Polyether-Segmented Nylon Hemodialysis Membrane. V. Evaluation of Blood Compatibility of Polyether-Segmented Nylons

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ABSTRACT: The biocompatibility of poly(propylene oxide)-segmented nylon610 (PPO-Ny610), poly(ethylene oxide)-segmented nylon610 (PEO-Ny610), poly(ethylene oxide)-segmented nylonM10 (PEO-NyM10), and poly(ethylene oxide)-segmented nylon69/M10 (PEO-Ny69/M10) hollow fibers were investigated in terms of the transient leukopenia by the extracorporeal circulation in a rabbit. PPO-Ny610 and PEO-Ny610 hollow fibers showed that the minimum leukocyte counts during the circulations were $> 80\%$ against the initial count of leukocyte. These results indicate that these polymers have good blood compatibility. In PEO-NyM10 and PEO-Ny69/M10, the remarkable decreases of the leukocyte count were observed and the minimum counts were in the range of 45–50%. From the evaluation results of homo nylons (Ny610 and NyM10) hollow fibers, the low blood compatibilities observed in PEO-NyM10 and PEO-Ny69/M10 are not attributed to the chemical structure of the nylon blocks. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 1253–1257, 1998

Key words: polyether-segmented nylon; hollow fiber; blood compatibility; extracorporeal circulation; rabbit; transient leukopenia

INTRODUCTION

Recently, a special attention has been paid to synthetic hemodialysis membranes because of their good blood compatibility. However, the representative materials of the membranes are polysulfone, ethylene–vinylalcohol copolymer, polymethylmethacrylate, and polyacrylonitrile, which are produced for industrial use, and are not designed to improve the biocompatibility. We have already reported[1–4] the permeability characteristics and membrane morphologies of the membranes prepared with several kinds of polyether-segmented nylons (PE-Nys), some of which are known as nonthrombogenic materials. The polyether-segmented linear aliphatic nylons such as poly(propylene oxide)-segmented nylon610 (PPO-Ny610) and poly(ethylene oxide)-segmented nylon610 (PEO-Ny610) give the solid–liquid phase separation at the top surface of the membrane in the membrane formation process, and give skin layers composed of rigid crystalline spherulite due to their high crystallinity. This skin layer brings about the low permeability characteristics as a hemodialysis membrane.[1,2] To improve the solid–liquid phase separation, new polyether-segmented nylon, poly(ethylene oxide)-segmented nylonM10 (PEO-M10) and poly(ethylene oxide)-segmented nylon69/M10 (PEO-Ny69/M10) were synthesized, which had rather low crystallinity and dissolved in a common organic solvent such as dimethylsulfoxide.[3] PEO-Ny69/M10 gives the finger-like or sponge-like structures in the cross sections of the membranes and does not give the

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skin layer structure composed of spherulite at the top surface of the membranes. The membrane exhibits high permeability characteristics for urea and vitamin B_{12}.[4]

For the polyether-segmented nylons, PPO-Ny610, PEO-Ny610, PEO-NyM10, and PEO-Ny69/M10, the nonthrombogenecity in terms of platelet adhesion onto the polymer surfaces was investigated and the good results in *in vitro* test were obtained.[1–3] This article will report the investigation of the nonthrombogenicity of the polymers in terms of the *ex vivo* extracorporeal circulation in a rabbit model.

EXPERIMENTAL

Materials

The polyether-segmented nylons, PPO-Ny610, PEO-Ny610, PEO-NyM10, and PEO-Ny69/M10 were synthesized according to the methods described in the previous articles.[1–3] The abbreviations for these PE-Nys are as follows. PPO-Ny610 consists of 25 wt % of PPO ($M_n = 2000$) block and 75 wt % nylon 610 block. PEO-Ny610 consists of 15 wt % of PEO ($M_n = 2000$) block and 85 wt % of nylon610 block. PEO-NyM10 consists of 10 wt % PEO ($M_n = 2000$) block and 90 wt % of nylonM10 block. PEO-Ny69/M10 consists of 10 wt % PEO ($M_n = 2000$) block and 90 wt % of nylon block, where Ny69/M10 block is a random copolyamide prepared by the polycondensation of nylon69 salt and nylonM10 salt and the weight ratio of nylon69 salt to nylonM10 salt is 1 : 9. 6, 9, M, and 10 present hexamethylenediamine, azelaic acid, *m*-xylenediamine, and sebacic acid, respectively.

