A Review on Membranes for Clinical Treatment and Drug Delivery in Medical Applications

Catherine Charcosset*

Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne; LAGEP, UMR 5007, CNRS, CPE, 43 bd du 11 novembre, 69100 Villeurbanne, France

Abstract: Membrane processes are used extensively in biomedical applications. This state of the art review presents the main applications including renal kidney, blood filtration, blood oxygenator, artificial liver, artificial pancreas, and drug delivery devices. For well-established treatments like dialysis, plasmapheresis, and blood oxygenator, the techniques are summarized by presenting membranes used, devices, configurations and treatments. The artificial liver and the artificial pancreas are not clinically used and some main aspects related to the development of these devices are given, including configurations and liver or pancreatic cells. Finally, drug delivery devices based on membranes, which are an important area in pharmaceutics, are summarized by focusing on diffusion and transdermal delivery systems, as well as colloids like liposomes and nanocapsules. These colloids with nanometric size are surrounded by a lipidic or polymeric thin membrane which controls drug transfer to the surrounding medium.

Keywords: Membrane processes, Biomedical applications, Bioartificial organs, Dialysis, Blood oxygenator, Drug delivery.

1. INTRODUCTION

Biomedical membrane processes are among the first successful membrane techniques [1, 2]. They include biomedical techniques like renal kidney, blood filtration, blood oxygenation, artificial liver, artificial pancreas, and drug delivery devices. Among these techniques, dialysis, plasmapheresis, and blood oxygenation are routine clinical treatment. Dialysis is defined as the diffusion of molecules in solution across a semipermeable membrane along an electrochemical concentration gradient [3-5]. Over the past half century, the widespread use of dialysis to prolong life for people without kidney function has been a remarkable achievement. Plasmapheresis, or plasma exchange, is an extracorporeal blood-purification process whereby plasma is removed from the patient and artificially replaced [6, 7]. It is widely used to remove large molecular weight pathogens from the patient's blood, including antibodies, immune complexes, monoclonal proteins, endotoxins, drugs, and cholesterol containing lipoproteins. The last technique, the membrane blood oxygenation is clinically used to oxygenate the patient's blood during open-heart surgery [8]. Gas (usually oxygen or oxygen/nitrogen mixtures) diffuses from the gas phase through the gas-filled membrane pores into the blood, and carbon dioxide diffuses from the blood into the gas for disposal.

Bioartifical organs, like artificial liver and bioartificial pancreas have also been extensively studied, although their applications remain more limited. Artificial liver systems have been developed to bridge patients with severe liver failure to liver transplantation or liver regeneration [9-11]. These devices include artificial organs, based on hemodialysis, plasmapheresis, specific or non-specific adsorption, and bioartificial organs. Bioartificial pancreases are dedicated to the treatment of diabetes which represents a major public health problem in industrialized countries [12, 13]. Three major types of bioartificial pancreas have been developed: diffusion chamber, hollow fiber unit and microcapsules.

Finally, drug delivery systems enable the introduction of a therapeutic substance in the body and improve its efficacy and safety by controlling the rate, time, and place of its release. Drug delivery systems are commercialized in various forms and applied to various medical treatments. Drug delivery membrane systems include osmotic membrane systems, diffusion controlled membrane systems (pills, implants, patches), and transdermal drug delivery [1, 2]. Liposomes and nano and microcapsules, which are spherical shaped colloids, may also be considered as membrane drug delivery systems, as their aqueous core is surrounded by a lipidic or polymeric membrane.

In this state of the art review, we focused on medical applications of artificial membranes including artificial organs and drug delivery systems based on membranes. Several membrane artificial organs are described in detail: artificial kidney, blood oxygenation,

*Address correspondence to this author at the Université de Lyon, F-69622, Lyon, France; Université Lyon 1, Villeurbanne; LAGEP, UMR 5007, CNRS, CPE, 43 bd du 11 novembre, 69100 Villeurbanne, France;
Tel: + 33 4 72 43 18 34; Fax: + 33 4 72 43 18 99;
E-mail: charcosset@lagep.univ-lyon1.fr

E-ISSN: 2410-1869/16

artificial liver, and artificial pancreas. For drug delivery, diffusion controlled systems include pills, implants and patches; and transdermal delivery systems include passive as well as iontophoretic systems. Colloidal formulation are also detailed such as liposomes [14-16] and nanocapsules [17, 18] with a lipidic or polymeric membrane surrounding an aqueous core.

2. DIALYSIS - ARTIFICIAL KIDNEY

Several reviews on specific aspects related to dialysis and artificial kidney are available. For example, Himmelfarb and Ikizler [3] describe the medical, social, and economic evolution of hemodialysis therapy. Treatment time, frequency of dialysis, hemodialysis in the United States, patient safety and technical advances, controlled trials of dialysis therapy and evaluation of cardiovascular risk are detailed. Hoenich [19] presents the current state of the art of hemodialysis membranes and discuss their biocompatibility and the role played in morbidity and mortality associated with dialysis treatment. Ronco *et al*. [4] analysed the evolution of synthetic membranes for blood purification, by focusing on Polyflux membranes, originally derived from polyamide. Waniewski [5] described current approaches to modeling of transport processes in dialysis, including the urea model, sodium model, models of peritoneal transport, which combine the general physiological knowledge with information about individual patients yielded by clinical measurements. Alternative and complementary approaches to modeling of transport processes in dialysis are also discussed.

Hemodialysis

In hemodialysis, dialysis is applied to blood purification of patients with end-stage renal disease. The primary goal of hemodialysis is to restore the intracellular and extracellular fluid environment that is characteristic of normal kidney function [3]. By replacing kidney excretory function, dialysis is intended to eliminate the symptom complex known as the uremic syndrome. Hemodialysis is accomplished by the transport of solutes such as urea, creatinine, and glucose from the blood into the dialysate and by the transport of solutes such as bicarbonate from the dialysate into the blood. Solute concentration and molecular weight are the primary determinants of diffusion rates. Small molecules, such as urea, diffuse quickly, whereas larger molecules, such as phosphate, β2-microglobulin, and albumin, and protein bound solutes diffuse much more slowly. In addition to diffusion, solutes may pass through pores in the membrane by means of a convective process driven by hydrostatic or osmotic pressure gradients.

The usual hemodialysis treatment time is about 4 h and frequency is 2 or 3 times per week [3]. The blood flow rate is usually between 200 to 400 mL/min, and depends on the type and quality of vascular access. Increasing blood flow increases solute removal; however, increased flow resistance will eventually limit the augmented clearance. The usual dialysate flow rate is twice the blood flow rate in order to obtain optimal solute clearance. The dialysate is typically composed of sodium, potassium, calcium, magnesium, alkaline buffers, predominantly bicarbonate with a small amount of acetate, chloride defined by prescribed cations and alkaline buffers in dialysate, glucose, and anticoagulation heparin or other agents.

Hemofiltration and Hemodiafiltration

Different other modes of dialysis treatment are commonly used. Hemofiltration is a fully convective treatment in which large amounts of ultrafiltrate are produced and replaced by an ultrapure substitution fluid, and no dialysis fluid is present [4]. This treatment offers the advantage of excellent clearances for large molecules. However, it has only been clinically applied in Europe because of the need for large quantities of substitution fluids and their high costs.

Hemodiafiltration is a treatment in which diffusive and convective transport occurs simultaneously [4]. The amount of ultrafiltration exceeds the patient weight loss, and therefore the final fluid balance is achieved by the infusion of appropriate amounts of substitution fluid in the arterial or venous line. Due to high costs related to substitution fluids, new approaches have been proposed in Europe for performing hemofiltration and hemodiafiltration. Part of the fresh dialysate is diverted from its line to the dialyser, is filtered and used as an ultrapure substitution fluid. These on-line treatments have the advantage of reduced costs, unlimited production of substitution fluid and no need for commercially prepared fluids in bags. The microbiological and chemical purity of the on-line produced fluid have been proved to be sufficient.

Dialysis Membranes

Cellulose and its derivatives are the first generation of polymers which were used in dialyzers in the 1970s. However, the low membrane permeability and molecular weight cutoff near 2000 daltons of cellulose-

based materials limits their use. From the 1980s, synthetic polymers such as polysulfone, polyethersulfone, polyacrylonitrile, polyamide and polymethylmethacrylate have been widely developed and can be considered as the second generation of dialysis membranes. Compared to cellulose membrane, synthetic materials offer a higher permeability and can be tailored to different molecular weight cutoffs. However, the hydrophobicity of synthetic polymer has adverse effect on biocompatibility. Therefore, surface grafting, coating or blending are usually needed to render the membrane hydrophilic and improve biocompatibility.

The Polyflux membranes are examples of dialysis membranes made from polymers blend, originally derived from the synthetic polymer polyamide (Figure **1**) [4]. Three different polymers are used: polyamide, which provides endotoxin retention due to the hydrophobic sites and improved biocompatibility due to minimal interaction with blood components; polyarylethersulfone, which provides mechanical strength and resistance to heat sterilization; and polyvinylpyrrolidone frequently used in pharmaceutical formulations, which contributes to the hydrophilic domains in the surface and the enhanced diffusive permeability.

