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REVIEW

Glycosylated Membranes: A Promising Biomimetic Material

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ABSTRACT: Development in the area of glycosylated membranes has been actively pursued in the past few years. This kind of promising biomimetic material is inspired by cell membranes. The recent surge of interest in these glycosylated membranes stems from their widespread number of applications to many areas in science and technology. With the glycosylation strategy, membrane separation properties, such as flux and antifouling, are greatly improved. Moreover, the ability to modulate biocompatibility, protein recognition, separation of biomolecules, enzyme immobilization, cell culture, and microorganisms capture are important in a variety of biological and medical applications. This review focuses on the recent progress in the preparation of these glycosylated membranes and highlights their applications. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39658.

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INTRODUCTION

As one of the core technologies of the 21st century, materials science has got rapid development during the past years.[1–3] Currently, a challenging task for the development of materials science is high selectivity mimicked from nature of synthetic materials.[4–6] Merging the disciplines, mimicked from nature, allows us to take advantage of the existing physical, chemical, or biological methods to generate new biomimetic materials and, conversely, to apply such biomimetic materials and physicochemical techniques to solve practical application problems.[7,8]

Membrane is an important branch of materials science, and membrane technology has obtained a huge importance in the last 50 years, competing with long established technologies for ultrapure water production, waste water treatment, food processing, and biotechnical applications.[9,10] The outstanding advantages of the membrane technology are low energy consumption, easy operation, highly efficient technique, and benefit for substance recycle. In the recent years, biomimetic membrane research has been paid much attention due to the requirements of other technologies, especially in biotechnology.[11–13] Among all of the studies for biomimetic membranes, glycosylated membrane is a promising one which has been rapidly developed in the last 10 years.[14–18]

As the name implies, the glycosylated membrane is one of the developing biomimetic membranes, which combines the separation functionality of membranes with the biological functionality of cell surface saccharides. These saccharides, located on the surfaces of various cells, are usually called "*glycocalyx*". They not only protect the cells avoid attacking by other species but act as recognition sites involving many molecular recognition processes, including virus invasion, cancer cell metastasis, bacterial infection, and specific enzymes or lectin recognition.[19–22] Inspired by the cell surface *glycocalyx*, researchers designed such biomimetic membranes with *glycocalyx*-like surface, namely glycosylated membranes. Unsurprisingly, the glycosylated membranes not only have excellent surface hydrophilicity, biocompatibility, but have specific recognition to target biomacromolecules because of the unique chemobiological properties of saccharides.

This article provides a comprehensive overview on the glycosylated membranes, including the fabrication methods and the potential applications. The fabrication methods, including physical,[23–27] chemical,[28–32] and biochemical methods (enzymatic glycosylation or glycosylation by biological interactions),[33–36] are first discussed and compared. The advantages and disadvantages of each of the methods are pointed out in detail. Then in the section thereafter, the potential applications of the glycosylated membranes will be presented, focusing on the fields of membrane separation and biological applications.

GLYCOSYLATION METHODS

Over the past years, several traditional methods, such as physical method, chemical method, and biochemical method, have been developed for the preparation of glycosylated membranes.

A detailed review of the various preparation methods of glycosylated membranes follows.

Physical Method

Physical Adsorption. Physical adsorption is the simplest method for surface modification of membranes, as it requires just the incubation of the adsorbate with a stationary surface.[37] The adsorption relies on the physical interactions between the adsorbate and the surface. The interactions may be hydrogen bonding, hydrophobic interaction, van der Waals force, and electrostatic interaction. The glycosylated membranes prepared by physical adsorption are divided into two categories: (1) physical adsorption based on electrostatic force and (2) physical adsorption based on hydrophobic interaction.

It is well-known natural polysaccharides, such as chitosan, heparin, and dextan sulfate, are polyelectrolytes with different charges. Self-assembly of natural polysaccharides has proved to be a good method for the preparation of gylcosylated membranes. Through this method, Yu et al. has prepared glycosylated poly(L-lactic acid),[38] poly(tetramethylene adipate-*co*-terephthalate),[39] and polysulfone (PSF) membranes[40] with these natural charged polysaccharides, respectively.

A question is that, for the electroneutral polysaccharides with extreme hydrophilicity, how can they adsorb on the membrane surface? To realize adsorption, they should have high molecular weight or need hydrophobic modification. Feizi and coworkers[25] is the first one to investigate the lipid-linked oligosaccharide adsorbed on nitrocellulose membrane by jet spray. Then neoglycolipids were arrayed on the membrane with the same method.[41] Similarly, Moller et al.[42] immobilized 50 cell wall glycans on the surface of nitrocellulose membrane. This method was then quickly developed for glycoarrays on other materials surface, such as glass and gold slides, and polystyrene microtiter plate. Lu et al.[43] synthesized hydrophobic galactose-derived Pluronic F68 (F68-Gal), which was adsorbed on poly(vinylidene difluoride) (PVDF) membrane through hydrophobic interaction between the membrane surface and the polypropylene oxide segment in Pluronic. The gylcosyl density increased with the concentration of the F68-Gal solution.

Dip Coating. Dip-coating method was widely utilized to prepare glycosylated membranes with polysaccharides. Through this way, chitosan was coated on the zeolite-filled regenerated cellulose membrane,[44] polyacrylonitrile (PAN) hollow fiber membrane,[45] polyethylene terephthalate (PET) membrane,[38] and ceramic membrane.[46] The chitosan-coating membranes showed long-term stability with the assist of cross-linking or plasma treatment, due to the good film-forming character of chitosan.[47]

Chemical Method

Chemical method provides the most stable glycosylation and gradually become an important process for fabricating glycosylated membranes. It can be divided into three kinds, as demonstrated later.

Chemical Synthesis of Glycopolymers for Membrane Preparation. This method is based on the synthesis of glycopolymers and the subsequent membrane preparation. The glycoside density on the membrane surface can be regulated by the saccharide content in the glycopolymers during polymerization process. However, not all of the glycosyl residues are exposed on the membrane surface. Most of them are embedded in the inward of membranes. Therefore, it is of great importance for inducing the hydrophilic glycosyl residues to be enriched on the membrane surface.

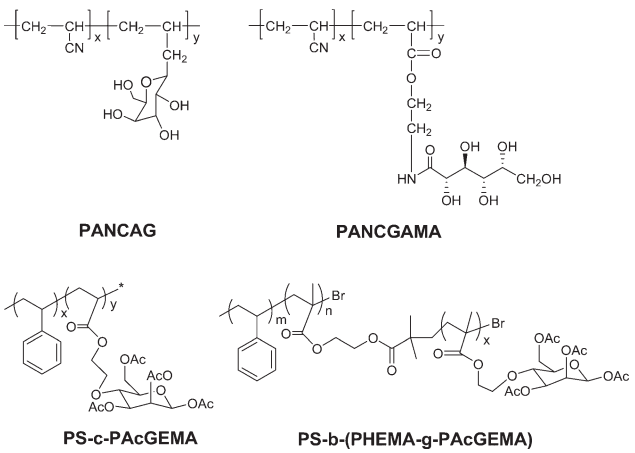

Figure 1. Chemical structures of PANCAG, PANCGAMA, PS-c-PAcGEMA, and PS-b-(PHEMA-g-PAcGEMA).

