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Initial platelet adhesion and platelet shape on polymer surfaces with different carbon bonding characteristics (an *in vitro* study of Teflon, Pellethane and XLON intravenous cannulae)

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Two commercially available intravenous catheters (i.d. 1.2 mm), made of polytetrafluoroethylene (PTFE, Teflon) and a thermoplastic polyurethane–polytetramethylene glycol block-copolymer (TPEU, Pellethane D-65), and a catheter made of a new test material, a polyamide–polyethylene glycol block-copolymer (XLON D-60), were compared with respect to surface chemistry, platelet adhesion and platelet shape change *in vitro*. Surface chemistry was evaluated by X-ray photoelectron spectrosopy. High-resolution carbon peaks were obtained at 15 and 90° take-off angle. The hydrophilicity was determined by measuring capillary rise in the catheters with distilled water. Platelet adhesion and platelet shape changes on the catheter surfaces were examined *in vitro*, in a system without blood–air interface, and visualized by scanning electron microscopy. The degree of hydrophilicity of the materials appeared to be related to the relative ether carbon content of their outermost surfaces. The platelet adhesion was low on hydrophilic surfaces (XLON) and increased with decreasing hydrophilicity on the more hydrophobic surfaces (TPEU and PTFE). The degree of shape change of the adhered platelets also increased with decreasing hydrophilicity. Changes in surface chemistry caused by toluene extraction of the TPEU significantly decreased platelet adhesion and the degree of shape change in parallel with an increased ether carbon content of the surface.

1. Introduction

Insertion trauma is inevitable when a cannula is introduced into a vein. Trauma triggers interactions between plasma coagulation factors (proteins) and platelets. The platelets adhere to the exposed collagen, releasing platelet factors, subsequently forming a thrombus, thereby obstructing the orifice in the vein wall. If platelets adhere to, and are activated on, the surface of the inserted cannula, a thrombus forms around it. The blood flow in the vicinity of the catheter is thereby reduced. The release of inflammatory agents from the platelets spread on the catheter surface may also initiate an inflammatory reaction on the adjacent vein wall. Thus, the conditions of "cannula thrombophlebitis" [1] are fulfilled.

To a large extent, the interaction of platelets with a biomaterial is determined by the nature of the plasma protein layer which adsorbs on to the surface. The nature of the protein layer is related to the hydrophilicity of the surface. Blood proteins adsorb rapidly on to hydrophobic surfaces [2], undergo conformational changes [3] and cause thrombus formation. Positively or negatively charged hydrophilic surfaces are also known to cause conformational changes to proteins or adhered platelets or both [4–6]. On the other hand, several investigators [7–11] have shown that proteins or other macromolecules do not readily adsorb to neutral hydrophilic surfaces, neither do they change their conformation. Various researchers have demonstrated that processing aids and non-polymerized components, present on polymers especially designed for clinical use, also influence the surface composition and the adhesion [12].

The aim of this work was to test two commercially available and one new material (whose surfaces did not contain any charged groups and had a different hydrophilicity) with respect to surface chemistry, platelet adhesion and platelet shape change *in vitro*.

2. Materials and methods

2.1. Materials

The following materials were investigated: (1) polytetrafluoroethylene (PTFE, Teflon, Dupont) a non-thermoplastic, strongly hydrophobic material with no loosely bonded additives; (2) a thermoplastic polyurethane (TPEU, Pellethane-2363, Shore D65, Dow Chemical), less hydrophobic than PTFE, with polytetramethylene glycol (PTMG) as a soft segment,

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butanediol as a chain extender, a phenolic compound as an antioxidant and ethylene-bis-stereamide as an extrusion lubricant; and (3) a polyamide-12-polyethylene glycol (PEG) block-copolymer (XLON, a new test material, ATO CHEM), hydrophilic, containing a small amount of PEG oligomers.

Two additional samples (derived from TPEU and XLON by solvent extraction) were also investigated in order to study the effects of extracting the above additives and loosely bound components from TPEU and XLON. The TPEU was treated with toluene at 80° C (3 × 10 min) in order to remove the extrusion wax, and the XLON treated with ethanol at room temperature in order to remove oligomers. These two samples are referred to as "extracted" in the text.

All catheters were extruded with an internal diameter of 1.20 mm. Only one batch was used for each polymer.

2.2. Methods

2.2.1. Experimental 1: comonomers

The following methods were used.