Characteristics of membranes used in dialyzers include water flux or water permeability. The water permeability of hemodialysis membranes is typically obtained from experiments in which bovine blood is ultrafiltered at varying transmembrane pressures (TMP). The relationship between plasma ultrafiltration rate and TMP is linear at relatively low TMP values, whereas a plateau occurs at higher TMP values. The permeability is defined by the slope of the linear portion of the permeate rate versus TMP curve. Low-flux dialysis favoring the removal of low-molecular-weight solutes may be performed using membranes manufactured from cellulose, modified cellulose, or synthetic polymer blends. Hemodiafiltration or hemofiltration require the use of high flux membranes, predominantly manufactured from synthetic materials. High-flux dialysis offering enhanced removal of small molecules and reduced treatment times (when used in combination with high blood and dialysate flow rates) can be performed with modified cellulose or synthetic membranes. In this case, convective transport dominates.

Biocompatibility related to the repeated contact of blood of patients treated by dialysis in the extracorporeal circuit is an important clinical issue [19]. The membrane in a dialyzer represents a large non-physiologic surface to which the blood is exposed during each treatment. This exposure results in a number of events such as the deposition of proteins onto the membrane surface, activation of the complement system, kinin, and coagulation and fibrinolytic pathways, as well as activation of the cellular elements of blood on each occasion (Figure **2**). A variety of factors governs the magnitude of these events, including chemical composition and surface character, with synthetic materials outperforming cellulose-based materials. However, it remains unclear if membrane biocompatibility may have a role in patient survival and recovery of renal function in acute renal failure. There seems to have no association between membrane type and recovery of renal function and that factors other than the membrane such as comorbid conditions and dose of dialysis are more important [19].

Membrane Modules

The first dialysis modules were flat plate- and frame-modules and coil type modules [1]. The flat plate-and frame-modules contained sheets of cellophane or cuprophane membranes. The coil type modules consisted of cellophane tubes, which were flattened, placed on a nylon open mesh “spacer” material and rolled into a coil. The coil was then held in a cartridge which was open at each end. Today, most dialysis modules are in hollow fiber configuration

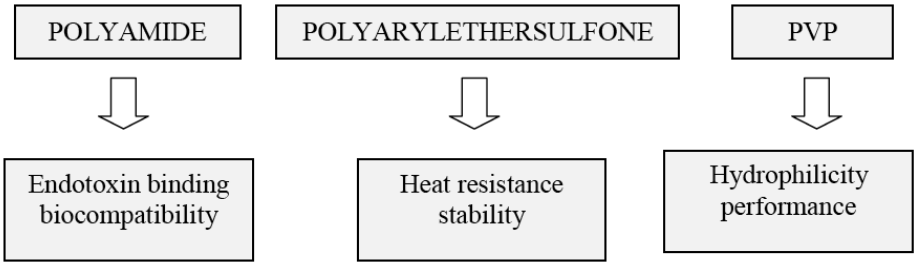

Figure 1: The Polyflux membrane results from a blend of chemical components prevalently present in the different layers of the membrane wall. (redrawn from [4]).

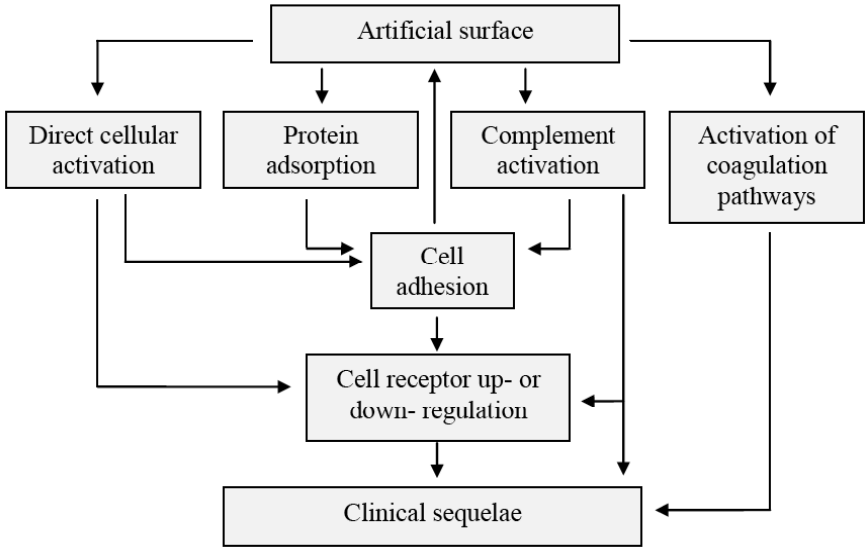

Figure 2: Blood pathway activation following material contact (redrawn from [19]).

modules. The fiber length is approximately 30 cm and the modules contain thousands of fibers (up to 15,000, membrane surface area up to 2.2 m^2). The inner diameter is typically between 180 and 220 μm and wall thickness between 20 and 50 μm.

The design and the construction of the dialysate compartment are of major importance for the performance of a dialyser to ensure even distribution of the dialysate [1]. For this purpose, different configurations have been proposed such as spacer yarns, fiber crossing or waved fibers. The wavy shape of the undulated fiber prevents dense packing and ensures optimum dialysate circulation. In the Polyflux dialysers, waved fibres are additionally crossed and, by combining these two steps, a three-dimensional network guarantees stable and high mass transport rates in the whole bundle [4]. A homogeneous distribution of the flow can then achieved as well as a significant increase in solute clearances. In addition, the blood ports cannot be considered simply as arterial and venous ends of the unit [4]. They must have a minimal stagnation of flow and should guarantee a homogeneous distribution of blood flow in all the fibres of the bundle. For this purpose, different types of flow distributors have been proposed (conical, spiral, etc.) with reduced space between the cap and the potting.

Urea Kinetic Modeling

Several models has been proposed for solute transport in hemodialyzer which have been extensively reviewed [5]. For example, the solute flows in blood and dialysate channels can been obtained by using the mass balance in the slice from x to x+d of the membrane [20]. Urea is usually used for quantifying dialysis adequacy through mathematical modelling based on changing blood concentrations [3, 5]. The most well-known parameter is “Kt/V”, an index of small solute (like urea) removal. It was introduced in the 1970s, and then linked to the morbidity and mortality of hemodialysis patients based on the results of the National Cooperative Dialysis Study [21]. From the 1990s, it was increasingly used and investigated in many studies.

In human kidney and dialyzer, waste solute removal is proportional to solute concentration [22]. In the steady state when mass of solute is constant, the generation and removal rates are equal. The mathematical description of the balance of total urea mass is given by:

$$0 = G - K_r C \quad (1)$$

where G is generation rate (mg/min), K_r is renal clearance (ml/min), and C is concentration (mg/mL). Eq. 1 can be solved as:

$$C = G / K_r \quad (2)$$

Eq. 2 describes the relationship between generation, clearance and concentration for many solutes excreted by the human kidney. When steady state is not more realized, such as in intermittent dialysis therapy, the mass balance is written as the product of solute distribution volume (V) and rate of change in concentration (dC/dt):

$$V(dC/dt) = G - K_d C - K_r C \quad (3)$$

where K_d is the intermittent dialyzer clearance and K_r is the remaining continuous renal clearance. Integration of eq. 3 over a complete treatment gives:

$$C_t = C_0 \exp(-K_d t/V) + (G/K_r)(1 - \exp(-K_d t/V)) \quad (4)$$

where C_0 and C_t are the initial and final solute concentrations at the beginning and end of each dialysis.

Eq. 4 can be applied to any solute targeted for removal and concentration control by intermittent dialysis therapy [22]. In Eq. 4, $K_d t/V$ is an exponential term which controls the magnitude of drop in solute concentration over a complete dialysis treatment. It quantifies the mechanisms by which solute removal is achieved by either dialyzer clearance or renal clearance; the concentration of solute being a function of both $K_d t/V$ and the generation rate, G. Nowadays, $K_d t/V$ is the basic parameter to prescribe the dose of dialysis (clearance K_d, time of dialysis t) for patients, represented by their total body water volume V. The clearance depends on the hemodialyzer used and operating conditions of dialysis (blood and dialysate flows, ultrafiltration rate) and it can be predicted from a mathematical model of dialyzer performance [5].

Several models have been derived from the simple Kt/V model, such as $spKt/V$, eKt/V, $(Kt/V)dp$, of single-pool, effective and double-pool Kt/V model, respectively [23]. Nevertheless, it has been pointed out that Kt/V and associated parameters should not be the only ones to be taken into account for the assessment of dialysis adequacy based on small solute removal. Many clinicians demonstrated that the dose parameter must be associated with medical outcome, *e.g*. mortality, morbidity, or medical well-being.

