Beyond separation: Membranes towards medicine

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ABSTRACT

Due to the global epidemic outbreak in recent years, membrane research and membrane-derived products have been of increasingly wide interest for medical applications. Currently, a new but important development direction of membranes in medicine goes beyond the separation function of the membrane itself to realize multifunctional integration. With the introduction of additional functions such as scaffold, responsiveness, and sensing, membranes have exhibited excellent performance in the areas of tissue engineering, drug delivery and disease diagnosis. From this perspective, we will review the recent progress made by membranes in the medical field and emphasize the principles of function integration and separation. Possible challenges will be proposed, and future development directions for medicine-related membranes will be discussed.

Introduction

Natural membranes have existed widely in organisms since the origin of life and dominate the basic metabolism of living organisms. As shown in Fig. 1, for a long time, humans made extensive efforts to decipher the behaviour and function of membranes in various physiological processes but were unable to prepare an artificial material mimicking the properties of natural membranes until the 19th century. Research on membranes originated in life science. In 1748, Abbé Nollet discovered the phenomenon of water penetration through the bladder of pigs, which became the first documented experiment describing membrane separation (Fane et al., 2011). The first artificial semipermeable membrane was prepared by Traube in 1867 by precipitating copper ferrocyanide in a thin layer of porous porcelain (Strathmann et al., 2011). Nearly a hundred years later, the membrane-based hemodialyzer was first successfully applied to clinical haemodialysis in 1944 (Kolff et al., 1944), beginning its application in medical science. However, due to the limits of the membrane ability and high research investment, great breakthroughs in membrane techniques and applications in medicine have been rare for a long time. In contrast, membranes have ushered in rapid development in various fields, such as energy, agriculture, food, and industry, during this period (Hugo et al., 2018; Vitola et al., 2021; Zhang et al., 2021; Liu and Jin, 2021). In the last twenty years, with the outbreak of global epidemics such as H1N1 influenza, SARS, and COVID-19, biomedical membranes have suddenly attracted attention again, and the application of membranes to human medicine has resumed.

Currently, most work on membranes in clinical medicine is mainly related to the artificial lung and kidney, which mostly rely on the sieving ability of membranes. Moreover, with increasing information on diseases and treatment processes, membranes are required to perform more functions than separation alone. Generally, biocompatibility is a necessary property for almost all medical membranes, as medical applications involve interactions with living systems. In addition, biodegradability, bioavailability, blood compatibility, scaffold, responsiveness, and electrocatalytic capacity are considered necessary properties for different applications. Do membranes possess these abilities? In terms of the geometric structure, a membrane has a porous surface and abundant internal channels, and its pore size, length, and degree of curvature can be freely adjusted through nanostructure methodology. The surface characteristics, hydrophilicity, hydrophobicity, or other physicochemical properties are controllable by grafting or modification. All of these properties can affect the separation rate of the membrane, and further special changes or designs will give the membrane other functions. In addition to structure and property control, the relevant membrane material may have external or internal responsiveness, facilitating its use in practical medical applications. At present, medical membranes are an emerging direction, and their applications in tissue engineering, drug delivery and disease diagnosis are gradually maturing. From this perspective, we will focus on medicine-related membranes for the above three medical applications, discuss the functions beyond separation, and examine the expected future application prospects of membranes into medical devices.

Abbreviations: 3D, three-dimensional; DDS, drug delivery system; HFM, hollow fibre membrane; NP, nanoparticle; PANI, polyaniline; PB, Prussian blue; PPY, polypyrrole; VEGF, vascular endothelial growth factor.

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Functions of medical membranes beyond separation

In addition to the traditional properties of permeability and selectivity, membrane materials or membrane processes used in medical applications often have new properties to satisfy different requirements of various clinical operations. In this review, we will discuss the functions of membranes beyond separation in three medical-related applications: tissue engineering, drug delivery, and disease diagnosis (Fig. 2).

