

Note

SUPPRESSION OF CELL ADHESION ON POLYACRYLONITRILE-BASED MEMBRANES BY THE ANCHORING OF PHOSPHOLIPID MOIETIES*

Xiao-jun Huang^a, Xiao-dan Huang^b, Ai-fu Che^a, Zhi-kang Xu^{a**} and Ke Yao^b

^a*Institute of Polymer Science, Zhejiang University, Hangzhou 310027, China*

^b*Medical College, Zhejiang University, Hangzhou 310027, China*

Abstract In this work, the membrane surface of poly(acrylonitrile-co-2-hydroxyethyl methacrylate) (PANCHEMA) was chemically modified by anchoring of phospholipid moieties. The process involved the reaction of hydroxyl groups on the membrane surface with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) followed by the ring-opening reaction of COP with trimethylamine. Chemical differences between the original and the modified membranes were characterized by FT-IR and XPS. It was found that the amount of macrophage adhered on the modified membrane surface is substantially lower than that on polyacrylonitrile (PAN) and PANCHEMA membranes under the same condition. The morphological change of the adherent cell is also suppressed by the generation of phospholipid moieties on the membrane surface.

Keywords: Acrylonitrile copolymer; Membrane; Surface modification; Phospholipid moieties; Cell adhesion.

INTRODUCTION

PAN-based homopolymer and copolymers, which possess excellent membrane forming property and high mechanical and chemical stability, have been successfully used as membrane materials in the fields of water treatment, pervaporation, biochemical product purification and biomedical applications^[1–3]. However, the moderate hydrophilicity and relatively poor biocompatibility for this type of membrane limit its further applications in hemodialyzers. It is for this reason that much attention has been paid to the development of PAN-based membranes with improved biocompatibility or antifouling property or both in recent years^[3–8]. In our previous papers^[9–11], in order to prepare PAN-based membranes possessing excellent hydrophilicity and outstanding resistance to protein adsorption and cell adhesion, sugar moieties were incorporated into PAN by the water-phase precipitation copolymerization of α -allyl glucoside (AG) with acrylonitrile. It was found that the number of adhered macrophage could be significantly decreased by increasing the AG content in the copolymer. However, when the AG content in the copolymer exceeded 20%, the macrophage adhesion enhanced again due to the “cluster effect” of the sugar moieties. In this note, in order to create biomimetic surface on the PAN-based membrane and to avoid the specific interaction between the sugar moieties and the cells, as schematically described in Fig. 1, phospholipid moieties, which are another principal component of the natural biomembrane and have been widely recognized to possess remarkable biocompatible and biologically inert properties^[12–19], were chemically anchored on the membrane surface. Macrophage adhesion on these phospholipid-modified PAN copolymer (PMANCP) based membrane was preliminarily studied.

* This work was financially supported by the National Natural Science Foundation of China (No. 50273032).

** Correspond author: Zhi-kang Xu (徐志康), E-mail: xuzk@zju.edu.cn

Received June 13, 2005; Revised August 25, 2005; Accepted August 30, 2005

adherent cell density on the membrane surface was quantified on the basis of measurements obtained visually from at least five randomly selected fields using an Olympus TE300 phase contrast optical microscope.

RESULTS AND DISCUSSION

Figure 2 shows the FT-IR/ATR spectra of the studied membranes. It can be seen that the characteristic peak at 2243 cm^{-1} was due to the stretching vibration of cyano group ($-\text{C}\equiv\text{N}$). The bands of asymmetric and symmetric C—H stretching of the polymer main chain at $2850\text{--}2980\text{ cm}^{-1}$ and the C—H deformation vibration at 1452 cm^{-1} can also be found in all spectra. Compared with the FT-IR spectrum of PANCHEMA membrane, there were a series of new adsorption peaks at 1475 , 1244 , and 967 cm^{-1} in the spectrum of the PMANCP membrane, which were ascribed to the stretching vibrations of $-\text{CH}_3$, $-\text{O}-\text{P}-\text{O}-$ and $-\text{N}^+(\text{CH}_3)_3$ groups in the phospholipid moieties, respectively. To further verify the chemical structure of PANCHEMA and PMANCP, XPS spectra were measured from the membrane surface. In the case of the PANCHEMA membrane (Fig. 3), two strong peaks at 285 and 525 eV were observed, which were attributed to C_{1s} and O_{1s} , respectively. Another peak at 401 eV attributed to N_{1s} in the nitrile group was also obvious. For the PMANCP membrane, on the other hand, an additional peak was observed at 137 eV , which was ascribed to P_{2p} in the phospholipid moiety. All these results confirmed that the process described in Fig. 1 could be used to generate phospholipid moieties on the PAN-based membrane surface.