2.2.1.1. X-ray photoelectron spectroscopy (XPS). The chemistry of the inner and outer surfaces of the catheters was evaluated by XPS using a model 206 spectrometer from Surface Science Instruments. Incident X-ray spot sizes were 150 or 300 μm. High-resolution carbon peaks were obtained under conditions yielding a full width at half-maximum (FWHM) of about 0.8 to 0.9 eV for gold $4f_{7/2}$. Wide-scan data were acquired at a poorer resolution for better count rate and signal-to-noise ratio. An electron flood gun was used for charge compensation. Data were obtained at two angles, 15 and 90° relative to the surface, the latter angle providing a deeper sampling volume. Carbon peaks were fitted assuming a 100% Gaussian shape. Only hydrocarbon (–C–H), ether (–C–O), amide (–C(=O)–N(H)–), urethane (–O–C(=O)–N(H)–), and fluorocarbon (–C(F)(F)–) bondings were assessed. The results were expressed as means of two measurements on each external and internal surface for all materials except Teflon (only one measurement).

2.2.1.2. Capillary rise. The relative degree of hydrophilicity was determined by measurement of capillary rise in the catheters. A 1 cm thick poly(methylmethacrylate) (PMMA) disc (diameter 74 mm), with 10 holes (each of diameter 2.3 mm) in a circle (diameter 60 mm), supported by a 12 mm stainless steel rod was placed in a clean glass beaker. The beaker was filled with double distilled water, and together with the PMMA disc assembly, placed in a heating chamber at 37° C. The water level rise in the catheters was measured with two graded rules placed 5 and 10 cm from the beaker in order to obtain parallelism. Ten tubes of each material, warmed to 37° C, were inserted through the drilled holes. The capillary height in each tube was measured at t = 0, 5 and 10 min, and then every 10 min for 1 h.

2.2.2. Platelet adhesion and shape changes

Scanning electron microscopy (SEM) was used to study both platelet adhesion statistics and shape. Catheters were cut into 6 cm sections and then cut longitudinally, exposing 1 to 2 cm of the interior. Five tubes (one of each material) were affixed to the top and the centre of the cover of a 100 ml polypropylene beaker with the cut "U-shaped" opening facing downward. About 80 ml fresh blood from one donor with a normal platelet count in the peripheral blood was directly collected in 12% citrate–phosphate–glucose adenine (CPD-adenine Fenwal, Travenol Laboratories SA, Castlebar, Ireland) and transferred to the beaker. Hence, the samples were totally immersed in the anticoagulated blood without an air–blood interface (Fig. 1). The beaker with blood and catheters was placed in a heating chamber at 37° C and tilted 15 times min^{-1} with a blood mixer (Lic. Instrument, Solna, Sweden). Segments, removed after 10 min, were immediately rinsed and fixed in 2.5% glutaraldehyde in phosphate buffer at pH 7.2 for 2 h and then rinsed in buffer five to seven times. Dehydration proceeded as follows: twice 10 min in 25% ethanol; twice 15 min in 50% ethanol; twice 15 min in 75% ethanol; twice 15 min in 96% ethanol; twice 30 min in absolute ethanol; 1 h in 50% ethanol–50% freon; refrigeration in freon overnight and drying in a CO_2 critical-point dryer. Specimens were sputter-coated with gold and examined in a Jeol JSM T330 SEM at 15 to 20 kV accelerating voltages.

In order to determine platelet populations, micrographs were taken primarily at 1500×. However, as the platelets were unevenly distributed and it was difficult to select respresentative areas, videorecording equipment was used. Platelets were counted from 47 to 104 screens (each screen about 6500 μm^2) of the internal surface for each material.

The morphology of adhered platelets was classified according to the criteria described by Goodman *et al.* [13], simplified to the following three groups: group I

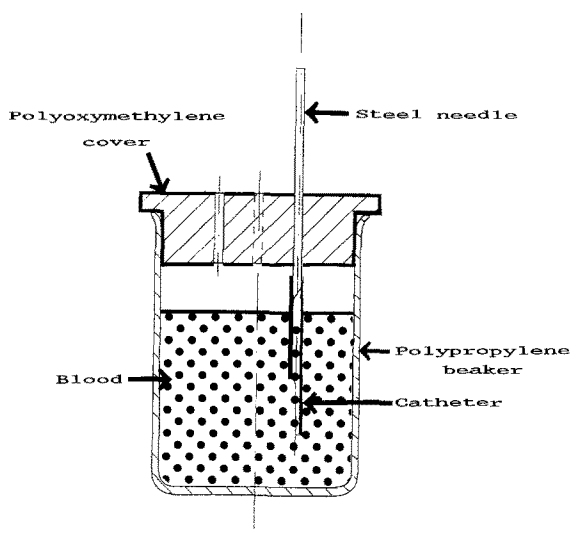

Figure 1 Diagram of the system used to keep the specimens of the materials in contact with donor blood *in vitro*.

