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Blood interactions with plasticized poly(vinyl chloride): relevance of plasticizer selection

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An investigation has been made of blood interactions with plasticized poly(vinyl chloride) (PVC) biomaterials in tubular form, taking into account the influence on the blood response of the polymer, antithrombotic agent, blood condition and test procedure. *In vitro* and *ex vivo* procedures were used to achieve a comparison between PVC plasticized with di-(2-ethylhexyl)phthalate (DEHP) and with tri-(2-ethylhexyl)trimellitate (TEHTM). The blood response was monitored in terms of the measurement of fibrinogen adsorption capacity, thrombin–antithrombin III complex (TAT) and the complement component C3a. Surface characterization of the polymers was performed by X-ray photoelectron spectroscopy (XPS). The data obtained indicate that in comparison with DEHP-PVC, there is a higher reactivity for TEHTM-PVC, which correlates with the plasticizer distribution at the polymer surface.
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1. Introduction

Plasticized poly(vinyl chloride) (PVC) is the most widely utilized blood-contacting material for the production of blood and blood-component storage bags, catheters, and tubing for extracorporeal circulation devices [1, 2]. Previous studies of the blood response to plasticized PVC indicate that the blood compatibility of plasticized PVC is influenced not only by the poly(vinyl chloride) itself, but also by the nature of plasticizers employed [3, 4] and the concentration of plasticizer in the PVC formulation [5]. The most widely used plasticizer is di-(2-ethylhexyl)phthalate (DEHP), which is incorporated into PVC at a level between 30 and 40% in order to achieve the required flexibility. Leaching of DEHP during blood contact has aroused concern about the implication of leaching for toxicity and an alteration to physical properties [6, 7]. In order to reduce the extractability of plasticizer, alternatives to DEHP such as tri-(2-ethylhexyl)trimellitate (TEHTM) [8], *n*-butyryltri-*n*-hexyl citrate (BTHC) [9] and polymeric adipate (PA) [3] have been utilized. These substances are of higher molecular weight than DEHP and less susceptible to extraction [10]. However, a higher level is required during plasticization in order to achieve a flexibility comparable to that of DEHP plasticized PVC [11].

In considering the blood response in the clinical utilization of a biomaterial, it is convenient to represent this as coming under the influence of different factors, such as the biomaterial, antithrombotic agent, blood condition and nature of the application [12]. Because blood interactions take place at the outer surface of the biomaterial, in this study, the evaluation of blood compatibility was correlated with the surface characterization of biomaterials. The biomaterial selected was medical-grade plasticized poly(vinyl chloride) (PVC) tubing and the influence of the biomaterial was studied with respect to the plasticizer selection and the biomaterial surface was characterized using X-ray photoelectron spectroscopy. The antithrombotic agent influence was examined *in vitro* by performing blood–biomaterial contact with and without the addition of the anticoagulant heparin. The influence of the blood condition was controlled by the use of blood from healthy donors. The test procedure influence was examined by the utilization of *in vitro* and *ex vivo* procedures. The blood response was monitored by measurements of three parameters. These were fibrinogen adsorption, thrombin–antithrombin III complex (TAT), an index of thrombin formation, and the complement component C3a, an index of complement activation. The overall objective of the blood compatibility assessment of the plasticized PVC is represented in Fig. 1.

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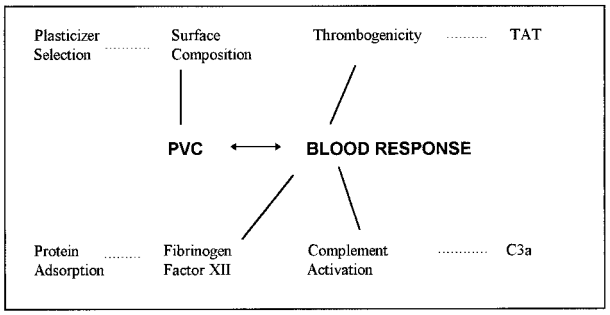

Figure 1 The objective of this study.

2. Materials and methods

2.1. Materials

Two medical-grade plasticized PVC tubing materials were evaluated to achieve a comparison between PVC tubing plasticized with di-(2-ethylhexyl)phthalate (DEHP) (PVC 1) and PVC tubing plasticized with tri-(2-ethylhexyl) trimellitate (TEHTM) (PVC 2). PVC 1 and PVC 2 were supplied by Sis-Ter S.P.A with an internal diameter of 4.70 mm. The plasticizer contents for PVC 1 and PVC 2 were 37 and 40.5%, respectively. ^{125}I-Fibrinogen was purchased from ICN Biomedical Ltd (High Wycombe, UK).

