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Original Article

Vitamin E-Coated Polysulfone Membrane-Based Hemodiafiltration Attenuates Inflammation in a Rat Model of Lipopolysaccharide-Induced Systemic Inflammation

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What's Known

- Many reports suggest that vitamin E-coated polysulfone dialyzers have antioxidant capacity and anti-inflammatory functions in dialysis therapy for chronic renal failure patients. However, no reports exist on the use of vitamin E-coated polysulfone dialyzers for hemodiafiltration (HDF) in patients with severe inflammatory disease.

What's New

- Relative to polysulfone membrane-based HDF, survival rates improved and inflammation was reduced early due to the antioxidant activity and early attenuation of inflammation associated with vitamin E-coated polysulfone membrane-based HDF.

Abstract

Background: Acute blood purification (ABP) therapy is used regularly in the clinical setting and reportedly alleviates organ failure associated with severe systemic inflammatory responses, leading to reduced mortality. The present study aimed to determine whether there is a difference in efficacy between polysulfone (PS) membranes, which are currently used regularly in the clinical setting, and vitamin E-coated polysulfone (VEPS) membranes, which are anticipated to exhibit the antioxidant and anti-inflammatory properties of vitamin E.

Methods: Male Wistar rats (n=15/group) were intravenously administered 10 mg/kg of lipopolysaccharide (LPS) to establish a systemic inflammatory response model. Six hours after LPS administration, hemodiafiltration (HDF) was performed for 30 minutes using a PS or VEPS membrane under general anesthesia. Blood was collected at various time points, lung tissue was evaluated histologically, and 24-hour survival was assessed.

Results: The rats in the VEPS group tended to have a higher survival rate than those in the PS group when undergoing HDF, although the difference was not significant. With respect to lung tissue, the inflammatory response was suppressed to a greater extent in the VEPS group than the PS group. Serum interleukin (IL)-6 levels were reduced at an early stage, plasma antioxidant activity was increased, and oxidative stress was reduced in the VEPS group compared to the PS group.

Conclusion: Relative to PS membrane-based HDF, the survival rate tended to improve and inflammation was subdued earlier due to the antioxidant activity and early attenuation of inflammation associated with VEPS membrane-based HDF.

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Keywords ● Vitamin E ● Inflammatory response ● Polysulfone ● Hemodiafiltration ● Rats

Introduction

Systemic inflammatory responses in intensive care settings lead to subsequent multiple organ failure, which can, in turn,

become uncontrollable and lead to death. Acute blood purification (ABP) therapy is associated with satisfactory outcomes for such severe conditions.[1] Several types of membranes currently exist for blood purification, and while these membranes are regularly used in this context, few studies have compared their efficacy.

While various factors such as sepsis may contribute to severe organ failure, recent studies point to the importance of oxidative stress and mediators such as cytokines. Excessive cytokine production is an important factor that can eventually lead to organ failure, and various studies have suggested the importance of controlling their levels.[2] Oxidative stress is also important, with some studies suggesting its involvement in the development of organ failure in patients with severe conditions.[3,4] Moreover, oxidative stress exacerbates inflammatory responses, and thus controlling oxidative stress during acute systemic inflammatory responses may be important.[5,6]

Numerous strategies such as treatment with pharmaceuticals have been reported as potential methods of controlling inflammatory responses and oxidative stress. Among these is vitamin E, which has anti-inflammatory and antioxidative effects.[7] Given its primary use in the nutritional context, vitamin E is a substance with established safety. This aspect is currently being examined closely, and vitamin E-coated blood purification hollow-fiber dialyzer membranes are under development.[8-10] Studies using these hollow-fiber dialyzer membranes have reported anti-inflammatory and antioxidative effects.[11-13] However, no study has addressed the efficacy of these columns for ABP.

This study aimed to assess the therapeutic efficacy and underlying mechanism of vitamin E-coated polysulfone (VEPS) membranes in ABP using a rat model of systemic inflammation, as well as clarify the utility of VEPS membranes in ABP in intensive care settings.

