Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 327 (2006) 117–125
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Platelet adsorption and hemolytic properties of liquid crystal/composite polymers

Mei-Fen Shih [a], Min-Da Shau [a], Meng-Ying Chang [a], Se-Kai Chiou [a], Jiunn-Kae Chang [a], Jong-Yuh Cherng [b,*]

[a] *Chia Nan University of Pharmacy and Science, 60 Erh-Jen Rd., Sec. 1, Jen-Te, Tainan 717, Taiwan, ROC*
[b] *National Chung Cheng University, 168 University Rd., Chia-Yi 621, Taiwan, ROC*

Received 26 January 2006; received in revised form 13 June 2006; accepted 25 July 2006
Available online 29 July 2006

Abstract

The aim of this study is to investigate how the presence of liquid crystal, cholesteryl oleyl carbonate, embedded into polymers (PMMA, Eb270, PU) affects the biocompatibility of composite membranes with human blood. The effects of different surface textures of composite membranes on platelet adhesion and platelet activation were evaluated as well. The adhesion and geometric deformation of platelets were demonstrated by SEM. The quantitative assay of platelet activation was determined by measuring the production of P-Selectin, and by measurement of the blood clotting index when PRP blood was incubated with pure polymer films and composite membranes. Moreover, the hemolysis studies on the damage to red blood cells were performed to gain information on the hemocompatibility of these biomaterials. The results showed that inclusion of cholesteryl oleyl carbonate (COC) embedded in composite membranes, improves their biocompatibility with respect to a substantial reduction of platelet adhesion and the controlled decrease of platelet activation. As the COC content of composite membranes was increased, the value of the blood clotting index increased and the production of P-Selectin decreased. The results also showed that the presence of COC resulted in a decrease of hemolysis ratios. Comparing among three different composite membranes, the best biocompatibility is achieved when PU/COC $\geqq$ Eb270/COC > PMMA/COC. The in vitro studies performed in this work suggest that it may be reasonable to use liquid crystal COC as a mean of surface modification to improve the blood compatibility of biopolymers.

Keywords: Polymer; Liquid crystal; Cholesteryl oleyl carbonate; Platelet; Blood compatibility

1. Introduction

Polymeric materials used in medical and related applications have safety issues that are related to their compatibility with blood, notably by the occurrence of thrombosis (Gorbet and Sefton, 2004). Many efforts have been employed to improve the blood compatibility of biomaterials via surface modification, e.g. DNA-blend polysulfone (Zhao et al., 2003) or inclusion of polyethylene oxide (PEO) or negatively charged side chains (Lee and Oh, 2002; Kim et al., 2003). The key point of these modifications is to prevent thrombosis initiated by platelet adhesion in normal and pathologic states of hemostasis.

Hemostasis is a physiological process for blood coagulation involving plasma coagulation factors, platelets and endothelial cells of blood vessels. The coagulation of blood consists of a cascade of reactions dividing into two pathways: the extrinsic and intrinsic pathways. Both pathways finally lead to the formation of an active form of factor X (factor Xa). The factor Xa catalyzes the conversion of prothrombin to thrombin that cleaves the fibrinogen to fibrin monomer. The polymerization and subsequent cross-linking of fibrin monomer produce fibers that enmesh platelets to form hemostatic clots. During this process of blood coagulation the platelet adhesion on the tissue surface is very important. Platelets can accelerate thrombosis through several actions: (1) secretion of bulk phase agonists (e.g. ADP, Thromboxane A2) which attract more platelet adhesion, (2) fibrinogen-mediated platelet–platelet aggregation and (3) by the acceleration of thrombin production (Grunkemeier et al., 1998). Moreover, P-Selectin excreted by activated platelets

* Corresponding author. Tel.: +886 958074911; fax: +886 5 2721040.
E-mail address: jycherng@yahoo.com (J.-Y. Cherng).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.07.043

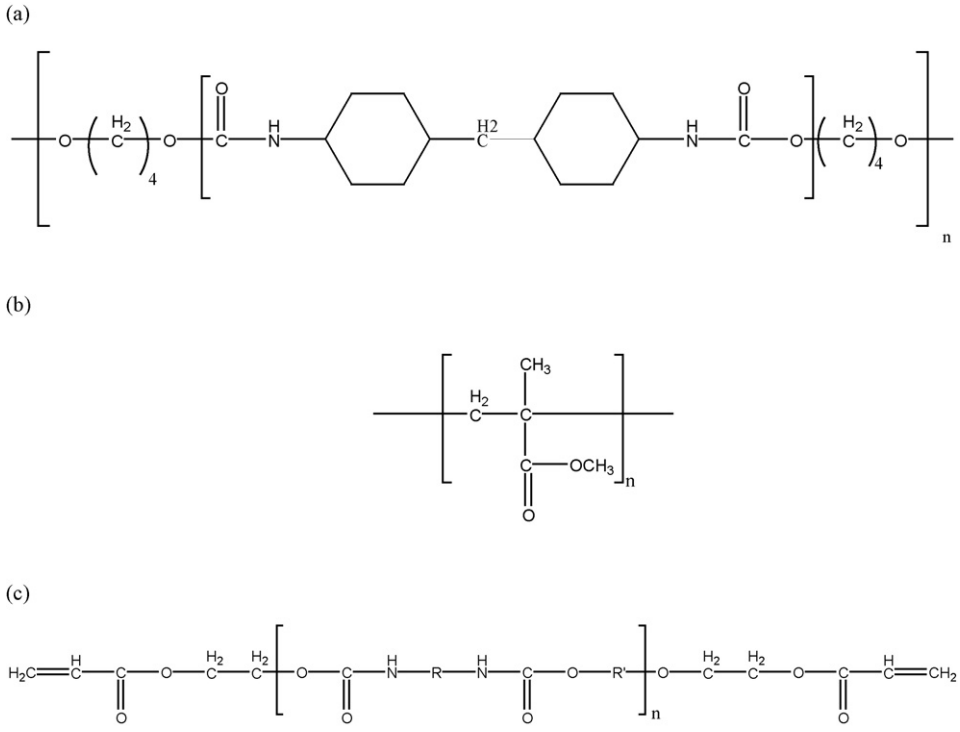

Diagram 1. The structures of tecoflex polyurethane (PU), poly-methyl methacrylate (PMMA) and aliphatic urethane diacrylate (Eb270): (a) Structure of tecoflex polyurethane, PU; (b) poly-methyl methacrylate, PMMA; (c) aliphatic urethane diacrylate, Eb270.

is also involved in the adhesion of platelets to monocytes and neutrophils, playing a central role in neutrophil accumulation within thrombi (Tedder et al., 1995). Therefore, the extent of platelet adhesion to biomaterials is often used as an index of blood compatibility (Aldenhoff and Koole, 2003; Skarja and Brash, 1997).