2.2. Surface analysis

X-ray photoelectron spectroscopy (XPS) was employed for analyzing surfaces of PVC tubing. Internal surfaces of each PVC tubing, size of about 3×3 mm, were removed with fresh scalpel blades and mounted on the standard sample holder with small squares of double-sided adhesive tape. A nickel mesh was placed 1–2 mm above the sample surfaces to control electrostatic charge build-up. The analysis was performed using the Fisons SSI M -probe XPS instrument (VG Scientific Ltd., UK). Monochromatized (200 w) AlK_α X-rays were focused into an elliptical spot size of 400×1000 μm on the material with a standard take-off angle of 35° to the sample surface. Survey scan analysis and high resolution analysis of Cls, Ols regions were recorded. All spectra were referenced to the Cls peak at 285.0 ev binding energy. The surface compositions were analyzed by peak area measurements followed by the application of Scofield based sensitivity factors. High resolution data were subject to Shirley background subtraction prior to peak synthesis using the instrument software.

2.3. *In vitro* fibrinogen adsorption

The exposure of protein to PVC tubing was achieved by a syringe pump system (Model 915A, Harvard Apparatus). Twenty milliliters of the fibrinogen solution with the concentration of 0.031 μg ml^{-1} were placed into a 30 ml syringe, which was then mounted in the syringe pump. The solution was perfused through the tubing at a flow rate of 1.2 ml min^{-1} for 15 min. After that period, the system was rinsed with phosphate-buffered saline (PBS) solution (0.5 M, pH = 7.4) for 10 min at the same flow rate and three segments (1.0 cm each) of the tubing were cut from the beginning, middle and end. The radioactivity of the working solution and the segments was measured with a gamma counter (Panax Ltd, UK).

2.4. *In vitro* blood response assessment

In vitro blood–material contact was achieved by the same syringe pump system. A length of the tubing material was fixed at each end within a rigid plastic cylinder, which in turn was mounted onto the cross-head of a syringe pump (Model 915A, Harvard Apparatus). A large polypropylene syringe (30 ml) was attached to one end to push the blood and two small syringes (5 ml) were attached to the other end via a three-way stopcock for sample collection. By fixing the large syringe plunger to the pump body, blood is perfused through the tubing by displacement of the cross-head. The flow rate can be controlled by changing the speed of the pump and the size of the syringe. This method of perfusion eliminates blood–air interfaces that may occur with discrete reservoir/roller pump circuitry and any blood trauma induced by roller pump application. Unsterilized PVC tubing with a length of 15 cm was tested. The system was filled with physiological saline prior to contact with blood. Blood was taken from healthy donors who had not taken any medication for the preceding 14 days. PVC 1 and PVC 2 were evaluated for blood without anticoagulant and blood containing heparin (1.0 IU ml^{-1}). Blood was exposed to the tubing by single pass flow at a rate of 1.2 ml min^{-1}). Blood was exposed to the tubing by single pass flow at a rate of 1.2 ml min^{-1}. Blood samples were collected before perfusion and at 3, 6, 9 and 12 min after commencement of perfusion. All the experiments were performed at room temperature.

2.5. *Ex vivo* blood response assessment

An *ex vivo* system employing human blood [13, 14] was modified to permit testing of tubing. Blood was obtained from an antecubital vein using a special designed catheter which allows the blood to be heparinized (0.5 IU ml^{-1}) immediately when it enters the catheter tip. Blood flow from the catheter was diverted via a Y-piece to two channels of a peristaltic pump (Ismatec, Switzerland) to allow simultaneous perfusion of tubing segments located at the outlet of the pump. Blood flow rate was monitored periodically by timed volume collection at the outlet of each segment. One meter lengths of unsterilized PVC tubing were tested. Prior to the test, the system was rinsed with 1.0 l of sterilized physiological saline. The other components (i.e. cannula and heparin infusion line) were supplied presterilized by the respective manufacturers. Blood from healthy donors was perfused through the tubing at a flow rate of 10 ml min^{-1} for 20 min. Blood samples were taken before the perfusion (with 0.5 IU ml^{-1} heparin) and at the outlet of the tubing at 5, 10, 15 and 20 min after blood–material contact.

