FIBRINOGEN PLATELET INTERACTION: CHANGES DUE TO SURFACE TREATMENTS ON POLYVINYL CHLORIDE

GEORGE JOSEPH and C. P. SHARMA
Division of Biosurface Technology,
Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum 695 012, India.

A LARGE variety of synthetic polymers are increasingly used in biomedical applications. Polyvinyl chloride is one among them which finds a broad application in blood-contacting devices. One of the initial events taking place when blood comes in contact with an artificial surface is adsorption of plasma proteins[1, 2] followed by the adhesion of platelets leading to thrombus formation[3, 4]. Various techniques such as autoclaving, γ-irradiation and glow-discharge treatment (GDT) are employed for sterilization as well as surface modification of polymers. In this paper we have attempted to study the fibrinogen adsorption and platelet adhesion changes on PVC due to such treatments. The PVC surface is further modified with polyelectrolyte by irradiation and glow-discharge treatment γ-irradiation was limited to a total dose of 0.5 MR since it had been found sufficient for grafting polyelectrolyte and higher dosage may affect the PVC. Protein adsorption and platelet adhesion on these surfaces are studied. The antiplatelet activity of the polyelectrolyte is discussed in the light of fibrinogen adsorption.

Polyvinyl chloride (Polymer Technology Division, SCTIMIST), Polyelectrolyte (PE) from natural poly *cis*-1, 4 isoprene (source: Havea Brasiliensis "Para rubber") was synthesized in our laboratory as discussed elsewhere[5]. Albumin (human, essentially fatty acid-free, fraction V, Sigma Co.) fibrinogen (human fraction I, over 95% clottable, Sigma Co.) γ-globulin (human Cohn fraction II, Sigma Co.) Iodinated (^{125}I) human albumin and iodinated (^{125}I) human fibrinogen (Amersham, England).

Preparation of surfaces

The PVC sheet was cut into pieces of size 2 × 1.5 cm. They were cleaned with 0.1% soap solution (Teepol), washed thoroughly with distilled water several times and dried. The following surfaces were prepared. I. Untreated bare PVC. II. Bare PVC autoclaved (At 121°C, 15 lbs for 15 min) III. Bare PVC irradiated with CO^{60} γ-rays under nitrogen atmosphere with a total dose of 0.5 MR (dose rate 0.2 MR/hr, exposure time 2.5 hr). IV. Bare PVC glow-discharge treated for 5 minutes. V. Bare PVC samples were exposed to polyelectrolyte (500 mg %) for 96 hr at room temp (~ 30°C) under vacuum. They were taken out, dried under vacuum and irradiated under nitrogen atmosphere with a total dose of 0.5 MR. After irradiation the samples were rinsed in distilled water for 24 hr to remove unbonded PE VI. Similarly prepared PE exposed samples as above, were glow-discharge treated (GDT) for 5 min instead of irradiation. After the treatment they were rinsed in distilled water for 24 hr to remove unbonded PE.

Glow-discharge treatment:

Edwards vacuum coating unit E306A was employed for the glow-discharge treatment. The samples were kept in a flat container placed on the base plate of the plasma reactor. Plasma glow was generated with nitrogen gas at a pressure of 10^{-1}m bar and the treatment was given for 5 min.

Platelet adhesion studies:

Fresh citrated bovine blood was prepared into a platelet-rich plasma (PRP) as described elsewhere[6]. The surfaces were exposed to the platelet suspension for 15 min and rinsed with buffer under controlled flow rate. The platelets were fixed with 2.5% glutaraldehyde and stained with Coomassie Blue G. The platelet density was estimated with an optical microscope.

Trace labelled studies:

For studying the adsorption kinetics of albumin and fibrinogen, the surfaces were exposed to protein mixture containing 25 mg % albumin, 15 mg % γ-globulin and 7.5 mg % fibrinogen, with a known amount of single-labelled protein in phosphate buffer pH 7.4. The exposure was carried out at 37°C and the experiments run over period of 24 hr. The samples were rinsed thoroughly thrice in buffer taken in separate beakers and then in running buffer. The amount of protein adsorbed was estimated with a γ-counter using the following relation: $\Gamma = C_p R_f / A\, Rs$ where Γ is surface concentration ($\mu g\, cm^{-2}$), C_p, the bulk concentration ($\mu g\, ml^{-1}$), R_f, the count rate of surface, A, the total surface area (cm^2) and Rs the count rate per ml of protein solution. The desorption kinetics were studied over a period of 24 hr at 37°C.

