Artificial Organs
16(2):146–150, Blackwell Scientific Publications, Inc., Boston

Antithrombogenicity of Polyacrylonitrile–Polyethyleneoxide Hollow Fiber Membrane Developed for Designing an Antithrombogenic Continuous Ultrafiltration System

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Abstract: To develop a continuous arteriovenous hemofiltration (CAVH) system, which does not need systemic anticoagulation, for patients of acute renal failure having bleeding tendencies, a totally antithrombogenic continuous ultrafiltration system (ACUS) was designed, which consists of an antithrombogenic polyacrylonitrile–polyethyleneoxide (PAN–PEO) hollow fiber membrane and ionically heparin-bound catheter, tubing, and module header. Antithrombogenicity of PAN–PEO membrane, which occupies more than 90% of total inner surface area of ACUS, was considered to be due to highly concentrated PEO near the inner surface of the membrane and the finely dispersed (less than 500 Å) microstructure of the inner surface. ACUS was applied to 24 patients without systemic anticoagulation, and one filter worked for an average of 32 h without deteriorating their bleeding tendencies. Any significant changes in major parameters of biocompatibility during those treatments were not observed. More than 200 ml/h of ultrafiltrate was obtained even under very low mean blood pressure, less than 70 mm Hg. Based upon these results, ACUS was concluded to be suitable for mild and sustained treatment to control fluid and electrolyte balance in patients of acute renal failure with bleeding complications. **Key Words:** Antithrombogenicity—Biocompatibility—Hemofiltration—Heparinized surface–PAN–PEO membrane.

Continuous arteriovenous hemofiltration (CAVH), proposed by Kramer et al. (1), has been demonstrated to be one of the most effective and practical procedures for controlling fluid and electrolyte balance in patients with acute renal failure. However, continuous administration of an anticoagulant during long CAVH treatment is inherently risky, especially for patients with bleeding tendencies or complications. We attempted, therefore, to develop a totally antithrombogenic continuous ultrafiltration system (ACUS), which scarcely needs systemic anticoagulation (2,3). In this system, the hollow fiber membrane made with polyacrylonitrile–polyethyleneoxide (PAN–PEO) copolymer blended with PAN homopolymer, occupies more than 90% of its total inner surface area. The purpose of this paper is twofold: to clarify the mechanism of the antithrombogenicity of PAN–PEO membrane, and to evaluate its biocompatibility in clinical settings in details.

Received October 1991.
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This work was presented in part at the VIIIth World Congress of the International Society for Artificial Organs, held August 19–23, 1991, Montreal, Canada.
Dedicated to Dr. Willem J. Kolff.

MATERIALS AND METHODS

Designing of ACUS

Elementary technologies in the designing of ACUS were classified into the following two categories: selection of two antithrombogenic materials, PAN–PEO membrane and heparinized material; and manipulation of smooth blood flow in the extracorporal circuit, including introduction of a tangential

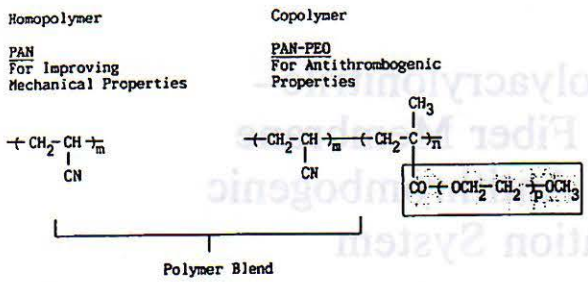

FIG. 1. Chemical structures and major functions of polymer materials that were used for preparing an antithrombogenic PAN–PEO hollow fiber membrane. The letters *m*, *n*, and *p* denote the unit numbers.

header, smooth connection of parts, and shallow and fixed insertion of catheters (2). As shown in Fig. 1, PAN–PEO hollow fiber membrane, which occupies more than 90% of the total inner surface area of ACUS, was prepared by blend spinning of two polymers, PAN–PEO copolymer and PAN homopolymer.