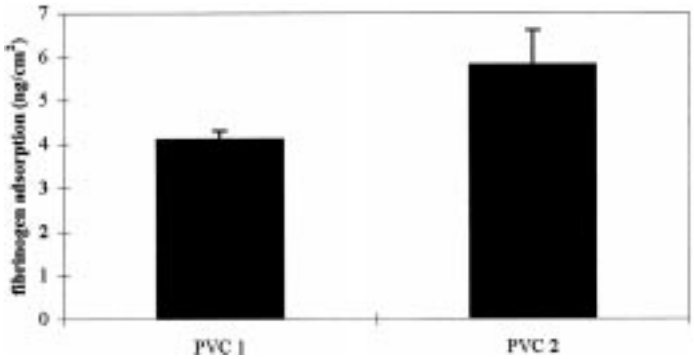

Figure 2 Fibrinogen adsorption on PVC 1 and PVC 2 ($n = 5$).

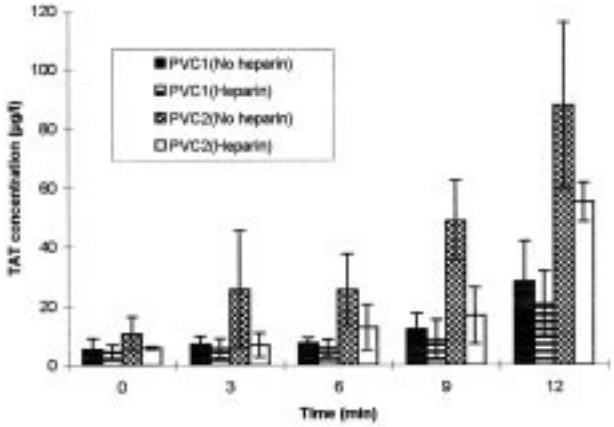

Figure 3 In vitro TAT levels for PVC 1 and PVC 2 in contact with blood with and without heparin ($n = 5$).

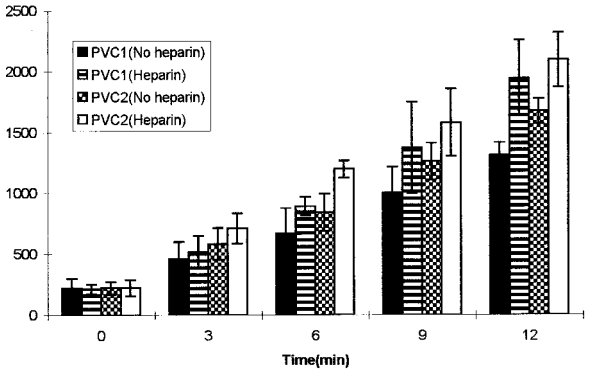

Figure 4 In vitro C3a levels for PVC 1 and PVC 2 in contact with blood with and without heparin ($n = 5$).

2.6. Assays

2.6.1. Thrombin–antithrombin III complex (TAT)

Blood samples were collected into tubes containing trisodium citrate and centrifuged at 2000 ***g***, 4 °C for 15 min. Aliquots of the plasma obtained were snap frozen in dry ice and stored at −50 °C until assay. TAT was measured by an enzyme-linked immunosorbent assay (ELISA) with commercially available kits (Behringwerke AG, Germany).

2.6.2. Complement C3a

Blood samples were collected into tubes containing disodium ethylenediamine tetra-acetic acid (EDTA) and centrifuged at 2000 ***g***, 4 °C, for 15 min. Aliquots of the plasma obtained were snap frozen in dry ice and stored at −70 °C until assay. C3a was measured by radio-immunoassay kits (Amersham International Plc, UK).

2.7. Statistics

Statistical analyses were performed with the Minitab package (version 8.0). Comparisons of the different groups were carried out by analysis of variance. All statistically significant differences are reported at 95% confidence intervals ($P < 0.05$).

3. Results

3.1. Protein adsorption

Protein adsorption occurs within seconds of blood–material contact and plays an important role in the subsequent blood response. In this study, the adsorption of the coagulation protein fibrinogen on plasticized PVC tubing was investigated as an initial index for assessing blood compatibility. The results are presented in Fig. 2.