included round to dendritic platelets with one or more pseudopodia present but no flattening evident; group II spread dendritic and spread platelets; and group III fully spread platelets with hyaloplasma extensively spread and no distinct pseudopodia. Groups I, II and III indicated (an increasing order) the degree of activation of the platelets by the contact with the surfaces. The platelet shape change was evaluated from 5000 × micrographs. At least 50 platelets were analysed on each sample.

2.2.3. Statistics

The data were processed with a 512$^+$ Statview (Brain Power Inc.) statistical program [14]. Descriptive and inferential statistics, including one-factor analysis of variance for repeated measures and linear regression analysis were used. The results were tested for significance with Fisher's PLSD test, the Scheffé test, Wilcoxon's signed-rank test, and r, the coefficient of correlation. $P < 0.05$ was accepted as significant.

3. Results and discussion

3.1. Surface chemistry

3.1.1. Surface composition

The surface compositions obtained from XPS wide-scan spectra are listed in Table I. Toluene extraction of TPEU catheters modified the surface chemistry by increasing the oxygen concentration by at least a factor of 2 and decreasing the carbon concentration by about 10%. Ethanol extraction of XLON led to only a small decrease in oxygen content of the outermost internal surfaces while the carbon and nitrogen content varied little.

The relative amounts of the different types of carbon bonding, obtained from curves fitting the carbon 1s XPS peaks, are shown in Fig. 2 for each of the materials investigated. The values represent the average of all measurements for internal and external surfaces. The as-received TPEU catheter carbon 1s spectra showed no evidence of urethane carbon, even at the higher angle. Instead, a peak typical of amide bonding, at a shift of 2.8 eV, was present and represented the bis-stearamide wax-coating. Together, the absence of urethane chemistry and the presence of the amide peak indicated that the amide-based wax-coating was > 10 nm thick. Extracted TPEU, in contrast to the as-received TPEU, had carbon 1s spectra indicating the presence of urethanes and reduction of amides.

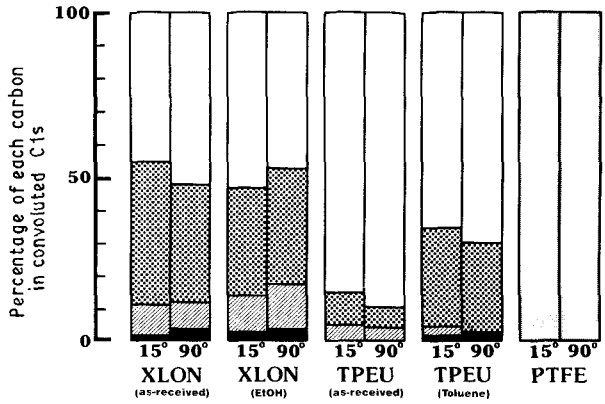

Figure 2 The relative amount of (□) hydrocarbon (–C–H), (▨) ether carbon (–C–O, chemical shift 1.5 eV), (▨) amide carbon (–C(=O)–N(H)–, chemical shift approximately 2.8 eV), (■) urethane carbon (–O–C(=O)–N(H)–, chemical shift approximately 4.1 eV) and (▨) fluorocarbon (–C(F)(F)–) from the deconvoluted carbon 1s peak for different materials expressed as the average of all (four) measurements for internal and external surfaces except Teflon (only two measurements). EtOH, Ethanol.

The relative percentages of ether carbon on the external and internal surfaces at both 15° (the more surface-sensitive angle) and 90° are shown in Figs 3a and b. A comparison of the ether content at the two angles indicated that the most superficial layers of the external surfaces of all of the materials (except the as-received TPEU) were enriched in ether linkages. The degree of enrichment depended on the material, its treatment and the location (internal or external) of the surface. For the as-received TPEU, no ether was identified on the external surface, because of the amide-based wax layer, but XPS spectra of the internal surface showed evidence of a small ether content. Toluene extraction of TPEU increased the ether composition on both surfaces. The XLON catheters had the highest ether levels, and PTFE, as expected, had no ether. Ethanol extraction of XLON lowered the ether content on the internal surface, but did not lead to major changes on the external surface.