Xu et al.[48] synthesized a glycopolymer poly(acrylonitrile-*co*-(α-allyl glucoside)) (PANCAG) by copolymerization of acrylonitrile and α-allyl glucoside (AG) for the first time through water-phase precipitation copolymerization (WPPCP). Glycosylated membranes were then prepared by casting the glycopolymers solution (7 wt %) onto glass plates followed by drying to completely remove the residual solvent. Taking advantages of the WPPCP method, both the AG content in the copolymers and the AG conversion for WPPCP are higher than those of solution copolymerization. Afterward, as shown in Figure 1, PANCAG and poly[acrylonitrile-*co*-(D-gluconamidoethyl methacrylate)] (PANCGAMA) containing cyclic and linear glucose residues, were, respectively, synthesized and fabricated into nanofibrous membranes by electrosponning.[49] With their nanoscale structures, these glycosylated nanofibrous membranes exhibit very high saccharide content to volume ratio. It is possible to control the fiber diameter and subsequently, the saccharide density, by varying the electrospinning parameters.

Recently, glycopolymers poly(styrene-c-2-(2-,3-,4-,6-tetra-O-acetyl-β-D-glucosyloxy) ethyl methacylate) (PS-c-AcGEMA) with well-defined linear (PS-c-AcGEMA) and/or comb-like structures poly(styrene-b-(2-hydroxyethyl methacrylate-g-2-(2-,3-,4-,6-tetra-O-acetyl-β-D-glucosyloxy) ethyl methacylate)) (PS-b-(PHEMA-g-PAcGEMA); shown in Figure 1) were synthesized by atom-transfer radical polymerization (ATRP) and were used to fabricate pattern films by the breath figure method.[50] The breath figure method is based on evaporative cooling and subsequent water-droplet templating to form an ordered array on the film or membrane surface. Therefore, the hydrophilic part in polymers will be enriched on the pore surface. In this work, highly ordered glycosylated pattern films were prepared from the comb-like glycopolymer and the linear block glycopolymers with relatively long PAcGEMA segment.

Chemical Immobilization of Polysaccharides on the Membrane Surface. Chemical immobilization is one commonly used glycosylation strategy for immobilizing natural and synthetic polysaccharides on the membrane surface. To realize glycosylation, the membranes needs possess reactive groups capable of combining polysaccharides. Therefore, some membranes need to be modified before immobilization and numerous modification routes can be adopted. Generally speaking, this method is easy to operate. However, because the polysaccharide molecules are directly immobilized on the surface without long spacer, the sterical hindrance will be high and the glycoside density will be relatively low. With the same reason, the biological functions of the immobilized polysaccharides could be restricted sometimes. These problems can be solved by bringing long spacer between the polysaccharides and the membrane surface.

When reactive groups exist on the membrane surface, the polysaccharide molecules can be bound directly. For example, chitosan and/or gelatin were immobilized on asymmetric membranes fabricated from poly(acrylonitrile-*co*-maleic acid) (PANCMA) by the reaction between the carboxyl groups of PANCMA and the amino groups of chitosan in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC).[51] Besides, Che et al.[52] also immobilized chitosan on poly(acrylonitrile-*co*-acrylic acid) (PANCAA) nanofibrous membranes by a coupling reaction between the carboxyl groups of PANCAA and the primary amino groups of chitosan. Similarly, glucose ligands were bound on poly(acrylonitrile-*co*-hydroxyethyl methacrylate) (PANCHEMA) nanofibrous membranes through a chemical reaction between the –OH groups of PANCHEMA and glucose pentaacetate under mild condition followed by a deacetylation process.[53] A glucose density up to 11.12 mg/g nanofibrous membrane can be facilely achieved.

Most of the membranes lack reactive groups for coupling reaction. Pretreatments are needed in these cases. For example, PSF membranes were activated with successive treatments of chlorodimethyl ether and ethylenediamine, and subsequent chemical binding of heparin with bifunctional linker molecules.[54,55] A heparin density up to 0.86 $\mu g/cm^2$ was achieved on the PSF membrane surface. Besides, PSF membranes were also treated with ammonia plasma, and then heparin was bound on the membranes through the reaction between the amino groups of membranes and the carboxyl groups of heparin in the presence of EDC and NHS.[56]

Gao and coworkers[57] modified polycaprolactone membranes by immobilizing chitosan on the membrane surface with a cross-linking agent glutaraldehyde. Miyagawa et al.[58,59] prepared glycosylated cellulose membranes by immobilizing the glycoconjugate polymer on carboxymethylated membranes through condensation reaction between the amino group of the glycoconjugates and the carboxyl group of the cellulose. Keusgen and coworkers[60,61] pretreated polytetrafluoroethylene (PTFE) membrane with elementary sodium followed by oxidation using ozone or hydrogen peroxide, and then mannose ligands were immobilized on the membrane surface through coupling reagent, 1,4-butanediol diglycidyl ether. Tabary et al.[62] modified PVDF membranes by impregnating them with the reactants of saccharide derivate (cycldextrin, maltodextrin, and citric acid) in a thermofixation oven. They indicated that the pretreatment method, such as chemical reaction and high-energy radiation, may disrupt the membrane bulk, which results in reduced mechanical property.

Coupling Reaction of Saccharides With Grafted Polymers on the Membrane Surface. This kind of glycosylation includes the grafting polymerization of monomers with reactive groups on the membrane surface and the coupling reaction between the saccharides and the reactive groups. Compared with directly immobilization of saccharides on the membrane surface, high glycoside density can be achieved with this method due to the introduction of huge reactive groups on the grafted polymer chains. And the glycoside density can be controlled in a wide range by the reaction degree. The reaction degree could be regulated through the grafting parameters, including the used solvent and the type of coupling reaction, such as conventional chemical reaction and/or new click chemistry reaction.

