CA). The fluorescence of adhered platelets was observed through a fluorescent microscope (IMT-2, Olympus, Japan) equipped with a fluorescent filter set (excitation wavelength 450-490 nm, emission wavelength > 510 nm).

The microscopic images were projected to a SIT-CCD camera (VE-1000SIT, Dage-MTI, Inc., Michigan, IN). The fluorescent image of adhered platelets was separately monitored using 20 X objective lens. The microscopic images were directed to a computer for image analysis and analyzed using an image processing software (BiPS, Biomedlab Co., Seoul, Korea).

RESULTS AND DISCUSSION

The fluorescence images in Figure 1 show the platelets adhered to RGDF-patterned surface. It shows that the extent of platelet adhesion to the surface is different according to the state of platelet activation. While the activated platelets treated with ADP or PMA adhered to RGDF-patterned surface extensively, nonactivated platelets treated with PGE₁ adhered to the surface much less than the activated platelets. Activated platelets adhered selectively to the surfaces immobilized with peptide, but not to the surface coated with fibrinogen. The results demonstrated that the adhesion of platelets to RGDF-patterned surface depended on platelet activation.

Platelet analysis by RGDF peptide array provides an alternative rapid diagnostic procedure for platelet activation as well as for the detection of other functional defects caused by an altered expression of platelet surface components.

As a result of this study, a simple and rapid method

for the analysis of platelet activation has been developed, using the peptide arrays bound to silicon wafer. A synthetic peptide derivative (RGDF), one of the platelet recognition sites of fibrinogen, was covalently immobilized on a micro-patterned surface using photolithography technology.

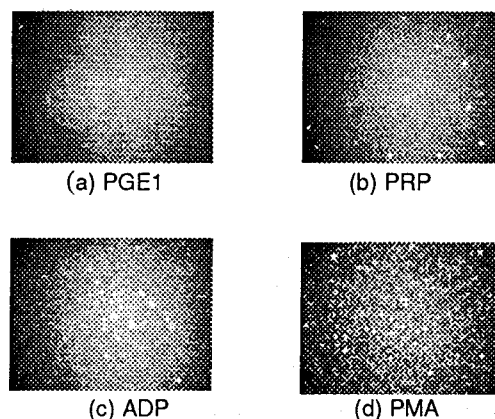


Figure 1. Fluorescence microscope images of adhered platelets on an RGDF micro-patterned chip (pattern width 5 μ m). Platelets were treated with (a) 10 μ M PGE₁ to inhibit activation; (b) no addition; (c) 20 μ M ADP; (d) 0.2 μ M PMA

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