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Ex-vivo blood compatibility of silicone-containing biomaterials

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The acute blood-contacting properties of five silicone-containing elastomers and a poly(vinyl alcohol) coated silicone elastomer were assessed using a canine *ex vivo* shunt model. The silicone-containing elastomers studied included two thermoset amorphous silica reinforced dimethyl methylvinyl siloxane-based polymers which were extruded as Silastic® RX-50 Medical Grade Tubing (RX-50) and Silastic® Medical Grade Tubing H.P. (HP). They also included three experimental thermoplastic silicone–urea urethane copolymers received as X7-4074 (SP-1), X7-4037 (SP-2), and X7-4943 (SP-3). The RX-50 tubing material showed less thrombus deposition compared to the silicone–urea urethane copolymers. This suggests that the blood-contacting response of a silicone elastomer is strongly affected by the incorporation of the urea urethane segments. Among the silicone–urea urethane copolymers, the SP-3 material showed higher levels of platelet and fibrinogen deposition than the SP-1 and SP-2 materials, whereas the SP-1 and SP-2 samples had similar levels of deposition. These results indicate that the blood-contacting properties of the silicone–urea urethane copolymers were influenced more by the molecular weight of the polydimethylsiloxane than by the type of diol used in the urea urethane segments. The maximal platelet deposition on the poly(vinyl alcohol)-coated silicone was approximately an order of magnitude greater than those on the silicone-containing elastomers indicating that the PVA coating was more thrombogenic.

1. Introduction

Silicone elastomers have been used in a wide range of biomedical applications in the past three decades, due to their good blood compatibility, low toxicity, good thermal stability, low modulus, water-repellency, and anti-adhesive properties [1, 2]. Medical devices fabricated using silicone elastomers include blood pumps, cardiac pacemaker leads, contact lenses, oxygenators, medical adhesives, finger joints, etc. [1–3].

Due to their wide range of applications, great attention has been focused on the responses of silicone elastomers when in contact with blood and living tissue. The formulations of silicone elastomers used in medical applications typically include the base polymer polydimethylsiloxane (PDMS), other copolymer units, and various components. For example, amorphous silica fillers, curing agents, and urethane or other polymer segments have been incorporated into PDMS in order to improve its mechanical properties [2, 3]. Studies have shown that the presence of these components influence the biocompatibility or hemocompatibility of PDMS. Weatherby *et al.* [4] showed that the addition of amorphous silica filler and curing agents caused changes in the *in vitro* clotting time of blood in contact with PDMS. *In vitro* lipid adsorption studies on PDMS-containing materials showed that lipid adsorption increased with the degree of cure and decreased with silica content [5]. Nyilas *et al.* [6] suggested that the presence of silica-rich areas in silicone rubber adversely influenced its *in vitro* clotting characteristics. Recently, incorporation of heparin and poly (ethylene oxide) segments into PDMS has been shown to decrease *in vitro* platelet adhesion and serotonin release [7].

We are interested in studying the effects that chemical structure variation and filler addition have on the blood compatibility of silicone-containing elastomers. In this study, the blood-contacting properties of five

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silicone-containing elastomers, including two silica reinforced dimethyl methylvinyl siloxane copolymer based elastomers and three experimental silicone–urea urethane copolymers, were investigated using a canine *ex vivo* shunt model. In addition, the blood-contacting response of a poly(vinyl alcohol) coated silicone elastomer was studied and compared with that of the silicone-containing materials.

2. Materials and methods

2.1. Materials

The materials used in this study were provided by Dow Corning Corp. (Midland, MI). Two dimethyl methylvinyl siloxane copolymer based elastomers reinforced with similar high surface area treated fumed silica (26% and 28% by weight) and similar cure systems (platinum catalyzed addition cure) were provided as extruded tubing (3.0 mm inside diameter). These products are commercially known as Silastic ® RX-50 Medical Grade Tubing (RX-50) and Silastic® Medical Grade Tubing H.P. (HP). The primary difference in the tubing materials is that different proprietary treating agents are used in the processing of the reinforcing fillers in the respective material formulations.