3.1.2. Capillary rise experiments.

Typically, there was a gradual rise of the water level as a function of time which became stable after 10 min

TABLE I The mean surface composition (and range) from XPS wide-scan spectra (internal surfaces)

Material	Angle of emission (degrees)	Obs. (n)	Carbon (%)		Nitrogen (%)		Oxygen (%)		Fluorine (%)	
			Mean	(Range)	Mean	(Range)	Mean	(Range)	Mean	(Range)
XLON (as-received)	15	2	80.0	(78.6–81.3)	3.0	(2.7–3.2)	17.1	(15.5–18.7)		
	90	2	78.5	(77.0–80.0)	4.4	(3.6–5.1)	17.2	(16.4–18.0)		
XLON (ethanol-extracted)	15	2	81.8	(77.8–85.8)	3.6	(2.5–4.7)	14.6	(9.5–19.7)		
	90	2	77.1	(75.5–78.7)	2.9	(2.1–3.7)	20.0	(17.6–22.4)		
TPEU (as received)	15	2	92.5	(91.6–93.3)	3.3	(3.2–3.3)	4.3	(3.3–5.2)		
	90	2	87.9	(86.3–89.4)	4.9	(4.8–4.9)	7.3	(5.7–8.9)		
TPEU (toluene-extracted)	15	2	82.2	(81.3–83.1)	3.4	(3.3–3.5)	14.4	(13.6–15.2)		
	90	2	74.7	(73.7–75.7)	3.8	(3.4–4.2)	21.6	(21.0–22.2)		
PTFE	15	1	33.6						66.4	
	90	1	34.5						65.5	

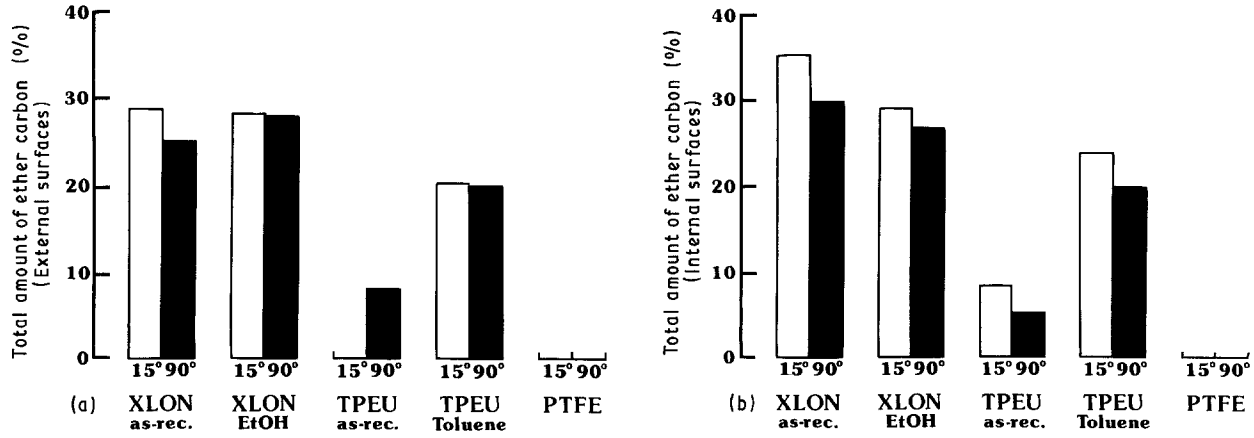

Figure 3 The total amount of ether carbon expressed as the mean of two measurements: (a) on the external surfaces of the tubes and (b) on the internal surfaces of the tubes.

for all tested materials except the as-received TPEU, which had a slow and continuous change in capillary rise, from -10 to -2 mm. The as-received TPEU and PTFE, the materials with the lowest surface ether contents, had negative capillary rises (Fig. 4). A linear correlation ($r = -0.99$) between the capillary rise of water at $t = 0$ and the ether carbon content of the surfaces was found (Fig 5).

