Evaluation of the Biocompatibility of Dialysis Membranes

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Key Words

Biocompatibility · Dialysis membrane · Platelet activation · Membrane surface property · In vitro Evaluation

Abstract

Background: Improvements in the biocompatibility of dialysis membranes have reduced biological responses elicited by blood–membrane interactions. In this article, recent technological developments in dialysis membranes with regard to biocompatibility and recent progress in the evaluation of the biocompatibility of dialysis membranes are reviewed.

Summary: The focus of investigation into dialysis membranes in recent years has focused on not only membrane materials, but also their surface textures, which have been changed, for example, by coating with vitamin E or by changing the amount and type of hydrophilizing agents used. Research and development is directed at altering the chemical and physical properties of membrane surfaces to suppress biological responses that are particularly elicited as a result of platelet activation. To develop membranes with excellent biocompatibility, biocompatibility should be evaluated on a like-for-like basis under conditions that are similar to those in clinical settings. Evaluation using actual dialyzers can be performed using porcine blood, platelet-rich plasma isolated from porcine blood (and platelet-rich plasma with leukocytes), or suspension of neutrophils isolated from porcine blood or cultured human monocytes. **Key Messages:** Highly biocompatible dialysis membranes can be developed when the overall correlations among biological reactions are ex-

amined by integrating all data on biological responses elicited by blood–membrane interactions or mutual interactions among blood cells.

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Background

Various biological reactions in the body occur during hemodialysis, including those induced by exposure to membrane materials, removal of solute, exposure to dialysate components, or contamination of the dialysate [1]. These biological responses not only influence each other, but are also altered by blood flow conditions and filtration rate as well as the amount of dialysate flow into the blood. Accordingly, biological responses are affected by various factors during hemodialysis. To evaluate membrane biocompatibility, individual responses and their interactions should be evaluated.

In this article, we review the biocompatibility of dialysis membranes with a focus on biological responses elicited by membrane materials. First, we describe previously known biological responses elicited when the blood is exposed to dialysis membranes. In hemodialysis, the dialysis membrane is essentially the site where the blood has substantial contact with non-biological material, so it is where various biological responses are elicited. The material of the dialysis membrane is the primary determinant of biological responses. We then review recent technological developments in dialysis membranes. In addition

to membrane materials, changes in membrane surface textures, such as vitamin E coating on dialysis membranes and changing the amount and type of hydrophilizing agents, have been the focus of recent studies as factors that influence biological responses. Lastly, we review the experimental techniques that are used to evaluate the biocompatibility of dialysis membranes *in vitro*. To acquire the information needed to develop highly biocompatible membranes, it is necessary to evaluate membrane biocompatibility on a like-for-like basis under conditions that are similar to those in clinical settings. Therefore, we also introduce the evaluation methods, including those currently under investigation, used by our group.

Biological Responses Elicited by Blood–Membrane Interactions

Many studies, mostly clinical ones, have investigated the biocompatibility of dialysis membranes [1]. Because regenerated cellulose (RC) was the mainstream membrane material in the past, the mechanisms underlying phenomena that occurred while using the RC membrane, such as a temporary decrease in leukocyte count in the peripheral blood at the start of hemodialysis and downstream biological reactions involving the alternative pathway of the complement activation, have been elucidated [1]. Because the complement system is activated by the interaction with the -OH groups in the cellulose, the -OH groups were modified using acetic acid to develop cellulose triacetate (CTA). In addition, different synthetic polymers were developed for dialysis membranes that are currently in use. These synthetic polymers included the following: polysulfone (PS), polyether sulfone (PES), and polyester polymer alloy (PEPA), all of which use polyvinylpyrrolidone (PVP) as a hydrophilizing agent; polymethylmethacrylate (PMMA); ethylene-vinylalcohol copolymer; and acrylonitrile and sodium methallylsulfonate copolymer (AN69).