The results show that TEHTM plasticized PVC tubing adsorbed more fibrinogen than DEHP plasticized PVC tubing. The difference is statistically significant.

3.2. *In vitro* blood response study

The results for TAT and C3a induced by PVC 1 and PVC 2 are presented in Figs 3 and 4, respectively. For blood without anticoagulant, all the mean TAT values for PVC 2 were higher than those for PVC 1, with a significant difference at 9 min. For blood containing heparin, the mean values of TAT for PVC 2 remained higher than those for PVC 1, with a significant difference at 12 min. Addition of heparin reduced TAT levels for both materials. For blood without anticoagulant, all mean C3a values induced by PVC 2 were higher than those by PVC 1, with a significant difference at 12 min. For blood containing heparin, PVC 2 induced higher C3a levels than PVC 1, with a significant difference at 6 min. In contrast to TAT, C3a values were increased for both materials when 1 IU ml^{-1} of heparin was added to the blood.

3.3. *Ex vivo* blood response study

The *ex vivo* results of TAT and C3a are illustrated in Figs 5 and 6, respectively. With respect to PVC 1 and PVC 2, all the mean TAT values for PVC 2 at all time intervals

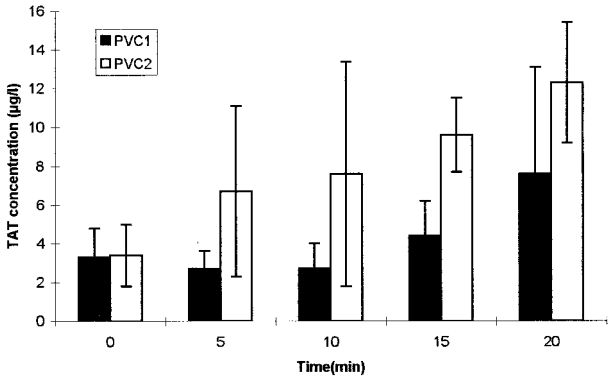

Figure 5 *Ex vivo* TAT levels induced by PVC 1 and PVC 2 ($n = 4$).

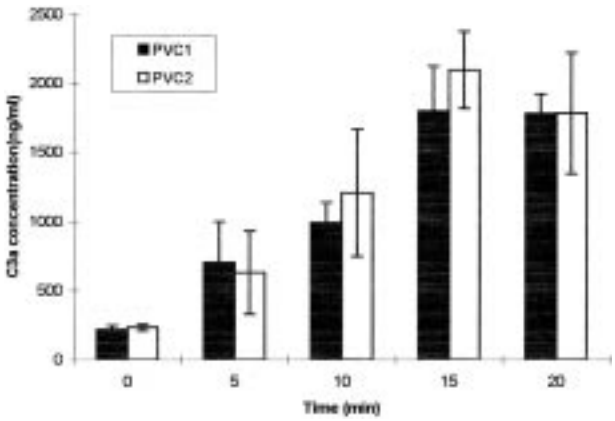

Figure 6 *Ex vivo* C3a levels induced by PVC 1 and PVC 2 ($n = 4$).

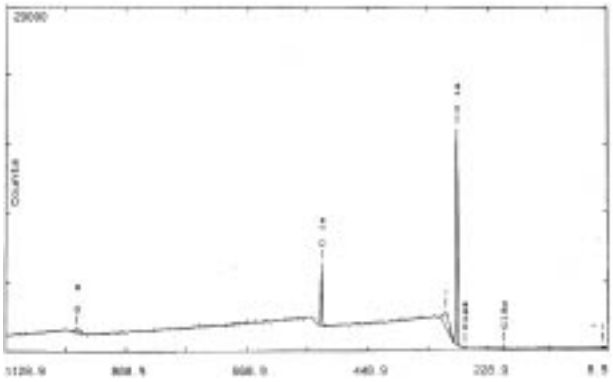

Figure 7 XPS survey scan record of PVC 1.