Three experimental silicone–urea urethane copolymers, X7-4074 (SP-1), X7-4037 (SP-2), and X7-4943 (SP-3), were received in solution form. These materials were prepared using the two-step reaction of a secondary amine-terminated polydimethylsiloxane (PDMS), an aliphatic diisocyanate, and a diol (Fig. 1). An aliphatic diol ($HO–(CH_2)_4–OH$) was used in preparing all three materials. However, SP-2 had an additional diol component, poly(ethylene oxide) $(H–(O–CH_2CH_2)_{33})_2$, with a 1:2 hard to total soft segment ratio. PDMS of similar degrees of polymerization ($x = 30$ and 35, respectively) were used for SP-1 and SP-2, respectively. A higher D.P. ($x = 50$) PDMS was used for SP-3. A poly(vinyl alcohol) (PVA) coated tubing was prepared by solution coating PVA onto the inner surface of the RX-50 tubing. This material was stored in sterile deionized water.

2.2. Sample preparation

The three silicone–urea urethane materials were prepared by separately coating an approximately 10% polymer solution onto chromic acid-oxidized polyethylene tubing (Intramedic, Clay-Adams, 3.18 mm I.D.) [8]. These coated tubes were dried under nitrogen at room temperature for seven days and then under vacuum at 50 °C for at least 48 h. The PVA coated tubing was dried under vacuum at 50 °C for a minimum of 24 h. The RX-50 and HP extruded tubing were used as received. Surface characterization and blood compatibility evaluation were carried out on the extruded and coated polymer tubings.

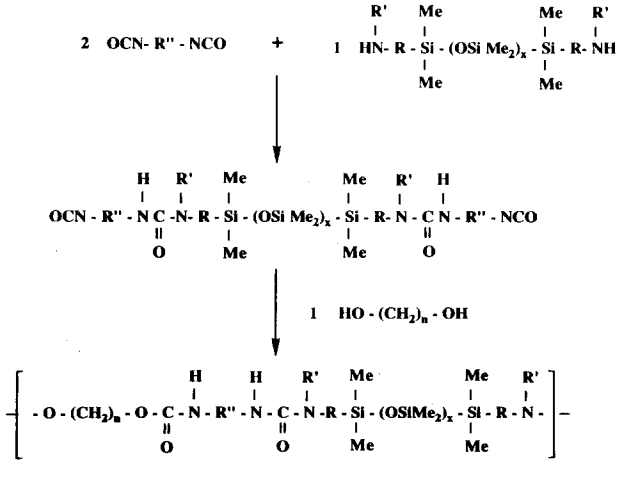

Figure 1 Schematic of the synthesis of silicon–urea urethane copolymers.

2.3. Surface property characterization

X-ray photoelectron spectroscopy (XPS) spectra were obtained using a Physical Electronics PHI 5400 spectrometer. A magnesium anode operating at 300 W and 15 kV and a photoelectron emission angle of 45 degrees was used. The relative atomic percentage of each element at the surface was estimated from the peak areas using atomic sensitivity factors specified for the PHI 5400. The high resolution C_{1s} spectra were deconvoluted and curve-fitted to analyse the chemical bonding states of the carbon atoms. A combination of Gaussian and Lorentzian peak shapes were used to fit the three peaks which correspond to the aromatic/aliphatic carbon at 285 eV, the ether carbon and/or urea carbon at 286.5 eV, and the carbonyl carbon at approximately 289 eV.

2.4. Blood compatibility evaluation

A canine *ex vivo* series shunt experiment, which has been previously described [9], was used to evaluate the blood compatibility of these materials (Fig. 2). The extruded and coated polymer tubings were cut into 1.5 inch lengths and assembled into shunts. Each polymer was present in triplicate in each shunt.

Following hematological screening, adult mongrel dogs were injected with autologous ^{111}In-labelled platelets and ^{125}I-labelled purified fibrinogen. No anticoagulants were used in this experiment. The femoral artery and the vein in one leg were cannulated with the shunt. Prior to surgery, the shunt was filled with a divalent cation-free Tyrodes (pH = 7.4) solution overnight to prevent blood–air contact and to hydrate the test polymer surfaces. A branch artery proximal to the shunt cannulation site was connected

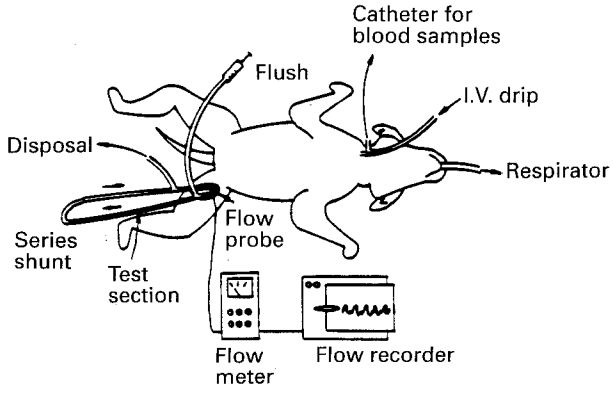

Figure 2 Schematic of the canine *ex-vivo* shunt model for blood compatibility evaluation.