Morphological analysis of PAN–PEO membrane

The morphological structure of the inner surface of the membrane was studied by field emission scanning electron microscopy (FE-SEM), and the cross-sectional structure of the membrane stained with osmic acid was observed by transmission electron microscopy (TEM). PEO content at 50 Å from the inner surface of the membrane was measured by x-ray photoelectron spectroscopy (XPS). The distribution profile of PEO in the cross-section of the membrane was observed by secondary ion mass spectrometry (SIMS).

Evaluation in animal models

Animal experiments were performed with unheparinized beagle dogs (body weight around 10 kg) under intravenous pentobarbital sodium anesthesia. Connecting a system for evaluation between the femoral artery and vein, extracorporal circulation without a blood pump was attempted. The blood flow rate was monitored by using an ultrasonic flowmeter (T101: Transonic Systems Inc., U.S.A.).

Clinical evaluation

ACUS was applied to 24 patients, listed in Table 1, without systemic heparinization or a blood pump by connecting a total system between the femoral artery and vein, after obtaining the informed consent. A total of 36 PAN–PEO filter modules were used in those 24 patients.

Data analysis

All data were expressed as mean ± standard deviation (SD), unless otherwise stated, and statistical analysis was performed by Student's *t*-test.

RESULTS

Antithrombogenicity of PAN–PEO membrane

By changing the spinning conditions of the PAN–PEO hollow fiber membrane, various kinds of microstructures at the inner surface were made. Some typical cases are shown as scanning electron micrographs in the upper panel of Fig. 2. The relationship of the microstructure size thus measured by SEM with the antithrombogenicity of those membranes expressed in terms of extracorporal circulation time in the unheparinized dog model is shown in the lower left panel of Fig. 2. It is noted that the extracorporal circulation time was strikingly elongated once the microstructure size became less than 500 Å. Contrasting differences in the adhesion of platelets and other cellular components on the membranes in the dog experiments are shown in the scanning electron micrographs of lower magnification in the lower right panel of Fig. 2. Again, the microstructure size is demonstrated to be a key determinant and marker of antithrombogenicity.

In order to clarify the microstructure of the inner surface, the cross-section of the membrane was studied by TEM, and the result is shown in the upper right panel of Fig. 3. This micrograph demonstrates the presence of three kinds of regions: high contrast black PEO region stained with osmic acid, white PAN region, and the gray part considered to be pores by comparing it with the scanning electron micrograph of the cross-section of the membrane (not shown here). Note that PEO is aggregated near the inner surface. PEO concentration at the depth of 50 Å from the inner surface was analyzed by XPS,

TABLE 1. *Primary diseases of 24 patients with bleeding tendencies*

	Patients	Filters
Congestive heart failure	6	10
Cancer	5	7
Hematological neoplasma	3	4
Valvular heart disease	3	5
Liver cirrhosis	2	2
Abdominal aortic aneurysma	2	2
SLE	1	3
Cerebral bleeding	1	1
Trauma	1	2
Total	24	36

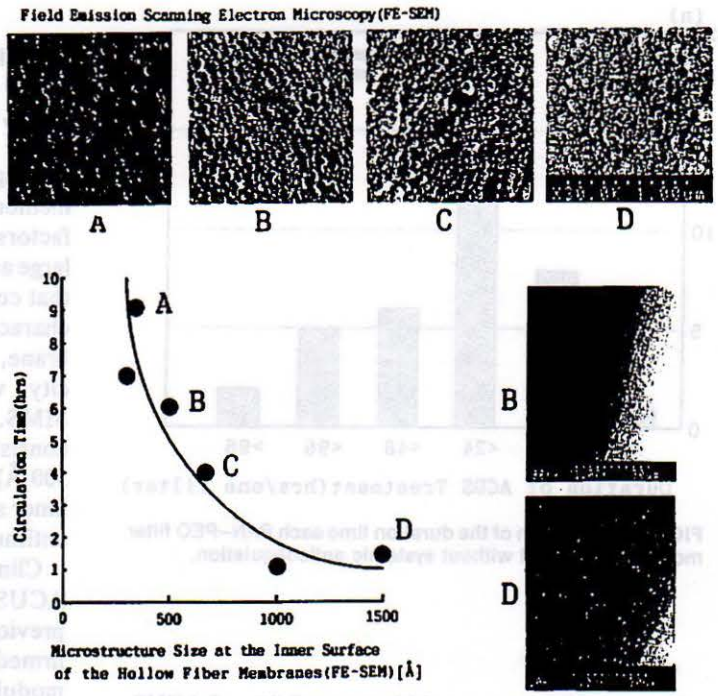

FIG. 2. Relationship between the surface microstructure of the membranes and their antithrombogenicity (see the text).