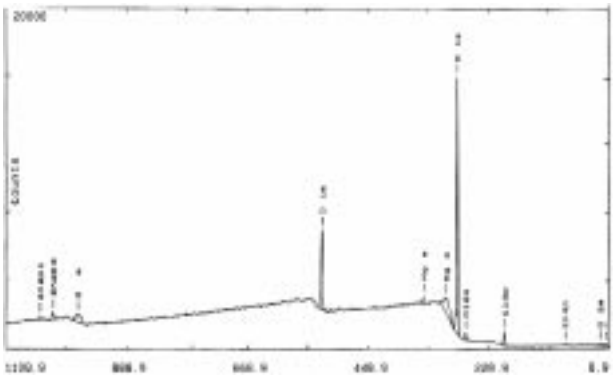

Figure 8 XPS survey scan record of PVC 2.

were higher than those for PVC 1, with a significant difference at 15 min. Both PVC 1 and PVC 2 induced considerable levels of C3a reaching a peak level at 15 min and decreasing at 20 min. The mean values of C3a for PVC 2 at 10, 15 and 20 min after blood–material contact were higher than those for PVC 1, although the differences were not significant.

3.4. Surface characterization of plasticized PVC tubing

Table I gives the surface compositions of PVC 1 and PVC 2 from XPS analysis as shown in Figs 7 and 8 in association with the theoretical compositions of plasticized PVC. High resolution spectra give the information about chemical state and these are summarized in Table II.

From high resolution spectra results, there is found a higher level of plasticizer in PVC 2 than PVC 1, since a higher level of ester bond (O═C—O) was present in PVC 2. In addition, the data in Table I enable the determination of the molecular distributions, which are shown in Table III. Results revealed a plasticizer-rich surface for both plasticized PVC tubings. DEHP accounted for 68% of molecules at the surface and TEHTM accounted for 85%.

4. Discussion

With respect to the *in vitro* evaluation, fibrinogen adsorption can be regarded as an initial index of subsequent blood reactions on PVC tubing. Higher fibrinogen adsorption normally leads to a higher thrombogenicity and this was reflected in the values of TAT. The influence of heparin was interesting in that it reduced levels of TAT but increased those for C3a with both TEHTM plasticized PVC and DEHP plasticized PVC. This supports a belief in the importance of the overall relationship among blood, biomaterial and antithrombotic agent [15] and the complex effect of heparin on complement activation [16]. In the *ex-vivo* evaluation, TAT and C3a values were higher for TEHTM-PVC, indicating the possibility of obtaining consistency between *in vitro* and *ex vivo* assessment.

Knowledge of the nature of a biomaterial surface is important for understanding the relationship between surface properties and biological response, since only the outermost few atomic layers of surfaces can interact with living system. In this study, it was found that the blood compatibility of plasticized PVC was strongly influenced by the selection of plasticizer and in particular the plasticizer distribution at the surface. The information derived in the assessment programme indicated a pattern, with TEHTM plasticized PVC presenting a more reactive surface than DEHP plasticized PVC. The ESCA surface characterization of TEHTM-PVC and DEHP-PVC reveals that the level of TEHTM at the surface is higher than that of DEHP. Attributing the higher reactivity of TEHTM-PVC to a higher plasticizer level at surface is consistent with the fact that both plasticizer removal from a PVC surface [17] and the reduction of plasticizer level in a PVC compounding system [5] reduce the blood response. We believe that for DEHP and TEHTM, the primary influence on the blood arises from the plasticizer

TABLE I Surface compositions for plasticized PVC tubing (%)

Samples	ESCA measured					Theoretical calculated		
	C	O	Cl	Zn	Mg	C	O	Cl
PVC	—	—	—	—	—	67	—	33
DEHP	—	—	—	—	—	86	14	—
TEHTM	—	—	—	—	—	85	15	—
PVC 1	89.2	10.1	0.7	—	—	—	—	—
PVC 2	84.0	12.6	2.4	0.4	0.6	—	—	—

— = Not measured/not calculated

TABLE II High resolution spectra results for plasticized PVC tubing

Elemental state	Binding energy (ev)	Elemental content (%)	
		PVC 1	PVC 2
C—H			
C—C	285.0	80.6	76.0
CH_2	286.0	4.2	8.8
C—O	286.5	7.4	4.8
CHCl	287.0	3.8	2.6
O=C—O	289.0	4.0	7.9
C=O	532.0	52.4	60.5
C—O—C	533.0	47.6	39.5

TABLE III Molecular distribution at the surfaces of plasticized PVC tubing

Samples	Poly(vinyl chloride)	Molecule distribution (%)		Carbon–hydrogen
		DEHP	TEHTM	
PVC 1	2	68	–	30
PVC 2	6	–	85	9

distribution at the surface rather than the chemical nature of the plasticizer.

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