TABLE I X-ray photoelectron spectroscopy analysis

Material	Si/C_{total}	O/C_{total}	N/C_{total}	$C\text{-}Si/C_{total} + C\text{-}C/C_{total}$	$C\text{-}O/C_{total}$	$C=O/C_{total}$
RX-50	0.41	0.58	0.01	1.00	—	—
HP	0.40	0.60	0.01	0.97	0.03	—
SP-1	0.27	0.43	0.04	0.88	0.08	0.03
SP-2	0.21	0.41	0.04	0.75	0.23	0.03
SP-3	0.28	0.44	0.03	0.89	0.07	0.03
PVA	0.28	0.54	0.01	0.87	0.10	0.02

to a flushing system. The blood flow rate was continuously monitored during blood exposure using an electromagnetic flow probe. The initial flow rate was controlled at 280 ± 20 ml/min. Blood samples were collected hourly to determine bulk radioactivity, platelet and fibrinogen concentrations, hematocrit, blood gas analysis and hematological function tests. Three separate surgeries were performed.

Shunts were run for 1, 2, 5, 10, 15, 20, 25, 30, 45, and 60 min of blood contact. At the end of each blood contact interval, the femoral artery was clamped, and the bulk blood was flushed out of the shunt at a shear rate much lower than that during blood contact. Immediately following flushing, the test sections were removed, and the tubing contents were fixed with 2% glutaraldehyde. Then, each test section was subdivided into sections for gamma scintillation counting and for evaluation by scanning electron microscopy (SEM).

Platelet and fibrinogen deposition profiles were determined by counting the segments in a gamma counter (Gamma 5500, Beckman) and converting the number of counts into the number of platelets or mass of fibrinogen. The average and standard deviation of the nine data points (three data points for each of three surgeries) was obtained. Outlier data points among the nine points were rejected at the 95% confidence level. Selected samples were prepared for scanning electron microscopy using the procedure previously described [10]. These samples were examined using a JEOL JSM-35C SEM at 15 kV accelerating voltage. This technique does not permit direct assessment of embolysis.

3. Results and Discussion

3.1. Surface properties

Table I shows the surface atomic ratios determined using XPS. Silicon (Si) is associated with the PDMS, methyl vinyl siloxane, and the silica fillers. Nitrogen resides primarily in the urethane component, whereas oxygen is associated with PDMS, methyl vinyl siloxane, poly(ethylene oxide) (PEO), and the urethane bond. For the silicone–urea urethane copolymers, the ether carbon (C–O) and urea carbon (N–C–N) are associated with the PEO and urea linkages, respectively, while the carbonyl carbon (C=O) resides in the urethane linkages.

As expected, the silicon/carbon (Si/C_{total}) atomic ratios of the RX-50 (= 0.41) and HP (= 0.40) materials were greater than SP-1 (= 0.27), SP-2 (= 0.21), SP-3 (= 0.28), and PVA (= 0.28), due to the PDMS and silica filler used in these materials. The small amount of nitrogen detected on the RX-50 and HP tubings was unexpected. It may be due to contamination during the sample preparation procedure.

The N/C_{total} ratios detected on the SP-1, SP-2, and SP-3 sample surfaces were 0.04, 0.04, and 0.03, respectively. This indicates the presence of the urea urethane segments at the air–polymer interface, *in vacuo*, for these polymers. A larger $C\text{-}O/C_{total}$ ratio was detected on SP-2 (= 0.23) compared to SP-1 (= 0.08) and SP-3 (= 0.07). Since the SP-2 material contained both a polyethylene oxide soft segment and an aliphatic diol, while the SP-1 and SP-3 materials both used only an aliphatic diol in the synthesis, the higher ether carbon content on the SP-2 surface was expected.