Several models has been proposed for solute transport in hemodialyzer which have been extensively reviewed [5]. For example, the solute flows in blood and dialysate channels can been obtained by using the mass balance in the slice from x to x+d of the membrane [20].

3. PLASMAPHERESIS

This state of the art review focuses on therapeutic plasmapheresis as a medical treatment, although plasmapheresis can also be conducted as donor plasmapheresis. Therapeutic plasmapheresis has been reviewed extensively. For example, Nakanishi *et al*. [6] described the state of the art of various therapeutic plasmapheresis (plasmapheresis with addition of a substitution fluid, double-filtration plasmapheresis (DFPP), and plasma adsorption) by presenting their modalities, representative diseases, and complications. Baweja *et al*. [7] reviewed current indications of plasmapheresis, or plasma exchange, for treatment in renal disease, such as anti-glomerular basement membrane disease, cryoglobulinemia, multiple myeloma, and in case of some renal transplantation.

Plasma Exchange

Plasmapheresis, or plasma exchange, is an extracorporeal blood-purification process whereby plasma is removed from the patient and artificially replaced [7]. The process was first described in the dog in 1914 and the first therapeutic plasmapheresis was performed in two patients with macroglobulinemia in the 60's. Since then, it has been widely used to remove large molecular weight pathogens from the patient's blood, including antibodies, immune complexes, monoclonal proteins, endotoxins, drugs, and cholesterol containing lipoproteins.

Several plasmapheresis techniques are currently available, such as plasma exchange, DFPP, and plasma adsorption [6]. Each modality has its own advantages and disadvantages based on the efficacy and specificity of removing the target substance, cost, and complications. Plasma exchange was developed in the 1970s and is currently widely used. In this treatment, the whole plasma is discarded using a plasma separator, which contains essential and pathogenic substances, and the substitution fluid of equal volume to the discarded plasma is infused back to the patient. Plasma exchange is used to treat many diseases, such as thrombotic microangiopathy, hepatic failure, and neurological diseases. In addition to the removal of pathogenic substances, essential factors, such as albumin and coagulation factors can be supplied to the patient. The whole blood flows into a plasma separator and approximately 0.6-1.2 L/h of plasma is separated from the whole blood for 3-4 h. Usually, the substitution fluid is fresh frozen plasma or 4–6 % albumin solution, depending on the coagulation and/or immune state of the patient.

Double Filtration Plasmapheresis

The principle of DFPP was first introduced by Agishi *et al*. [24] to reduce the amount of substitution fluid, such as albumin solution. Two types of membrane filters with different pore sizes are used for DFPP: the plasma separator and the plasma fractionator with

smaller pore size. The plasma separator separates plasma from the whole blood, while the plasma fractionator fractionates the smaller molecular substances, including albumin, from relatively higher molecular weight (MW) proteins, such as immunoglobulins, associated with the pathogenesis of the disease. The albumin-rich plasma, filtered with the plasma fractionator, is returned to the patient with the substitution fluid to maintain the plasma volume. Thus, DFPP can selectively remove macromolecules and reduce the use of substitution fluid, compared to plasmapheresis in the plasma exchange configuration. DFPP is mainly used for conditions in which relatively larger molecules in the plasma are responsible for the pathogenesis (*e.g*., hyperviscosity syndrome and several neurological diseases) [6]. Furthermore, DFPP has been used to remove antibodies due to blood group ABO-incompatible kidney transplantation.

Several types of plasma fractionators are available for adequate removal of the targeted substance with a more specific MW depending on the specificity of the pathogens in each disease [6]. The membrane of the plasma fractionator has a microporous structure similar to the membranes used for plasma separation, but the pore size is 0.01–0.03 μm compared to the plasma separator, which is 0.3 μm. Therefore, the pore size of the plasma fractionator should be selected according to the MW of the target substance. The Cascadeflo EC-20w or EC-30w (Asahi-Kasei Medical Co., Ltd., Tokyo, Japan) is designed for removing immunoglobulin G (IgG) and/or immunoglobulin A (IgA), the EC-40w is used for removing immunoglobulin (IgM) and/or macroglobulin, and the EC-50w is used for removing low-density lipoprotein and/or hepatitis C virus. The Cascadeflo devices contain ethylene vinyl alcohol copolymer hollow fibers. The inside diameter is 175 μm, with overall dimensions of 280 mm (length) and 57 mm (diameter) which gives a total surface area of 2.0 m^2.

A common complication of plasmapheresis using membrane filtration is hemolysis [25], especially in DFPP and occurs in approximately 20% of the treatments. Hemolysis often causes early termination of the treatment. It is usually attributed to increased TMP, which causes alteration in the plasma flow, shearing forces, and sieving.

Plasma Adsorption

The third technique, plasma adsorption, can specifically remove pathogenic agents, such as autoantibodies, immune complexes, low density lipoprotein (LDL), and bilirubin, from the plasma using an adsorption column with selectivity for each substance [6]. Plasma adsorption does not require substitution fluids because of its minimal loss of essential plasma proteins. Therefore, the advantage is the lower risk of an anaphylactic reaction or viral infection compared to plasma exchange and DFPP. Plasma adsorption includes LDL-apheresis, immunoadsorption plasmapheresis, and bilirubin apheresis. Several plasma adsorption columns are available such as the Immusorba TR and PH, Plasorba BRS (Asahi Kasei Medical, Japan), Liposorber LA and Selesorb (Kaneka Medix, Japan), and Medisorba BL (Kawasumi, Japan) [6]. For example, the immunoadsorption column of the Immusorba TR device contains 300 mL of a tryptophan immobilized polyvinylalcohol gel and is designed specifically for the treatment of neurological diseases; the Immusorba TR device is made from 300 mL of phenylalanine immobilized polyvinylalcohol gel for treatment of autoimmune diseases (*e.g*. systemic lupus erythematosus, malignant rheumatoid arthritis, Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, multiple sclerosis).

Plasma adsorption can also be realized using affinity membranes instead of adsorption columns. Affinity membranes exhibit short treatment time and excellent selectivity for toxins due to elimination of diffusional resistance and high specific binding. They have been introduced in the 1980's [26] and then used to remove successfully pathogenic agents such as antibodies, bilirubin and endotoxin. The removal of bilirubin and endotoxin using affinity membranes is detailed below.

Bilirubin, one of the common metabolites of hemoglobin, is a pathogenic substance which is released into blood due to the normal or abnormal destruction of red blood cells. At high bilirubin concentration, jaundice occurs, indicating hepatic or biliary tract dysfunction. High bilirubin concentration in blood (*i.e*. hyperbilirubinemia) may also cause brain damage or death in more severe cases. Several affinity membranes have been proposed for bilirubin removal. For example, arginine as model ligand for affinity adsorption of bilirubin was immobilized on a composite membrane to obtain arginine-immobilized affinity membrane, to remove bilirubin from plasma [27].

Endotoxin, the lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria, produces serious biological effects when it enters into

the human blood system, even at extremely low concentration. It is considered as a key factor in the pathogenesis of sepsis. Some surface-modified hemodialysis hollow fiber membranes have been reported to have ability to remove LPS from blood. For example, Zhang *et al*. [28] proposed affinity membrane modules for extracorporeal elimination of endotoxin from blood by grafting l-serine ligand onto polyvinylidene fluoride (PVDF) hollow fiber membranes. The affinity membranes were then used to remove endotoxin from sepsis patients' plasma *in vitro*. At an initial concentration of 0.42 EU/mL, the endotoxin adsorption capability was 0.058 EU/cm^2 and the removal efficiency was almost 100% for 15 mL, 93.5% and 48.3% for 20 mL and 40 mL samples, respectively.

Affinity membranes have been proved to be successful for plasma treatment *in vitro,* as shown in the two examples detailed above. However, to our knowledge, treatments coupling plasmapheresis to membrane adsorption have not been applied clinically.

4. STEM CELLS PURIFICATION

In clinical applications, stem cell therapy is a promising technology in which the target cells are recovered and purified from autologous or allogenic sources [29]. The cells are then cultured *in vitro* or directly transplanted to regenerate damaged or impaired tissue. Stem cell therapies have been applied to the treatment of several pathologies like musculoskeletal, hematopoietic, neurological, and cardiovascular disorders. The separation and purification of stem cells exploits the characteristics of the cells, which include size, density and surface properties (*i.e*. charge, markers and adhesiveness). Purification techniques may be classified into three main categories: centrifugation, which is based in size, density and adhesiveness properties, immunochemical techniques for cell sorting, which require a suitable surface marker to use as a tag, and other techniques like membrane filtration [29].