Liquid crystals are a phase of matter whose molecule order is intermediate between that of a liquid and that of a crystal. This means that a liquid crystal may flow like a liquid but have the molecules in the liquid arranged and oriented in a crystal-like way. Liquid crystal has been used in medical applications such as embedding liquid crystal molecules in membranes for an analysis of thermo-responsive systems (Lin et al., 2001). Similarly, several surfactants with liquid crystal properties are used for prolonged drug delivery, as well as topical delivery systems via liquid crystals (Makai et al., 2003; Nesseem, 2001). Due to the phase transition property (solid to cholesteric phase) of liquid crystal molecules, they can behave like mobile plasma membranes. Also, thermotropic nematic liquid crystal molecules, whose thickness is just twice the length of lipid molecules, are like the structural units of cell membranes (Tu et al., 2001). These characteristics of liquid crystals [especially cholesteryl oleyl carbonate (COC)] make them biocompatible and useful as anticoagulant biomaterials (Tu et al., 2001; Lin et al., 2002). In this work, the blood compatibility of three series of polymer/liquid crystal composite membranes is investigated. The extent of platelet adhesion and platelet activation on polymer surfaces was determined for establishing a correlation to the hemocompatible properties of liquid crystal-embedded polymers.

2. Materials and methods

2.1. Materials

Chemical structures of three polymers were plotted and are shown in Diagram 1. Tecoflex polyurethane (PU) was purchased from Fluka, Buchs, Switzerland. Polymethyl methylacrylate (PMMA) with molecular weight of 15,000 Da and cholesteryl oleyl carbonate (COC) were obtained from Aldrich chemicals, Milwaukee, WI. Aliphatic urethane diacrylate was supplied by Double Bond Chem. Ind. Co. Ltd. 1-hydroxy-cyclohexyl-phenyl-ketone (HCPK) was obtained from Ciba-Geigy, Basel, Switzerland. For the quantitative determination of human soluble P-Selectin in plasma, an immunoassay kit was purchased from R&D systems, Inc., MN, USA. All other chemicals and reagents used were of analytical grade.

2.2. Preparation of polymer/liquid crystal composite membrane via photo-polymerization

A polymer, Eb270, was synthesized by photo-polymerization of aliphatic urethane diacrylate in the presence of liquid crystal COC. The aliphatic urethane diacrylate (prepolymer) was first mixed with 3% radical initiator (HCPK). A clear melting solution was formed around 60°–70° and various amounts of COC were added to obtain weight ratios of COC from 0 to 30%. A hollow spacer (80 μm in depth) on a bottom lining (all composed of polypropylene) was set on a glass plate and then the mixed solution was cast into a mold. After a polypropylene cover lining was placed over the mold, a glass cover was put on and attached to

another glass plate with clips. Subsequently, the whole assembly was moved into a UV-lamp box for photo-polymerization and composite membranes were formed after 60 s of exposure time. After polymerization was finished, the polymer was vacuum dried in dessicator.

2.3. Preparation of polymer/liquid crystal composite membrane via solvent evaporation

Composite membranes composed of polymer (PU or PMMA) and liquid crystal (COC) were prepared by a solvent-casting method. Briefly, the solution of polymer (PU or PMMA) containing COC in various ratios (0, 10, 20 and 30%, w/w) was prepared by stirring the components in solvent chloroform for 1 h at room temperature. The resulting solution is clear and homogeneous. The solution was cast into an aluminum mold and after 48 h in a clean environment the solvent was evaporated to form composite membranes.

2.4. Observation of platelet adhesion by scanning electron microscopy (SEM)

Fresh blood in addition with the anticoagulant citrate dextrose (ACD) (9:1) was centrifuged at 100× for 10 min at 4 °C to obtain platelet-rich plasma (PRP). The composite membranes were rinsed three times with deionized water and then covered with 3 ml PRP (average platelet number is 5.4×10^5 ml^{-1}), pre-warmed to 37 °C. After 1 h of incubation at 37 °C, membranes were washed with PBS to remove non-adherent platelets. The adhered platelets were fixed with 2% (w/v) glutaraldehyde/PBS for 5 min at 4 °C. After thorough washing with PBS, the platelet-attached membranes were vacuum dried prior to SEM studies.

Non-treated composite membranes and platelet-attached membranes were shadowed with Pt–Pd alloy at 15 mA for 100 s. Base on the observation of electron scanning microscope (Hitachi S-3000N), the distribution of COC on the surface of composite membranes was evaluated. Also, the degree of platelet adhesion on different membranes and the morphology of adhesive platelets were investigated to understand the influence of the COC-embedded polymer surface on the blood compatibility.

2.5. In vitro blood compatibility test: blood clotting measurement

The prepared polymer/liquid crystal composite membranes were placed into flat-bottom bottles. These bottles were thermostated in water bath at 37 °C for 5 min. Then 0.27 ml from blood sample (0.3 ml ACD-whole blood by addition of 0.024 ml $CaCl_2$ (0.2 mol/l)) was slowly dropped on the surface until completely covered. The bottles containing blood samples were further incubated in a thermostate incubator at 37 °C. After 10 min, 10 ml of deionized water were carefully added by dripping water down the inside wall of the bottles without disturbing the clotted blood. Subsequently 10 ml of solution were taken from bottles and centrifuged at $100 \times g$ for 30 s. The supernatant was decanted into a tube with additional 40 ml deionized water and kept in 37 °C for 60 min. The blood clotting test was carried out by spectrophotometric measurement of the relative absorbance of blood samples that had been diluted to 50 ml at 542 nm. The absorbance of 0.25 ml ACD-whole blood in 50 ml deionized water at 542 nm was assumed to be 100 as a reference value. The blood clotting index (BCI) of biomaterials can be quantified by the following equation:

$$\text{BCI index} = \frac{100 \times (\text{abs of blood which had been in contact with sample at 542 nm})}{\text{abs of ACD whole blood in water at 542 nm}}$$

It is clear that as the BCI index rises, blood clotting decreases.