Some membranes made from synthetic polymers are negatively charged and may therefore activate the complement system. However, the complement components C3a and C5a [2], inflammatory mediators, and the complement factor D [3], a 24-kilodalton molecule needed for complement activation, are adsorbed by these membranes, thereby minimizing biological reactions triggered by complement activation. In addition, cytokines and the complement factor D are efficiently removed using highly permeable membranes or a high efficiency treatment mode such as hemodiafiltration, which also suppress some biological responses resulting from blood–membrane interactions.

Consequently, many types of synthetic polymeric membranes have a low incidence of bioincompatible responses.

A well-known example of bioincompatible responses elicited by negatively charged membranes is the response that begins with coagulation factor XII activation and subsequently produces bradykinin [4]. Because bradykinin is broken down by angiotensin-converting enzyme (ACE), negatively charged membranes may cause anaphylactic-like shock in patients taking ACE inhibitors, due to the accumulation of bradykinin [5]. Therefore, dialysis treatment with AN69 membranes is contraindicated for patients using ACE inhibitors.

Blood–membrane interactions also directly activate blood cells such as leukocytes, platelets, and red blood cells [1] or indirectly activate them through the pathway that activates the complement system or coagulation factors, etc. Furthermore, blood cells regulate each other through activation and/or inhibition. For example, platelets and leukocytes directly [6, 7] or indirectly [8, 9] activate or inhibit each other. Platelets activated upon exposure to a dialysis membrane adhere to and aggregate on the membrane as well as form clots. Activated platelets also bind to leukocytes, thereby activating the leukocytes [10, 11]. On the other hand, reactive oxygen species released from activated leukocytes also activate platelets [12]. In hemodialysis, blood coagulation pathways are suppressed due to the use of anticoagulants during the treatment, which cannot inhibit the activation of platelets. Since platelet activation induces the activation and inhibition of various blood cells and biological reactions, the suppression of platelet activation is the key target to improving membrane biocompatibility.

When evaluating membrane biocompatibility, it is necessary to develop an experimental model that reveals the effect of the membrane on individual blood cells and their interactions, and facilitates understanding of the overall correlation between biological reactions. However, because this cannot be achieved by a single experimental model, the evaluation performed by combining several experimental models will be a good option.

Newly Developed Dialysis and Filtration Membranes

Membranes with improved permeability and biocompatibility have been developed over the past few decades. The membranes commercialized in recent years still use the materials that have been in use for many years, but the blood–contact surface of some membranes have been modified. In particular, surface modifications that are in-

tended to suppress platelet activation and subsequent biological responses are the current trends in membrane development.

Membranes made from materials containing the sulfonyl group (PS, PES, and PEPA) are most common today because it is easy to manipulate the pore size of these membranes and thus generate highly permeable membranes. However, because these membranes are basically hydrophobic, PVP is needed to make the membranes hydrophilic. In these membranes, the internal surface of hollow fiber membranes, where PVP is present, was further modified to improve their biocompatibility. For example, vitamin E-coated PS membranes (VPS; Asahi Kasei Medical Co., Ltd., Japan) [13] reduce oxidative stress [14, 15], whereas dialysis membranes with both the novel hydrophilic NV polymer (Toraylight™ NV; Toray Medical Co., Ltd., Japan) and PVP reduce the adhesion of platelets [16]. For continuous renal replacement therapy, filtration membranes with reduced platelet adhesion and fouling have been developed by altering the length and amount of the PVP used [17].

PMMA-based dialysis membranes are developed by controlling the stereocomplex structures that are formed from a mixture of syndiotactic PMMA and isotactic PMMA polymers, and they are known for their low cell-adhesive and blood-coagulant properties [18]. Particularly, the modification of the surface structure led to the development and commercialization of new PMMA membranes with low adhesion of platelets and the coagulation protein fibrinogen (Filtrizer NF™; Toray Medical Co., Ltd.) [19].