In tissue engineering, membranes act primarily as scaffolds, and the prepared biocompatible scaffolds should support cell growth and differentiation, degrade at an ideal rate, and allow the regeneration of newly cultured tissues. As a result, suitable mechanical capacity and biodegradability are advantageous characteristics. In summary, the design and control of membranes to mimic the macro- and microstructures of natural tissues or organs are important for biological scaffolds, cell transport, or tissue regeneration. Furthermore, although drug delivery systems (DDSs) depend heavily on membrane permeability, through modification and improvement, membranes can exhibit enhanced bioavailability, provide selective cytotoxicity, enable targeted recognition, and thus achieve smart on-demand drug release. More importantly, due to the development of membranes with different stimulus responsiveness, the release rate of the corresponding DDS can be controlled by external trigger or feedback information, which is conducive to studying the relationships amongst membrane structure and properties and the intelligent control of drug release and will further promote research on complex DDSs. Moreover, to achieve the rapid reporting of key physiological indicators and develop an online dynamic monitoring method that indicates the fluctuation of the required analyte, disease diagnosis in medical treatment requires the membrane to have good blood compatibility, anti-interference ability, and high electrocatalytic activity. In the following sections, relevant examples, unique membrane properties and new techniques are briefly discussed.

Separation with scaffold (tissue engineering)

As an interdisciplinary field, tissue engineering was first proposed in 1987 to develop functional substitutes for damaged tissues (Langer and Vacanti, 1993). Its scope includes but is not limited to artificial scaffolds, cell culture, and artificial organs. Since the process involves the separation of cells or the penetration of nutrients, membranes with separation functions played a role in early research. In the middle of the 20th century, artificial kidneys based on cellophane tubing membranes were successfully applied in the clinic (Kolff et al., 1944). Since then, research on membrane-related tissue engineering has received extensive attention (Fig. 3).

In addition to biocompatibility, suitable mechanical properties and biodegradability, a tissue-engineering scaffold needs a 3D skeleton with controllable pore size and structure to support tissue formation and deliver sufficient nutrients. Of course, the applied membrane material is also required to satisfy the above requirements. Early in 1999, biodegradable scaffolds made of organic polymers were used to inoculate autologous cells and successfully used in cardiac surgery (Matsumura et al., 2003). In recent years, with the development of new technologies, more possibilities have emerged in scaffold design (Cipitria et al., 2011). Based on 3D printing technology, interconnected perfusable microchannel networks within polymer nanofibrous scaffolds were prepared for the engineering vascularized bone tissue (Gu et al., 2021). The connected microporous structure of the scaffold facilitates the penetration of nutrients, and the vascular endothelial growth factor (VEGF)-loaded hydrogel is conducive to repairing bone defects. Addi-
tionally, based on porous microchannels made of polycarbonate membranes, a semicircular microfluidic blood vessel scaffold was developed to reconstruct vascularized tissue (Kappings et al., 2018). Moreover, the combination of organic or inorganic membrane-related tissue engineering scaffolds and conductive elements can bridge the heart and neuronal scar tissues, resulting in macroscopic improvements in organ function and body behaviour (Burnstine-Townley et al., 2020).

Cell culture in tissue engineering requires the generation of high-density cell tissue in vitro. In this process, the surface topography and pore structure of the scaffold will impact the production, proliferation and migration of cells (Abbott and Kaplan, 2015). Because of the characteristics of the membrane itself, such as adjustable pore size and high specific surface area, and with the selection of appropriate materials and optimization by modification, membranes have been widely adopted in cell culture. In 1972, human choriocarcinoma cells were successfully cultured on polymeric hollow fibres (Knezek et al., 1972). Gradient membranes can be designed with different pore sizes, and different types of cells can grow on different sides of the permeable 3D-printed soft membrane and used for guided tissue regeneration applications (Tayebi et al., 2018). Recently, a nanofibrous cell culture insert device composed of a free-hanging polymer nanofibrous membrane was designed to mimic the extracellular matrix and better stimulate tissue production (Kumar et al., 2021). However, the environment of cells cultured in vitro for a short time may be different from that of cells in the body, leading to deviations in relevant physiological data. Therefore, it is of great significance to create a suitable environment, combine novel technologies, and conduct long-term and stable in vitro cell culture for research on tissue regeneration and the evaluation of drug results.