Preparation of Hollow Fibers

Hollow fibers of the PE-Nys were prepared by two methods. PPO-Ny610, PEO-Ny610, and PEO-NyM10 hollow fibers were prepared by melt compression (melt spinning). The hollow fibers of PEO-NyM10 and PEO-Ny69/M10 were prepared by wet spinning. The wet spinning method is as follows. The polymer was dissolved in formic acid at 80°C and the polymer solution was spun using water as a coagulant and then immersed in ultrapure water for 24 h to remove the solvent and to keep the membrane structures. The inner diameters of these hollow fibers were in the range of 185–260 μm. The hollow fibers of polysulfone and regenerated cellulose were obtained from commercialized hemodialysis membrane modules.

Ex Vivo Evaluation of Nonthrombogenicity

The nonthrombogenicity of these PE-Nys was evaluated by using their hollow fiber modules applying to extracorporeal circulation in a rabbit model. The module had 300 cm^2 surface area, and the effective length of the fiber was 12 cm. The outside of the hollow fiber in the module was filled with saline to prevent the permeation of blood. The rabbit used was a Japanese white rabbit weighing 2–3 kg. After cervical intubation and connection to extracorporeal circuit, the blood samples were taken from the outlet ports at 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min during the circulation. The changes of leukocyte counts were measured by the blood cell counter (Sysmex NE8000, Toa Iyou Denshi, Japan). Heparin or other anticoagulants were not used.

RESULTS AND DISCUSSION

It is well known that in hemodialysis treatments the significant transient leukopenia is observed when a regenerated cellulose membrane is used. The reason for this phenomenon has been explained by the acute modulation of phagocyte adhesion receptors mediated via membrane-induced complement activation leading to cell aggregation mainly in the pulmonary vasculature, and it has been established that the degree of the transient leukopenia correlates with the degree of the activation of complement.[5–9] Craddock et al.[10] reported that the transient leukopenia in peripheral blood occurring in the clinical hemodialysis treatment corresponds to the transient leukopenia observed in a rabbit. Hence, the transient leukopenia observed in the extracorporeal circulation in a rabbit model can be used as an index of the blood compatibility to evaluate the nonthrombogenicity of the dialysis membrane.

We have been investigating the new hemodialysis membranes by using various polyether-segmented nylons.[1–4] The transient leukopenia, the activation of the complement, is one of the most important items to develop the blood compatible membrane. Therefore, to clarify the blood compatibility of these polymer, the extracorporeal circulation in a rabbit was carried out by using the hollow fibers of these polymers. The polymers used were PPO-Ny610, PEO-Ny610, PEO-NyM10,

Table I Minimum Counts of Leukocyte in Melt Spinning Hollow Fibers

Polymer	Minimum Count (%)	Experiment Numbers
PPO-Ny610	89.2 ± 8.9	4
PEO-Ny610	85.0 ± 2.5	3
PEO-NyM10	47.1 ± 11.3	4
Polysulfone[a]	82.6 ± 8.7	4
Regenerated cellulose[a]	33.0 ± 3.5	3

[a] Modules were prepared by using the hollow fibers obtained from commercialized hemodialyzers.