Membrane filtration is a simple method which does not necessitate the use of antibodies targeting stem cells. Antibodies are generally produced using animal-derived proteins and cells, which might cause contamination from viruses or prions. For example, Higuchi *et al*. [30] purified hematopoietic stem cells (HSCs) from peripheral blood and umbilical cord blood by membrane filtration. Different membranes were used (*e.g*. polyurethane foaming membranes and Nylon mesh filters) with large pore size of 11 μm. HSCs from blood were found to adhere more strongly to membranes than red blood cells, platelets, T cells, or B cells. Cunha *et al*. [31] optimized the clarification and concentration of human mesenchymal stem cells using membrane fibers devices. Human mesenchymal stem cells (hMSCs) are expanded using microcarrier-based stirred culture systems from one to hundreds of liters of culture volume to guarantee the required cell numbers. Culture suspension needs then to be clarified, to remove microcarriers, and to be concentrated without modifying the cells' properties. Polypropylene filters with pore sizes higher than 75 μm could ensure the removal of microcarriers from the cell suspension bulk, without compromising cells' recovery or viability. Furthermore, hMSCs were concentrated up to a factor of ten, allowing the recovery of more than 80% of viable cells, at optimal conditions of initial cell concentration higher than 2 × 10^5 cell/mL, and polysulfone membranes with pore sizes higher than 0.45 μm

Specific devices are commercialized for cell concentration or dilution, supernatant or culture media exchange, and cell washing. For example, in the LOVO cell processing system (Fresenius Kabi), a cell solution is pumped through a spinning membrane filtration device to allow for rapid fluid management and fast, efficient cell processing. This spinning membrane separation device uses a 4 μm track-etched polycarbonate membrane, and allows for separation of target cells through size discrimination, as well as supernatant removal and product concentration or dilution. The StemQuick^{TM}E system (Asahi Kasei Medical, Japan) is a filtration apparatus including a filter for collecting white blood cells containing hematopoietic stem cells from cord blood [32].

5. MEMBRANE BLOOD OXYGENATORS

Membrane blood oxygenators have been used used over 50 years to oxygenate a patient's blood during open-heart surgery [8, 33]. The first membrane blood oxygenator was proposed in 1955 by Kolff's group, followed in 1956 by the first disposable membrane oxygenator to eliminate the need of cleaning before re-use. Microporous blood oxygenation has been presented in a very complete chapter including history, design (flat sheet and hollow fiber, effects of Non-Newtonian blood rheology, effects of oxygen binding to hemoglobin), computational fluid dynamics methods and future trends [8].

In a membrane blood oxygenator, a microporous membrane is used to separate blood and gas phases.

Gas (usually oxygen or oxygen/nitrogen mixtures) diffuses from the gas phase through the gas-filled membrane pores into the blood, and carbon dioxide diffuses from the blood into the gas for disposal. Hydrophobic flat sheets and hollow fibers can be used. The hollow fibers geometry is more popular. A typical hollow fibre blood oxygenator (Cobe Optima XP, Cobe Cardoivascular Inc., Arvada, USA) contains 14,500 hollow fibres, with 200 μm inner diameter, 300 μm outer diameter, with a total surface area of 1.9 m^2. The hollow fibres are woven together to form a mat. The mats are then wound around a central tube in order to form a fibre bundle. Microporous hollow-fiber membranes are primarily used for short-term cardiopulmonary bypass application, whereas nonmicroporous hollow-fiber membranes are used for long-term extracorporeal membrane oxgenation.

Blood oxygenators are designed to include mixing of the blood to disrupt the concentration boundary layer formed at the membrane surface and thus increase the gas-transfer efficiency. In hollow-fiber blood oxygenators, the blood flows outside and across the fibers rather than inside the fibers. Thus the fibers themselves induce mixing of the blood. In addition, this induces lower pressure resistance in the module and causes less hemolysis. In the flat-sheet blood oxygenators, a screen is placed in the blood flow channel to prevent the flat-sheet membranes from collapsing on top of each other. This configuration induces also mixing of the blood.

Since the membranes used are hydrophobic (*e.g*. polypropylene, Teflon), the pores are gas filled resulting in a negligible membrane mass transfer resistance. The major resistance to oxygen transfer from the gas phase to the blood (and carbon dioxide transfer in the opposite direction) is due to the blood side concentration boundary layer. Several studies have focussed on developing mass transfer and friction factor correlations for flat sheet and hollow fibre blood oxygenators and comparing with experimental results using blood analogue fluids [33-35]. For example, Wickramasinghe *et al*. [33] have proposed mass-transfer and friction-factor correlations for flat-sheet and hollow-fiber blood oxygenators. Generalized Graetz, Reynolds, and Schmidt numbers are used to account for the shear thinning behavior of blood. Because oxygen not only dissolves in plasma but also binds to hemoglobin, a mass-transfer enhancement factor based on film theory has been developed to account for this event. Experimental results for oxygen transfer to bovine blood and both non-Newtonian and Newtonian blood analogue fluids indicate that the correlations developed may be used to predict the performance of blood oxygenation.

6. BIOARTIFICIAL LIVERS

In a bioartificial liver (BAL) device, the patient plasma is circulated extracorporeally through a bioreactor that houses metabolically active liver cells (hepatocytes) sandwiched between artificial plates or capillaries (Figure **3**). Several reviews are available on BAL devices, to present their current developments and future trends. For example, Strain and Neuberger [9] provided a state of the art of the BAL by focusing on the type of cell for a BAL bioreactor, clinical trials and trends. Pless and Sauer [10] summarized the current status of artificial liver assist devices such as hemodiafiltration, hemoperfusion, plasmapheresis, and hemodialysis, bioartificial liver assist devices and further development in BAL assist devices. Allen *et al*. [11] focused on advances in extracorporeal BAL devices, cellular components, stabilization of primary hepatocyte phenotype, bioreactor design, regulation and safety, and clinical trials.

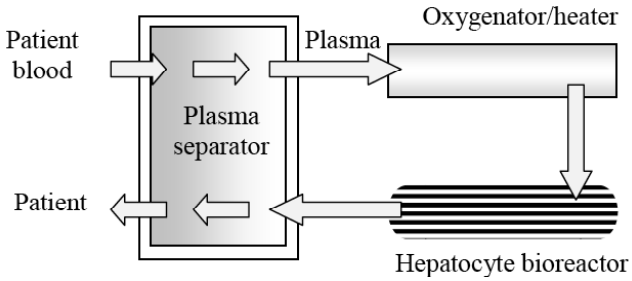

Figure 3: A bioartificial liver (BAL) bioreactor (redrawn from [9]).

The liver has a number of important functions that are principally carried out by hepatocytes [9]. These cells synthesize many proteins, including clotting factors; they produce bile and regulate carbohydrate, fat, and protein metabolism; they detoxify the ammonia product of nitrogen metabolism and break down alcohol and drugs. In addition, the liver has Kupffer cells that are part of the immune system. The healthy liver is able to regenerate itself after acute injury, but once damaged by fibrosis and cirrhosis, caused by a variety of chronic conditions such as alcohol abuse or infection with hepatitis virus B or C, it can no longer regenerate normally [9]. Among currently available therapies, liver transplantation is an accepted and effective way for the treatment of acute hepatic failure. However, it is not applicable for many patients due to limited organ availability. Artificial liver systems have been developed to bridge patients with severe liver failure to liver

transplantation or liver regeneration. In the past two decades, several devices have been developed, which can be classified into two main categories: artificial organs, based on classical methods such as hemodialysis, plasmapheresis, specific or non-specific adsorption, and bioartificial organs.

Artificial Organs

To clear the blood of protein-bound substances such as bilirubin, bile acids, short chain fatty acids, and aromatic amino acids, with dialysis techniques, adsorbent or acceptor substances are necessary to enhance mass exchange. The molecular adsorbent recirculation system (MARS*,* Gambro Hospal GmbH, Germany) and the single pass albumin dialysis (SPAD) were introduced to improve removal of protein-bound substances [1, 10, 36]. In the MARS system, the albumin solution is circulated in a closed circuit separated from patient blood by a high-flux hemodialysis filter. The albumin acts as the acceptor for the toxins. It is partly regenerated by passing an anion exchanger and a charcoal adsorber in a closed circuit, which is itself dialyzed. The noncommercial technique SPAD uses standard renal replacement therapy machines without an additional perfusion pump system. The patient's blood flows through a hollow fiber hemodiafilter, identical to that used in MARS. The other side of this membrane is cleaned with an albumin solution in counter directional flow, which is, instead of being regenerated as in MARS, discarded after passing the hemodiafilter. The Prometheus® system (Fresenius Medical Care AG, Germany) combines the fractionated plasma separation and adsorption (FPSA) method with high-flux hemodialysis of the blood in an extracorporeal detoxification system [36]. In the FPSA system, a specific albumin-permeable polysulfone membrane with a cut-off of approximately 250 kDalton is used. Thus albumin and the protein-bound toxins pass through the membrane and are then removed from the blood by an adsorber within the secondary circuit.

Treatments with these non-biological liver systems have shown some benefits for short-term liver support in patients with acute liver failure. However, most of the clinical studies were conducted on a small number of patients and thus provided limited clinical data.