An unexpectedly high Si/C_{total} atomic ratio was detected on the PVA coated tubing samples. Since PVA itself did not contain any Si and the coated tubing was prepared by coating PVA on to the inner surface of the RX-50 tubing, the detected Si signal may be due to an uneven PVA coating on the tubing or due to diffusion of the Si-containing components from the RX-50 tubing to the coating layer.

3.2. Blood-contacting properties

A material is considered to be thrombogenic if a relatively large number of platelets and fibrin/fibrinogen molecules adhere to the surface during blood contact. A surface coverage of approximately 70–100 platelets/1000 μm^2 represents a monolayer of platelets on the surface, although the degree of platelet spreading and activation can cause this number to vary [11].

Fig. 3 shows the transient platelet deposition profiles for the materials tested during the initial hour of blood exposure. The platelet deposition profiles show a peak between 10 and 30 min of blood contact. The same trend was previously reported for different polymeric materials in the same canine series shunt experiment [9–12]. This peak reflects thrombus growth in competition with thrombus detachment. The RX-50 material shows the lowest level of platelet deposition with a maximum of approximately 90 platelets/1000 μm^2 after 5 min of blood contact being observed. The HP, SP-1, and SP-2 materials all showed relatively low levels of platelet deposition (< 200 platelets/1000 μm^2) during blood contact. The SP-3 had a higher level of platelet deposition with a maximum of 450 platelets/1000 μm^2 at 15 min. The PVA

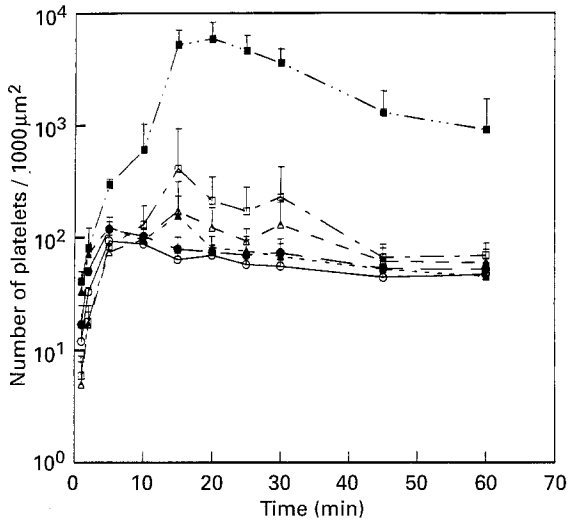

Figure 3 Transient platelet deposition profiles during the initial hour of blood contact (○ RX-50, ● HP, △ SP-1, ▲ SP-2, □ SP-3, ■ PVA)

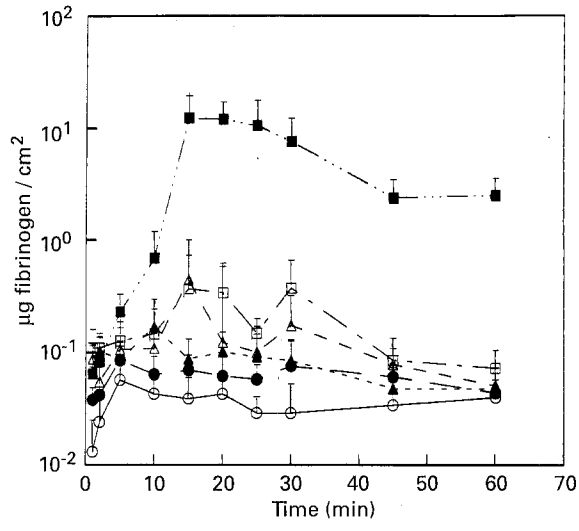

Figure 4 Transient fibrinogen/fibrin deposition profiles during the initial hour of blood contact (key as for Fig. 3).

material had a maximum deposition of 5900 platelets/1000 μm², which was approximately an order of magnitude larger than that observed on any of the silicone-containing materials.

The transient fibrinogen/fibrin deposition profiles during the initial hour of blood contact are shown in Fig. 4. The fibrinogen/fibrin profiles closely parallel the platelet deposition profiles for all of the materials tested. The similarity in the trends of the platelet and fibrinogen/fibrin deposition profiles indicates fibrinogen incorporation into the platelet thrombi by possible binding to specific receptor sites, or by fibrinogen incorporation into the thrombi as fibrin strands linking the platelets together [13]. Overall, PVA showed the highest level of fibrinogen/fibrin deposition, followed by SP-3, SP-1, SP-2, and HP, with RX-50 having the lowest level.