and PEO-Ny69/M10, and the contents of the polyethers in the polymers were 25, 15, 10, and 10 wt %, respectively. In the previous articles,[2,4] it was reported that PPO-Ny610 and PEO-Ny610 membranes did not exhibit good permeability characteristics, but PEO-Ny69/M10 membrane showed good permeability characteristics. First of all, the extracorporeal circulations were carried out for PPO-Ny610, PEO-Ny610, and PEO-NyM10 hollow fibers prepared by melt spinning, where these PE-Nys had a homo nylon block to evaluate simply the influence of the polyether and nylon blocks on the blood compatibility. The results of the transient leukopenias are shown in Table I, where the minimum leukocyte counts based on the count at the start of the circulation are listed. The representative changes of the leukocyte counts during the extracorporeal circulation are shown in Figure 1, where the results of the PEO-NyM10 and PEO-Ny69/M10 fibers described below are shown together. The patterns of the changes of leukocyte counts for the other fibers (PEO-Ny610 and PEO-NyM10 fibers) are similar to the ones shown in Figure 1. The minimum counts of leukocyte for all the hollow fibers appear at 10–20 min after the start of the circulation and then the counts rebound to 160–200% after 120 min. The time of the minimum point and the rebound behavior of the leukocyte in the rabbit model agree with the behaviors of the transient leukopenia observed in clinical hemodialysis treatment.[10] The PPO-Ny610 and PEO-Ny610 hollow fibers have the minimum counts of leukocyte in the range of 85–89%, and these values are close to that observed in polysulfone membrane, which is a negative control. It is known that polysulfone dialyzer is one of the membranes that induce the least complement activation. In the case of the regenerated cellulose membrane (a positive control), which is known to bring about significant transient leukopenia in clinical use, the leukocyte count falls to 33%. These results suggest that PPO-Ny610 and PEO-Ny610 have good compatibilities for complement system and leukocyte and that the difference of chemical structures of polyether blocks does not affect the transient leukopenia. On the other hand, the PEO-NyM10 hollow fiber exhibits the remarkable leukopenia, and the minimum count is about 50%. This value is significantly lower than those of PPO-Ny610 and PEO-Ny610. The comparison of the result of PEO-Ny610 with the one of PEO-NyM10 predicts that the difference of chemical structure in the nylon blocks influences the blood compatibility because PEO does not affect the leukopenia as mentioned above.

Considering these results, PEO-Ny69/M10, which gives the membrane with the good permeability characteristics when it is prepared in phase inversion method,[4] has a possibility to bring about the significant transient leukopenia. Hence, the comparison of blood compatibilities of PEO-Ny69/M10 hollow fiber with that of PEO-NyM10 were carried out to investigate the effects of the composition of the nylon block. At the same time the effect of the spinning methods (melt or

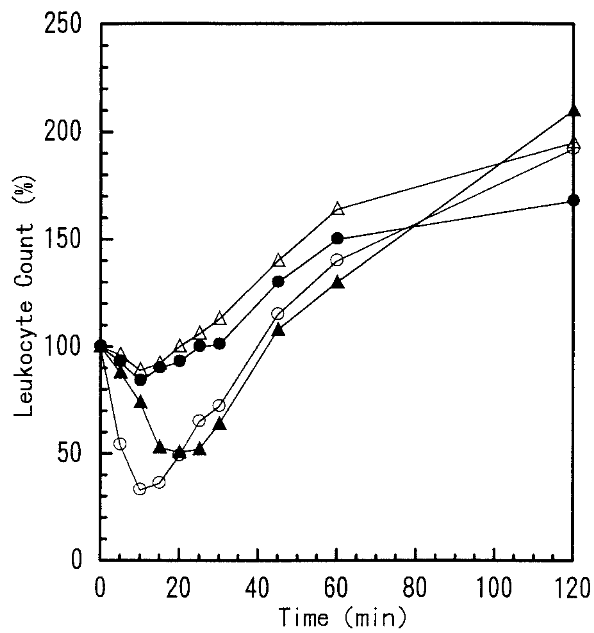

Figure 1 Representative changes of leukocyte counts during extracorporeal circulation in rabbit. (○) regenerated cellulose (commercialized membrane); (●) polysulfone (commercialized membrane); (△) PPO-Ny610 (melt spinning); (▲) PEO-Ny69/M10 (wet spinning). The leukocyte count (%) was based on the initial count at the start of the circulation and was the average in three or four experiments for each fiber.