Materials and Methods

The current study was performed at the Department of Anesthesiology, Oita University Faculty of Medicine, Oita, Japan, between 2011 and 2013. Male Wistar rats (n=15/group) weighing 250 to 300 g (Kyudou, Saga, Japan) were used in all the experiments. The rats had unlimited access to food and water before and after treatment. This study was approved by the Ethics Committee of Animal Research of our institution. All the protocols conformed to the National Institutes of Health's guidelines, and the rats received humane care in compliance with the Principles of Laboratory Animal Care.

The rats were randomly assigned to 1 of 3 groups. 1) Control group: The rats received an intravenous injection of 0.9% saline solution (1 mL/kg) 6 hours prior to a sham operation under general anesthesia (4% sevoflurane) without hemodiafiltration (HDF). 2) Polysulfone (PS) group: The rats received an intravenous injection of lipopolysaccharide (LPS) (10 mg/kg body weight) 6 hours prior to the following. No fluids were administered to the rats prior to HDF. Before starting HDF, we secured the blood access line from the femoral artery and vein using a 24-G catheter under general anesthesia (4% sevoflurane). HDF was performed via a dialyzer using a PS membrane at the rates of QB 1 mL/min, QD 8 mL/h and QF 0-0.3 mL/min (mean=0.14 mL/min) for 30 minutes. After 30 minutes of HDF, the same volume as saline filtrate was supplied to the rats for fluid compensation. Total membrane surface and priming volume for these mini-module dialyzers were 50 cm^2 and 0.6 mL, respectively. 3) VEPS group: The rats received an intravenous injection of LPS (10 mg/kg body weight) 6 hours prior to the following. No fluids were administered to the rats prior to HDF. Before starting HDF, we secured the blood access line from the femoral artery and vein using a 24-G catheter under general anesthesia (4% sevoflurane). HDF was performed via a dialyzer using a VEPS membrane at the rates of QB 1 mL/min, QD 8 mL/h, and QF 0–0.3 mL/min (mean=0.13 mL/min) for 30 minutes. After 30 minutes of HDF, the same volume as saline filtrate was supplied to the rats for fluid compensation. Total membrane surface and priming volume for these mini-module dialyzers were 50 cm^2 and 0.6 mL, respectively.

Histologic Analysis

The animals were sacrificed 24 hours after LPS administration under pentobarbitone anesthesia; lungs were quickly removed and processed as indicated below. The tissues were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm thick sections. Lung tissue was stained with hematoxylin and eosin (HE).

Plasma Antioxidant Potential

Total plasma antioxidant potential (PAO) was evaluated with a commercially available PAO-U kit (Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd.) according to the manufacturer's instructions (n=7/group). The method employed by this kit is not affected by

uric acid levels. The colorimetric assay is based on the reduction of Cu^{2+} to Cu^{+} to form a stable Cu^{+}–bathocuproine complex, which absorbs light at 480 to 490 nm. PAO was estimated from a standard curve generated from samples of known uric acid concentrations. The sensitivity of this assay was 22 mM, intra-assay variability was 3%, and inter-assay variability was 5%.

Measurement of Interleukin (IL)-1β, IL-6, Tumor Necrosis Factor (TNF)-α, Interferon (INF)-γ, and Macrophage Inflammatory Protein (MIP)-3α

Levels of cytokines and chemokines in blood serum (n=9/group) and filtrate (n=6/group) were measured before, after, and 24 hours after therapeutic treatments using commercially available Bio-Plex kits (Research & Development, California, USA), in accordance with the manufacturer's recommendations.

Statistical Analysis

Comparisons between 2 independent groups were performed with the Mann–Whitney *U*-test and the log-rank test using Mini StatMate (ATMS CO., LTD. Tokyo, Japan). The continuous data are expressed as means±SEs. $P<0.05$ was considered significant.

Results

Survival Rate after Hemodiafiltration in the Polysulfone and Vitamin E-Coated Polysulfone Groups

Figure 1 shows the survival rate after HDF in the PS and VEPS groups after LPS administration. While no difference in the survival rate was observed between the 2 groups at 15 hours after LPS administration, the survival rate tended to be lower in the PS group after 24 hours, although the difference between the groups was not significant.