Bioartificial Livers

The principle of BAL devices is closer to that of a real liver and therefore these systems are expected to be more efficient than non-biological artificial organ. In a BAL device, the patient plasma is circulated extracorporeally through a bioreactor that houses metabolically active liver cells (hepatocytes) sandwiched between artificial plates or capillaries. The basic BAL bioreactor consists of a hollow-fiber membrane device [9]. Up to 10^{10} human or porcine hepatocytes are housed into the extracapillary space of the bioreactor either alone or attached to microcarrier beads. In the primary circuit, the patient is connected by venous catheters to a plasma separator. In the secondary circuit, the plasma is separated, warmed, oxygenated, and then perfused through the lumen of the bioreactor capillaries. This allows free exchange of molecules between hepatocytes and patient plasma: hepatocytes extract oxygen and nutrients and detoxify chemicals in the plasma, and their metabolites pass into the plasma. Blood cells are then added back to the plasma before it is returned to the patient.

Several BAL devices have been proposed including the following ones [1, 10, 11]:

- The extracorporeal liver assist device (ELAD, Vitagen) uses around 200 g of cells of the human hepatoblastoma cell line C3A (derived from HepG2) in modified dialysis devices. The cells are housed in the extracapillary space separated from plasma by the capillary membranes. Prior to entering the bioreactor, the plasma passes a charcoal adsorber and a membrane oxygenator. In a similar setting, the HepatAssist (Circe Biomedical) system uses 5 - 7 10^9 cryopreserved porcine hepatocytes.

- The modular extracorporeal liver support (MELS) system is based on the Cell Module, a unit consisting of 3 interwoven capillary bundles in a polyurethane housing. One of the bundles serves as decentralized oxygenation; 2 bundles are used for perfusion with patient plasma. It is operated with primary porcine hepatocytes or with human hepatocytes isolated from discarded donor organs. In the MELS system, the Cell Module is combined with SPAD and continuous veno-venous hemodiafiltration.

- Within the BAL device developed by the Amsterdam Medical Center (AMC-BAL), the capillary membranes serve oxygenation, in contrast to previous systems. The cell compartment of the device is loaded with about 200 g of primary porcine hepatocytes. During therapy, the polymeric matrix of the device is directly perfused by patient plasma.

These systems use various cell sources, having different advantages and limits [10]. Porcine hepatocytes are available in large quantities, but bear the risk of zoonoses (such as porcine endogenous retrovirus or herpes species) and metabolic incompatibility. Human tumor cell lines can be easily expanded to large quantities, but have the disadvantage of poor metabolic performance and potential metastatic ability. Primary human cells are biocompatible but are not available in large quantities and originate from histologically impaired organs.

Various membranes are used which function as scaffold for cell attachment and as permselective barriers allowing transfer of oxygen and nutrients [1]. The hollow fiber membranes are made from different polymeric membranes, such as cellulose acetate in ELAD (MWCO 70 kDa) or polyethersulfone (molecular weight cut-off (MWCO)> 400,000) in MELS. Hydrophilic membranes are used for cell attachment and mass exchange, while oxygenation membrane capillaries are hydrophobic (*e.g*. polypropylene in AMC-BAL). Ideally, the semi-permeable membrane has a nominal MWCO that is high enough to allow diffusion of small molecules and proteins (*e.g*. albumin) but small enough to exclude cellular material, as well as immunoglobulins and viral pathogens.

BAL devices have been tested *in vitro* and in animal models in several studies [9]. Different parameters have been measured as indicators of hepatocyte viability and function, including clotting factor production, urea synthesis, conjugation of the bile pigment bilirubin, drug metabolism, and a variety of other metabolic processes. The first clinical trial was realized in 1987 to treat a single patient with acute liver failure. Although the feasibility of BAL devices was easily established, the efficacy of the treatment was much more difficult to demonstrate with only few clinical trials performed.

Further developments in BAL devices include finding an appropriate cell source [10]. Research on human hepatic progenitor cells, embryonic stem cells, or fetal stem cells might offer the necessary sources. In addition, the combination of artificial and bioartificial organ is a promising area. For example, the HepatAssist system (bioreactor + charcoal adsorber) and MELS (bioreactor + SPAD) combine bioreactors with detoxification techniques.

7. BIOARTIFICIAL PANCREAS

In a bioartificial pancreas, islets are encapsulated within a semi-permeable membrane and transplanted into a patient. The islets are isolated from the patient's immune system, whereas oxygen, glucose, and nutrients are supplied through the membrane. Insulin secreted by the islets in response to changes in blood glucose levels can diffuse from the bioartificial pancreas to the patient to maintain blood glucose metabolism. Kizilel *et al*. [12] reviewed the recent progresses and challenges remaining for the successful development of the bioartificial pancreas. The review focuses on historical development, impediments to the progress of cell encapsulation technology, different forms of bioartificial pancreas, and techniques in microencapsulation. Teramura and Iwata [13] focused on the principle of the bioartificial pancreas, conventional bioartificial pancreas (types of bioartificial pancreas, islet encapsulation with agarose beads, and clinical applications), and conformal coating of cell surfaces.

Diabetes represents a major public health problem, as it is the most frequent endocrine disease in industrialized countries. In 2005, the number of people suffering from diabetes worldwide was 177 million, and this number is expected to double by the year 2025 [12]. The complications of diabetes include several medical problems related to ophthalmic, renal, neurological, cerebrovascular, cardiovascular, and peripheral vascular disease. Type 1 diabetes (insulin-dependent diabetes) occurs when pancreatic islet cells are unable to produce insulin. Consequently, the blood glucose concentration becomes high while tissues are starving for metabolic fuel. In Type 2 diabetes (non–insulin-dependent diabetes), the body continues to make at least some insulin, but is unable to respond properly to the action of insulin produced by the pancreas. Nowadays, the treatment for Type 1 diabetes include exogenous insulin with external glucose monitoring, whole-organ pancreas transplantation, islet cell transplantation, the artificial pancreas, and the bioartificial pancreas. Three major types of bioartificial pancreas have been developed: diffusion chamber, hollow fiber unit and microcapsules (Figure **4**) [13].

The mechanical artificial pancreas consists of a glucose sensor, an insulin pump, and a computer, which determines the rate of insulin delivery [12]. In contrast, a bioartificial pancreas is a device that substitutes for the endocrine portion of the pancreas. It contains functional islets encapsulated within a semipermeable membrane to protect cells from the host immune response. In order to meet the requirement of immunoisolation, the membrane has to

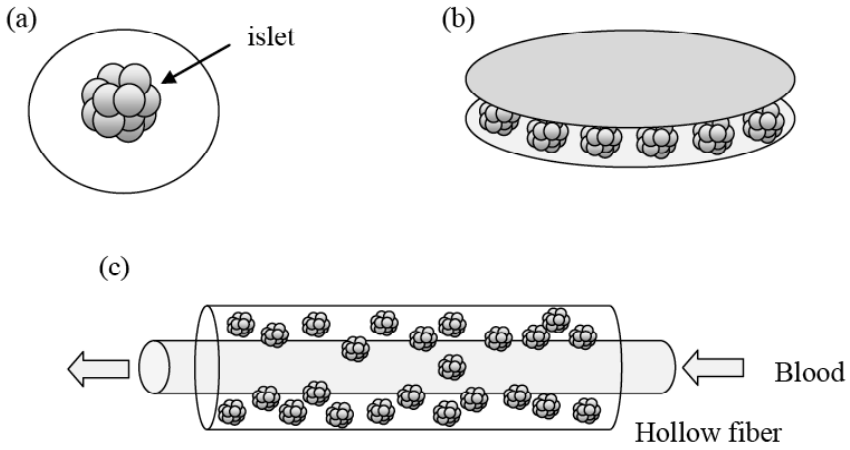

Figure 4: The conventional bioartificial pancreas types. (**a**) microcapsule, (**b**) diffusion chamber, and (**c**) hollow fiber-based diffusion chamber (redrawn from [13]).

reject antibodies, complements, some cytokines, and other cytotoxic substances. For islets survival, the membrane has to allow the free permeation of substances, such as oxygen, necessary nutrients, and cellular metabolites (Figure **5**).

In a diffusion chamber pancreas, Nuclepore™ membranes with a pore size of 0.05–1 μm are used as an immunoisolation membrane [13]. For example, Hirotani *et al*. [37] reported that normoglycemia was maintained for 30 weeks in streptozotocin (STZ)-induced diabetic rats by implanting a device that encapsulated mice pancreatic beta cell lines (MIN6) (xenotransplantation). Recently, Lee *et al*. [38] reported human fetal pancreatic islet like cell clusters (18–24 weeks) enclosed in devices (TheraCyte) made of polytetrafluorethylene (PTFE) with a pore size of 0.4 μm that were transplanted at a subcutaneous site. After transplantation into in the non-obese diabetic mouse model of type 1 diabetes, the blood glucose level was normalized, indicating efficacy. A limit of the diffusion chamber devices is the aggregation of islets in the space [13]. When islets form aggregates, cells in the center are necrotic due to oxygen shortage. In addition, the size of the device (evaluated to 30 cm in diameter) would be a major obstacle when applied to patients.