In our group, five different routes were used to prepare glycosylated microporous polypropylene membrane (MPPM), as schemed in Figure 2. (1) 2-Aminoethyl methacrylate hydrochloride (AEMA) was grafted on MPPMs by ultraviolet (UV)-induced graft polymerization to generate an amino-functionalized surface. Then saccharide moieties were bound with the grafted functional layer to form glycopolymer by the reaction between amino groups on the membrane surface and gluconolactone.[63] (2) Acrylamide was grafted onto the MPPMs by UV-induced graft polymerization. The amide groups of grafted poly(acrylamide) were then transformed to primary amine groups by the Hofmann rearrangement reaction. Saccharide moieties were introduced on the membrane surface by the reaction between primary amine groups and gluconolactone.[64] (3) HEMA was grafted on MPPMs by UV-induced graft polymerization. Saccharide moieties were bound on the membrane surface through the reaction between the acetylated saccharides and the hydroxyl groups of poly(HEMA). After the acetyl groups of acetylated saccharides were deprotected, four different glycosylated membranes were gained.[14,65] (4) Acrylic acid (AAc) was grafted on MPPMs by UV-induced graft polymerization. The poly(AAc)-grafted membranes were then rendered to react with propargylamine to give terminal alkyne-modified MPPMs. Subsequently, azide-containing glucose pendants were linked to the membrane surface by click chemistry. Profiting from the high efficiency of click chemistry, the surface glycoside density can be well controlled over a wide range and the maximum value is over 10 $\mu mol/cm^2$.[17] (5) In addition, the alkyne-modified MPPMs were also glycosylated via thiol-yne click reaction in the presence of 50% 2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl thiol solution in tetrahydrofuran (THF). The result showed that THF was a beneficial solvent for increasing the glycosylation efficiency.[18] Comparison of these results reveal that higher glycoside density results from larger amount of the grafted polymers introduced on the membrane surface if the conventional chemical reactions are adopted. However, exorbitant grafting degree changes the bulk properties of membranes, like mechanical property. In contrast, the high glycoside density can be achieved with low grafting degree of polymers grafted on the membrane surface when using click chemistry.

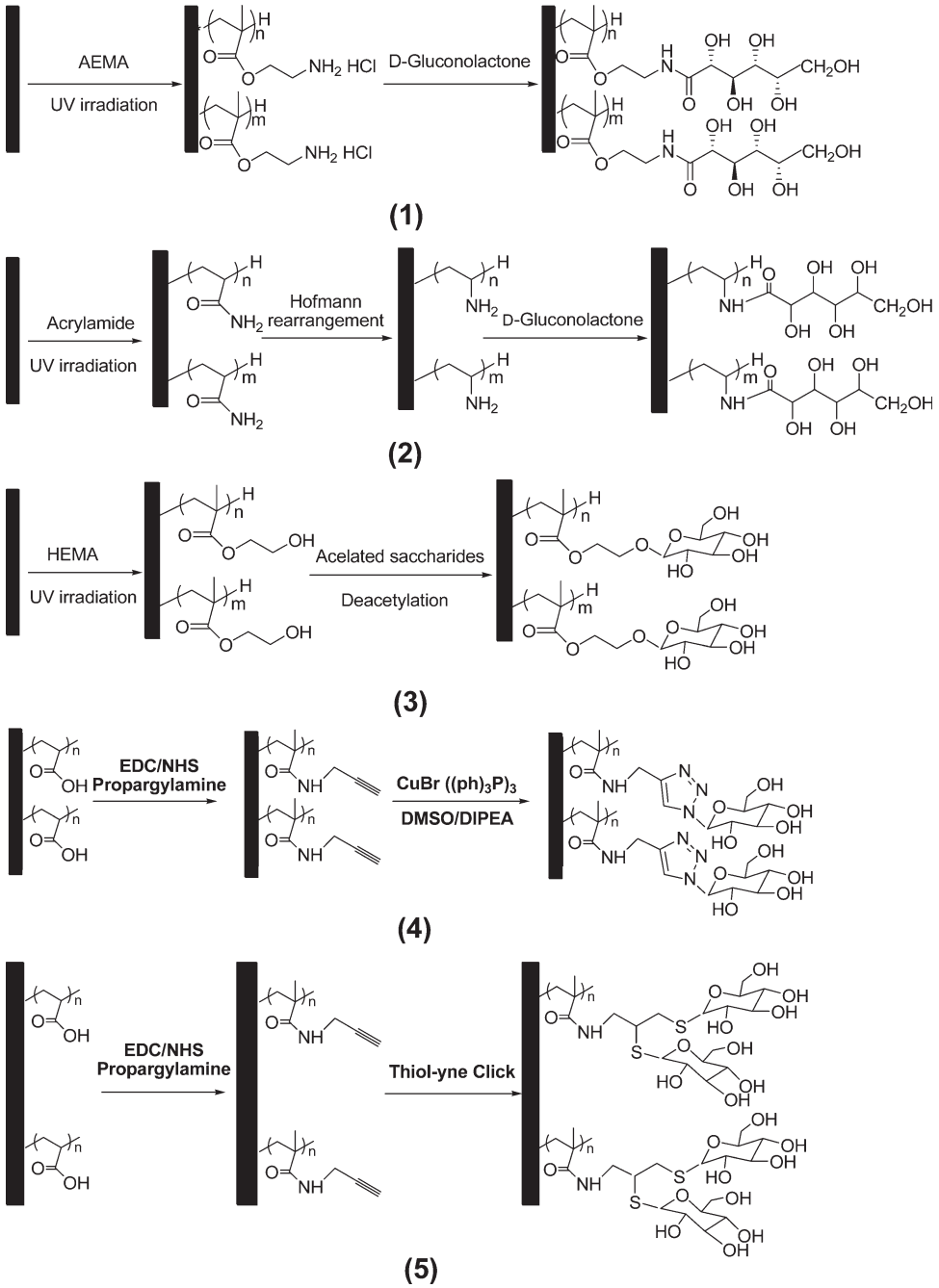

Figure 2. Schematic representation of five different surface gylcosylation methods by coupling reaction of saccharides with grafted polymers on the membrane surface.

Ying et al.[66] modified the argon-plasma-pretreated PET films via UV-induced graft polymerization with AAc. Galactosylated surfaces were then obtained by coupling a galactose derivative to the AAc graft chains with the aid of a water-soluble carbodiimide, and *N*-hydroxysulfosuccinimide (sulfo-NHS). Chu et al.[67] reported a similar glycosylation protocol.