2.6. Quantitative evaluation of activation of platelets by P-Selectin measurements

The P-Selectin assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for P-Selectin was pre-coated onto a microplates. Standards, samples and control were pipetted into microwells and then added to a polyclonal antibody specific for P-Selectin, which had been conjugated with horseradish peroxidase. After removal of unbound conjugated antibody, a substrate was added and color developed, which is proportional to P-Selectin concentration. PRP without incubation of polymers is taken as a control for the comparison of samples in presence of polymers. A known concentration of P-Selection (36.86 ng/ml) included in kit was measured at wavelength at 450 nm as a standard and for establishing of a standard curve. Experimental procedures were followed as listed in the kit brochure.

2.7. In vitro blood compatibility test: hemolysis ratio measurement

The prepared polymer/liquid crystal composite membranes were rinsed three times with deionized water and normal saline before being transferred and placed into flat-bottom bottles. Then 10 ml normal saline were poured into the bottles and kept at 37 °C in a shaking water bath (shaking rate = 100 times per hour). After 60 min incubation, 0.2 ml of diluted ACD-whole blood (8 ml ACD-whole blood was diluted by addition of 10 ml normal saline) was dropped into the bottles allowing the test membranes to be soaked in the blood solution for another 60 min. Next, the solutions were aspirated and centrifuged at $100 \times g$ for 5 min. The supernatant was measured at the absorbance of 542 nm by spectrophotometer. The hemolysis ratio (HR) was obtained by the equation $HR = 100 \times (AS - AN)/(AP - AN)$, where AS is the absorbance of sample supernatant. AP and AN are the absorbance of the positive controls (10 ml pure water + 0.2 ml diluted ACD-whole blood), and the negative control (0.2 ml

Table 1
Contact angle (°) and surface energy (mN/m) on PMMA and PU composite polymers

	PMMA	PMMA + 10% COC	PMMA + 30% COC	PU	PU + 10% COC	PU + 30% COC
Contact angle in water	95.2 ± 0.8	78.7 ± 0.5	67.1 ± 1.6	96.2 ± 5.6	84.9 ± 0.4	83.9 ± 0.6
Contact angle in diiodomethane	27.3 ± 2.8	21.5 ± 1.6	18.5 ± 1.2	42.4 ± 6.6	37.5 ± 3.3	25.2 ± 2.2
SE (surface energy)	46.1 ± 0.3	49.9 ± 0.3	54.5 ± 0.5	38.6 ± 0.3	42.6 ± 1.0	47.5 ± 1.0

Values are means of at least 10 measurements ± standard deviation.

diluted ACD-whole blood in presence of 10 ml normal saline), respectively.

2.8. Contact angle and surface energy determination

Contact angles methods were used to determine the wetting ability and surface energy of PMMA and PU composite films. The contact angles subtended by two kinds of liquid (water and diiodomethane) were measured with a sessile drop method and surface energy was then calculated (DSA100, Krüss, Germany). At least five drops of each liquid were used on each sample surface to get good statistical results.

2.9. Statistical analysis

Data from at least three independent polymers in each group were analyzed. Results are expressed as mean values (±S.D.) of three experiments. Results of SEM experiments are shown with representative micrographs.

3. Results and discussion

3.1. General properties of the composite membranes

The pure polymer films and the polymer/liquid crystal composite membranes in which the liquid crystal (COC) content is less than 10% are colorless and transparent at room temperature. The composite membranes were observed to be translucent when the COC content is more than 10%. With the increase of COC these composite membranes become more hydrophlic and higher wetting ability (Table 1). This might be due to the fact that the presence of amphipathic COC molecules in a polymer would shield the biomaterial surface with their polar moieties face outwards to the hydrophilic environment (non-polar moieties of COC are more close to the hydrophobic polymer) as indicated in contact angel studies. The higher wetting ability of a surface causes a decrease in contact angle and an increase of surface energy. The transition temperature of COC from crystal state to liquid state is lower than room temperature. This is because the temperature of smectic–cholesteric transition and the cholesteric–isotropic transition of COC are 18.9 and 38 °C, respectively (Lin et al., 2001). Therefore, the liquid crystal molecules were mobile in the composite membranes. It is likely that these COC molecules are also mobile, behaving in vivo like the phospholipid component of cell membranes.

3.2. Observation of surface morphology and platelet adhesion on pure polymer films and composite membranes

SEM micrographs were employed to assess the effect of COC contents, embedded in artificial membranes, on human blood compatibility. The extent of adhesion and deformation of platelets were observed and correlated to the blood–membrane interaction. Fig. 1A and B shows that the morphology of platelets on pure PMMA films, spreads flatten and extends into irregular shapes indicating activation of platelets. Also, coagulated platelets were found to be less compatible with human blood. When COC content is increased to 10% in PMMA composite membranes, the number of adhesive platelets on the surface decreases (Fig. 2A and B). However, the shape of most adhesive

