NANOPOROUS MEMBRANE FOR IMMUNOPROTECTION BIOFILTER

S. Lee[1], Y. Kim[1], M. Park[1], H. S. Park[2], S. Cho[1], J. H. Cho[2], J. Park[1], and W. Hwang[1]*
[1] Department of Mechanical Engineering, POSTECH, Pohang, Republic of Korea
[2] Dept. of Endocrinology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea
* Corresponding author(whwang@postech.ac.kr)

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1 Introduction

For the microencapsulation of pancreatic islets, the encapsulating membranes must be adequately permeable to insulin and glucose with anti-fouling properties, and simultaneously impermeable to immunoglobulin G (IgG), which could lead to destruction of the transplanted cells.[1] Human IgG is a large molecule of about 150 kDa composed of four peptide chains, and the hydrodynamic radius is approximately 7.5 nm.[2] Among immunoglobulins, IgG is the smallest; therefore, once IgG is isolated, most immune rejection including humoral as well as cellular immune rejection could be prevented. It is therefore extremely important that a suitable pore size is designed for the biofilter, such that it acts to block the entrance of IgG while allowing reasonable free passage of insulin and glucose for abundant nutrient supply. To date, various nanofabrication techniques and materials have been investigated, but a remaining challenging is to determine the ideal tradeoff among the effective geometries such as pore size and porosity, mechanical stability for robustness, and high-throughput fabrication to achieve cost effectiveness.[3] Alternative approaches therefore should be proposed for attaining high-throughput, cost-effective and self-organized straight nanochannel membranes that are chemically and mechanically more stable; the membranes must minimize the diffusion of IgG while maximizing the diffusion of nutrients of curable molecules, such as glucose and insulin.

2 Method

The nanofilter was fabricated by two-step anodization and one-step PEO coating method. A pure Al sheet (99.999%) was electropolished in a mixture of perchloric acid and ethanol in order to remove surface irregularities. Highly ordered porous aluminum oxide was obtained from two-step anodization. The anodization solution is 0.3 M sulfuric acid. During anodization the solution was maintained at 0℃ by circulator (Lab. Copmpanion, RW-0525G) with constant voltage of 25 V, using a power supply (Digital Electronics Co., DRP-92001DUS). The pore size of the aluminum oxide was simply adjusted by etching in a 0.1M phosphoric acid solution at 30℃. The aluminum bottom layer was removed in a $CuCl_2$-based solution at room temperature and the alumina barrier layer was etched in 0.1 M phosphoric acid at 30℃ for 70 min. To modify the surface of the nanofilter, PEO was added to alumina nanofilter in 2 mM toluene and the mixture was stirred at room temperature for 24 hr.

Fig. 1. shows a schematic diagram and optical images of a PEO-coated alumina nanochannel array with large-area uniform and straight channel geometry. The nanochannel is shown to be sufficiently permeable to glucose and insulin while blocking IgG. The diameter of the nanochannel was reduced from 18 nm to 14.6 nm by PEO coating onto the alumina surface. Scanning electron microscopy (SEM) images were obtained with a JEOL JSM-7401F SEM (Field Emission Scanning Electron Microscope, NCNT).

We investigated how the diffusion of glucose and IgG varies with the pore size of the nanochannel and with time. To determine the permeability of nanofilters, the concentrations of nutrients and immune molecules of interest were measured on

either side of the nanofilters. We designed an artificial immunoprotection system in vitro.

A bypass-type apparatus for connecting the PEO-coated nanofilters to arteries and veins was designed and machined from transparent polycarbonate. Instead of real porcine islets for insulin secretion, the insulin was inserted between nanochannel arrays simply to determine the diffusion coefficient of glucose, insulin, and IgG in a mixed condition in such an artificial system.