Chemical immobilization was also used to fabricate glycosylated membranes from polysaccharides rather than monosaccharides mentioned above. As an example, dextran was immobilized on the poly(AEMA)-grafted MPPMs.[68]

Grafting Polymerization of Glycomonomers on the Membrane Surface. This glycosylation approach needs to synthesize glycomonomers at first. Corresponding glycopolymers are then grafted on the membrane surface by various initiation steps to construct glycosylated membranes. Compared with the coupling reaction of poly-/oligo-/monosaccharides, this method has the following advantages: (1) the pretreatment of membranes is avoided, (2) the structure of grafted glycopolymer is definite, and (3) the glycoside density is controllable to a certain extend. By the application of new grafting technologies

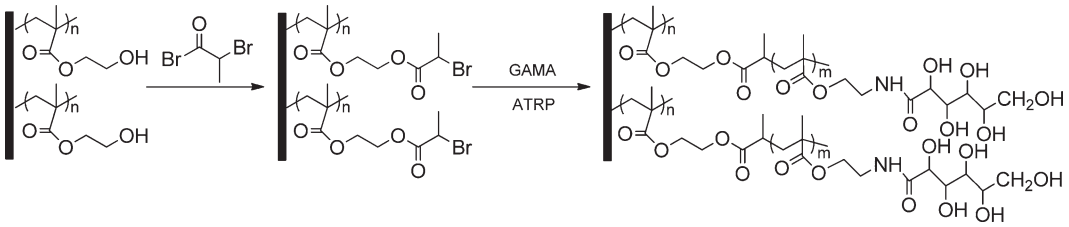

Figure 3. Schematic representation of gylcosylation method by ATRP on the membrane surface.

such as surface-initiated controlled/living polymerization, it is possible to exactly control the chain length and sequence distribution of the grafted glycopolymer chains, which is of crucial importance to the intensive study of surface glycosylation.[69]

In our laboratory, we grafted saccharide-containing monomer AG on MPPM by dipping the membranes in a monomer solution followed by N_2-plasma-induced graft polymerization for the first time.[70] The glycoside density is affected by both the monomer concentration and the plasma radiation.. Besides, MPPMs in hollow fiber form was glycosylated with the same process.[71] It is worth to notice that long time of plasma radiation cause obvious etching of the membrane surface. To avoid this problem, the glycomonomer was grafted on MPPMs by UV-induced graft polymerization to generate glycosylated surface. Compared with plasma radiation, UV irradiation is more moderate for membrane materials.[16] It was found that the AG grafting degree increases reasonably with the increase in AG monomer concentration and the UV irradiation time. We used 40 g/L AG concentration and 20–25 min UV irradiation to gain the maximum glycoside density of 187.76 $\mu g/cm^2$. After that, MPPMs were glycosylated with the same method by using a novel saccharide-containing monomer (D-gluconamidoethyl methacrylate (GAMA)).[72] Similarly, Roger and coworkers[73] synthesized a new UV-reactive saccharide molecule, azidophenyl lactamine, and photografted it on PET fibers.

For satisfying the saccharide-based biological demands, the grafted glycopolymers becomes largely expected with well-defined chain structure and appropriate saccharide density. However, these structure details can not be well controlled with traditional chemical methods. Development of controlled/living grafting polymerization makes it possible. Under the above consideration, the surface of MPPMs was modified with comb-like glycopolymer brushes by a combination of UV-induced graft polymerization and ATRP in our laboratory.[74] As shown in Figure 3, HEMA was first grafted onto the MPPM surface by UV-induced graft polymerization. The reaction between the hydroxyl group and 2-bromopropionyl bromide gave the immobilization of the ATRP initiator. Then, the surface-initiated ATRP of GAMA was carried out and resulted in well-defined comb-like glycopolymer brushes on the membrane surface at ambient temperature in aqueous solvent. Water has a significant acceleration effect on the ATRP process while hampers the controllability. The addition of $CuBr_2$ largely increases the controllability at the cost of the polymerization rate. Subsequently, surface-initiated ATRP of glycomonomer AcGAMA was utilized to glycosylate the honeycomb-patterned films for constructing saccharide microarrarys.[75] The films were prepared from an amphiphilic block copolymer, poly(styrene-block-(2-hydroxyethyl methacrylate)), by a breath figure method. Therefore, the hydroxyl groups aggregate mainly inside the pores and initiate ATRP of glycomonomer.

Biochemical Method

Enzymatic Transglycosylation. Enzymatic transglycosylation provides a new method to fabricate glycosylated membranes. The main principle of this method is enzyme catalyzes the transfer of a monosaccharide from the donor to the acceptor immobilized on the membrane surface. Up to now, such method mainly has been used to fabricate glycosylated cellulose membranes. Hummel and coworkers[76] successfully used different glycosyltransferase enzymes, such as α-1,3-galactosyltransferase, α-1,3-fucosyltransferase, α-2,6-(*N*-)-sialyltransferase, and α-2,3-(*N*-)-sialyltransferase, to catalyze the transfer of different monosaccharides from the donors to *N*-acetyllactosamine immobilized cellulose membranes. In addition, Kitaoka et al.[34,77] used cellulase as biocatalysts to catalyze the transfer of lactose moieties onto the surface of the cellulose membranes. Recently, our group presented a detailed study on the enzymatic transglycosylation of poly(ethylene glycol) brushes by β-galactosidase (β-Gal). Quartz crystal microbalance (QCM) was used as a label free read-out method to evaluate the kinetics of such transglycosylation. Then, biomimetic MPPMs with glycocalyx-like surfaces were prepared by a chemoenzymatic method based on the above studies. As shown in Figure 4, poly (oligo(ethylene glycol) methyl methacrylate) (POEGMA) brushes was first grafted on the membranes surface by UV-induced grafting polymerization of

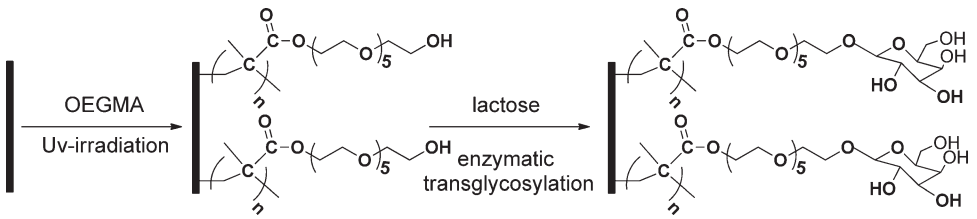

Figure 4. Schematic representation of chemoenzymatic method for preparing glycosylated membranes.

OEGMA. Subsequently, galactose moieties were transferred to the end hydroxyl group of POEGMA brushes by β-Gal catalyzed transglycosylation. This enzymatic transglycosylation is a promising (simple and "green") procedure for preparing biomimetic membranes with glycocalyx-like surfaces.

Above all, like most of the enzymatic reactions, the enzymatic transglycosylation has unique advantages, including high selectivity, mild reaction conditions, and environmental friendliness etc.

Glycosylation by Biological Recognition Interactions. Biological recognition interactions are the fundamental interactions between biological molecules in organisms. Particularly, avidin–biotin interaction is the most common ones, which is usually been used for the conjugation reaction. Bundy and Catherine[78] prepared a glycosylated membrane based on streptavidin–biotin interaction. The glycosylated membrane was constructed by first immobilizing streptavidin to the membrane surface, followed by attachment of a commercially produced biotin–saccharide polymer. Another example has been reported by Sun et al.,[79] a straightforward approach was first been adopted to synthesize biotin chain-terminated glycopolymers of low polydispersity, then such glycopolymers were attached to the surface of patterned PET membrane by streptavidin–biotin binding. The approach facilitates the production of glycosurface arrays with varying saccharides type and density. In brief, glycosylation technique involving avidin–biotin interaction is a simple and sensitive method to localize saccharides on the membrane surface.