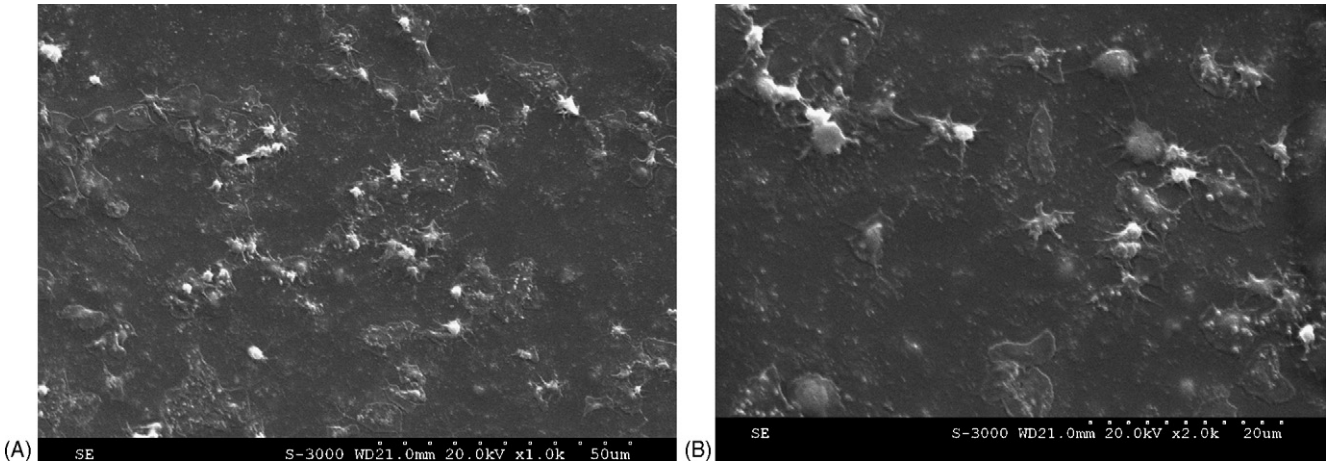

Fig. 1. SEM micrographs of platelets adhesion on the surface of PMMA film after 60 min: (A) 1000× magnification and (B) 2000× magnification. Pseudopodia and flattening of adhered platelets were observed.

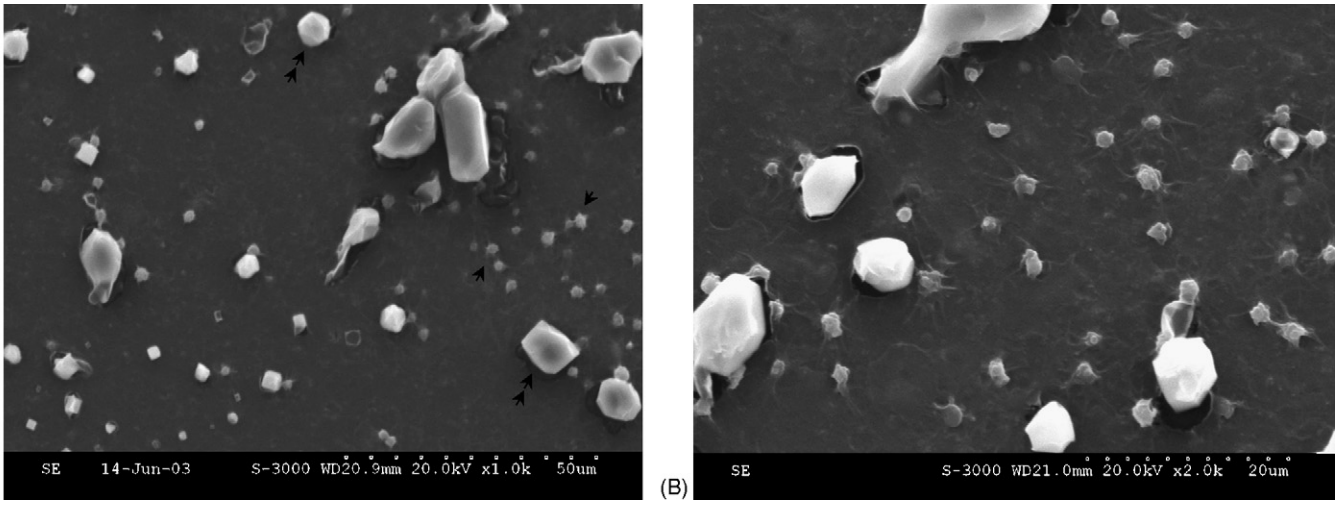

Fig. 2. SEM micrographs of platelets adhesion on the surface of PMMA–10%COC composite membrane after 60 min: (A) 1000× magnification and (B) 2000× magnification. Platelet (arrowheads) and crystalline of COC (double arrowheads).

platelets was shown to stay regular and did not flatten, indicating that the biocompatibility of the composite membrane to human blood is improved. The SEM micrographs of PMMA composite membranes with COC content of 20 and 30% could not be taken due to the motion of COC molecules on composite membranes, which is caused by high-energy electron beams.

Fig. 3A and B shows the morphology of platelets on pure PU membranes. It is clear in the figures there are fewer platelets on the membranes, but that they are more regular in shape. On the surface of PU composite membrane having more COC content (10%), far fewer adhesive platelets were observed (Fig. 4A and B). The more COC (20–30%) present in composite membranes, the fewer adhesive platelets were found (Fig. 5A and B). Vesicle-like objects are liquid crystal droplets or domains of COC molecules, which form on the surface texture of PU composite membranes (Figs. 4 and 5). Non-PRP treated PU composite membranes that exhibit vesicle-like objects. The Eb270 composite membrane is prepared by dissolving COC in a suitable prepolymer, and is followed by photo-polymerization instead of solvent evaporation, which is used for PU and PMMA composite membranes. In Fig. 6A and B, fewer platelets adhere on the Eb270 pure film in comparison with PMMA. Also, these adhesive platelets all remained in their regular shapes, indicating good biocompatibility. When 10 or 20% COC was embedded, far fewer adhesive platelets were found on Eb270 composite membranes (Figs. 7 and 8, respectively). Also, a distinct surface texture on Eb270 composite membranes was found, showing the existence of micelle-like domains of COC molecules. It was found that as the COC content that is embedded into composite membranes was increased, the hemocompatibility was improved via a marked drop in platelet adhesion. It could be asserted that the reason behind this phenomenon is that the existence of COC molecules acts like phospholipid moieties of plasma membranes, resulting in better hemocompatibility (Morimoto et al., 2002; Nakabayashi and Williams, 2003; Yang et al., 2003). Moreover, Kim et al. reported that grafting of polyethylene oxide on a material surface resulted in more blood compatibility, due to an additional chain motion (molecular cilia) effect