Hollow fiber and tubular membranes have been extensively studied in bioartificial pancreas. In one of the first studies, Maki *et al*. [39] developed a bioartificial pancreas that was suitable for implantation into large animals. The device consisted of a chamber passing a semi-permeable tubular membrane that was connected to vascular grafts. Islets were placed between the

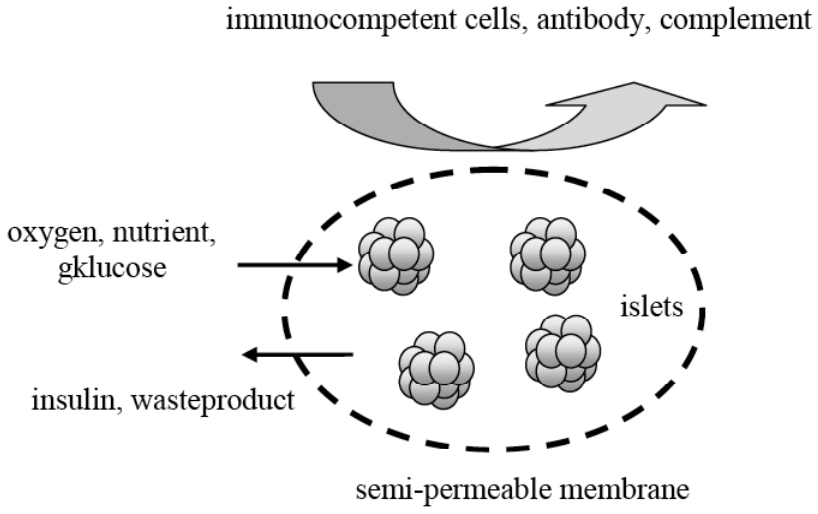

Figure 5: Concept of the immunoisolation membrane in a bioartificial pancreas (redrawn from [13]).

housing chamber and the tubular membrane (MWCO 80 kDa). The device was implanted in severely diabetic dogs that had undergone total pancreatectomies. Aspirin was systematically administered in order to control blood coagulation and inhibit thrombus formation, which could not be applicable in a clinical treatment due to the increase risk of bleeding. In addition, such devices have poor diffusive exchange between the enclosed islets and surrounding blood and are vulnerable to rupture, which have limited their application.

In order to improve these devices, recent studies on bioartificial pancreas focused on evaluation of new membrane materials. For example, Risbud *et al*. [40] investigated the *in vitro* biocompatibility and suitability of cellulose dialysis membrane (Spectra/Port 2, MW no larger than 12–14000) for the immunoisolation of pancreatic islets. The membrane allowed regulated transport of glucose and insulin in an *in vitro* diffusion assay. These islets also retained their functionality, as judged by insulin secretion. New configurations have also been proposed. For example, Ikeda *et al*. [41] developed a new type of bioartificial pancreas composed of polyethylene vinyl alcohol (EVAL) hollow fibers that were permeable to glucose and insulin and a poly-amino-urethane-coated, non-woven PTFE fabric that allowed cell adhesion. Porcine islets attached to the surface of the PTFE fabric, but not to the surface of the EVAL hollow fibers, allowing nutrient and oxygen exchange between blood flowing inside the fibers and cells outside. The bioartificial pancreas was inoculated with porcine islets and connected it to the circulation of totally pancreatectomized diabetic pigs. Blood glucose levels were found to be reduced to a normal range and general health was improved, resulting in longer survival times. In addition, regulation of insulin secretion from the bioartificial pancreas was controlled in response to glucose both *in vitro* and *in vivo*. Moreover, improved mathematical models of local oxygen transport have been developed. Dulong and Legallais [42] calculated the number of islets that needed to be implanted in order to obtain a correct response in terms of insulin secretion. In most configurations, it was found to be much higher than that of ultimately functional islets, because of hypoxia and necrosis. Fiber length should thus be adjusted accordingly. The authors concluded that the compromise to be found between the reduction of the number of implanted islets and fiber length and diameter did not correspond to realistic hollow fiber systems.

8. DRUG DELIVERY USING MEMBRANES

Drug delivery devices based on membranes consist basically of osmotic and diffusion controlled membrane systems [1, 2]. Stamatialis *et al*. [1] reviewed in details several aspects of drug delivery systems including osmotic membrane systems, diffusion controlled membrane systems (pills, implants and patches), transdermal drug delivery, passive diffusion, iontophoresis, etc. Membrane based formulations used in drug delivery consist of colloids like liposomes and nanocapsules. Liposomes are enclosed spherical vesicles with one or several concentric phospholipidic membrane bilayers with an aqueous phase inside. A large number of reviews focus on liposomes do to their widespread used in fields like pharmaceutics, cosmetics and foods *e.g*. [14-16]. Laouini *et al*. [15] reviewed the recent progresses in liposomal drug delivery systems including definition and classification of liposomes, methods of preparation, characterization, and pharmaceutical, cosmetic and food applications. Nanocapsules are other membrane based drug delivery systems. Nanocapsules have a polymeric shell membrane and an inner liquid core, the drug being dissolved in the inner core, or adsorbed at their surface. Several reviews *e.g*. [17, 18] are available on drug loaded nanoparticles, including nanocapsules to present the state on the art on preparation methods and applications in drug delivery.

A drug delivery system is a device or a formulation that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of its release [43]. It includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. A drug delivery system may be: (1) a device used to deliver the drug, or (2) a formulation of the drug which will be administered for a therapeutic purpose. Drugs may be introduced into the human body by various anatomical routes. The choice of the route of administration depends on the disease, the effect desired, and the product available. Drugs may be administered directly to the organ affected by disease or given systemically and targeted to the organ. Methods of systemic drug delivery are classified as follows: gastrointestinal system (oral, rectal), parenteral, transnasal, pulmonary (drug delivery by inhalation), transdermal drug delivery and intra-osseous infusion. The oral route of drug administration is the most used for both conventional as well as novel

drug delivery. The reasons for this preference are the ease of administration and widespread acceptance by patients.

Drug Delivery Systems Using Membranes

Membrane devices used to deliver the drug consist basically of a drug reservoir [1]. Two main types of systems can be distinguished: (1) osmotic membrane systems (Figure **6**), and (**2**) diffusion controlled membrane systems. An osmotic system consists of a reservoir made of a polymeric membrane permeable to water but not to the drug (semi-permeable membrane). The reservoir contains a concentrated drug solution. As water crosses the membrane due to osmotic pressure, the drug solution is released through the orifice. In diffusion controlled membrane systems, the drug release is controlled by transport of the drug across a membrane. The transport is dependent on the drug diffusivity through the membrane and the thickness of the membrane. These systems find broad application in pills, implants and patches.

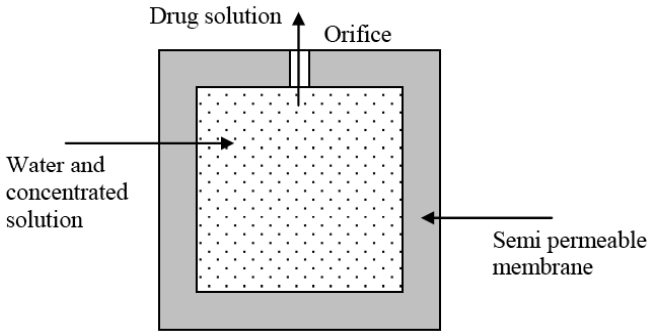

Figure 6: Schematic view of an osmotic drug delivery system (redrawn from [1]).

Drug delivery membrane systems include osmotic membrane systems, diffusion controlled membrane systems (pills, implants, patches), transdermal drug delivery (passive diffusion, iontophoresis and skin or device controlled delivery) [2]. These drug delivery applications concern mainly transdermal, oral and ocular delivery routes of administration.

In transdermal drug delivery [1], the drug is incorporated into a patch and delivered through the skin due to the concentration difference or electrical current (Figure **7**). Transdermal patches are used as a convenient and effective method of drug delivery through the human skin. A membrane may exclusively control or partially control drug transport together with other components (such as the adhesive layer). Patches are commercialized for example for delivery of nicotine for smoking cessation, estrogenor testosterone for hormonal therapy, and methylphenidate for attention deficit hyperactivity disorder. Ocular patches are membrane-controlled reservoir systems. The drug, accompanied by carriers, is captured in a thin layer between two transparent, polymer membranes, which control the rate of drug release. The device is placed on the eye where it floats on the tear film. The drug slowly diffuses to the target area.