The CTA-based membrane with less fouling (ATA™ membrane; Nipro Co., Japan) was also developed. Conventional CTA membrane has a homogeneous structure, but this newly developed membrane has an asymmetric structure and introduces a dense layer over the internal surface of the hollow fiber membranes. CTA membranes are characterized by their low protein adsorption capacity, but by making the internal surface of the membrane denser, less fouling of the CTA membranes appears to have been improved further. Few studies to date have investigated its biocompatibility because the membrane was commercialized only recently, but the change in the internal surface structure has probably improved biocompatibility.

The AN69-ST membrane (Baxter Ltd., Japan) was developed by treating the AN69 membrane with positively charged polyethylenimine to neutralize the internal surface of hollow fiber membranes [20]. AN69-ST suppresses biological responses such as activation of the complement system and the production of bradykinin [21], and is commercially available as a membrane for continuous renal replacement therapy in Japan.

It has been reported that platelet adhesion is associated with roughness of the membrane surface in cellulose-based membranes [22]. Similarly, the roughness of PS membranes is associated with albumin adsorption [23]. For membranes containing PVP, the smoothness, softness, and even physical properties of the membrane materials depend on the type and amount of PVP in the membrane [24]. Through coating and surface modification, not only the chemical composition of the membrane material, but also the physical properties such as smoothness and softness of the surface are altered, which in turn alters the effect on platelets and leukocytes. In recent technological developments of membranes, one of the aims is to optimize both the chemical and physical properties of the membrane surface that is exposed to blood in order to improve biocompatibility.

Assessment of Membrane Biocompatibility

The biocompatibility of dialysis membranes should ultimately be assessed in clinical settings. In fact, most studies of membrane biocompatibility have been conducted in clinical settings. However, it is sometimes difficult to interpret clinical data because of the involvement and mutual influence of a variety of factors. In addition, patient factors have a large effect on data because of inconsistent treatment and pathological conditions, making it difficult to perform proper assessment of membrane biocompatibility. On the other hand, in the basic research field of material sciences, materials are mostly evaluated using conditions different from clinical ones, thus dissociating the evaluation process from clinical settings. It is necessary, therefore, to perform the evaluation of biocompatibility on, to the extent possible, a like-for-like basis under conditions similar to clinical ones, by using *in vitro* or animal (*in vivo*) experiments. It is also important to evaluate the interaction between membrane materials and blood under flow conditions as well as under conditions that allow investigation of the mutual interactions among blood cells. These evaluation procedures will enable clinical data to be integrated into material evaluation data and then the combined data will be incorporated into the design of membrane materials.

It is also desirable to perform an evaluation using actual dialyzers. To compare actual dialyzers, it is necessary to prepare several liters of experimental solutions. For this, it is relatively easy to prepare platelet-rich plasma separated from porcine blood (and platelet-rich plasma with leukocytes), and suspension of neutrophils isolated

from porcine blood and cultured human monocytes. We introduce several evaluation methods that we use, including ones currently under investigation.

Experiments with platelet-rich plasma enable us to study the interaction between platelets and membrane materials. However, because leukocytes and platelets mutually activate and inhibit each other [8, 9], the use of only platelet-rich plasma is not enough, but if we use the platelet-rich plasma with leukocytes at the same time, it will provide greater information. In fact, when vitamin E-coated and non-coated PS dialysis membranes were compared in the presence of both leukocytes and platelets, vitamin E did not cause any difference in platelet activation. However, by comparing the results obtained from platelet-rich plasma and platelet-rich plasma with leukocytes, we found that platelet activation was enhanced more strongly when they were exposed to the non-coated membrane in the presence of leukocytes than when exposed to the vitamin E-coated membrane [25].

In experiments using neutrophil suspension, it is necessary to go through several steps to isolate neutrophils from porcine blood, but a relatively large amount of neutrophils can be prepared. We prepared neutrophils to measure the production of reactive oxygen species upon exposure to vitamin E-coated and non-coated PS dialysis membranes. Results showed that the production of reactive oxygen species is inhibited in neutrophils that were exposed to the vitamin E-coated membrane [26].