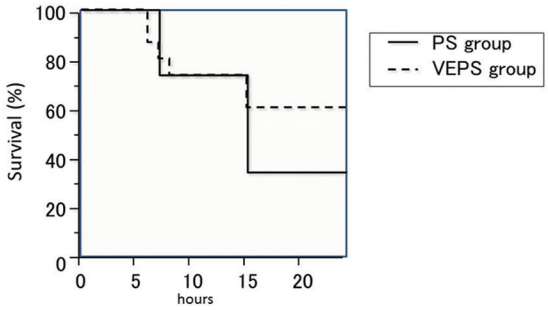

Figure 1: Survival after lipopolysaccharide (LPS) administration. The Kaplan–Meier method was used to assess survival for 24 hours after LPS administration. The log-rank test was used to compare the 2 groups (n=15/group).

Pulmonary Histologic Findings after Hemodiafiltration in the Polysulfone and Vitamin E-Coated Polysulfone Groups

Figure 2 shows HE-stained lung tissue sections following HDF using the PS or VEPS membrane after LPS administration. Compared to the control group (figures 2A and 2B), the PS group (figures 2C and 2D) showed evidence of acute lung injury, as reflected by alveolar edema and inflammatory cell infiltration. Meanwhile, acute lung injury was less frequent in the VEPS group than in the PS group (figures 2E and 2F).

Evaluation of Antioxidant Potential in Plasma and Lung Tissue after Hemodiafiltration in the Polysulfone and Vitamin E-Coated Polysulfone Groups

We used an antioxidant potential measurement kit, which takes advantage of the reduction of copper ions ($Cu^{2+} \Rightarrow Cu^{+}$), to assess changes in antioxidant potential in plasma and lung tissue in the PS and VEPS groups. PAO was higher immediately after HDF in the VEPS group than in the PS group (figure 3A). Antioxidant potential was also higher in the lung tissue of the VEPS group than that in the PS group 24 hours after LPS administration (figure 3B).

Changes in Levels of Serum Cytokines after Hemodiafiltration in the Polysulfone and Vitamin E-Coated Polysulfone Groups

We measured the serum levels of the following 5 inflammatory cytokines and chemokines: interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and macrophage inflammatory protein (MIP)-3α. While the IL-6, TNF-α, and MIP-3α levels increased immediately after HDF in the PS group, they significantly decreased in the VEPS group. Moreover, although not significant, the IL-1β and IFN-γ levels showed a decreasing trend in the VEPS group (figures 4A–E).

Filtrate Levels of Cytokines in the Polysulfone and Vitamin E-Coated Polysulfone Groups

We measured the filtrate levels of the inflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α, IFN-γ, and MIP-3α) during HDF. The measured levels were multiplied by the amount of the filtrate to calculate the total amount eliminated. The elimination of IFN-γ was significantly greater in the PS group than in the VEPS group, and the amount eliminated tended to be greater in the PS group for the other cytokines as well (figures 5A–E).

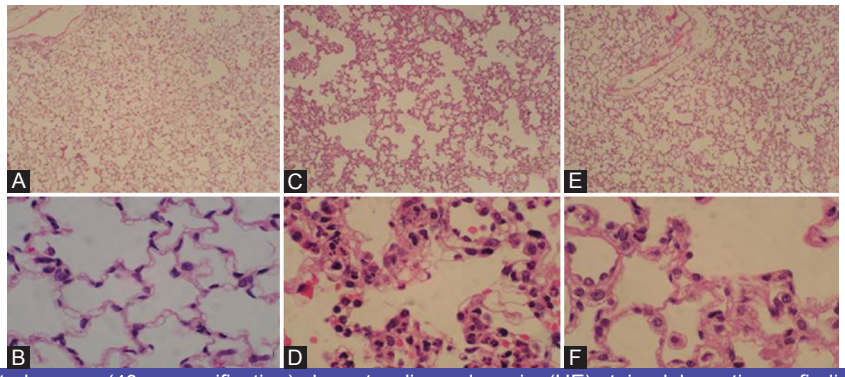

Figure 2: (A) Control group (40× magnification); hematoxylin and eosin (HE)-stained lung tissue findings 24 hours after lipopolysaccharide (LPS) administration. (B) Control group (400× magnification); HE-stained lung tissue findings 24 hours after LPS administration. (C) Polysulfone (PS) group (40× magnification); HE-stained lung tissue findings 24 hours after LPS administration. (D) PS group (400× magnification); HE-stained lung tissue findings 24 hours after LPS administration. (E) Vitamin E-coated polysulfone (VEPS) group (40X magnification); HE-stained lung tissue findings 24 hours after LPS administration. (F) VEPS group (400× magnification); HE-stained lung tissue findings 24 hours after LPS administration.