During organ development, cells combine with extracellular matrix in a specific direction to form a unique functional unit for each organ, and appropriate scaffold and/or cell culture methods in tissue engineering are essential to the preparation of artificial organs (Taylor, 2019). Taking artificial skins as examples, different modified membranes provide different functions. Antibacterial agent-modified graphene oxide-thermoplastic polyurethane composite wound dressing shows both excellent water vapour permeability and long-lasting antibacterial properties (Jian et al., 2020). However, the introduction of gelatine aerogel into graphene oxide gives wound dressings better haemostatic properties (Guajardo et al., 2021). In addition, based on superhydrophilic fibres and superhydrophobic copolymers, the obtained amphiphilic wound dressing presented unidirectional drainage and anti-adhesion capabilities, which can accelerate wound healing (Luo et al., 2021). However, these artificial skins still cannot perform all the functions of real skin. If we can fabricate smart membranes with electronic perception ability or pH perception ability, we can obtain better skin substitutes that more closely resemble real skin. In addition, there are also a large number of reports on the development of other artificial organs related to membranes with various properties, such as artificial corneas with anti-angiogenesis activity (Bakshandeh et al., 2021), and artificial livers with enhanced ultrafiltration performance and the ability to promote cell growth (Verma et al., 2019).

With the emergence of new technologies and advanced materials, membranes are increasingly widely applied in tissue engineering and have various functions close to those of the human body. For example, by integrating a thin porous membrane into a microfluidic device for cell culture, organ-level lung function can be reconstructed on a chip (Huh et al., 2010). As regeneration and reorganization of tissue cells or tissues are common in tissue engineering, thus, how to use tissue engineering methods to arrange different cells in an orderly manner on the membrane and form complex functional units in vitro is helpful for studying membrane-induced cell regeneration mechanism. In addition, we also need to further investigate whether the obtained artificial organs can mimic the development of complex diseases in vitro. Clinically, we need to design specific engineered tissues according to the different conditions of the patient. In order to more thoroughly understand the interaction between tissue engineered membranes and the human body, we need to make more efforts to study the biological effects of tissue engineered membranes and their interaction with the body’s immune regeneration system, etc.

Separation with responsiveness (drug delivery)

DDSs refer to the transport of drugs through various carriers to the target biological site for drug release and absorption (Li et al., 2019). To provide and maintain a therapeutic concentration of the drug at the desired location, the DDS needs to increase its pharmacological activity with fewer side effects while improving the solution and chemical stability of the active agent. Membranes have the following advantages in DDS applications: (1) increasing the solubility of bioactive substances, (2) protecting bioactive molecules from degradation, (3) providing continuous release of drugs, (4) improving the bioavailability of drugs, and (5) achieving targeted drug delivery. In addition to separation, the responsiveness of DDSs to external and internal stimuli (temperature, magnetism, ultrasound, pH, redox potential, enzymes, etc.) can enable site-specific release at the target location and regulation of the release rate with feedback. In a responsive DDS, at least one component of the membrane material will be easily affected by a specific physical stimulus, or will respond to a given stimulus, producing reactions such as protonation, hydrolysis, charge change, etc (Mura et al., 2013). Currently, many materials have been used to design the responsive DDS. For example, the common used temperature-responsive membrane materials are liposomes, polymer micelles or nanoparticles (NPs). Meanwhile, polymers, lipids and inorganic materials have been used in enzyme-responsive systems. Below, the application of membranes in transdermal drug delivery and NP-assisted drug delivery (Fig. 4) will be discussed.