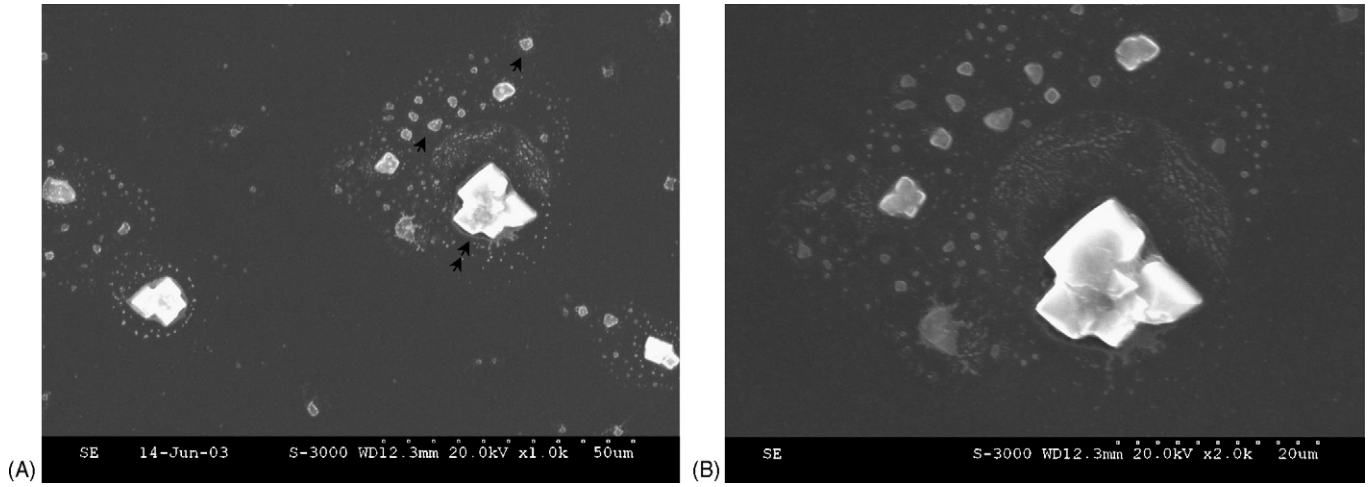

Fig. 3. SEM micrographs of platelets adhesion on the surface of PU film after 60 min: (A) 1000× magnification and (B) 2000× magnification. Platelet (arrowheads) and crystal of salts (double arrowheads).

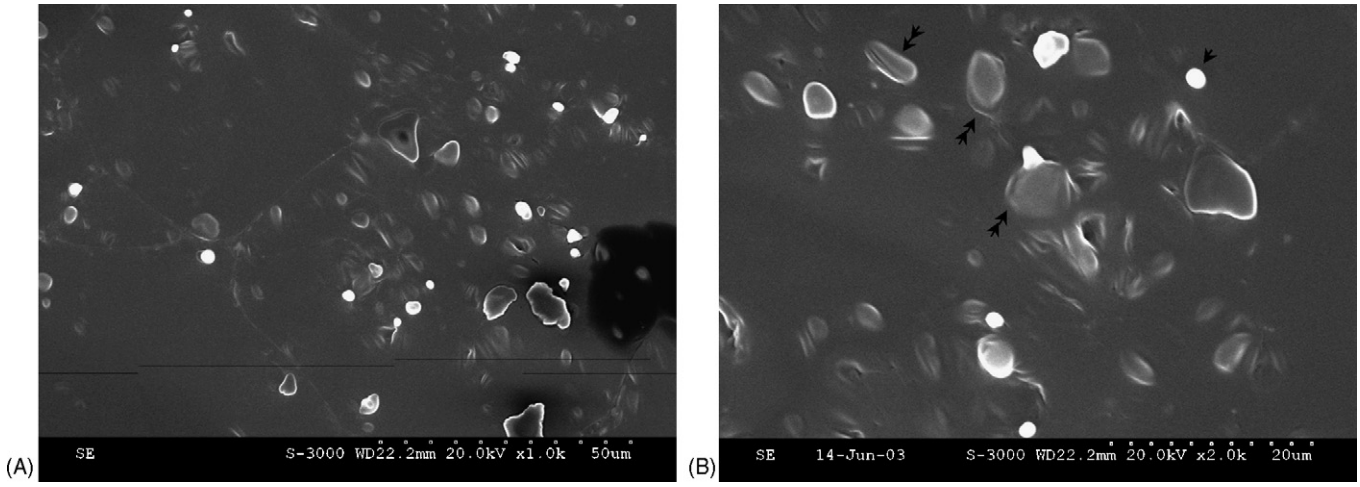

Fig. 4. SEM micrographs of platelets adhesion on the surface of PU–10% COC composite membrane after 60 min: (A) 1000× magnification and (B) 2000× magnification. Platelet (arrowheads) and vesicle-like objects (double arrowheads).

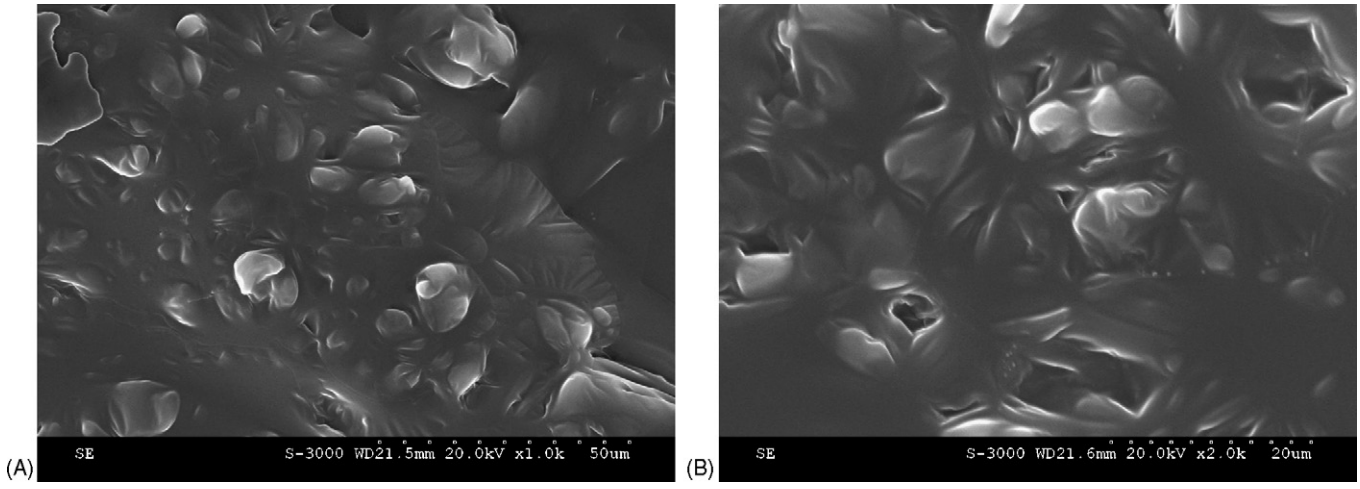

Fig. 5. SEM micrographs of platelets adhesion on the surface of PU–20% COC composite membrane after 60 min: (A) 1000× magnification and (B) 2000× magnification.