APPLICATIONS OF THE GLYCOSYLATED MEMBRANES

The glycosyl residues on the membrane surface endow the membrane with improved properties and the functions of glycosylated membranes are widely expanded. Definitely, the membranes show improved separation functions and/or novel biological functions after glycosylation.

Separation Applications

Saccharides usually possess multiple hydroxyl groups and then show strong hydrophilicity. As saccharides are introduced on the membrane surfaces, separation performance of the membranes is usually modified owing to the hydrophilicity and the glycosylated membranes show resistance to the nonspecific adsorption of proteins in most cases.[69]

Permeation. Permeation of a membrane is affected by both the physical structures and the chemical characters of membrane. For example, when the membrane is hydrophilic, the water flux is relatively high. Because the surface of glycosylated membranes is always hydrophilic devoting by the saccharide molecules, the permeation flux of aqueous solution is normally increased. Compared with the unmodified MPPM, the hydrophilicity of poly(AG)-grafted membranes was greatly improved and the pure-water flux increased sharply from 0.42×10^3 kg/(m^2·h) to 4.35×10^3 kg/(m^2·h) with the increase in the glycoside density.[70] Most importantly, the hydrophilicity was permanent, and no hydrophobic recovery was observed.

Antifouling. Membrane fouling may result from many processes and is the critical factor limiting the long-time application of a membrane. Until now, it is well recognized that the antifouling characteristics of hydrophilic membranes is usually better than that of the hydrophobic ones. As well known, a hydrated layer is created on the hydrophilic membrane surface, so the deposition of hydrophobic mass is energetically unfavorable on the premise that the hydrated layer must be destroyed firstly and the fouling is suppressed.

The poly(AG)-grafted membrane surface shows less susceptible to the adsorption of bovine serum albumin (BSA).[70] And the filtration results of BSA solution indicates that the glycosylated membrane gives low relative flux reduction and high flux recovery after cleaning, which mean the antifouling property of the membrane is improved. Besides, the ring-opening glycomonomers GAMA was grafted on PAN ultrafiltration membranes.[80] Static adsorption of fluorescein isothiocyanate-BSA is significantly inhibited on this kind of glycosylated membranes and the flux recovery ratio is also increased. When the poly(AG)-grafted[81] and poly(GAMA)-grafted[82] hollow fiber MPPMs were used in submerged membrane bioreactor for a continuous operation, these glycosylated membranes also showed good antifouling performance.

Pervaporation. Hydrophilic membranes are widely investigated for pervaporation dehydration, among which the natural polysaccharides such as chitosan, cellulose, and sodium alginate are commonly studied. To overcome the over swelling and poor mechanical stability of these membranes, composite membranes containing glycopolymers were constructed. The polysaccharides and/or glycopolymers have high affinity to water molecules by polarity interactions and hydrogen bonds,[83] and a dense network structure by the interchain and intrachain hydrogen bonds.[84] Therefore, the membranes containing these molecules have good selectivity and high flux during pervaporation.

A chitosan layer have been introduced on PAN,[45,85] cellulose,[86] and ceramic membranes[46] to prepare composite membranes. It was found that chitosan plays an important role in increasing the membrane permeation flux and the separation factor. Zhu et al.[46] found that in the dehydration of alcohol/water mixtures, the permeation flux increases without a decrease in separation factor. In the dehydration of ester/water mixtures, the membrane exhibits excellent pervaporation performance, especially in the dehydration of ethyl acetate/water mixture at 3.5 wt % water in feed with a flux of 1250 g/(m^2 h) and a separation factor larger than 10,000. When the chitosan layer on the membrane surface is cross-linked, the composite membranes display desirable stability during the long-term continuous operation. Pervaporation efficiency of the membranes can be maintained after 330 days of operation for 70 wt % aqueous *iso*-propanol solution at 25°C.[45]

Compared with the natural polysaccharides, glycopolymers are considered to be more versatile for the preparation of various composite membranes. We prepared glycopolymer-filled composite membranes by pore-filling strategy from MPPM using *in situ* copolymerization of AAc and GAMA. Swelling experiments in isopropanol/water mixture revealed that the glycosylated membranes show selective sorption of water. Influence by the sorption selectivity on the separation performance is more

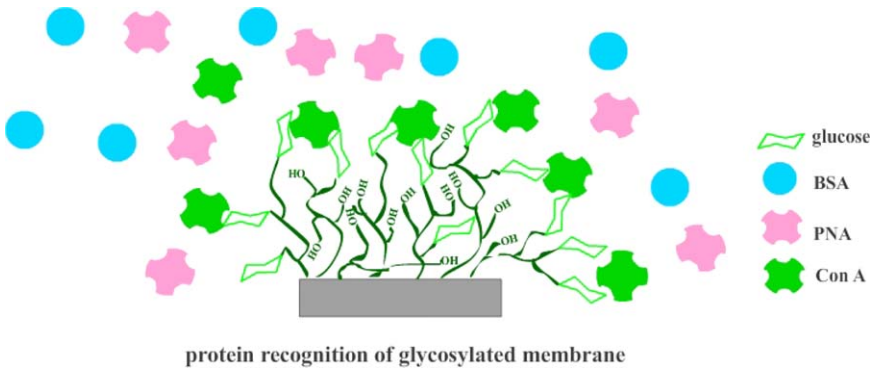

Figure 5. Scheme for the specific adsorption properties of MPPM glycosylated with glucose. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

significant than that by the diffusion selectivity. Compared with membranes from natural polysaccharides, the present membranes display an advantage on flux when normalized by thickness.[87]

Biological and Medical Applications

It is well known that glycoproteins and glycolipids on cell surfaces collectively form membrane-bound saccharide layers, often referred to as a *glycocalyx*. These saccharide layers on the cell membrane surface are critical mediators of molecular recognition events via the interactions of unique oligosaccharide sequences with specific protein epitopes that maybe found on bacteria, viruses, and other cells, as well as on a variety of soluble and matrix-bound factors.[88] Therefore, it is reasonable to believe that fabricating a saccharide-containing layer on the synthetic membrane surface will endow the membrane with some biological functions similar to *glycocalyx*.