Early in the 1950s, osmotic membrane systems based on rate-controlling semipermeable membranes were developed (Stamatialis et al., 2008). Relying on similar drug release mechanisms, the transdermal patch, which is currently the most common transdermal system, has been widely applied in the medical market. The first patch on the market was a hyoscine patch (Transderm Scop®; Novartis Consumer Health), which controls the drug release rate through a microporous organic membrane for the treatment of motion sickness (Pastore et al., 2015). The available transdermal patches generally use organic membranes as the material, such as natural polymers, elastomers, and synthetic polymers (Silva et al., 2014; Pichayakorn et al., 2012; Anirudhan and Nair, 2017). However, due to the high diffusion resistance of the lipophilic stratum corneum, to enhance skin penetration and increase the delivery efficiency of hydrophilic or macromolecular drugs, many transdermal drug delivery systems combined with noninvasive and minimally invasive technologies, such as electroperoration (Anirudhan and Nair, 2019), sonophoresis (Manikkath et al., 2017), iontophoresis (Fan et al., 2008), and microneedles (Waghule et al., 2019), have emerged. Simultaneously, compared with traditional transdermal patches, responsive transdermal patches can achieve a more controllable and safer release mode. For instance, mimic multienzyme MOF-based stimuli-responsive microneedles were designed for painless glucose-mediated transdermal delivery, and MOF was first designed as a catalase mimic carrier that can decompose H₂O₂ during insulin release (Yang et al., 2020). Because transdermal patches have the advantages of noninvasiveness and flexible dosing, related products have been continuously developed. At present, the transdermal patches on the market still have problems such as poor adhesion, low drug flux, and poor stability. The future trend will be to select suitable membrane materials combined with appropriate transdermal technology to carry out sufficiently effective drug delivery.

During the past few years, because NPs may improve the stability and solubility of encapsulated drugs and increase the safety and effectiveness of drug delivery, NP (lipid-based, polymeric and inorganic NP)-assisted drug delivery has received increasing attention (Mitchell et al., 2021). Liposomes are the most popular and most thoroughly studied nanocarriers for targeted drug delivery, as they have many advantages, such
as biocompatibility, self-assembly capability, and the ability to carry a large number of drug payloads (Sercombe et al., 2015; Crawford et al., 2011). Through temperature response or pH-related polymer modification, liposomes can deliver drugs to locally heated target tissues or intracellular spaces (Yuba, 2020). Among polymeric NPs, polymersomes are more stable and have a higher efficiency of cargo retention than liposomes (Rideau et al., 2018). Recently, Genexol-PM, a biodegradable Cremophor-free polymer micelle formulation of paclitaxel, has entered clinical trials (Lee et al., 2018). Additionally, the active functional groups on the exterior of dendrimers can bind to biomolecules while loading drugs into the interior, enabling their use as therapeutic diagnostic agents, transfection agents, topical gels and contrast agents. However, polymeric NPs still have the risk of increased particle aggregation and toxicity. In addition, inorganic NPs, such as Fe$_2$O$_3$ NPs, Fe$_3$O$_4$ NPs, calcium phosphate and mesoporous silica nanoparticles, are used in therapeutic delivery due to their optical, magnetic and/or physicochemical properties (Montaseri et al., 2021).

The use of nanomaterial-based membranes promises additional possibilities for drug delivery. Since the characteristics of NPs, such as size, shape, charge and surface characteristics, can be modified through design, DDSs can be specifically optimized and further combined with medical technology to achieve large-scale clinical application. Responsive DDSs can more effectively achieve on-demand release, improve treatment effects, and reduce adverse reactions, which provide opportunities for future medical applications.

**Separation with sensing (disease diagnosis)**

Due to the haemolysis and coagulation mechanism of whole blood, the clinical methods for blood assays always require the separation of whole blood for serum collection before testing. In this case, the online and dynamic monitoring of blood indices is difficult because of such independent operations. However, this technique is always desired and meaningful for risk prediction (such as complications and inflammation) before and during the surgery to guide point-of-care operation and reduce mortality. In many recent studies, although some biosensing methods have already achieved the direct analysis of whole blood, dynamic and real-time analysis is still a major challenge due to the discontinuous operations and instruments of blood separation and testing. Membranes have already been confirmed to enable the size sieving of different blood...
components and are capable of replacing traditional blood separation methods, such as centrifugation, for the continuous collection of serum from whole blood. Electrochemical biosensors have also proven that as long as serum is obtained, the detection signal can be dynamically reported. Therefore, some work has begun to examine this new area, seeking to combine membranes and biosensors for dynamic monitoring of the physiological indices of patients in surgery.