In order to evaluate the thrombogenicity of the materials tested, the materials were ranked for each time point from the highest to the lowest level of platelet deposition. This was repeated for the fibrinogen/fibrin deposition data. A relative rank was assigned (highest = 6 and lowest = 1) to each material at each blood-contacting time, and the relative ranks at all blood-contacting times were averaged for each material. Tables II and III summarize the platelet and fibrinogen ranks for the materials tested. These tables show the same trends as Figs 3 and 4, namely that PVA had the highest platelet deposition and RX-50 had the lowest levels. The HP, SP-1, and SP-2 all had similar levels of platelet and fibrinogen/fibrin deposition.

The poor blood compatibility of the PVA-coated silicone rubber as assessed by surface-adherent platelets and fibrinogen was somewhat surprising. Cholakis *et al.* [14] found fewer surface-adherent platelets on

TABLE II Platelet deposition relative rank

Material	RX-50	HP	SP-1	SP-2	SP-3	PVA
Average rank at all times ± standard deviation	1.7 ± 1.0	3.1 ± 0.9	3.0 ± 1.5	3.1 ± 1.3	4.1 ± 1.5	6.0 ± 0.0

(n = 10)

TABLE III Fibrinogen deposition relative rank

Material	RX-50	HP	SP-1	SP-2	SP-3	PVA
Average rank at all times ± standard deviation	1.0 ± 0.0	2.1 ± 0.3	4.0 ± 0.7	3.4 ± 1.0	5.0 ± 0.7	5.5 ± 1.1

(n = 10)

PVA and heparin-PVA hydrogels than on polyethylene *in vitro* using a Chandler loop system. ^{14}C-Serotonin release was also lower *in vitro* on these hydrogels than on polyethylene. The results of the present study are also in contrast to those found by Cholakis *et al.* in a chronic canine *ex vivo* shunt model [15]. Scanning electron micrographs showed that the surfaces of PVA and heparin-PVA hydrogels were somewhat less thromboadherent than polyethylene *ex vivo*, but the differences were not quantified [15]. However, a significant drop in platelet count was observed, along with an increase in platelet regeneration times as measured using a malondialdehyde (MDA) assay to assess platelet cyclooxygenase activity [15, 16]. Their conclusion was that PVA hydrogels were platelet consumptive, but not thromboadherent. However, in the present case, significant adherence of thrombi to the surfaces was observed. It is possible that the substrate underlying the PVA hydrogel may influence the thromboadherence of the hydrogel. In the present study, PVA was coated onto a silicone rubber, whereas Cholakis *et al.* cast onto a polyethylene substrate. The method of preparing the PVA hydrogel may also differ. Platelet consumption was not investigated in the present study.

Scanning electron micrographs (SEMs) show differences in the blood-contacting behaviours of these polymers that are not evident in the platelet and fibrinogen/fibrin deposition profiles. Fig. 5 shows micrographs of the RX-50 material after 5 and 60 min of blood exposure. At 5 min, a monolayer of adherent platelets with some small aggregates (5–10 platelets) were observed. The adherent platelets showed some extended pseudopodia which indicates that the activation of the platelets was not severe. After 60 min, the number of adherent platelets was similar and the morphology of the adherent platelets did not change extensively. Fig. 6 shows the SEMs for the HP material after 5 and 60 min of blood exposure. At 5 min, small thrombi which mainly consisted of platelets were observed, however, the number of the adherent platelets was relatively low and was close to monolayer coverage. The number of adherent platelets remained at low levels up to 60 min of blood exposure.