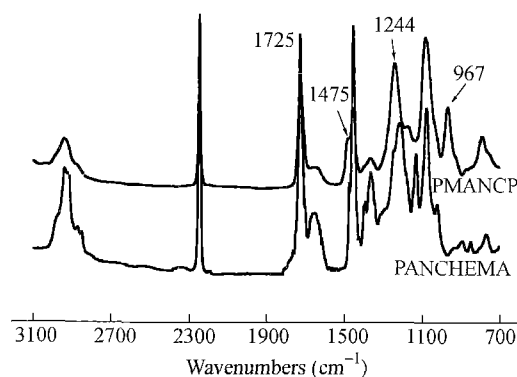


Fig. 2 IR spectra of PANCHEMA and PMANCP membranes

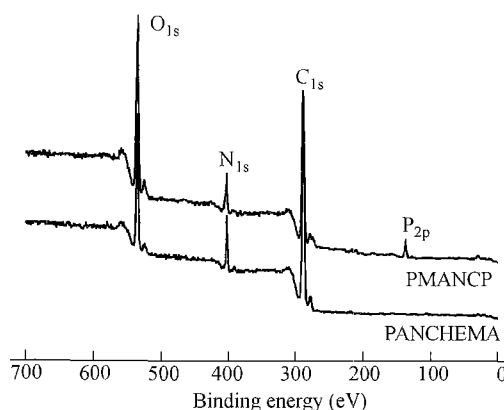


Fig. 3 XPS spectra of PANCHEMA and PMANCP membrane surface

The host-foreign-body response is a well-known interfacial phenomenon when polymeric materials come into contact with living organisms. A common component of this response is the presence of adherent macrophages, which fuse to form foreign-body giant cells (FBGCs) on the surface of the polymeric material. It has been shown that these adherent macrophages can cause biodegradation in the form of cracking and pitting through the release of bioactive agents^[22]. Based on this concept, an ideal biomaterial that is designed for blood-contacting applications should inhibit the adhesion, activation, and subsequent fusion of macrophages into FBGCs, thereby preventing the structural, mechanical, or functional failure of the biomaterial. Herein, Macrophage adhesion was measured to characterize the biocompatibility for the modified membranes and the typical results are shown in Fig. 4. It was clearly demonstrated that the number of macrophage adhered on all PMANCP membranes is significantly decreased as compared with that on the PAN and PANCHEMA membranes, which indicates that the phospholipid moieties on the membrane surface could induce the reduction of macrophage adhesion. This phenomenon is due to the phosphorylcholine group, a typical phospholipid polar group located at the outer cell membrane and could provide hydrated surface for the polymeric membrane in an aqueous medium^[12–15]. Thus, cell adsorption could be effectively inhibited and the conformation of cell does not change even when they are adsorbed on the membrane surface or contacted with the membrane surface. These results prove that the biocompatibility of the PAN-based membranes could be improved efficiently by anchoring phospholipid moieties on the membrane surface.

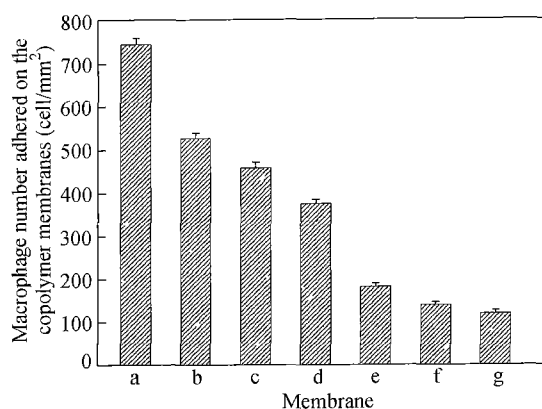


Fig. 4 Adhesion of macrophage on the PAN, PANCHEMA and PMANCP membrane surface
The HEMA mole fraction in the copolymer membrane is (a) 0%, (b) 6.4%, (c) 9.3% and (d) 17.8%; The mole fraction of phospholipid moiety on the copolymer membrane surface is (e) 6.09%, (f) 9.19% and (g) 17.1%.