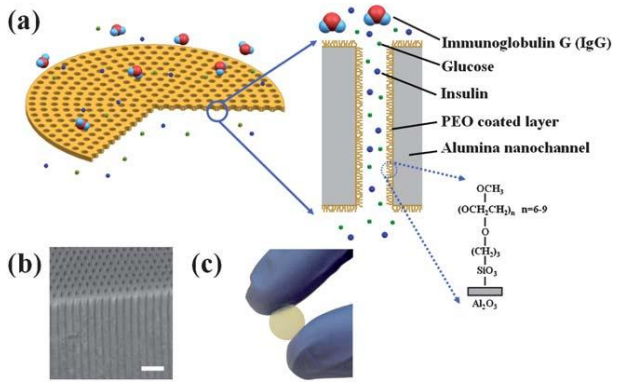

Fig. 1. (a) Schematic diagram of filtration behavior of the PEO-coated nanochannel array and unit of the PEO-coated nanochannel. (b) FE-SEM image of the PEO-coated nanochannel array. Scale bar: 200 nm. (c) Photographic image of the PEO-coated nanochannel biofilter.

3 Results

The PEO-functionalized straight nanochannel array was developed based on a self-organized porous alumina for a novel biofilter with antifouling and superior immunoprotection. In order to optimize the nanochannel geometry for achieving high permeability of glucose and insulin while blocking IgG, the length of PEO coated an alumina nanochannel array with a pore size of 18 nm was controlled to be around 1.7 nm, through which the pore size of a PEO-coated nanochannel (14.6 nm) can be slightly smaller than the hydrodynamic diameter (15 nm) of IgG (Fig. 2.).

As shown in Fig. 3b, the diffusion percentage of glucose and IgG are respectively 0.0008% and 28.8%, and the percentage of the insulin is 6.7% after six days. Our result is at least one order of magnitude less than that of the previous research.[4]

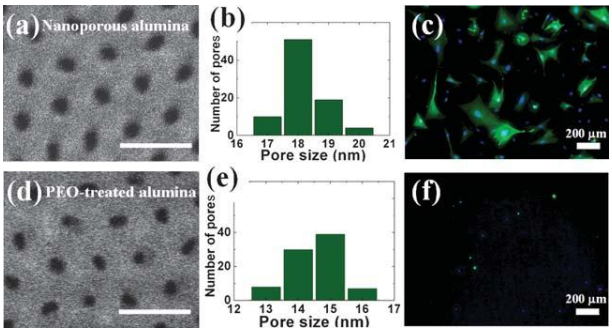

Fig. 2. Pore size distribution of nanochannel arrays. FE-SEM micrographs for: (a–c) top view, pore size distribution, and cell adhesion of the nanochannel array with pore diameter of about 18 nm: (d–f) top view, pore size distribution, and cell adhesion of the PEO-coated nanochannel array with pore diameter of about 14.6 nm. Scale bar: 100 nm.

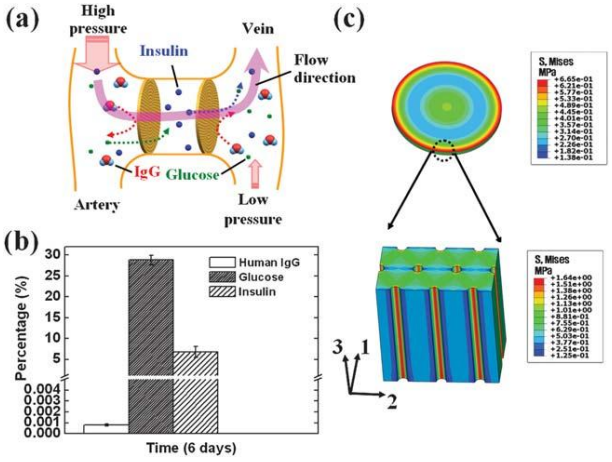

Fig. 3. Filtration behavior of PEO-coated nanochannel arrays in artificial arteries–veins flow system in vitro. (a) Scheme for connecting the nanochannel arrays to arteries and veins simultaneously. (b) The percentage of IgG and glucose entering through the nanochannels and the percentage of insulin released from the nanofilter system during six days. (c) Schematic diagrams of nanochannel arrays after pressure application.