To compare the biocompatibility of dialysis membranes, we are currently investigating gene expression in polymorphonuclear leukocytes before and after exposure to dialysis membranes by performing comprehensive gene expression analysis. This analysis readily generates large volumes of information, but when analyzing clinical data, it is difficult to understand whether the observed difference is due to a change in gene expression in the leukocytes or a change in fractions of subsets of leukocytes in the peripheral blood. On the other hand, in vitro experiments in which fractions are virtually the same would reveal changes in gene expression that are specific to leukocytes. We recently used bovine blood and vitamin E-coated and non-coated PS membranes to perform comprehensive gene expression analysis. After the blood circulated through the dialyzer, polymorphonuclear leukocytes were isolated from bovine blood, and mRNA were extracted, amplified, and analyzed using the GeneChip (Affymetrix Co., Japan) [27]. We are currently analyzing the data in detail to identify the phenomenon that triggers various biological reactions. Once we have established the analytical method, comprehensive gene

expression analysis will be an extremely effective way to evaluate the biocompatibility of dialysis membranes.

We are also currently using suspensions of cultured monocytes for evaluation. When using a monocyte cell line in experiments, we can use cultured cells with the same properties and we expect the experiment will be highly reproducible. We used THP-1, a commercially available cell line derived from patients with acute monocytic leukemia. We subcultured the THP-1 cells to generate a sufficient amount of monocytes and adjusted the concentration similar to that in actual blood. At present, we are analyzing the changes in immune functions, including the production of cytokines upon exposure to the membrane. This method is used to evaluate the biocompatibility of the membrane and could also be used to obtain fundamental data that can be examined to reveal changes in the immune functions of the patients after hemodialysis treatment.

These in vitro data can be compared on a like-for-like basis under conditions similar to those in clinical settings, and the data will be useful in the development of dialysis membranes. In clinical settings, interaction between the blood and membrane material was found to be changed in different operating modes (pre- or postdilution hemodiafiltration) even when the same membrane material was used [28]. Therefore, further studies are needed to accumulate a large number of cases in order to establish a highly biocompatible treatment.

Summary

In clinical settings, various factors are superimposed that lead to the final results on biocompatibility; thus, clarification of individual reactions involved in the final results is necessary. To achieve this, it is important to establish an appropriate evaluation method for biocompatibility, which can be used to evaluate the interaction between membrane materials and blood not only under flow conditions, but also under conditions that allow investigation of the mutual interactions among blood cells. Although the evaluation requires a large volume of experimental solution, it can be performed using porcine blood, platelet-rich plasma isolated from porcine blood (and platelet-rich plasma with leukocytes), or suspension of neutrophils isolated from porcine blood and human cultured monocytes. In addition, various experimental tools such as analysis of cell surface markers using flow cytometry, quantitation of proteins using ELISA, and gene expression analysis may be used to investigate changes in blood cells to reveal membrane biocompatibility.

The blood is inevitably exposed to foreign materials in blood purification therapy. Therefore, it is important to elucidate what actually happens when the body (blood) is exposed to membrane materials. To this end, it is important to investigate the effect of the dialysis membrane on individual blood cells and their mutual interactions, and to integrate all of the experimental findings to create an overall correlation of biological responses elicited by blood-membrane interactions and mutual interactions among materials and blood cells. Furthermore, different dialysis treatment settings (blood, dialysate, and filtration flow rate, and operating mode) will certainly cause different interactions between the blood and membrane mate-

rial even when the same material is used. Therefore, further study is warranted to address this issue. We expect that all of the experimental systems discussed in this review will contribute to the development of highly biocompatible dialysis membranes and to the establishment of optimum dialysis treatment settings.

Conflicts of Interest

Hirosuke Kobayashi and Kenichi Kokubo received research funding from Asahi Kasei Medical Co., Toray Medical Co., Nipro Co., and Gambro Japan (now part of Baxter Ltd.). There are no other conflicts of interests to declare in regard to this manuscript.

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