Fig. 7 shows SEMs of the SP-1 after 15 and 60 min of blood exposure. At 15 min, adherent platelets spread extensively and formed thrombi. Additionally, adherent leukocytes were observed. After 60 min, white thrombi (mainly platelets and leukocytes) were observed. Similar blood-material interactions were observed on the SP-2 material (Fig. 8). Thrombi containing platelets and leukocytes were observed after 15 and 60 min of blood exposure. The SP-3 material showed slightly greater adherence of blood components as shown in Fig. 9. At 15 min, large thrombi consisting of platelets and leukocytes were observed. After 60 min, adherent leukocytes and platelets were observed. Leukocyte attachment and spreading has been observed previously on surfaces that were considered to be thrombogenic using the same *ex vivo* canine model [13]. The PVA material showed the largest amounts of thrombus deposition. As shown in

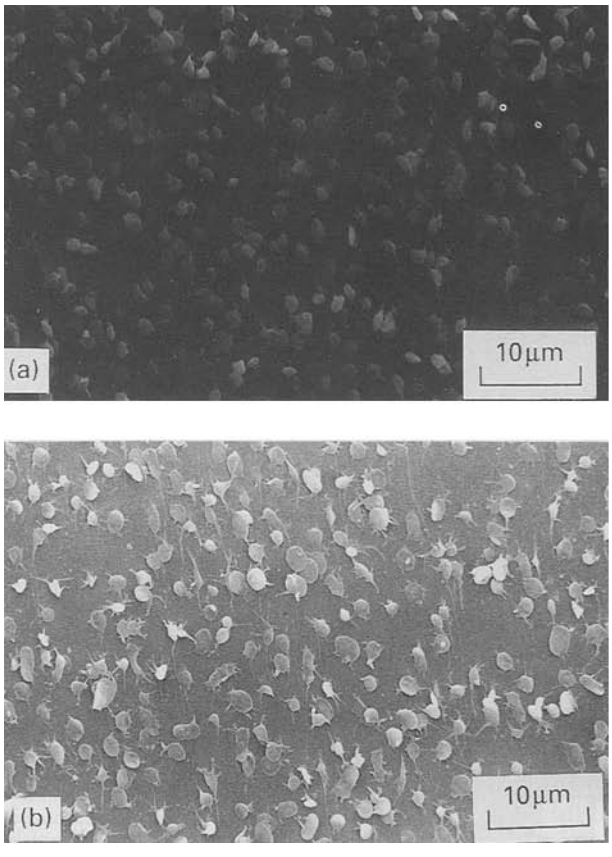

Figure 5 Scanning electron micrographs of the surface of RX-50 tubing after (a) 5 and (b) 60 min of blood exposure.

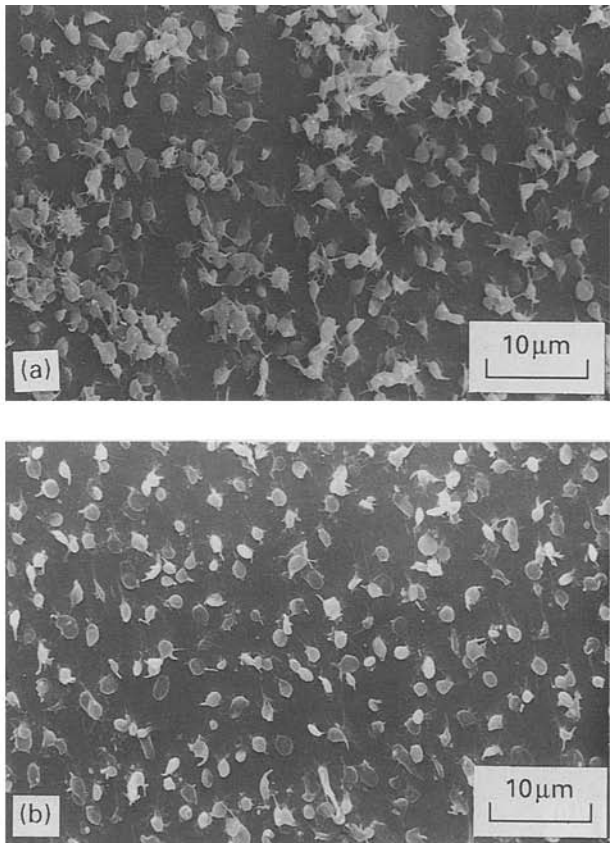

Figure 6 Scanning electron micrographs of the surface of HP tubing after (a) 5 and (b) 60 min of blood exposure.