Fig. 4a shows the diffusion of glucose (MW 180 Da) through our nanochannels of different pore sizes for 24 h. The amount of passed glucose was gradually reduced as the diameter of the nanochannels decreased. In other words, the amount of passed glucose was proportional to the porosity of the nanochannels because the size of glucose (hydrodynamic radius is 0.35 nm) is much smaller than the pore size of the nanochannels. The glucose

diffusion percentage (10.2% over 24 h) from nanochannels with a pore size of 14.6 nm was slightly smaller than that (10.7% over 24 h) from nanochannels with an 18 nm diameter. Fig. 4b shows the diffusion of human IgG (MW 150 kDa) through membranes with different pore sizes. The amount of passed IgG was progressively reduced as the diameter of the nanochannels decreased. Interestingly, we observed the abrupt decrease of IgG diffusion when the pore size of the nanochannel array was changed in diameter from 26 nm to 18 nm. Although we assume that the diameter of IgG is about 15 nm, real human IgG is continuously deformed and takes random shapes in blood, so that we consider that the nanochannel array with 18 nm diameter can offer highly effective blocking of human IgG. Again, when we apply the PEO-coated nanochannel array with 14.6 nm diameter, we found a significant decrease (over three times) in the diffusion percentage of IgG compared to that of the nanochannel with a diameter of 18 nm. Thus, the PEO-coated nanochannel array with 14.6 nm diameter not only displays an excellent IgG blocking effect but also provides high permeation of glucose.

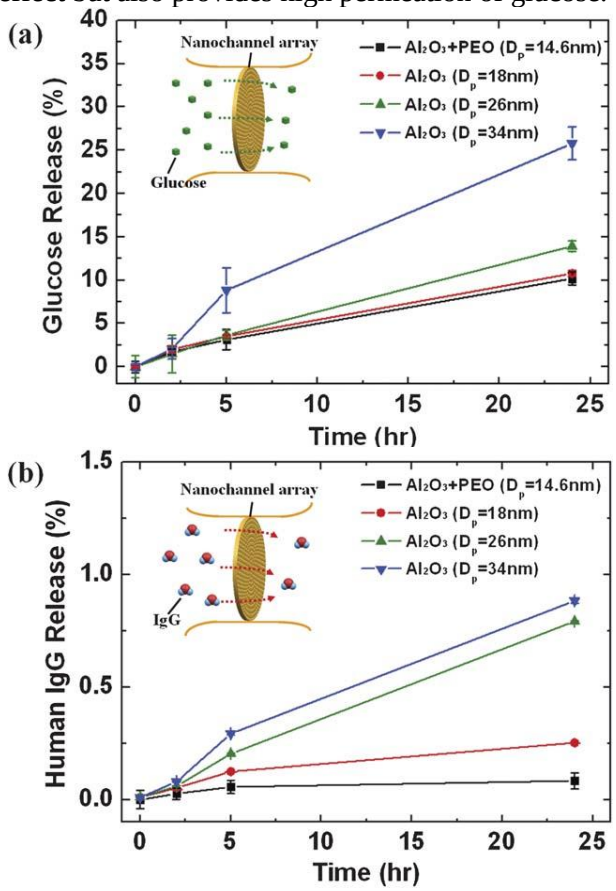

Fig. 4. Glucose and IgG diffusion through nanochannel arrays of different pore sizes. (a and b) The released percentage of glucose and IgG through nanochannel arrays. (D_p is the pore diameter.)

It is critically important that nanochannels should block IgG from entering for immunoprotection, but how much glucose relative to IgG can penetrate the nanochannels to stimulate insulin secretion. To investigate the effectiveness of the nanochannels, the $D_{Neff,glucose}/D_{Neff,IgG}$ was calculated using the normalized effective diffusion coefficients (D_{Neff}). The effectiveness of the PEO-coated nanochannel array was about 2765, which is over four times higher than for previous membranes (the ratio for micromachined membranes was about 298 through 7-nm membranes and 592 through 13-nm membranes).[5] The PEO-coated nanochannel arrays can minimize immune reaction caused by IgG while maintaining the rapid diffusion of low molecular-weight molecules such as glucose and insulin.

4 Conclusions

We have demonstrated the superior immunoprotection of a PEO-coated nanochannel array as a novel biofilter. Based on the hydrodynamic size of IgG and the employment of PEO, we were able to design successfully an ideally optimized biofilter to block IgG and to be permeable to nutrients such as glucose and insulin. Since the alumina nanochannel array provides a large-area uniform and straight channel geometry, metabolic shadows and hypoxia could be avoided. Furthermore, the excellent in-vivo mechanical stability of the nanochannel biofilter was demonstrated by numerical simulation, and the safety factor was capable of being tailored by varying the geometry of our biofilter. Thus, our strategy may provide great advantages in novel membrane biotechnologies such as biofiltration and artificial cells.

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