Glycoarray. In the past few years, glycoarrays have become a standard tool to screen large number of saccharide–biomolecule interactions and investigate the role of saccharides in biological systems. The most important advantages of glycoarray technology over conventional approaches, such as enzyme-linked lectin assay, surface plasmon resonance, QCM, or isothermal titration calorimetry, are the ability to screen several thousand binding events on a single slide and the miniscule amounts of both analytes and ligands required for one experiment. Additionally, glycoarrays are ideal platforms to detect interactions that involve saccharides because of the multivalent display of saccharides on a surface highly mimicked from *glycocalyx* on cell surface.[89,90]

Currently, most of the glycoarrays are fabricated on 96-well plates (PS, U-bottom, Costar),[23,91] glass,[92,93] and gold surfaces.[94] Membranes are also promising substrates for fabricating glycoarrays. Membranes have unique advantages such as high specific surface area, diverse available membrane materials (natural, synthetic polymers, etc.), and flexible modification methods compared to the standard nonporous solid support materials (96-well plates, glass, gold, etc.). These advantages will further improve the sensitivity and applicability of glycoarrays. Fukui et al.[25] described microarrays of oligosaccharides as neoglycolipids and their robust display on nitrocellulose membrane. Saccharide-recognizing proteins single out their ligands not only in arrays of homogeneous oligosaccharides but also in arrays of heterogeneous oligosaccharides. Jobron et al.[76] have previously reported that *N*-acetyllactosamine immobilized cellulose membranes can be successfully adapted for high-throughput screening glycosyltransferase assays. Moller et al.[42] have also constructed saccharide microarray for high-throughput screening of monoclonal antibodies against plant cell wall glycans on nitrocellulose membrane.

Biocompatibility. The term "biocompatibility" indicates that the material does not produce a toxic, injurious, or immunological response in living tissue, including hemo- and cytocompatibility.[95] Because the glycosylated surface only interacts with the recognized biomolecules and the nonspecific adsorption is drastically inhibited, the glycosylated membrane shows good biocompatibility. Heparin and dextran sulfate, natural polysaccharides with great anticoagulation, were usually immobilized on the membrane surface to improve the membrane biocompatibility. The dextran sulfate-modified membranes have been confirmed to suppress or even eliminate platelet adhesion and human plasma fibrinogen adsorption on the surfaces, thereby prolonging effectively the blood coagulation times.[38] In addition, the glycosylated membranes exhibit noncytotoxic properties. On the heparin-modified membrane surface, the amount of adhered platelets and macrophages decrease significantly, indicating the fine anticoagulation.[96] Furthermore, the glycosylated membranes show excellent cytocompatibility. The group of Gao[57,97] found that chitosan existing on the surface improve the attachment, activity, and proliferation human endothelial cells on membranes. Yu et al.[98] described the similar results.

Besides, it has been found that the synthetic glycopolymers such as poly(GAMA)-modified membranes also show good biocompatibility.[72]

Protein Recognition. Specifically recognizing proteins is one of the most important biological functions of saccharides. Therefore, the glycosylated membranes can be used for protein recognition according to the kind of glycosyl groups.

Because of the specific interactions of concanavalin A (Con A) with α-D-mannose and α-D-glucose residues, the poly(AG)-grafted MPPMs exhibit recognition capability toward Con A.[16] When the glycoside density exceeds a critical value, the amount of Con A adsorbed to the membrane surface obviously increases

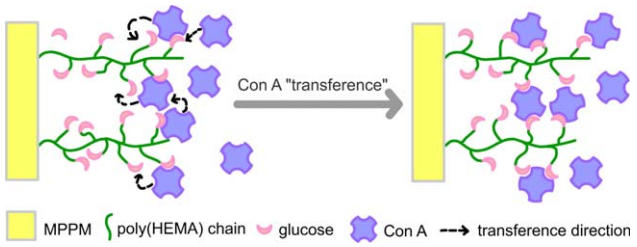

Figure 6. Scheme for the Con A "transference" mechanism (Reproduced from Ref. 15, with permission from Copyright Elsevier). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

as a result of the "glycoside cluster effect". Another work further confirms that the glycosylated membranes with glucosyl residues greatly inhibit the nonspecific adsorption of BSA and peanut agglutinin (PNA), but can selectively recognize Con A (as schemed in Figure 5).[14] Based on these results, a series of glycosylated membranes were prepared from galactose, lactose, glucose, and maltose (denoted as MPPM-Gal, MPPM-Lac, MPPM-Glc, and MPPM-Mal, respectively) and compared to recognize and adsorb specifically one of the two lectins, Con A and PNA. MPPM-Glc and MPPM-Mal adsorb Con A, whereas MPPM-Gal and MPPM-Lac adsorb PNA.[65] MPPM-Lac has enhanced affinity to PNA as compared with MPPM-Gal having similar glycoside density, whereas MPPM-Mal shows no enhanced affinity to Con A in comparison with MPPM-Glc as the "glycoside cluster effect" occurs. The reason for this phenomenon is that the nonreducing residue of some kind of disaccharide occupies the monosaccharide combining site with the additional contacts to the lectin provided by the reducing residue of disaccharide.

Apart from these traditional membranes, the nanofibrous membranes glycosylated with chitosan[52] and glucose[99] selectively recognize Con A while show almost no affinity binding with PNA.

Bioseparation. Based on the reversible interaction between saccharides and proteins or ligands, the glycosylated membranes could be used for bioseparation. This procedure belongs to affinity separation with highly specific and mild separation condition. It is meaningful for susceptible protein separation. Sarti and coworkers[100] applied amylase-modified cellulose membranes for affinity separation of lectins. It was found that the amylase affinity membranes are suitable for the separation of maltose-binding protein (MBP)-fusion proteins and the salt concentration in the feed solution affects the adsorption. A high concentration of NaCl reduces the affinity interaction between protein and ligand. From the results of dynamic adsorption and elution, they found that the kinetic constant decreases when the protein molecular weight increases. In addition, they compared the separation performances of cellulose membranes glycosylated with arabinogalactan, guar gum, and *N*-acetyl-D-galactosamine, respectively.[101] Among those membranes, that with *N*-acetyl-D-galactosamine shows the best separation performances, whilst membrane with arabinogalactan gives the highest binding capacity. Miyagawa et al.[58] have compared the separation properties of the glycosylated membranes with glycoconjugate polymers having lactose and mannose. The membrane glycosylated with mannose-containing polymer adsorbed 53% of the applied Con A and the membrane with lactose-containing polymer adsorbed 83% of the applied RCA.

To increase the binding capacity of protein, glycopolymer brushes were introduced on the membrane surface.[15] The glycopolymer brushes not only increase the specific area of membrane but also are benefit to adsorb multilayer proteins, which can enhance the binding capacity of protein on the affinity membrane. As schematically shown in Figure 6, Con A molecules adsorbed on the outer layer of flexible polymer brushes can be transferred into the inner layer through the reversible binding between Con A and glucose residues. This transference will be affected by the glycoside density, flow rate of protein solution, and degree of saturation.