Prussian blue (PB), due to its different metal valence states and low energy gap, has good electrocatalytic ability. During the redox process of \( \text{H}_2\text{O}_2 \), PB is a good electron carrier, which can transport electrons from the electrode surface to \( \text{H}_2\text{O}_2 \). In addition, the working potential of PB is very low, which can avoid the interference of other substances. Therefore, PB has been widely used as a sensing material. Early in 2016, our group first achieved cell sieving and detection with a synchronous device. Through a self-assembly method, we prepared a double-nanostructured blood separation-sensing film using PB as the membrane material and alumina hollow fibre as the support (Fig. 5a) (Chu et al., 2016). The amorphous porous PB separation layer formed on the surface can be used for serum extraction, and the sensing layer consisting of regular PB crystals, formed in the inner channel, can detect physiological indices in the separated serum. Recently, we presented a new separation-sensing platform and further achieved the online and dynamic detection of blood sugar, lactic acid, glutamate, aminotransferase and cancer targets. By adjusting the reaction rates of polypyrrole (PPy) and PB, we successfully obtained a separation-sensing membrane with heterogeneous nanostructures. First, the PB precursor solution was filled in the hollow fibre. Later, Py was diffused into the pores of the hollow fibre. Since Fe\textsuperscript{3+} was used as an oxidant to initiate the polymerization of Py, the formation of PB occurs after the formation of PPy. By compositing PB and PPy to optimize the separation-sensing membrane material and its detection performance, the obtained membrane can integrate the processes of blood drawing, serum testing and analysis in traditional blood testing, shortening the time from more than 30 min to 1 min (Fig. 5b) (Chu et al., 2020). This separation-sensing membrane can achieve non-destructive separation and stable sensing under the conditions of extended surgery, revealing the great potential of the membrane in medical applications. Recently, with the deposition of polyaniline (PANI) and Pt NPs on polysulphone hollow fibre membrane (HFM) scaffolds, a gradient porous 3D conductive gradient PANI/Pt-HFM-based biosensor platform was proposed, which could achieve capillary-driven selective blood separation and \textit{in situ} electrochemical detection (Wu et al., 2021b).

In addition to the above two-in-one devices, membrane separation and sensor integration can also be achieved by building a multicomponent platform, which has already been applied to wearable devices. Due to the growing demand for chronic disease prevention and treatment, wearable devices capable of continuous and real-time monitoring will have very broad medical application prospects. Through the combination of membrane separation and biosensing, wearable devices can be used for physiological index detection and drug release (Jqbal et al., 2021). By integrating drug reservoirs, strain sensors for force detection, and microneedles for effective transdermal delivery, a touch-actuated transdermal delivery patch was developed for controllable drug delivery, and quantitative skin permeation control could be observed through the mathematical model of relevant parameters (Kim et al., 2018). Recently, based on silicone hydrogels, a smart contact lens comprising a biosensor, a flexible drug delivery system, a wireless power transmission system from a transmitter coil to a receiver coil, an ASIC chip, and a remote communication system was fabricated to detect glucose levels in tears and deliver drugs for the treatment of diabetic retinopathy (Keum et al., 2020).

By using membrane separation and biosensing synchronously instead of traditional centrifugal biochemical analysis techniques in the clinical assay, the two-in-one membrane enables us to solve problems that require a long time for reporting, high-cost instruments for operation, and non-real-time feedback for diagnosis. This technique has begun to exhibit advances, distinguishing state-of-the-art assay methods in the onsite and online monitoring of key blood contents, which promises to play important roles in emergencies, surgeries, blood transfusions and wearable devices. In the future, more detectable blood components, much higher separation precision and device miniaturization are the main challenges to extend this research area to whole-period medical care from early disease screening to treatment and recovery.