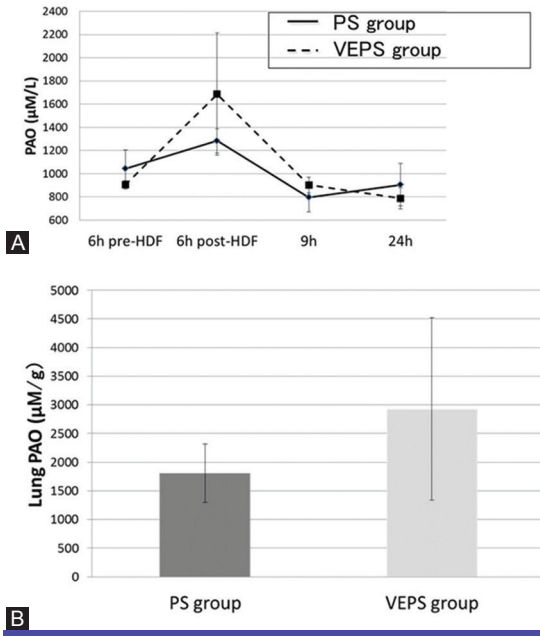

Figure 3: (A) Changes in plasma antioxidant potential (PAO) in the lung tissue. (B) Changes in PAO in the lung tissue after lipopolysaccharide (LPS) administration. All the data are expressed as means±SEs. The Mann–Whitney *U*-test was used to compare the 2 groups (n=7/group). (n=9/group).

Discussion

Compared to PS membranes, which are conventionally used for ABP in intensive care settings, VEPS membranes exhibited superior anti-inflammatory and antioxidative effects, and thus may improve survival rate.

ABP is currently used as an effective therapeutic measure against severe organ failure in intensive care settings.[1,14] While many types of membranes are used in ABP, only a few studies have compared them for efficacy based on membrane material. Our findings suggest the possibility that the use of VEPS membranes in HDF contributed to an improved survival rate and reduced organ failure compared to PS membranes following severe systemic inflammatory responses. Thus, the use of VEPS membranes in ABP may be advantageous when treating severe conditions.

A major difference between the PS and VEPS membranes used in this study is that the VEPS membrane surface is covered with vitamin E. As reported by numerous studies, vitamin E possesses anti-inflammatory and antioxidative effects[7] and the antioxidant capacity of the vitamin E-coated dialyzer membrane is significantly higher than that of the PS membrane.[15] In the present study as well, a decrease in antioxidant potential was not observed in the VEPS group, particularly after HDF, and antioxidant potential was maintained even in serum. We also found that relative to the PS group, the VEPS group had a high antioxidant potential, not only in plasma but in the lung tissue as well. Repeated contact between patient blood and extracorporeal circulation materials causes the formation of oxidative stress biomarkers.[15] However, some studies have shown that columns coated with vitamin E exhibit the same properties as vitamin E.[11,12] Thus, the antioxidant potential of the VEPS group was comparable to that of other dialyzer membranes.

Systemic inflammation is also known to increase oxidative stress.[16] The present study found that the VEPS group had significantly decreased inflammatory responses (e.g., cytokines and chemokines) relative to the PS group. The radical scavenger vitamin E has been used to neutralize harmful reactive species and to mimic the lipid structures of blood cell plasmalemma and lipoprotein particles when used in the vitamin E-coated dialyzer membrane.[9,10] Indeed, the inhibition of various