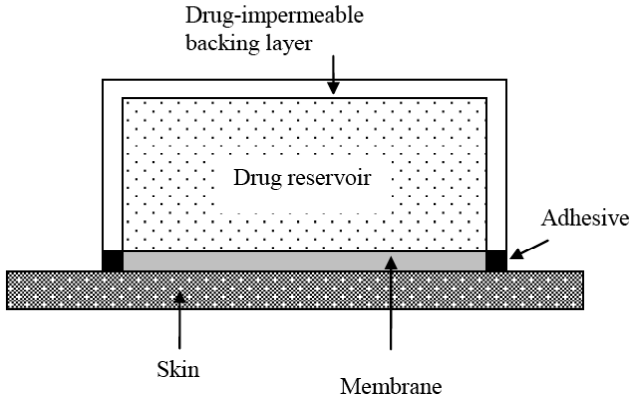

Figure 7: Schematic view of transdermal membrane delivery systems (redrawn from [1]).

As a non-invasive transdermal drug delivery method, iontophoresis applies electrical current to deliver drugs through the skin to the underlying tissue or capillaries and then to the whole circulating system [44]. A voltage applied between two electrodes immersed in a drug solution causes the drug to be moved from the donor part into the skin. The positively charged electrode (anode) attracts the negatively charged drug ions; the negatively charged electrode (cathode) attracts the positively charged ions. These devices are commercialized for example for delivery of lidocaine and epinephrine in local analgesia.

In oral delivery, the drug is pressed into tablet which is coated with a non-digestible hydrophilic membrane such as cellulose esters like cellulose acetate, cellulose acetate butyrate, cellulose triacetate and ethyl cellulose [1, 2, 45]. Once the membrane gets hydrated, a viscous gel barrier is formed, through which the drug slowly diffuses. The release rate of the drug is determined by the type of membrane used. The key feature of these systems is that drug release is independent of pH and hydrodynamics of the external dissolution medium. The result is a robust dosage form for which the *in vivo* rate of drug release is comparable to the *in vitro* rate, producing an excellent *in vitro*/*in vivo* correlation. Another key advantage is that they are applicable to drugs with a broad range of aqueous

solubility such as diltiazem HCl, carbamazepine, metoprolol, oxprenolol, nifedipine, and glipizide.

Another example is implants which consist of a membrane reservoir containing a drug in liquid or powder form [2]. The drug slowly diffuses through the nondegradable membrane that usually consists of silicone and ethylene-*co*-vinyl acetate. By properly choosing membrane thickness and permeability, release kinetics can be controlled and designed for a specific therapeutic target. For example, an implantable 5 years contraceptive permits the long term release of levonorgesterol.

Membrane Based Formulations

Membrane based formulations used to deliver the drug consist of carriers that are biodegradable, biocompatible, targeting, and stimulus responsive like liposomes and nanocapsules [45]. Liposomes are enclosed spherical vesicles that are organized in one or several concentric phospholipidic membrane bilayers with an aqueous phase inside. Liposomes are usually classified on the basis of their size (small, large and giant vesicles), number of bilayers (uni-, oligo and multi-lamellar) and phospholipid charge (neutral, anionic or cationic). Liposomes have also been categorized with respect to their function such as conventional, stealth, ligand-targeted, long-release, and triggered-release.

Liposomes can entrap hydrophilic pharmaceutical agents in their internal aqueous compartment and lipophilic drugs within the lipid membrane. Over the past few years, liposomal drug preparations have been developed from laboratory research to clinical applications, such as Doxil, Myocet and DepoCyt [16]. Not only the pharmaceutical area benefits from liposomes, but also the food and cosmetic industries. Liposome function is strongly dependent on properties such as liposome size, shape, lamellarity and surface charge. Other parameters that characterize liposomes are the encapsulation efficiency, the ratio of phospholipids to drug concentration, the surface charge through zeta potential measurement, phase transitions through differential scanning calorimetry and quantification of residual solvents through gas chromatography.

Since the pioneering discovery of liposomes by Bangham *et al*. [46], many techniques have been reported for liposome preparation including mechanical methods (preparation by film methods, homogenization techniques such as sonication, microfluidization, extrusion), methods based on replacement of organic solvents by aqueous media (ethanol injection method, proliposome-liposome method, reverse-phase evaporation), and methods based on detergent removal [14]. The ethanol injection method is an interesting technique for production of liposomes. It offers several advantages, *e.g*., simplicity, fast implementation, and reproducibility. The ethanol injection method was first reported in the early 1970s by Batzri and Korn [47] as one of the first alternatives for the preparation of small unilamellar vesicles (SUVs) without sonication. By the immediate dilution of the ethanol in the aqueous phase, the lipid molecules precipitate and form bilayer planar fragments [48]. Through energy dissipation in the system (by stirring and/or ultrasonication), the fragments of these lipid bilayers tend to decrease the exposure of the hydrophobic parts of their molecules to the aqueous environment, resulting in the curvature of these fragments which take a quasi-spherical structure.

Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids, or the synthetic polymer polyethylene glycol (PEG) [49]. The presence of PEG on the surface of the liposome has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake (stealth liposomes). PEG modified liposomes have then been developed for encapsulation of active molecules, with high target efficiency and activity. In addition, by modification of the terminal PEG molecule, liposomes can be actively targeted with monoclonal antibodies or ligands.

Nanocapsules are also membrane based formulations used in drug delivery. They are ranging in size from about 10 to 1000 nm. Nanocapsules have a polymeric shell membrane and an inner liquid core, the drugs being dissolved in the inner core, or adsorbed at their surface. Nanocapsules differ from nanospheres that have a matrix type structure with drugs adsorbed at their surface, entrapped in the particle or dissolved in it. Nanocapsules have been investigated for the entrapment of a wide variety of drugs, for applications ranging from ophthalmic delivery to carriers in chemotherapy. They are characterized in terms of morphology (transmission electron microscopy and scanning electron microscopy), size and size distribution (*i.e*. by photon correlation spectroscopy), zeta potential and density (*i.e*. by isopycnic centrifugation) [18]. Important properties of nanocapsules are their drug entrapment efficacy (amount of drug loaded in the nanocapsules expressed as the percentage of the total amount of drug added in

the process), and their drug release behaviour (*in vitro* and *in vivo*) [18].

Several methods for the preparation of nanocapsules are available, involving either a dispersion of preformed polymers or a polymerization of dispersed monomers [17]. Nanocapsules prepared by dispersion of preformed polymers involve the use of purified natural molecules or preformed synthetic polymers, and for nanocapsules prepared by polymerization reaction the following polymers may be useed: polyacrylamide, poly(alkyl methacrylates), poly(alkyl cyanoacrylates) and polyglutaraldehyde. The nanoprecipitation method developed by Fessi *et al*. [50] is an easy and reproducible method involving dispersion of preformed polymers. It is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. The organic phase (solvent, polymer, eventually oil, and drug) is added drop wise under moderate stirring into the aqueous phase (water, and surfactant). Another method to prepare nanocapsules is the interfacial polymerization technique in which two monomers, one oil-soluble and the other water soluble, are employed and a polymer is formed on the droplet surface [18]. The organic phase (solvent, monomer, eventually oil, and drug) is added into the aqueous phase (water, co-monomer and surfactant).

CONCLUSION

Membrane based techniques are extensively used for biomedical applications. Current techniques include dialysis and blood oxygenation used in daily treatments. Applications of artificial liver and bioartificial pancreas are more limited and clinical trials are still under way. Drug delivery using membrane based systems like liposomes and nanocapsules has been increasingly reported with commercial products now available in particular for cancer treatment. All these membrane techniques involve a large range of polymeric or inorganic membranes, at very large scales (*e.g*. dialysis and blood oxygenation) or at very small scales (*e.g*. the membrane surrounding the liposomes or nanocapsules), in flat sheet or hollow fiber configurations. Different mechanisms of transfer are associated to these processes like molecules diffusion (*e.g*. in dialysis and drug delivery devices) or convection (*e.g*. in hemofiltration), in the liquid phase (*e.g*. in dialysis, hemofiltration, etc) or gas phase (*e.g*. in blood oxygenation). Further developments are related to membrane materials with improved biocompatibility and mass transfer (*e.g*. in bioartificial pancreas), new biological developments (*e.g*. new cell sources for BAL devices), device and process design (*e.g*. in dialysis and BAL devices), characterization and modelling of the systems, and intensive clinical trials based on appropriate and well controlled methodologies.

ABBREVIATIONS

BAL: bioartificial liver

DFPP: double-filtration plasmapheresis

EVAL: polyethylene vinyl alcohol

FPSA: fractionated plasma separation and adsorption

HCl: hydrochloric acid

hMSCs: human mesenchymal stem cells

HSCs: hematopoietic stem cells

IgA: immunoglobulin A

IgG: immunoglobulin G

IgM: immunoglobulin M

LDL: low density lipoprotein

LPS: lipopolysaccharide

MELS: modular extracorporeal liver support

MW: molecular weight

MWCO: molecular weight cut-off

PEG: polyethylene glycol

PTFE: polytetrafluorethylene

PVDF: polyvinylidene fluoride

STZ: streptozotocin

TMP: transmembrane pressure

SYMBOLS

C: concentration (mg/mL)

C_0 and C_t: initial and final solute concentrations at the beginning and end of each dialysis (mg/mL)

G: generation rate (mg/min)

K_d: intermittent dialyzer clearance (mL/min)

K_r: continuous renal clearance (mL/min)

t: time (min)

V: solute distribution volume (L)

REFERENCES

[1] Stamatialis DF, Papenburg BJ, Gironés M, Saiful S, Bettahalli SNM, Schmitmeier S *et al*. Medical applications of membranes: Drug delivery, artificial organs and tissue engineering, J Membr Sci 2008; 308: 1-34. http://dx.doi.org/10.1016/j.memsci.2007.09.059

[2] Grassi M. Membranes in drug delivery. In: Pabby AK, Rizvi SSH, Sastre AM (eds) Handbook of membrane separations, CRC Press, NW, 2009; 427-492.

