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Trypsinated (immobilized) nylon surfaces: antithrombogenicity

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Abstract. An attempt is made to immobilize low molecular weight trypsin on nylon surface and to evaluate how such surfaces affect blood compatibility by investigating variations in surface energy parameters, plasma recalcification time and platelet adhesion using calf's blood.

Keywords. Trypsin; nylon; blood compatibility; platelet adhesion.

1. Introduction

To achieve a truly antithrombogenic surface the attachment of a benign layer that does not initiate blood coagulation or damage blood elements is essential. It is known that albumin coated surfaces do not seem to adhere platelets (Lyman 1974) whereas γ-globulin coatings not only cause platelet adhesion but also aggregation and release of platelet constituents (Packham *et al* 1969). An attempt has been made to demonstrate that enzymated surfaces (*e.g.* trypsin) have improved blood compatibility.

2. Experimental

Nylon-6 (5 g% (w/v)) was dissolved in M-Cresol (BDH, Poole England). The films were cast on glass slides (2·5 × 7·5 cm), dried in hot air oven at 100°C for 2 hr, dipped in 10% nitric acid for 1 min, and finally rinsed thoroughly with distilled water and again kept for drying at room temperature. Nylon is a polyamide containing $-NH-\overset{O}{\overset{\|}{C}}-$ groups. When treated with an acid *e.g.* HNO_3, some of the amide bonds break and free amino ($-NH_2$) and acid groups ($-COOH-$) will be generated which in turn will link covalently with the acid groups and amino groups of the enzyme trypsin without affecting its active sites. The dried films were divided into two batches. The 1st batch was taken as bare surfaces. The second batch was immersed in 50 mg% trypsin (DIFCO Laboratories, USA, mol. wt. 24000) solution and left overnight in the refrigerator. The films were taken out, rinsed with phosphate buffer (pH = 7·4) and stored in the refrigerator for more than 4 hr. Later they were used for contact angle and platelet adhesion studies.

The contact angles for each set of films (bare and trypsinated) were measured using a Goniometer (Kernco Instruments Co. Inc., Texas) and spectroscopically pure solvents. From the contact angles values for γ_{SV} surface energy and γ_{SL} interfacial energy were obtained from the conversion tables (Neumann *et al* 1980) and are shown in table 1.

Table 1. Surface energy components on immobilised trypsinated nylon surface.

		Bare nylon surface (HNO_3 treated)			Trypsinated nylon surface		
Liquids	γ_{LV} (ergs/cm^2)	Contact angle ± SD	γ_{SV} (ergs/cm^2)	γ_{SL} (ergs/cm^2)	Contact angle ± SD	γ_{SV} (ergs/cm^2)	γ_{SL} (ergs/cm^2)
Glycerol	63	50 ± 2	45.08	4.59	35 ± 1	53.03	1.42
Formamide	58	37 ± 1	47.75	1.43	24 ± 1.5	53.29	0.30
Ethylene glycol	48*	25 ± 1	43.74	0.23	14 ± 1	46.60	0.03
		Mean $\gamma_{SV} = 45.52 \pm 2$.			Mean $\gamma_{SV} = 50.97 \pm 4$.		

Values expressed ± standard deviation.
*Since the γ_{LV} value for ethylene glycol is 47·7, which is not included in table value 48 has been used for γ_{SV} and γ_{SL} values as a fair approximation.

Table 2. Platelet adhesion and plasma recalcification time (PRT) to nylon and trypsinated nylon surfaces.

Surfaces	Mean platelets	PRT (sec) ± SD
Glass surface	—	86 ± 2
Bare nylon surface (HNO_3 treated)	5 ± 1	92 ± 9
Trypsinated nylon surface (immobilized)	3 ± 1	116 ± 11

Following the same procedure several tubes were also coated with nylon and some were trypsinated. They were used for studying plasma recalcification time (PRT) as described elsewhere (Chandy and Sharma 1983). Results are shown in table 2.

Washed calf platelets were prepared and suspended in tyrode solution and used for adhesion studies (Lee and Kim 1979). Different fields using an optical microscope were read randomly and averaged for all samples. The results are shown in table 2.

3. Results and discussion

The major advantage of immobilising an enzyme on a surface is not only to cover the surface and make it passive but also to keep the enzyme in a biologically active form. For successful immobilisation one should consider that the enzyme must be stable under the conditions required for reaction (Traven 1980). Delay in plasma recalcification time with a decreased number of adhered platelets (table 2), indicates its improved antithrombogenicity. From our surface energy parameters (table 1) the evaluated interfacial tension (γ_{SL}) (for all organic liquids used) tends to be lower for enzymated surfaces compared to bare surfaces, which will also be helpful in reducing the adverse interactions (Andrade *et al* 1973) relatively to blood components *e.g.* proteins. Effect of changes in activity of an enzyme on the surface towards antithrombogenicity is under investigation. Selected enzymes, are being used to

immobilize onto various polymer surfaces using suitable catalysts. Our above investigations, are only preliminary in nature, basically, to explore possibilities in this area which appears to be interesting for certain enzymes suitable towards blood compatibility.

References

Andrade J D, Lee M B, John M S, Kim S W and Hibbs J B 1973 *Trans. Am. Soc. Artif. Intern. Organs* **1** 19
Chandy T and Sharma C P 1983 *Bull. Mater. Sci.* **5** 103
Lee E S and Kim S W 1979 *Trans. Am. Soc. Artif. Intern. Organs* **25** 124
Lymann D J 1974 *Angew Chem.* **13** 108
Lymann D J, Knutson K, McNeil B and Shibatani K 1975 *Trans. Am. Soc. Artif. Intern. Organs* **21** 49
Neumann A W, Absolom D R, Francis D W and Vanoss C J 1980 *Sep. Purif. Methods* **9** 69
Packham M A, Evans G, Glynn M F and Mustard J F 1969 *J. Lab. Clin. Med.* **73** 686
Traven M D 1980 *Immobilized enzymes: An introduction and application in biotechnology* (New York: John Wiley)