wet) were investigated. The wet spinning method means the phase inversion process. The results of the transient leukopenia for the PEO-NyM10 and PEO-Ny69/M10 wet spinning hollow fibers are shown in Table II. The significant decreases of the leukocyte counts are observed in both hollow fibers, and the minimum values are in the range 47–51%, that is, both fibers have poor blood compatibility. The results of PEO-NyM10 hollow fibers shown in Tables I and II exhibit that the spinning method does not influence the transient leukopenia. From these results it is concluded that the poor blood compatibility is attributed to the original property of the block copolymers, PEO-NyM10 and PEO-Ny69/M10, and that PEO-Ny69/M10 is not suitable for the hemodialysis membrane material in terms of blood compatibility. The results that the PEO-Ny610 gives good compatibility while PEO-Ny69/M10 and PEO-NyM10 give poor compatibility seem to lead to the conclusion that the compositions of nylon block, NyM10 or Ny69/M10 blocks, cause the less blood compatibilities. To confirm this assumption, the hollow fibers prepared from the homo nylons, Ny610 and NyM10, by wet process were evaluated. The results are listed in Table III. The transient leukopenias are observed in both membranes, but the degrees are low and close to those of PPO-Ny610 and PEO-Ny610 hollow fibers. Consequently, it is concluded that the poor blood compatibility observed in PEO-NyM10 and PEO-Ny69/M10 hollow fibers will not be induced by the chemical structure of the nylon block. The reason for the low blood compatibility is now unclear. One of the reasons may be attributed to the PEO segment. Chaikof et al.[11] reported the effect of the PEO chain length in the interpenetrating networks by endlinking PEO to poly(glycidoxypropyl methyl-dimethyl siloxane) on the blood compatibility, and they concluded that only the network with PEO, the molecular weight = 2000, significantly activated the complement and the PEO with longer molecular chain did not cause the activation of complement. However, they did not give a clear reason for the activation by low molecular weight PEO. Now, further investigations to clarify the reason for the poor blood compatibility of PEO-Ny69/M10 membrane are now being carried out.

Table II Minimum Counts of Leukocyte in Wet Spinning Hollow Fibers

Polymer	Minimum Count (%)	Experiment Numbers
PEO-NyM10	49.4 ± 10.8	4
PEO-Ny69/M10	50.7 ± 6.6	3

Table III Minimum Counts of Leukocyte by Wet Spinning Hollow Fibers

Polymer	Minimum Count (%)	Experiment Numbers
Ny610	83.6 ± 2.0	4
NyM10	72.9 ± 6.1	3

CONCLUSION

The biocompatibility of PPO-Ny610, PEO-Ny610, PEO-M10, and PEO-Ny69/M10 hollow fibers were investigated in terms of the transient leukopenia by the extracorporeal circulation in a rabbit. PPO-Ny610 and PEO-Ny610 showed good compatibility for leukopenia and the minimum counts of the leukocyte are > 80% against the initial count of leukocyte. In PEO-NyM10 and PEO-Ny69/M10, the remarkable transient leukopenias were observed and the minimum counts are in the range of 45–50%. From the results of PPO-Ny610, PEO-Ny610, and PEO-NyM10, it is predicted that the PEO block does not affect the blood compatibility, and that nylon block causes the poor blood compatibility. However, the evaluation results of homo nylon (Ny610 and NyM10) hollow fibers conclude that the low blood compatibilities of PEO-NyM10 and PEO-Ny69/M10 are not attributed to the nylon block composition.

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