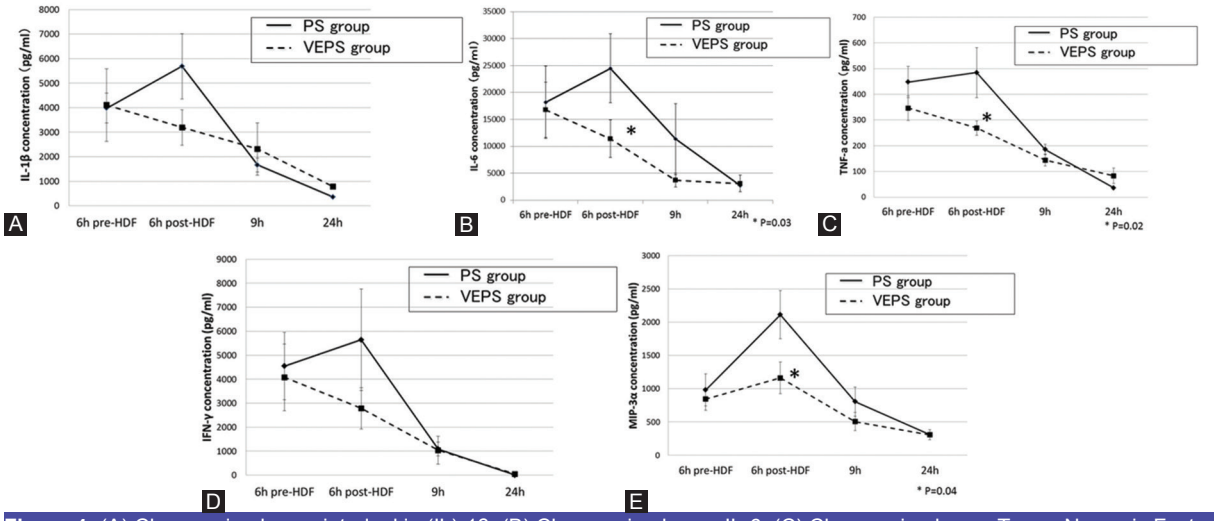

Figure 4: (A) Changes in plasma interleukin (IL)-1β. (B) Changes in plasma IL-6. (C) Changes in plasma Tumor Necrosis Factor (TNF)-α. (D) Changes in plasma interferon (IFN)-γ. (E) Changes in plasma macrophage inflammatory protein (MIP)-3α. All the data are expressed as means±SEs. The Mann–Whitney *U*-test was used to compare the 2 groups (n=9/group).