In addition to purifying protein, the gylcosylated membranes were constructed for clinical application, for example, removing undesired and harmful substances from blood. Because heparin is one of the most effective low-density lipoprotein (LDL) ligands, it was bound on PSF membrane to obtain a dialysis membrane for selective removal of LDL from the blood of chronic kidney disease patient.[54–56,102] Miyagawa et al.[103] synthesized glycoconjugate polymers containing globotriose as Shiga-toxin adsorbents. Even at high concentration of protein such as fetal calf serum, the glycoconjugate polymers immobilized membranes eliminate Shiga-toxin from solution excellently. This kind of glycosylated membranes was also used to eliminate verotoxins (VTs), which can effectively dilute the starting concentration of VT1 (1 μg/mL) and VT2 (1 μg/mL) to about one hundred thousandth and about one thousandth parts of the starting concentration, respectively.[59]

Enzyme Immobilization. Immobilization of enzyme is a useful strategy to improve enzyme thermal and operational stability and recoverability. However, the enzyme activity decreases after immobilization. Improving the activity retention becomes a crucial issue. Among different ways, improving the microenvironment for the immobilized enzyme has been adopted on the membrane surface. For example, different natural molecules, such as saccharides and protein, were bound on the membrane surface to change the microenvironment for enzyme because of their excellent biocompatibility. In the light of this reason, the glycosylated membrane possesses a biofriendly surface suitable for enzyme immobilization.

Lipase was immobilized on the poly(AG)-grafted MPPMs by adsorption.[104] It was found that, as for the glycosylated membrane, the adsorption capacity and the activity retention of lipases are lower than those of the nascent ones, but the thermal stability is improved to some degree. These results are due to the hydrophilicity of the poly(AG)-grafted MPPMs, which weakens the hydrophobic interaction between the membrane surface and the enzyme protein and reduces the adsorption capability of lipase. It is believed that the active center of lipase can be activated by the interfacial hydrophobic interaction between the support surface and the enzyme protein. Therefore, hydrophobically modified chitosan and Nafion membranes were used to immobilize this kind of enzyme.[105,106] The hexyl-

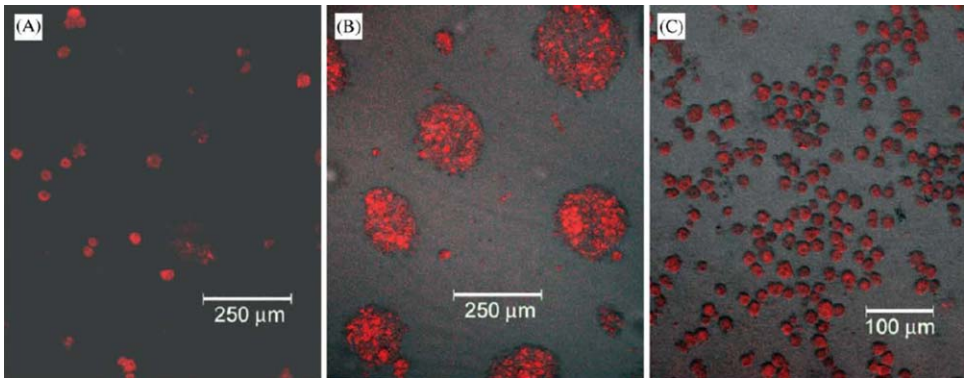

Figure 7. EROD assay for P450 1A1 activity of hepatocytes cultured on (A) PVDF membrane, (B) PVDF/F68-Gal membrane, and (C) PVDF/collagen membrane at day 5 (Reproduced from Ref. 43, with permission from Copyright Elsevier). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

modified chitosan membranes show a 2.69-fold enhancement in catalytic activity compared to that immobilized on unmodified chitosan.

Covalent immobilization is usually used to enhance the enzyme stability on membranes. Chitosan was tethered on the PANCMA membrane surface and lipase was then immobilized on these dual-layer biomimetic supports using glutaraldehyde.[11,107] It was found that both the activity retention of the immobilized lipase and the amount of bound protein on the dual-layer biomimetic membrane (44.5% and 66.5 mg/m^2) are higher than those on the nascent membrane (33.9% and 53.7 mg/m^2). The K_m values are similar for the immobilized lipases, whereas the V_{max} value of the immobilized lipase on the dual-layer biomimetic membrane is higher than that on the nascent membrane. Results indicate that the pH and thermal stabilities of lipase increase upon immobilization. Then a series of work of enzyme immobilization on chitosan-modified membranes were developed. Urease covalently immobilized on the PAN/chitosan composite membranes show a high activity (94%).[108] The relative activities and V_{max} of the immobilized acetylcholinesterase on the chemically bound chitosan membranes are higher than that on the physically bound chitosan membranes.[109] Similar results are from the thermal and storage stabilities of the immobilized acetylcholinesterase. All these studies show that the chitosan-modified membranes are suitable for enzyme immobilization. It may be ascribed to the biocompatible and hydrophilic microenvironment for the immobilized enzymes created by the chitosan layer on the membrane surface.

Moreover, the gylcosylated membranes can be used to immobilize suitable enzymes based on the saccharide-protein interactions. Compared with chemical immobilization, this method is benefit for the retention of enzyme activity. It has been reported that $\beta(1 \rightarrow 4)$ galactosyltransferase expressed as a fusion protein with binding MBP-galactosyltransferase was displayed specifically on a Langmuir–Blodgett membrane through maltotriose-MBP interaction.[110] Alliinase was also immobilized on PTFE membrane surface indirectly by a saccharide-lectin binding.[60] The saccharide mannan was bound to the membrane surface as an anchor for layers of Con A. Then alliinase was indirectly immobilized on the membranes by the lectin–enzyme interaction. Up to 0.2 $\mu g \cdot cm^{-2}$ of alliinase was immobilized as the highest enzyme loading. The best long-term stability was also achieved as compared with the covalent immobilization of alliinase.

Cell Culture. Saccharides on the cell membrane surfaces play an important role in recognition and regulation of the interactions between cells. By immobilizing specific saccharides onto synthetic membranes, it is possible to mimic the microenvironment for cells and provide multifunctional cell-adhesive surfaces.[111] Up now, galactose-modified materials for cell culture *in vitro* are the most studied glycosylated ones. They are usually used for hepatocyte culture. A large amount of asialoglycoprotein receptors (ASGPRs) are distributed on hepatocyte surface, which can selectively recognize and bond galactose and *N*-acetylgalactosamine.[112] So the galacosylated materials can enhance the hepatocyte adhesion on the surface.[113–117] Hepatocytes on the galacosylated surface maintain the round shape, whereas hepatocytes on collagen-coated surface spread out. With high galactose density, hepatocytes on the material surface are easy to form aggregates.[118] The hepatocyte behaviors are not only affected by galactose density, but also influenced by the orientation and microdistribution of the galactose ligand.[119,120] Compared with other materials, membranes are not only the substrates of hepatocytes *in vitro*, but also responsible for selective transport of metabolites and nutrients to cells and removal of catabolites and specific products from cells. Bartolo and coworkers[121] found that human hepatocytes maintain their liver-specific functions on the galactosylated PES membrane bioreactor for 21 days in terms of urea synthesis and albumin production as well as protein secretion. Hepatocytes develop aggregation and form tight junctions. Vinculin distributes into the cytoplasm and focal adhesions are visible. The gene expression of albumin and C-reactive proteins confirms the maintenance at the gene level of the specific functions of cells in the bioreactor. Lu et al.[43] found that the attached hepatocytes on PVDF/F68-Gal membrane self-assembly into multicellular spheroids after 1 day of culture, as shown in Figure 7. These attached hepatocytes in spheroids exhibit higher cell functions such as albumin synthesis and P450 1A1 detoxification function