and the result is shown in the upper left panel of Fig. 3. Concentration of PEO at that point was 45%, which was much higher than the average concentration of 18% in the whole membrane. Furthermore, according to the depth profile of PEO content observed by SIMS, PEO concentration near the inner surface of the membrane made with PAN–PEO and PAN blend polymers was higher than that with only PAN–PEO copolymer, as shown in the lower panel of Fig. 3.

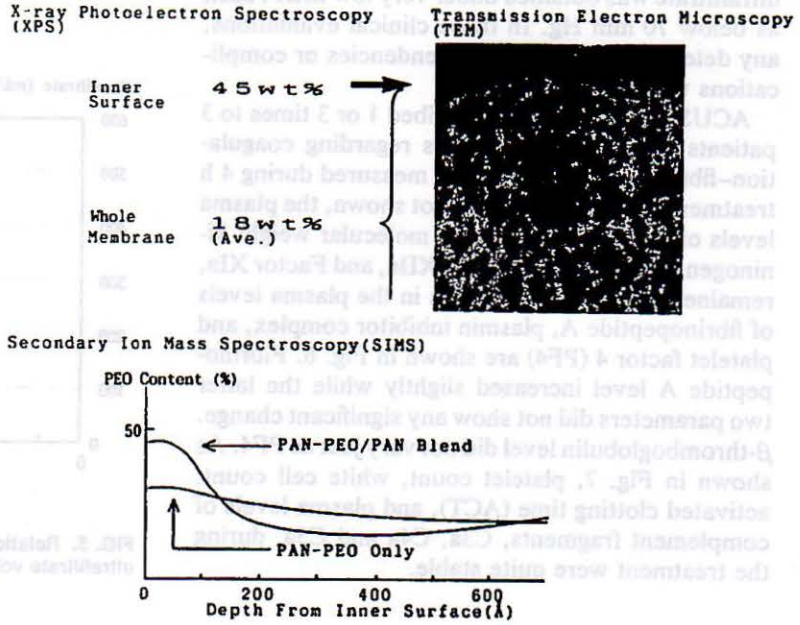

FIG. 3. Distribution of PEO in the membrane (see the text).

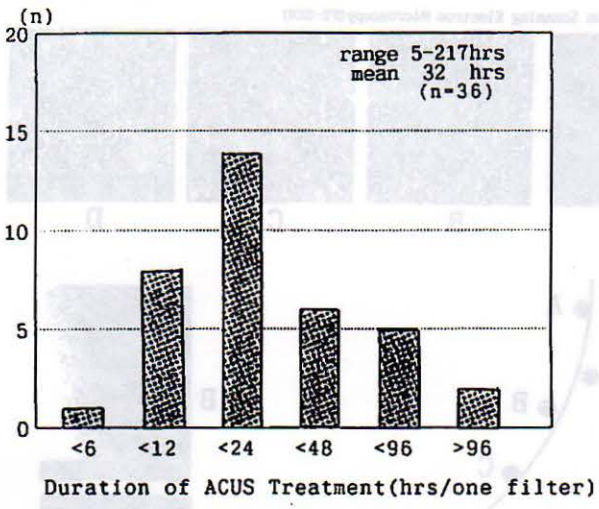

FIG. 4. Distribution of the duration time each PAN–PEO filter module functioned without systemic anticoagulation.