The results indicate that the adsorption of fibrinogen is relatively high in the case of glow-discharge treated PVC surfaces (table 1). The stability of fibrinogen on the surface is also high in this case as the desorbed amount

Table 1 *Adsorption and desorption of fibrinogen as a function of time*

Surfaces	Adsorption $\mu g\,cm^{-2}$				Desorption $\mu g\,cm^{-2}$ (After 3 hr. adsn.)			
	1′	15′	45′	3 hr	1′	15′	45′	3 hr
I. Bare PVC	0.32	0.59	0 68	0.94	0 84	0.74	0 65	0 57
II. PVC Autoclaved	0.33	0 50	0.58	0.98	0.78	0.61	0.51	0.46
III. PVC Irrad.	0 31	0.51	0.70	1.06	0 86	0 74	0.59	0 52
IV. PVC GDT*	0 48	0.88	0.97	1 29	1.13	1 05	1.00	0.93
V. PVC + PE Irrad.	0.61	0.70	0.96	1.27	1 11	0 97	0.83	0.73
VI PVC + PE GDT	0 52	0.98	1.27	1 68	1.36	1.22	1.15	1.07

* Glow-discharge treated

Table 2 *Adsorption and desorption of albumin as a function of time*

Surfaces	Adsorption $\mu g\,cm^{-2}$				Desorption $\mu g\,cm^{-2}$ (After 3 hr adsorption)			
	1′	15′	45′	3 hr	1′	15′	45′	3 hr
I. Bare PVC	0 06	0 07	0.07	0.09	0.07	0.05	0.04	0.03
V. PVC + PE. Irrad.	0.06	0 07	0 08	0.09	0.07	0 05	0 05	0 03
VI. PVC + PE. GDT	0.04	0.05	0.07	0.08	0.06	0.06	0 05	0.04

for 3 hr is small. Similar behaviour is observed with polyelectrolyte modified surfaces, although the pattern of albumin adsorption and desorption seems to be similar to that of bare PVC (table 2). It is obvious that glow-discharge treatment does make significant changes on the surface compared to irradiation (limited dose 0.5 MR) or autoclaving, causing higher fibrinogen adsorption and platelet adherence (table 3).

It is interesting to note that the adherence of platelets is less on the modified surfaces, despite the increased fibrinogen adsorption. It has been suggested that platelets adhere where they find adsorbed fibrinogen[7]. This is true in the case of bare PVC, but when it has been modified by polyelectrolyte, the phenomenon seems to be different. In the past we have observed that in the case of PGI_2 analog-immobilized polyurethane the fibrinogen adsorption was high while the platelet adherence remained negligible[8]. This is attributed to the potent antiplatelet activity of the PGI_2 analog. Similar phenomenon seems to emerge from the present experiment. It is also reported that adsorption of fibrinogen on to polyacrylonitrile did not increase the adherence of platelets[9]. This has been explained as a possible interaction of active sites of fibrinogen with the surface and its conformational variations.

The anticoagulant activity of polyelectrolytes has been demonstrated elsewhere[10, 11]. Our results suggest

Table 3 *Platelet adhesion data*

Surfaces	No. of Platelets/ $mm^2 \pm S.D.$
I. Bare PVC	16 4 ± 1.7
II. PVC Autoclaved	16.5 ± 1.3
III. PVC Irrad.	18 5 ± 1.3
IV. PVC GDT	23 5 ± 2 0
V. PVC + PE. Irrad.	11.9 ± 2 0
VI. PVC + PE GDT	13.0 ± 2 0
VII. PVC + PE	9.0 ± 1 25

that polyelectrolytes as well possess antiplatelet activity. Possibly the orientation of carboxylate and sulphamate groups of the surface bound polyelectrolyte is playing an important role in its antiplatelet activity. Further biological tests such as *in vivo* trials are needed for an indepth evaluation of the surfaces. Presently it seems that polyelectrolyte may have wide applications in developing antithrombogenic polymer surfaces.