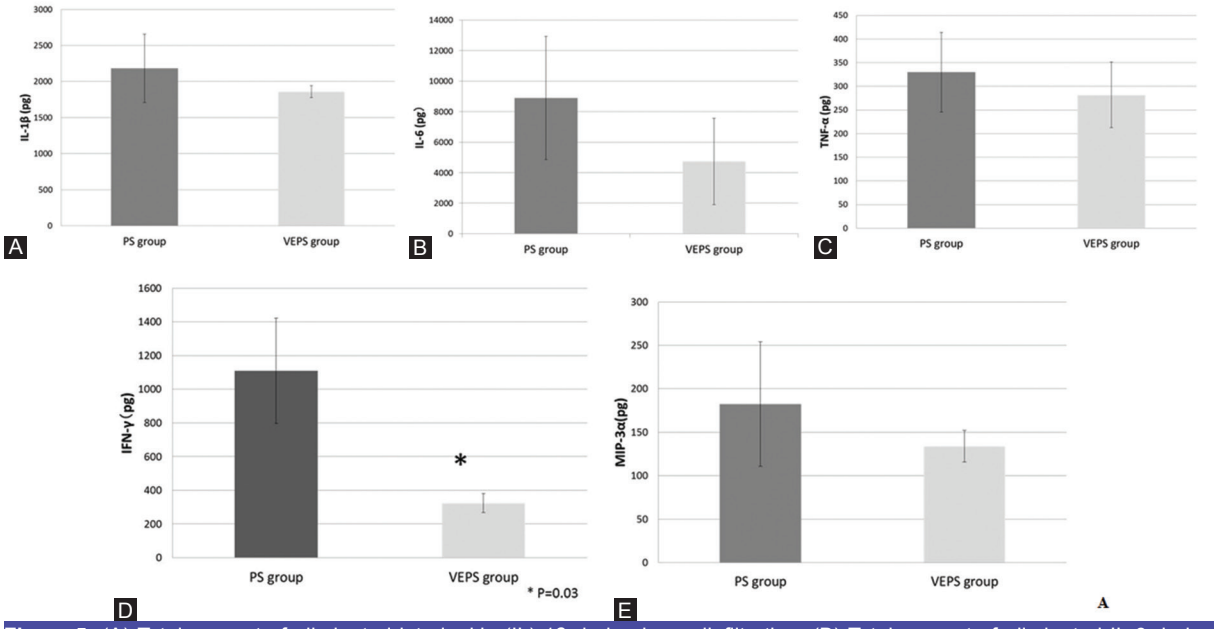

Figure 5: (A) Total amount of eliminated interleukin (IL)-1β during hemodiafiltration. (B) Total amount of eliminated IL-6 during hemodiafiltration. (C) Total amount of eliminated Tumor Necrosis Factor (TNF)-α during hemodiafiltration (HDF). (D) Total amount of eliminated interferon (IFN)-γ during HDF. (E) Total amount of eliminated MIP-3α during hemodiafiltration. All the data are expressed as means ± SEs. The Mann–Whitney *U*-test was used to compare the 2 groups (n=6/group).

inflammatory mediators such as IL-6 was observed in the VEPS group. These effects may be associated with the decreased inflammatory response. In addition, these effects may have collectively led to the attenuation of organ failure and increased survival.

Compared to the PS group, the total amount of cytokines that were eliminated was small in the VEPS group. One possibility is that the pores of the VEPS membrane became smaller due to the vitamin E coating, which led to reduced elimination of cytokines. Previous studies have shown that cytokine elimination plays an important role in improvements in organ failure resulting from ABP.[17,18] Yet, in the present study, we achieved satisfactory outcomes in the VEPS group, which showed less cytokine elimination than the PS group. The fact that the inhibition of oxidative stress leads to reduced inflammatory responses and organ failure raises the possibility that the inhibition of oxidative stress by HDF is also very important.[19,20] On the other hand, IFN-γ was significantly lower in the VEPS group. Some studies have suggested that vitamin E inhibits the production of IFN-γ.[21] Accordingly, the significantly decreased production of IFN-γ

in the VEPS group may have been due to the effects of vitamin E.

Chemokine induction after HDF was inhibited to a greater degree in the VEPS group than in the PS group, and the inflammatory response was subdued earlier in the VEPS group as well. A previous study demonstrated that inhibiting MIP-3α in a mouse model of sepsis increased survival.[22] Thus, the attenuation of organ failure and increased survival might be attributed to the inhibition of oxidative stress in the VEPS group.

With respect to cytokines, IL-1β and TNF-α are secreted from macrophages, and the excessive secretion of these cytokines is associated with the exacerbation of organ failure.[23,24] The inhibition of these cytokines with the VEPS membrane may be related to the effectiveness of this membrane during acute organ failure associated with inflammation. Another cytokine, IFN-γ, is required for systemic inflammatory responses,[25] and inhibiting its excessive production during severe systemic inflammatory responses (e.g., sepsis) leads to improved survival.[26] Accordingly, the efficacy of the VEPS membrane might be related to its ability to inhibit IFN-γ production. In addition, IL-6 has been reported to be an important prognostic factor for sepsis,[27,28] and in the present study, the IL-6 levels were lower in the VEPS group than in the PS group, suggesting that the VEPS membrane may be useful for treating sepsis.

This study has a number of limitations worth noting. First, a rat model was used, and thus actual clinical situations may not be accurately reflected. Second, the duration of HDF was short at 30 minutes. Accordingly, the full effects of long duration or continuous HDF may not have been reflected in the experiments. Finally, we only assessed the inflammatory response resulting from 1 round of LPS administration, which is possibly different from severe organ failure observed in actual clinical settings. While we were able to demonstrate the utility of the VEPS membrane via its anti-inflammatory and antioxidative effects in the systemic inflammatory response context, it will be necessary in the future to demonstrate the utility and efficacy of this membrane during ABP in humans.

Conclusion

This study is the 1st of its kind to report differences in the effects of PS and VEPS, which are 2 different types of PS-based hemodialyzer membranes used in ABP, on the inflammatory burden generated in an in vivo animal model of sepsis. Our findings suggest that when used during HDF, the VEPS membrane may serve as a more effective treatment than existing PS membranes in controlling the main events of septic comorbidities such as severe inflammation and multiple organ failure.

Conflict of Interest: Takaaki Kitano received research funding from ASAHI KASEI MEDICAL CO., LTD. (Tokyo, Japan). Yoshihiro Hatanaka and Satoru Inoue are employed by ASAHI KASEI MEDICAL CO., LTD. The remaining authors have no conflict of interest to declare.

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