Clinical performance and biocompatibility of ACUS

Figure 4 illustrates how long each of the 36 PAN–PEO membrane modules functioned as a continuous hemofilter without systemic anticoagulation. The duration time of each module varied from 5 to 217 h, and the average value was 32 h. More than 75% of the total modules worked for more than 12 h. The relationship between the ultrafiltration rate (UFR) and the mean blood pressure (MBP) is shown in Fig. 5. It is noted that more than 200 ml/h of ultrafiltrate was obtained under very low MBP, such as below 70 mm Hg. In those clinical evaluations, any deterioration of bleeding tendencies or complications was not observed.

ACUS treatment was prescribed 1 or 3 times to 3 patients and major parameters regarding coagulation–fibrinolysis cascade were measured during 4 h treatment. Although data are not shown, the plasma levels of contact factors, high molecular weight kininogen, prekallikrein, Factor XIIa, and Factor XIa, remained unchanged. Changes in the plasma levels of fibrinopeptide A, plasmin inhibitor complex, and platelet factor 4 (PF4) are shown in Fig. 6. Fibrinopeptide A level increased slightly while the latter two parameters did not show any significant change. β-thromboglobulin level did not vary just as PF4. As shown in Fig. 7, platelet count, white cell count, activated clotting time (ACT), and plasma levels of complement fragments, C3a, C4a and C5a, during the treatment were quite stable.

DISCUSSION

As previously described (2,3), ACUS was designed and developed based upon two key technologies, that is, selection of two kinds of antithrombogenic materials and hemodynamic modification. Among them, the antithrombogenicity of PAN–PEO membrane is one of the most essential and critical factors because the surface area of the membrane is large and occupies the major part of the inner surface that comes in contact with blood. In this report, the characteristic morphological structure of the membrane, which showed excellent antithrombogenicity, was clarified using SEM, TEM, XPS, and SIMS. The data obtained through these observations consistently suggest that finely dispersed (less than 500 Å) microstructure of PEO aggregated near the inner surface contribute to the materialization of the antithrombogenicity of PAN–PEO membrane.

Clinical performance and biocompatibility of ACUS were widely evaluated in this study, and the previous preliminary observations (2,3) were confirmed. Although the duration time of each filter module varied, partly due to the very variable physical conditions of the patients to whom ACUS was applied, one module functioned for an average of 32 h, and the desired level of UFR was attained even under very low blood pressure. Furthermore, a wide range of parameters regarding biocompatibility were measured in this study. No anticoagulant was administered; nevertheless, coagulation, fibrinolysis and complement systems were hardly activated. Thus, safety of ACUS as well as its efficiency were further confirmed.

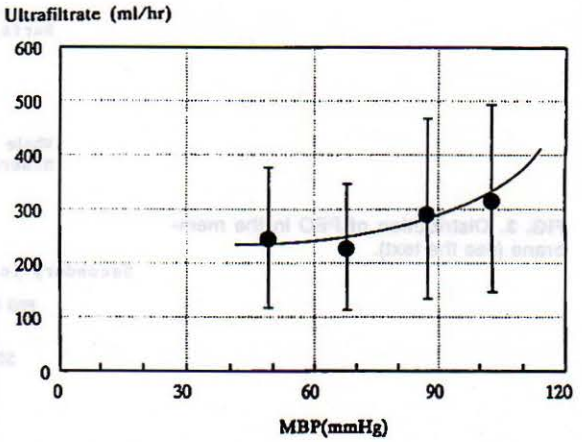

FIG. 5. Relationship between the mean blood pressure and ultrafiltrate volume obtained in clinical trials.