3.2. Platelet adhesion and shape changes

3.2.1. Platelet adhesion

A box plot diagram illustrating the distribution of adhered platelets on the different surfaces after a contact time of 10 min with normal donor blood at 37° C is shown in Fig. 6. The lowest platelet count was found for XLON, the most hydrophilic surface, and the highest count for PTFE, the most hydrophobic. Platelets on as-received TPEU surfaces were found to be unevenly dispersed, with some regions having a higher platelet density than the PTFE. Significant differences within the hydrophobic (PTFE and as-received TPEU, $P < 0.01$) and between the hydrophobic and the hydrophilic (toluene-extracted TPEU, ethanol-extracted and as-received XLON, $P < 0.001$) materials were found. No significant differences within the group of hydrophilic materials existed ($P > 0.05$). For each material that had a positive capillary rise, the mean number of platelets found for each 10 000 μm^2 was < 4. This platelet density represented $< 0.1\%$ of the surface.

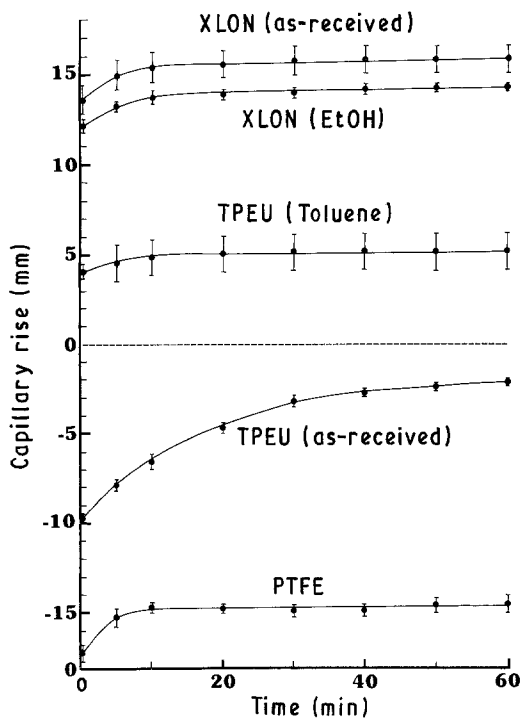

Figure 4 The capillary rise (means ± 1 s.d.) with double distilled water at 37° C plotted against time in catheters with an internal diameter of 1.2 mm.

We found a correlation ($r = 0.64$) between the number of platelets adhered at 10 min and the capillary rise at 10 min. Other authors have found correlations between platelet retention and surface chemistry. Sa Da Costa *et al.* [6] developed a "retention index" relating the ratio of the areas of the hydrogen-bonded carbon and oxygen-bonded carbon (associated with the hydrophobic and the hydrophilic properties of a surface, respectively). They could extrapolate zero platelet retention to a ratio value of zero. Hanson *et al.* [15] published a large amount of data correlating the alkyl concentration (related to the hydrophobic properties) of a surface and thrombus formation, as well as platelet consumption and hydrogel formation.

3.2.2. Platelet shape changes

The SEM micrographs also provided information on the morphology of adhered platelets and their activation state. The relative populations of three activated shapes are shown in Fig. 7. Platelets on PTFE were almost uniformly scattered over the surface, with about 30% of the platelets in the fully spread state. On the as-received TPEU surfaces the majority of platelets were rounded in the areas sparsely populated and partially spread in the regions with high platelet density. Toluene extraction of TPEU resulted in a surface with a more uniform coverage, with about 55% of the platelets in the rounded state and the remainder partially spread. These results might be explained by the fact that the as-received TPEU was more or less covered by extrusion wax, which substantially influenced the surface properties. Other researchers [16] have also found that processing aids and unpolymerized components present on polymers for clinical

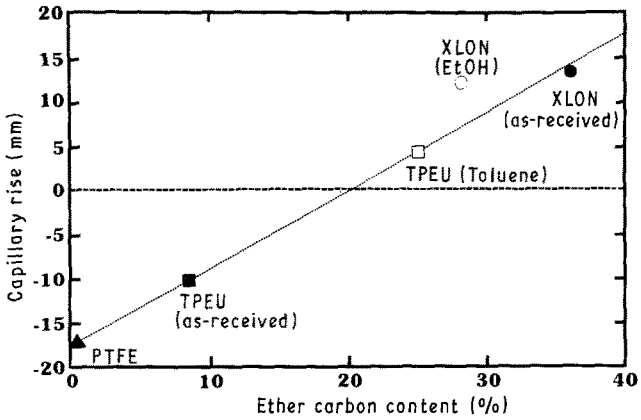

Figure 5 Initial (t = 0) capillary rise of water (T = 37°C) in catheters of i.d. 1.2 mm as a function of the ether carbon content of the internal surface at 15° take-off angle.