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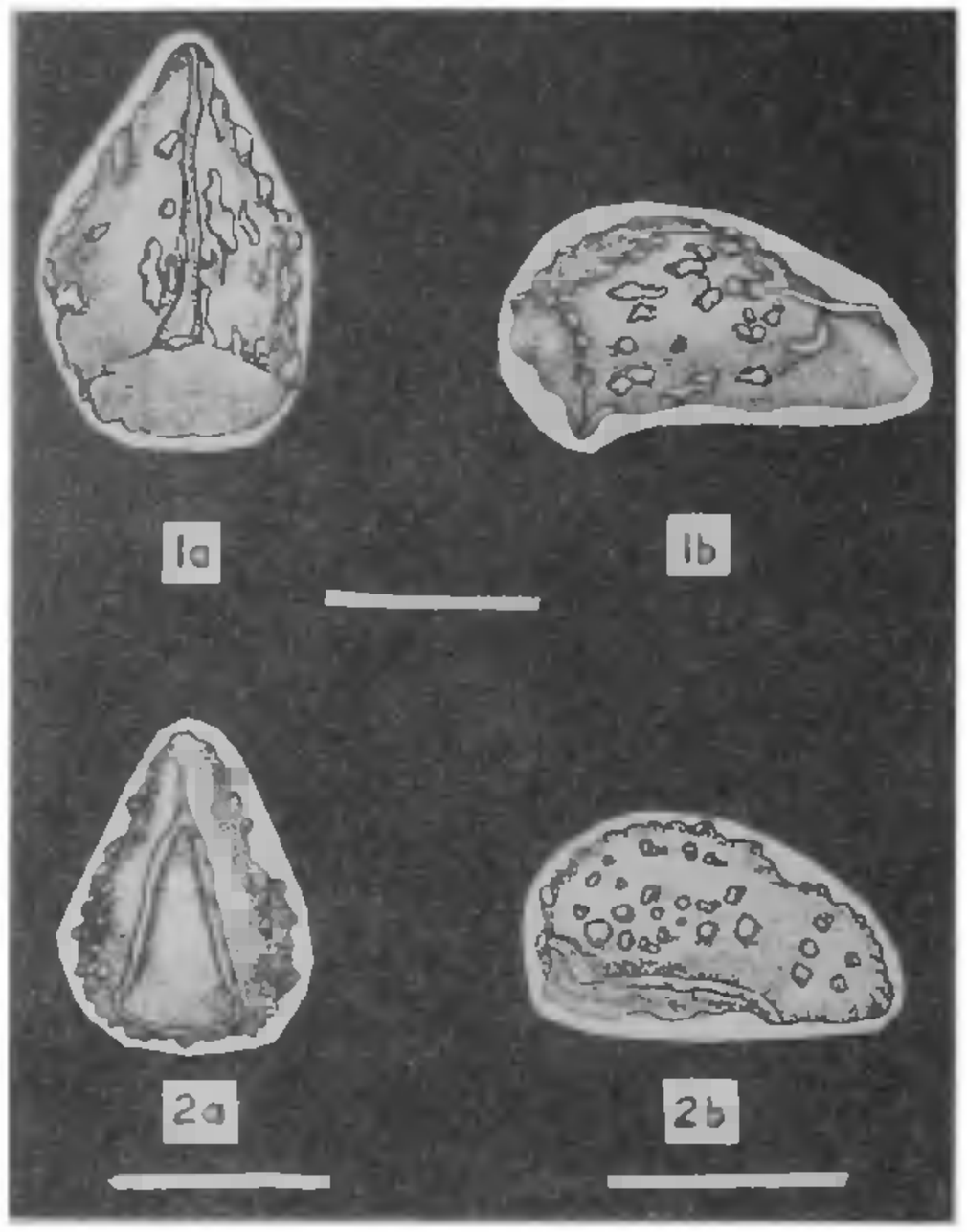

Figures 1, 2. 1. Boraginaceae (Angiosperm) seed, Lameta Beds (Upper Cretaceous- ? Palaeocene), Balasinor, Kheda district, Gujarat; **1a.** ventral view, **1b.** lateral view of another seed. **2.** *Boraginocarpus lakhanpalii* Mathur, Neogene, Chandigarh; **2a.** ventral view, **2b.** lateral view (scale represent 1 mm)

BORAGINACEAE (ANGIOSPERM) SEEDS AND THEIR BEARING ON THE AGE OF LAMETA BEDS OF GUJARAT

A. K. MATHUR and U. B. MATHUR
Geological Survey of India,
B-201, Rajendra Marg,
Jaipur 302015, India.

THE Lameta beds of Balasinor area, Kheda district, Gujarat have recently acquired great importance because of the finds of dinosaur eggs in them[1, 2]. During the course of search for microfossils in the limestone and marl of the dinosaur eggs-bearing locality, the authors recovered angiospermic seeds belonging to the family Boraginaceae (figures 1a, b). So far there is no record of such seeds from either the Lameta beds or from any of the other Cretaceous-Lower Tertiary sediments of India. However, from the Neogene sediments of Siwalik hills near Chandigarh, the Boraginaceae seeds have been described[3] (figures 2a, b). They have also been well documented from tropical American Neogene[4–9]. The oldest fossil Boraginaceae seeds are known from Palaeocene of England[10].

The angiospermic seeds from Kheda (figures 1a, b) are small, 1.5 to 2.5 mm long, ovoid trigonal in shape, having basal, semi-circular scar of attachment. A well developed keel is present all along the ventral margin. The nutlets are inflated posteriorly and narrow apically. The surface is rough with irregular ridges and prickles.

The above seeds are primitive in characters as compared with the Neogene seeds from Chandigarh (*Boraginocarpus lakhanpalii* Mathur) with which they show resemblance in overall shape and rough prickled surface (figures 1, 2). However, the basal, semi-circular scar of attachment and a well-developed keel all along the ventral margin (figure 1a) are very distinctive in the Kheda specimens. Among the present-day Boraginaceae, the nutlets of *Anchusa officinalis* Linneaus bear a similar semi-circular scar of attach-