Materials and properties of medical membranes

As mentioned above, emerging medical applications are no longer limited to traditional properties but involve higher requirements. As one of the necessary important properties, biocompatibility of medical membranes is generally evaluated by a series of standard tests (Ghasemi-mobarakeh et al., 2019), such as cytotoxicity test, genotoxicity test, carcinogenicity test, and implant test. A membrane with good biocompatibility is required not only to cause insignificant immune response in body fluids or organs, but also to tolerate the normal metabolism behaviour with high biological and physical stabilities. Illustrative examples of membranes that have been utilized in medical applications are summarized in Table 1. In tissue engineering, membranes can simulate the biological microenvironment; for example, block copolymer scaffolds with anatomical-like architecture were produced to realize architecture-guided cell distribution, thereby providing more possibilities for the biomanufacturing of autologous tympanic
membrane replacements (Mota et al., 2015). Villi-like micropatterned porous membranes can promote cell differentiation and culture cells with improved physiological relevance (Gommers et al., 2019). In particular, certain parts of a tissue may require specific functions, and the most obvious is that membrane transparency is necessary for artificial cornea-related applications (Xu et al., 2018). In drug delivery, the use of materials with different responsiveness will enable DDSs to better achieve on-demand drug release. For instance, the use of temperature-responsive nanogels enables transdermal delivery to provide long-term drug release in a temperature-controlled manner (Carmona-Moran et al., 2016). Simultaneously, the sonoactivatable liposomes obtained by introducing pyrophosphoribide respond to low-intensity focused ultrasound (Wang et al., 2018). In addition, drug-coupled dendritic macromolecules could exhibit pH-controlled targeted drug delivery (Kaur et al., 2017). For medical diagnosis via separation and sensing, a one-time blood assay could be achieved by combining capillary-driven separation and enzymatic reaction principles to simplify the entire blood processing process through trace sampling (Wu et al., 2021a). Moreover, our group has verified that the separation-sensing membrane as a platform technology is able to achieve the dynamic collection of various biotargets during surgery and emergency procedures (Chu et al., 2020). Moreover, immune and aptamer responses have been introduced for the first time, which greatly expands the detection categories, enabling application not only to the detection of some small molecular physiological substances but also to protein dynamic detection, proving the reliability of this approach in the clinic.

Conclusion

In current medical care, the process of disease treatment can be roughly divided into three stages: diagnosis, treatment and rehabilitation, which are related to tissue engineering, drug delivery, and sensor analysis. Membrane technology has made various advances in these aspects, illustrating its unique and irreplaceable advantages in medical science. An increasing number of membranes have performed roles beyond separation, exhibiting various functions for extended application. In addition to separation, scaffold, responsiveness, biocompatibility, etc., have been continuously considered for grafting to the native separation properties for applications in tissue engineering, drug delivery, and disease diagnosis. Nevertheless, membrane research in medicine is a new direction that must face many challenges for its clinical applications:

1. The match between the preparation environment of tissue engineering-related membranes and the real, constantly changing environment requires membranes with good biological stability. Furthermore, through in-depth study of the surface functionalization, activation and intelligence of scaffold materials, the prepared new generation of tissue engineering membrane can regulate cell growth, differentiation and tissue regeneration, which will promote the further application of membrane in organ transplantation and clinical treatment.

2. More smart drug delivery systems are required to selectively increase the bioavailability of the drug at the target site and maintain its stability in the blood circulation. More in-depth exploration of membranes with more precise responsiveness, much higher targetability, and more specific control rates is needed. Simultaneously, the future DDS membranes will be multi-component, multi-stimulus responsive, and predictable. More attentions should be paid to how to reduce biological toxicity and improve pharmacokinetic efficiency in clinical development.

3. The online and long-term monitoring of blood indices during surgery requires membranes with improved antipollution and blood compatibility with rare cell damage to maintain steady separation and sensing. In order to develop wider clinical applications of separation-sensing membranes, it is necessary to consider new methods or technologies to increase the number and speed of detection of physiologic indicators, and to improve the analytical compatibility of membranes with clinical instruments.

Overall, future development will not be limited to the membrane but will be further extended to the device, focusing on the development of miniaturized, intelligent, and visual instruments. This will require cost reduction and the subsequent large-scale manufacturing of supporting instruments to be sustainable. With the advent of new materials and technologies, the intersection of membrane science and other disciplines has become increasingly close, and we believe that one day, membranes could be used on a larger scale in medical applications such as artificial retinal transplantation, highly precise drug delivery, and autologous blood purification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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