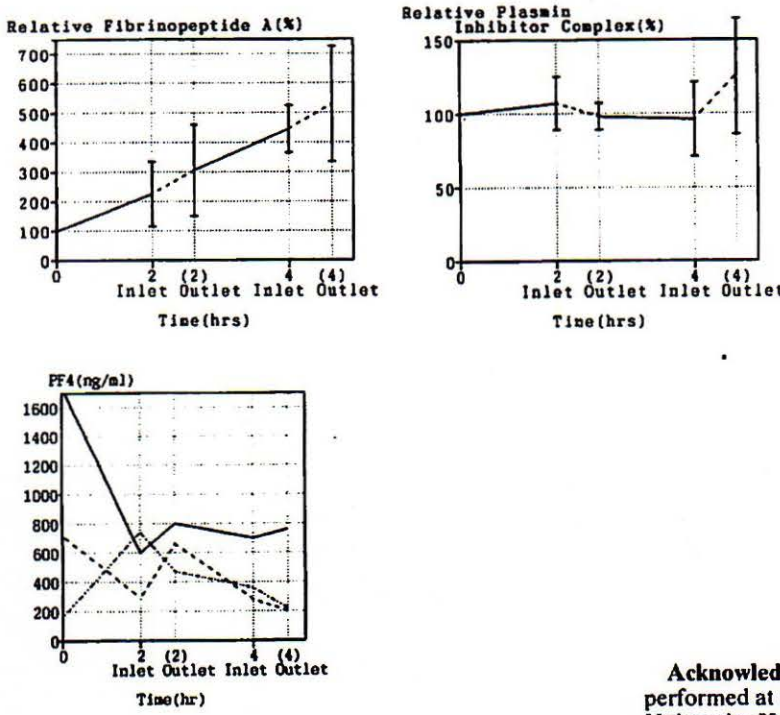

FIG. 6. Changes in three parameters regarding coagulation–fibrinolysis cascades in ACUS treatment.

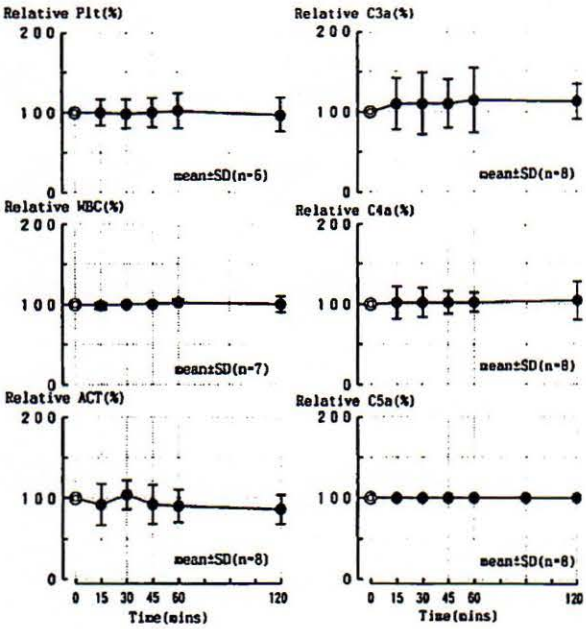

FIG. 7. Changes in major biocompatibility parameters during ACUS treatment.

Acknowledgments: Clinical evaluations of ACUS were performed at the following hospitals in addition to Niigata University Hospital. We acknowledge the contribution of medical staffs (principal investigator in the parentheses) of these hospitals:

Shinrakuen Hospital (Drs. Yoshihei Hirasawa and Masashi Suzuki); Niigatakenritu Shibata Hospital (Dr. Yoshiaki Miura); Kokuho Suibarago Hospital (Dr. Kazuya Kawada); Niigata Rinko General Hospital (Dr. Takashi Shimotori); Kido Hospital (Dr. Shogo Yada); Niigata Shimin Hospital (Dr. Masaaki Nakano); Kohseiren Sado General Hospital (Dr. Masanori Tajiri); Niigataken Kohseiren Sanjo General Hospital (Dr. Akira Kamimura); Nagaoka Red Cross Hospital (Dr. Shoji Miyamura); Chuetsu Clinic of Tachikawa General Hospital (Dr. Norio Obata); Niigataken Kohseiren Chuo General Hospital (Dr. Kazuo Kobayashi); Ojiya General Hospital (Dr. Toshiaki Ikeda); Niigataken Kohseiren Kariwagun General Hospital (Dr. Noriyuki Homma); Niigatakenritsu Chuo Hospital (Drs. Takao Yamakawa and Yuuichiro Maruyama); Shirone Kensei Hospital (Dr. Hiroyuki Kobayashi); Akita Red Cross Hospital (Dr. Tuyoshi Yamagishi).

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