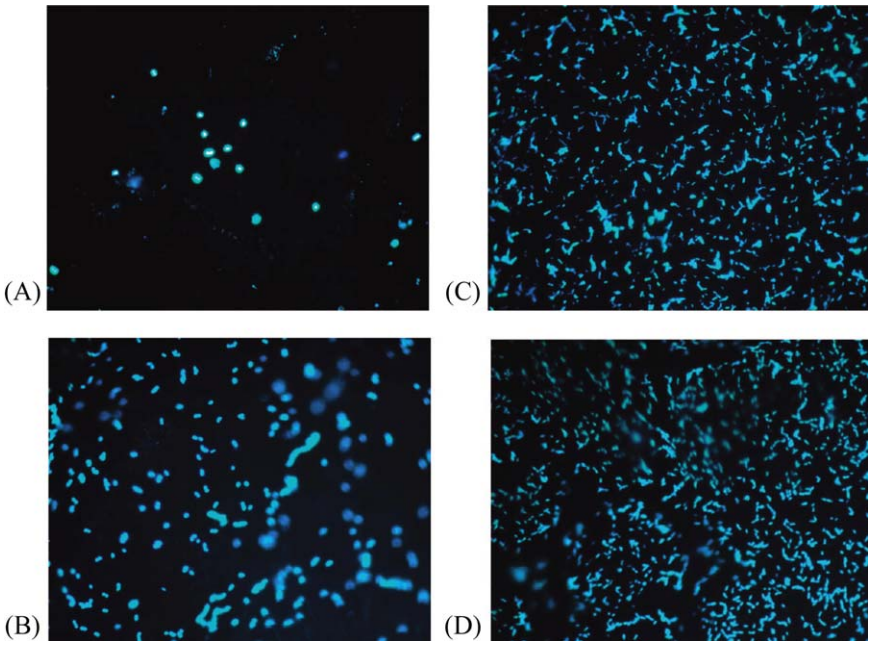

Figure 8. Fluorescence microscope images of DAPI stained *E. faecalis* and *Stenotrophomonas maltophilia* on MPPM surface. (A and B) Unmodified membrane with *E. faecalis* and *S. maltophilia*, respectively. (C and D) Poly(LAMA) grafted membrane with *E. faecalis* and *S. maltophilia*, respectively (Reproduced from Ref. 127, with permission from Copyright ACS). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

compared to unmodified PVDF membrane and collagen-coated membrane surface.

However, 3D hepatocyte spheroids cultured on the galactosylated surface eventually detach from the surface due to the relatively weak interaction between galalctose and ASGPRs.[43] To resolve this problem, galactosylated Si_3N_4 membranes were utilized to support hepatocyte attachment and function in the sandwich culture.[122] Compared to hepatocytes cultured in a collagen gel sandwich, diffusion studies confirm that the mass transport of this culture system is significantly better and can be configured by varying the porosity of the Si_3N_4 membranes. Besides, hepatocytes exhibit earlier apical repolarization and biliary excretion, improve differentiated functions and enhance drug sensitivity. Another method have been developed to stabilize the hepatocyte spheroids on the membranes by fabricating Arg-Gly-Asp and galactose hybrid membranes with an optimized hybrid ration of these two bioactive ligands.[123] Then the spheroid bottom can be firmly tethered to the membrane surface maintaining the spheroid.

Microorganisms Capture. Up to now, membrane surfaces are of great interest to have controlled bacterial adhesion properties. The glycosylated Gelman membranes were used to capture bacteria via microbial lectins expressed on their surfaces.[124] Polymers displaying five different saccharides (β-D-glucose, α-D-mannose, and the blood group antigens Lewis a, b, and x) were immobilized on the membrane surface. The glycosylated membranes provide greater sensitivity than the lectin-modified ones, due to the larger number of accessible saccharide ligands on the glycopolymers. Furthermore, saccharide capture surfaces maintain their affinity from microorganisms and are less readily blocked by contaminants, such as saccharide molecules containing in physiological buffers, urine, milk, and processed chicken samples. The glycosylated membranes can be used to capture and clean up microorganisms before matrix-assisted laser desorption/ionization mass spectrometry analysis.

In the last years, the synthetic glycoconjugates were also demonstrated to have the ability to recognize exposed molecules on bacteria surfaces.[125,126] Poly(2-lactobionamidoethyl methacrylate) (poly(LAMA)) was immobilized on the membrane surface to mimic the glyco-receptor on the cell surface for selective adhesion of bacteria.[127] All the results clearly demonstrate that the poly(LAMA) grafted membrane can selectively capture *Enterococcus faecalis* and that this selection is based on the interaction between galactose side groups on the grafted glycopolymer brushes and galactose-binding protein on the *E. faecalis* cell membrane (shown in Figure 8).

CONCLUSIONS

Although important progress on the glycosylated surface has been made in the past decade, much research is now focusing on the glycosylation on gold, glass, bead, and film surface. However, the glycosylated membranes combine the separation function of the membranes with the biological function of the saccharides in one system, the applications of which could be widely improved and expanded. To date, the glycosylated membranes are obtained by various methods including physical, chemical, and biochemical methods. Nonetheless, there are still some challenges. To mimic the *glycocalyx* layer of cell membrane, saccharides with more information and/or glycopolymers with controlled structures should be immobilized on membranes. Controlled/living grafting polymerization and click

chemistry provide the feasibility of controlled glycosylation. Moreover, development of gentle and efficient glycosylation method becomes impending. So the biological method, especially the enzymatic transglycosylation, is with unlimited potential due to its high selectivity, mild reaction conditions, and environmental friendliness.

Because the glycosylated membranes are highly hydrophilic, the water flux and antifouling performances of membranes are greatly enhanced. However, more and more attention has been attracted on the biological functions of these membranes. Based on the interaction between the glycosyl groups on membranes and protein, cell, or microorganism, the glycosylated membranes not only could be exploited as a tool for understanding the corresponding biological processes but also could be utilized to recognize and separate biomolecules. Until now, the biological and medical applications of the glycosylated membranes have a preliminary study with the introduction of the simplest glycosyl groups on membranes. Through the introduction of complex and special glycosyl groups with more bioinformation, the biological and medical functions of these membranes could be enriched, and the application could be extended.

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