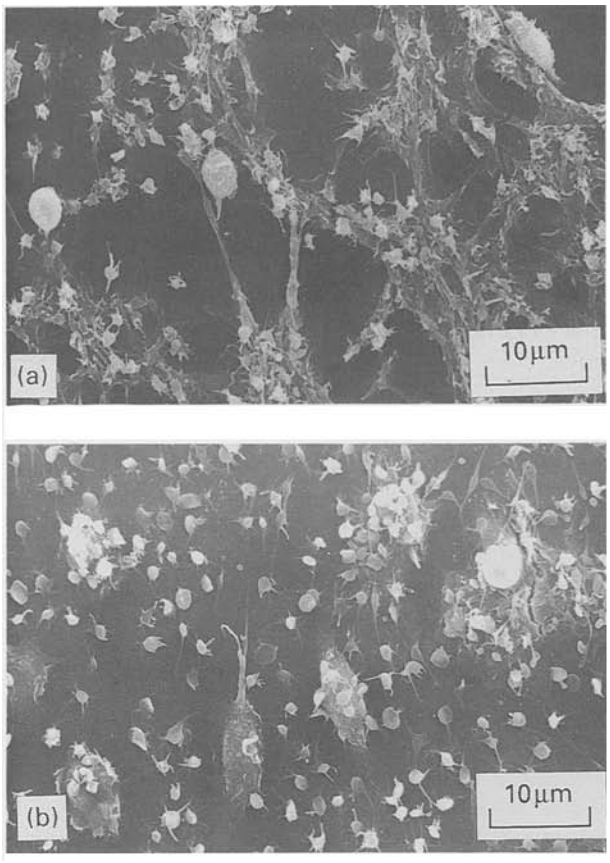

Figure 7 Scanning electron micrographs of the surface of SP-1 after (a) 15 and (b) 60 min of blood exposure.

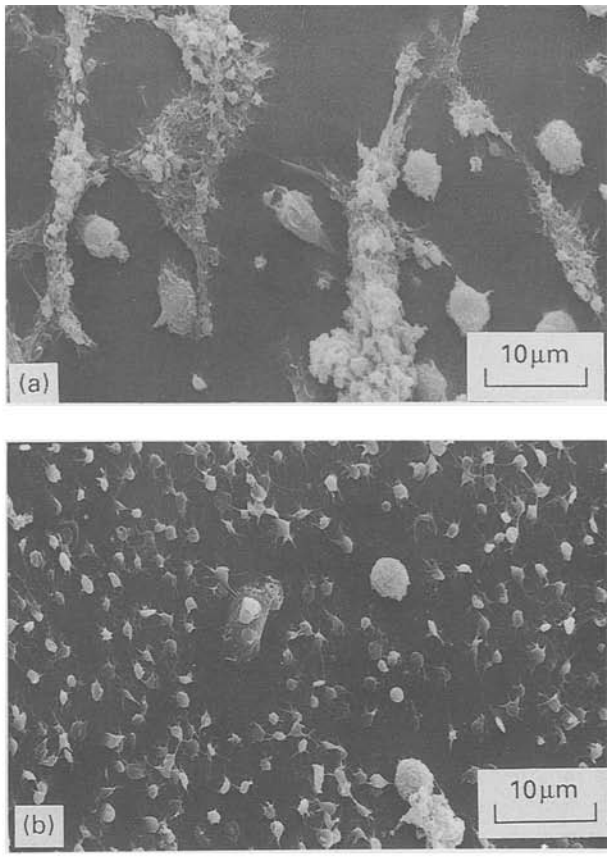

Figure 9 Scanning electron micrographs of the surface of SP-3 after (a) 15 and (b) 60 min of blood exposure.