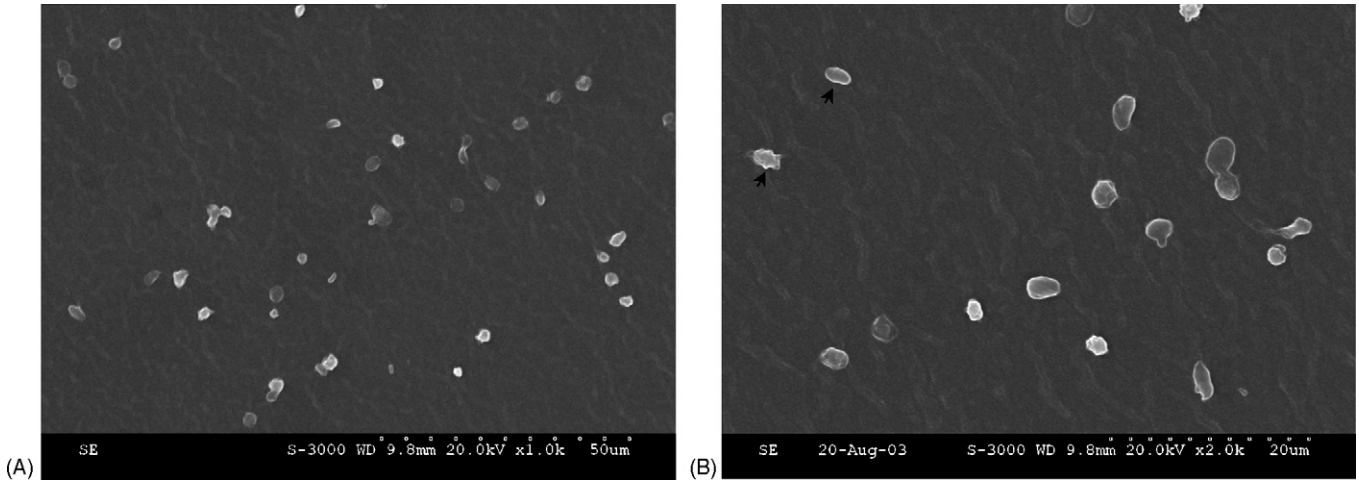

Fig. 6. SEM micrographs of platelets adhesion on the surface of Eb270 film after 60 min: (A) 1000× magnification and (B) 2000× magnification. Platelet (arrowheads).

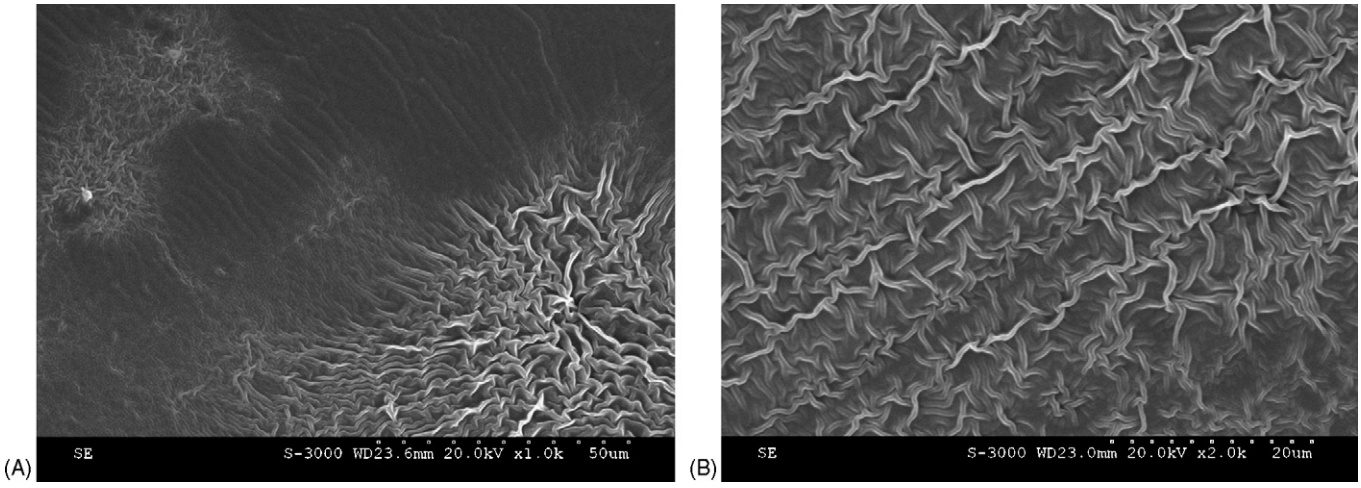

Fig. 7. SEM micrographs of platelets adhesion on the surface of Eb270–10% COC composite membrane after 60 min: (A) 1000× magnification and (B) 2000× magnification.