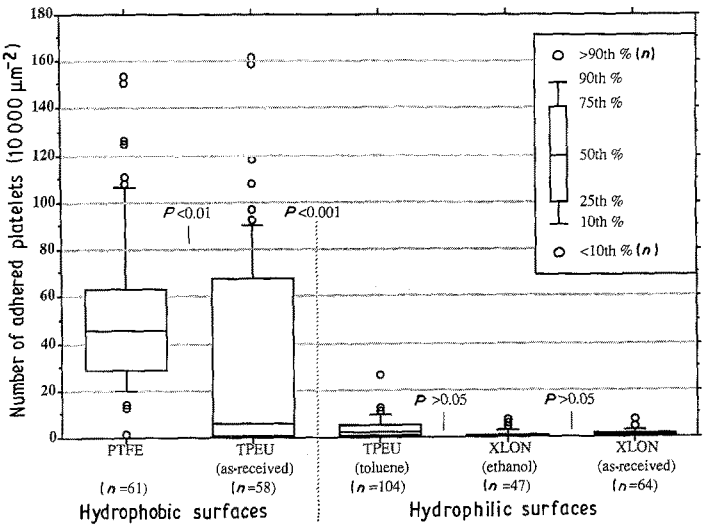

Figure 6 Box plot of percentiles (see legend on the right, upper corner of the figure) illustrating platelet populations on PTFE, as-received TPEU, toluene-extracted TPEU, ethanol-extracted XLON and as-received XLON, where n represents the number of screens. The number of adhered platelets counted on each screen (about 6500 μm^2) was corrected for 10 000 μm^2.

use influence the surface composition and the adhesion of proteins. In clinical practice the positive effect obtained by extraction of any material (e.g. TPEU) might be counteracted by the lubricant which necessarily must be used in order to facilitate insertion of peripheral venous cannulae. As-received XLON had the smallest density of platelets, with about 90% having a primarily rounded shape. Ethanol extraction of XLON affected neither the count of adherent platelets nor their shapes.

Thus, the percentages of round platelets and the total number of adhering platelets were related to the degree of hydrophilicity: the more hydrophilic the surface is (higher relative ether carbon content), the lower the number of platelets adhering to it, and the smaller the changes of platelet shape.

4. Conclusions

The degree of hydrophilicity of XLON, ethanol-extracted XLON, TPEU and toluene-extracted TPEU (investigated by the measurement of the capillary rise with distilled water) was related to the relative ether carbon content on the outermost surfaces of the materials.

The platelet adhesion was low on hydrophilic surfaces (XLON, ethanol-extracted XLON and toluene-extracted TPEU) and increased with decreasing hydrophilicity on the more hydrophobic surfaces (as-received TPEU and PTFE).

The degree of shape change of the adhered platelets increased with increasing hydrophobicity.

Changes in surface chemistry caused by toluene extraction of the wax-covered TPEU surfaces lead to

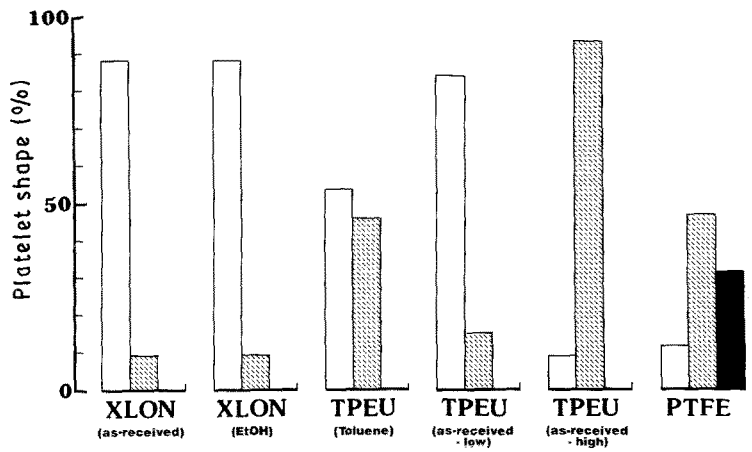

Figure 7 Platelet shapes on different surfaces (contact time 10 min). For as-received TPEU data are shown for surface areas with low, as well as high density of platelets (□) "rounded" = group I; (▨) "medium" = group II and (■) "fully spread" = group III, according to the text).

measurable and important differences in platelet adhesion and platelet shape changes *in vitro*.

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