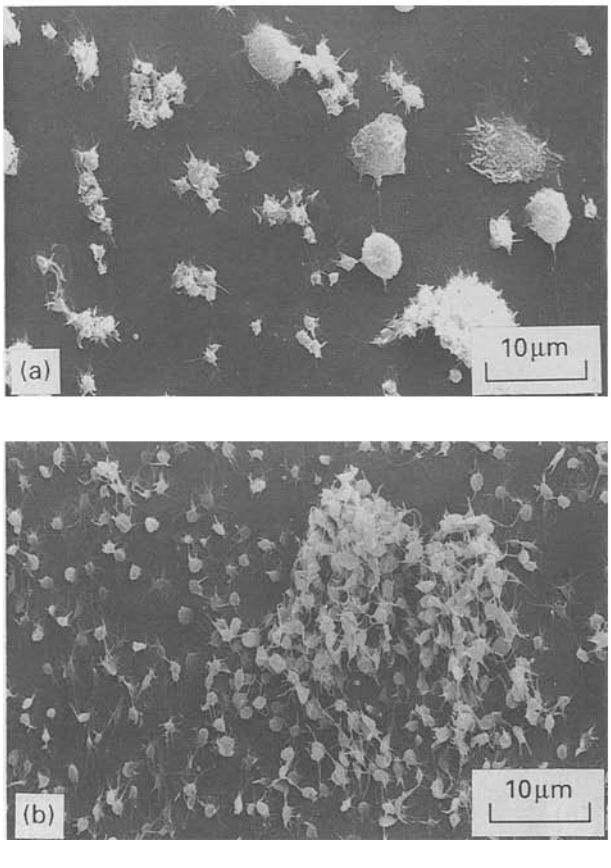

Figure 8 Scanning electron micrographs of the surface of SP-2 after (a) 15 and (b) 60 min of blood exposure.

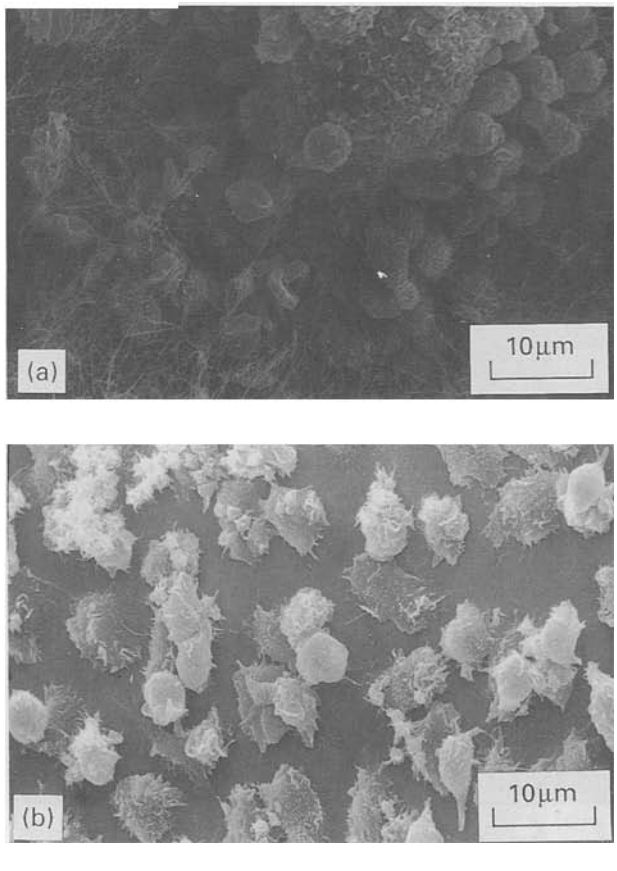

Figure 10 Scanning electron micrographs of the surface of PVA after (a) 15 and (b) 60 min of blood exposure.

Fig. 10, at 15 min, large complex mural thrombi consisting platelets, erythrocytes and leukocytes, and fibrin strands were observed. At 60 min, after significant thromboembolism a large number of activated leucocytes and thrombi were observed on the material surface.

4. Conclusions

Using a canine *ex vivo* shunt model, the structure of the silicone-containing elastomers has been shown to affect their blood compatibility. The RX-50 material showed lower thrombus deposition compared to the experimental silicone–urea urethane copolymers (SP-1, SP-2, and SP-3), while the HP material showed similar platelet deposition but less leukocyte adhesion and spreading compared to the SP-1 and SP-2 materials.

Among the silicone–urea urethane copolymers, the SP-3 material was slightly more thrombogenic than the SP-1 and SP-2 materials indicating that the blood-contacting properties of these materials were affected by the molecular weight of polydimethylsiloxane (PDMS) in these copolymers. Similar platelet and fibrinogen deposition levels were observed on the SP-1 and SP-2 materials suggesting that the type of diol used in preparing the silicone–urea urethane copolymers has little effect on their blood-contacting responses.

The poly(vinyl alcohol) (PVA) coated silicone tubing was the most thrombogenic among the materials studied. Scanning electron micrographs showed that large thrombi consisting of platelets, leukocytes and erythrocytes, and fibrin strands were observed on the material surface. The maximum level of platelet deposition on the PVA coating was approximately an order of magnitude greater than on any of the silicone elastomers.

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