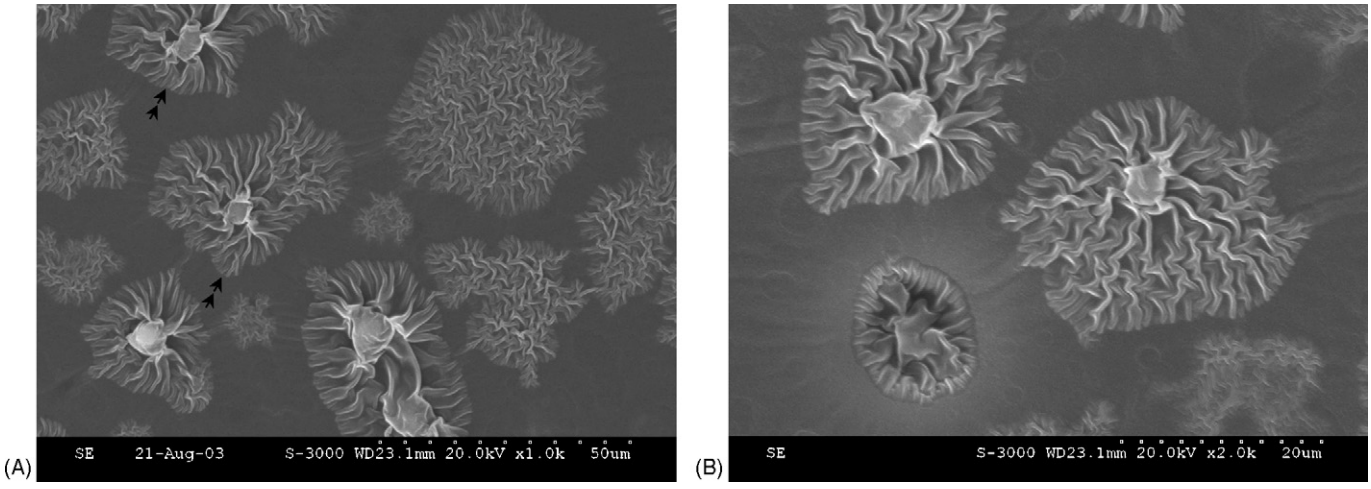

Fig. 8. SEM micrographs of platelets adhesion on the surface of Eb270–20% COC composite membrane after 60 min: (A) 1000× magnification and (B) 2000× magnification. Micelle-like objects (double arrowheads).

(Kim et al., 2003). By means of shielding material surfaces from platelet (e.g. platelet glycoprotein Iib-IIIa (Gp Iib-IIIa) receptors), thrombotic effects may be retarded (Skarja and Brash, 1997; Spijker et al., 2002). Furthermore, the inclusion of COC in composite polymers results in less platelet adhesion; this is also likely due to an increase of membrane hydrophilicity on surface (Zhao et al., 2003; Lee et al., 2002).

3.3. Blood clotting properties of polymer composite membranes

The blood clotting study evaluates the actions of antithrombogenic activity of a material on human blood. The antithrombogenic activity is quantitatively expressed by a relative parameter known as the blood clotting index (BCI). A larger BCI value indicates an increase of compatibility. Fig. 9 shows the influence of COC contents in composite membranes on BCI values. An increase in the BCI value was found for polymers embedded with higher COC content (from 10 to 30%). This indicates that the addition of liquid crystal into polymer

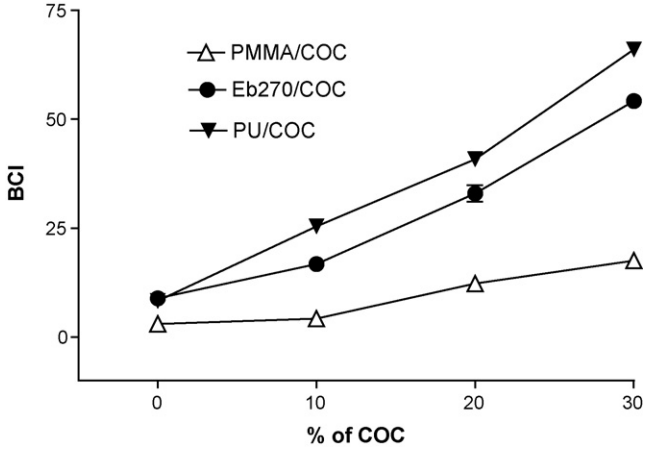

Fig. 9. Effects of COC content embedded in composite membranes on blood clotting index (BCI).

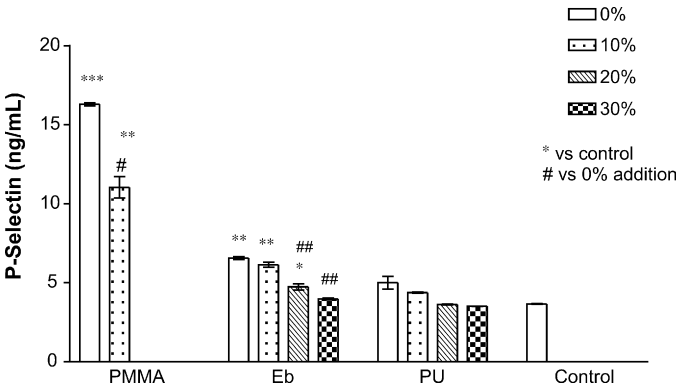

Fig. 10. Effects of COC content embedded in composite membranes on P-Selectin levels. Various ratios of COC to composite membranes (0, 10, 20 and 30%, w/w) were incubated with platelet-rich plasma (PRP). Statistics are shown for $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.005$, compared to control and $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared to the 0% addictive groups. The P-Selectin in PRP blood sample was taken as a control.

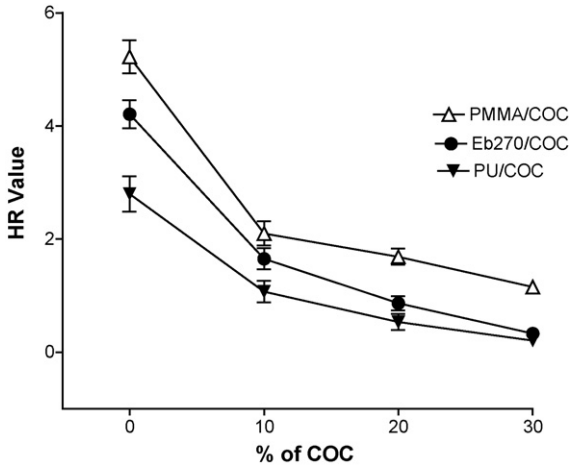

Fig. 11. Effects of COC content embedded in composite membranes on hemolysis ratio (HR) values.

improves biocompatibility with human blood. This result is clearly attributed to the presence of a liquid crystal phase on the surface of composite membranes that prevents the adhesion of platelets. Moreover, the addition of COC in PU or Eb270 composite membranes shows superior blood compatibility in comparison with PMMA composite membranes. This might be due to the formation of different surface textures on each polymer compound, as shown in SEM images.

3.4. Functional assay for activation of platelets: P-Selectin measurements

P-Selectin is a surface-component of platelet granules that appears when platelets are stimulated and activated (McEver et al., 1989). P-Selectin has a positive correlation with platelet count (Rand et al., 2003). Therefore, to directly quantify the antithrombotic effects mediated by platelets, P-Selectin measurements were performed to determine the level of platelet activation.