CONCLUSION

Phospholipid moieties could be chemically anchored on the PAN-based membrane surface with the described process. Cell adhesion on the modified membrane was effectively suppressed.

REFERENCES

- Shinde, M.H., Kulkarni, S.S., Musale, D.A. and Joshi, S.G., *J. Membr. Sci.*, 1999, 162: 9
- Mandal, S. and Pangarkar, V.G., *J. Membr. Sci.*, 2002, 209: 53
- Lin, W.C., Liu, T.Y. and Yang, M.C., *Biomaterials*, 2004, 25: 1947
- Wenzel, A., Yanagishita, H., Kitamoto, D., Endo, A., Haraya, K., Nakane, T., Hanai, N., Matsuda, H., Koura, N., Kamusewitz, H. and Paul, D., *J. Membr. Sci.*, 2000, 179: 69
- Nie, F.Q., Xu, Z.K., Huang, X.J., Ye, P. and Wu, J., *Langmuir*, 2003, 19: 9889
- Nie, F.Q., Xu, Z.K., Yang, Q., Wu, J. and Wan, L.S., *J. Membr. Sci.*, 2004, 235: 147
- Nie, F.Q., Xu, Z.K., Ye, P., Wu, J. and Seta, P., *Polymer*, 2004, 45: 399
- Lavaud, S., Canivet, E., Wuillai, A., Maheut, H., Randoux, C., Bonnet, J.M., Renaux, J.L. and Chanard, J., *Nephrol. Dial. Transpl.*, 2003, 18: 2097
- Kou, R.Q., Qu, C., Xu, Z.K., Xu, Y.Y. and Ke, Y., *Chinese J. Polym. Sci.*, 2003, 21: 373
- Xu, Z.K., Kou, R.Q., Liu, Z.M., Nie, F.Q. and Xu, Y.Y., *Macromolecules*, 2003, 36: 2441
- Xu, Z.K., Yang, Q., Kou, R.Q., Wu, J. and Wang, J.Q., *J. Membr. Sci.*, 2004, 243: 195
- Ishihara, K., Nomura, H., Mihara, T., Kurita, K., Iwasaki, Y. and Nakabayashi, N., *J. Biomed. Mater. Res.*, 1998, 39: 323
- Iwasaki, Y., Sawada, S.I., Ishihara, K., Khang, G. and Lee, H.B., *Biomaterials*, 2002, 23: 3897
- Uchiyama, T., Watanabe, J. and Ishihara, K., *J. Membr. Sci.*, 2002, 208: 39
- Iwasaki, Y., Tojo, Y., Kurosaki, T. and Nakabayashi, N., *J. Biomed. Mater. Res. Part A*, 2003, 65A: 164
- Xu, J.M., Yuan, Y.L., Shan, B., Shen, J. and Lin, S.C., *Colloids Surf. B-Biointerface*, 2003, 30: 215
- Wang, L., Yang, Z.M., Chen, L., Xu, J.M., Shen, J. and Lin, S.C., *Chem. J. Chinese Univ.*, 2002, 23: 2393
- Yang, Z.M., Wang, L., Yuan, J., Shen, J. and Lin, S.C., *J. Biomater. Sci. Polym. Ed.*, 2003, 14: 707
- Sun, S.D., Yue, Y.L., Huang, X.H. and Meng, D.Y., *J. Membr. Sci.*, 2003, 222: 3
- Huang, X.J., Xu, Z.K., Wan, L.S., Wang, J.Q. and Wang, J.L., *Macromol. Biosci.*, 2005, 5: 322
- Huang, X.J., Xu, Z.K., Wang, J.Q., Wan, L.S. and Yang, Q., *Polym. Prepr.*, 2004, 45(2): 487
- Zhao, Q., Topham, N., Anderson, J.M. and Hiltner, A., Payet, C. R., *J. Biomed. Mater. Res.*, 1991, 25: 177