In Fig. 10, we demonstrate the production of P-Selectin in PRP blood samples that were incubated with three pure polymer films, as well as P-Selectin formation on composite membranes. In pure polymer films, incubation with PMMA resulted in formation of extensive amounts of P-Selectin. This suggests that there is substantial platelet activation, making PMMA the poorest performer in biocompatibility. Fifty-percent less P-Selectin was produced in incubation with Eb270 and PU, indicating that the biocompatibility of Eb270 is close to PU. Moreover, the P-Selectin level was decreased proportionally with embedding of COC contents, again showing that liquid crystal COC can be utilized to improve the compatibility of a material with human blood.

3.5. The hemolysis properties of polymer composite membranes

Good biocompatibility with human blood not only concerns platelet adhesion and activation, but also involves the hemolysis of red blood cells (RBC). The hemolysis ratio (HR) represents the extent of RBC broken by the sample in contact with blood. The greater the value of HR, the more broken RBC is. Therefore, a smaller HR value translates into increased blood compatibility of biomaterials. It is known that the HR value of acceptable biomaterials, required for medical applications, must be below 5% (Zhou and Yi, 1999). The effects of COC content on HR ratios and comparisons among the three composite membranes prepared (PU, Eb270 and PMMA) are shown in Fig. 11. This study definitively shows that the HR value decreases in three different composite membranes as COC content increases. Greater COC contents result in lower HR values. The relationship of HR values and biocompatibility of polymers can be expressed as: PU/COC > Eb270/COC > PMMA/COC.

4. Conclusion

In this work, an analysis of biocompatibility properties of polymeric materials to human blood is demonstrated and shows good correlation between qualitative and quantitative data. The in vitro studies also suggest that it may be reasonable to use liquid crystal COC as a mean of surface modification to improve the blood compatibility of polymers.

References

Aldenhoff, Y.B.J., Koole, L.H., 2003. Platelet adhesion studies on dipyridamole coated polyurethane surfaces. Eur. Cell. Mater. 5, 61–67.

Gorbet, M.B., Sefton, M.V., 2004. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. Biomaterials 25, 5681–5703.

Grunkemeier, J.M., Tsai, W.B., Horbett, T.A., 1998. Hemocompatibility of treated polystyrene substrates: contact activation, platelet adhesion, and procoagulant activity of adherent platelets. J. Biomed. Mater. Res. 41, 657–670.

Kim, Y.H., Han, D.K., Park, K.D., Kim, S.H., 2003. Enhanced blood compatibility of polymers grafted by sulfonated PEO via a negative cilia concept. Biomaterials 24, 2213–2223.

Lee, J.H., Oh, S.H., 2002. MMA/MPEOMA/VSA copolymer as a novel blood-compatible material: effect of PEO and negatively charged side chains on protein adsorption and platelet adhesion. J. Biomed. Mater. Res. 60, 44–52.

Lee, H.J., Hong, J.K., Goo, H.C., Lee, W.K., Park, K.D., Kim, S.H., Yoo, Y.M., Kim, Y.H., 2002. Improved blood compatibility and decreased VSMC proliferation of surface-modified metal grafted with sulfonated PEG or heparin. J. Biomater. Sci. Polym. Ed. 13, 939–952.

Lin, S.Y., Ho, C.J., Li, M.J., 2001. Precision and reproducibility of temperature response of a thermo-responsive membrane embedded by binary liquid crystals for drug delivery. J. Control. Release 73, 293–301.

Lin, S.Y., Lin, H.L., Li, M.J., 2002. Manufacturing factors affecting the drug delivery function of thermo-responsive membrane prepared by adsorption of binary liquid crystals. Eur. J. Pharm. Sci. 17, 153–160.

Makai, M., Csanyi, E., Nemeth, Z., Palinkas, J., Eros, I., 2003. Structure and drug release of lamellar liquid crystals containing glycerol. Int. J. Pharm. 256, 95–107.

McEver, R.P., Beckstead, J.H., Moore, K.L., Marshall-Carlson, L., Bainton, D.F., 1989. GMP-140, a platelet alpha granule protein, is also synthesized by vascular endothelium and is located in Weibel–Palade bosies. I. Clin. Invest. 84, 92–99.

Morimoto, N., Iwasaki, Y., Nakabayashi, N., Ishihara, K., 2002. Physical properties and blood compatibility of surface-modified segmented polyurethane by semi-interpenetrating polymer networks with a phospholipid polymer. Biomaterials 23, 4881–4887.

Nakabayashi, N., Williams, D.F., 2003. Preparation of non-thrombogenic materials using 2-methacryloyloxyethyl phosphorylcholine. Biomaterials 24, 2431–2435.

Nesseem, D.I., 2001. Formulation and evaluation of itraconazole via liquid crystal for topical delivery system. J. Pharm. Biomed. Anal. 26, 387–399.

Rand, M.L., Leung, R., Packham, M.A., 2003. Platelet function assays. Transfus. Apheresis Sci. 28, 307–317.

Skarja, G.A., Brash, J.L., 1997. Physicochemical properties and platelet interactions of segmented polyurethanes containing sulfonate groups in the hard segment. J. Biol. Mater. Res. 34, 439–455.

Spijker, H.T., Busscher, H.J., van Oeveren, W., 2002. Influence of abciximab on the adhesion of platelets on a shielded plasma gradient prepared on polyethylene. Thromb. Res. 108, 57–62.

Tedder, T.F., Steeber, D.A., Chen, A., Engel, P., 1995. The Selectins: vascular adhesion molecules. FASEB J. 9, 866–873.

Tu, L., Li, M., Mou, S., Zhou, C., 2001. Preparation and blood compatibility of polysiloxane/liquid-crystal composite membranes. Biomaterials 22, 2595–2599.

Yang, Z.M., Wang, L., Yuan, J., Shen, J., Lin, S.C., 2003. Synthetic studies on nonthrombogenic biomaterials 14: synthesis and characterization of poly(ether-urethane) bearing a Zwitterionic structure of phosphorylcholine on the surface. J. Biomater. Sci. Polym. Ed. 14, 707–718.

Zhao, C., Liu, X., Nomizu, M., Nishi, N., 2003. Blood compatible aspects of DNA-modified polysulfone membrane-protein adsorption and platelet adhesion. Biomaterials 24, 3747–3755.

Zhou, C., Yi, Z., 1999. Blood-compatibility of polyurethane/liquid crystal composite membranes. Biomaterials 20, 2093–2099.