[3] Himmelfarb J, Ikizler TA. Hemodialysis, New England J Med 2010; 363: 1833-1845. http://dx.doi.org/10.1056/NEJMra0902710

[4] Ronco C, Crepaldi C, Brendolan A, Bragantini L, d'Intini V, Inguaggiato P *et al*. Evolution of synthetic membranes for blood purification: the case of the Polyflux family, Nephrology Dialysis Transplantation 2003; 18: vii10-vii20. http://dx.doi.org/10.1093/ndt/gfg1073

[5] Waniewski J. Mathematical modeling of fluid and solute transport in hemodialysis and peritoneal dialysis, J Membr Sci 2006; 274: 24-37. http://dx.doi.org/10.1016/j.memsci.2005.11.038

[6] Nakanishi T, Suzuki N, Kuragano T, Nagasawa Y, Hasuike Y. Current topics in therapeutic plasmapheresis, Clin Exper Nephr 2014; 18: 41-49. http://dx.doi.org/10.1007/s10157-013-0838-0

[7] Baweja S, Wiggins K, Lee D, Blair S, Fraenkel M, McMahon LP. Benefits and limitations of plasmapheresis in renal diseases: an evidence-based approach, J Artificial Organs 2011; 14: 9-22. http://dx.doi.org/10.1007/s10047-010-0529-5

[8] Wickramasinghe SR, Han B. Chapter 23, Microporous Blood Oxygenators, pp. 671-692, in Handbook of Membrane Separations: Chemical, Pharmaceutical, Food, and Biotechnical Applications, Eds Pabby AK, Rizvi SSH, Sastre AM. 2009, CRC Press.

[9] Strain AJ, Neuberger JM. A bioartificial liver - State of the art, Science 2002; 295: 1005-1009. http://dx.doi.org/10.1126/science.1068660

[10] Pless G, Sauer IM. Bioartificial liver: Current status, Transplantation Proceedings, 2005; 37: 3893-3895. http://dx.doi.org/10.1016/j.transproceed.2005.09.113

[11] Allen JW, Hassanein T, Bhatia SN. Advances in bioartificial liver devices, Hepatology 2001; 34: 447-455. http://dx.doi.org/10.1053/jhep.2001.26753

[12] Kizilel S, Garfinkel M, Opara E. The bioartificial pancreas: Progress and challenges, Diabetes Technol Ther 2005; 7: 968-985. http://dx.doi.org/10.1089/dia.2005.7.968

[13] Teramura Y, Iwata H. Bioartificial pancreas, Microencapsulation and conformal coating of islet of Langerhans, Adv Drug Delivery Rev 2010; 62: 827-840 http://dx.doi.org/10.1016/j.addr.2010.01.005

[14] Wagner A, Vorauer-Uhl K. Liposome technology for industrial purposes. J Drug Delivery 2011; 1-9. http://dx.doi.org/10.1155/2011/591325

[15] Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H, Preparation, characterization and applications of liposomes: State of the art, J Colloid Sci Biotechnol 2012; 1: 147-168. http://dx.doi.org/10.1166/jcsb.2012.1020

[16] Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications, Adv. Drug Delivery Rev 2013; 65: 36-48. http://dx.doi.org/10.1016/j.addr.2012.09.037

[17] Allémann E, Gurny R, Doelker E. Drug-loaded nanoparticles-Preparation methods and drug targeting issues, Eur J Pharm Biopharm 1993; 39: 173-191.

[18] Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review, Crit. Rev Ther Drug Carrier Syst 2002; 19: 99-134. http://dx.doi.org/10.1615/CritRevTherDrugCarrierSyst.v19.i2.10

[19] Hoenich NA. Update on the biocompatibility of hemodialysis membranes, Hong Kong J Nephrology 2004; 6: 74-78. http://dx.doi.org/10.1016/S1561-5413(09)60162-9

[20] Jaffrin MY, Gupta BB, Malbrancq JM, A one-dimensional model of simultaneous hemodialysis and ultrafiltration with highly permeable membranes, J Biomech Eng 1981; 103: 261-266. http://dx.doi.org/10.1115/1.3138290

[21] Waniewski J, Lindholm B. Fractional solute removal and KT/V in different modalities of renal replacement herapy, Blood Purif 2004; 22: 367-376. http://dx.doi.org/10.1159/000080033

[22] Gotch FA. Kt/V Is the best dialysis dose parameter, Blood Purif 2000; 18: 276-285. http://dx.doi.org/10.1159/000014449

[23] Lowrie EG.The normalized treatment ratio (Kt/V) is not the best dialysis dose parameter, Blood Purif 2000; 18: 286-294. http://dx.doi.org/10.1159/000014450

[24] Agishi T, Kaneko I, Hasuo Y, Hayasaka Y, Sanaka T, Ota K, *et al*. Double filtration plasmapheresis. Trans Am Soc Artif Int Organs 1980; 26: 406-11.

[25] Yeh JH, Chen WH, Chiu HC, Bai CH. Hemolysis in double-filtration plasmapheresis, Am J Clin Pathol 2007; 27: 76-80. http://dx.doi.org/10.1309/F8XMTALTTCE5H37U

[26] Brandt S, Goffe RA, Kessler SB, O'Connor JL, Zale SE. Membrane-based affinity technology for commercial scale purifications, Biotech 1988; 6: 779-782. http://dx.doi.org/10.1038/nbt0788-779

[27] Xue M, Y Ling, G Wu, X Liu, D Ge, Shi W. Surface-modified anodic aluminum oxide membrane with hydroxyethyl celluloses as a matrix for bilirubin removal, J Chromatogr B 2013; 912: 1- 7. http://dx.doi.org/10.1016/j.jchromb.2012.10.032

[28] Zhang M, Zhang L, Cheng LH, Xu K, Xu QP, Chen HL *et al*. Extracorporeal endotoxin removal by novel l-serine grafted PVDF membrane modules, J Membr Sci 2012; 405- 406: 104-112.

[29] González-González M, Vázquez-Villegas P, García-Salinas C, Rito-Palomares M. Current strategies and challenges for the purification of stem cells, J Chem Techn Biotech 2012; 87: 2-10. http://dx.doi.org/10.1002/jctb.2723

[30] Higuchi A, Chuang C-W, Ling Q-D, Huang S-C, Wang L-M, Chen H, *et al*. Differentiation ability of adipose-derived stem cells separated from adipose tissue by a membrane filtration method, J Membr Sci 2011; 366: 286-294. http://dx.doi.org/10.1016/j.memsci.2010.10.009

[31] Cunha B, Peixoto C, Silva MM, Carrondo MJT, Serra M, Alves PM. Filtration methodologies for the clarification and concentration of human mesenchymal stem cells, J Membr Sci 2015; 478: 117-129. http://dx.doi.org/10.1016/j.memsci.2014.12.041

[32] Aoki M, Yasutake M, Murohara T. Derivation of functional endothelial progenitor cells from human umbilical cord blood

mononuclear cells isolated by a novel cell filtration device, StemCells 2004; 22: 994-1002. http://dx.doi.org/10.1634/stemcells.22-6-994

[33] Wickramasinghe SR, Han B, Garcia JD, Specht R. Microporous membrane blood oxygenators AIChE J 2005; 51: 656-670 http://dx.doi.org/10.1002/aic.10327

[34] Vaslef SN, Mockros LF, Anderson RW, Leonard RJ. Use of a mathematical model to predict oxygen transfer rates in hollow fiber membrane oxygenators, ASAIO J 1994; 40: 990-996. http://dx.doi.org/10.1097/00002480-199440040-00016

[35] Matsuda N, Sakai K. Blood flow and oxygen transfer rate of an outside blood flow membrane oxygenator, J Membr Sci 2000; 170: 153-15. http://dx.doi.org/10.1016/S0376-7388(00)00331-8

[36] Rifai K, Ernst T, Kretschmer U, Bahr MJ, Schneider A, C Hafer *et al*. Prometheus - a new extracorporeal system for the treatment of liver failure, J Hepatology 2003; 39: 984-990. http://dx.doi.org/10.1016/S0168-8278(03)00468-9

[37] Hirotani S, Eda R, Kawabata T, Fuchinoue S, Teraoka S, Agishi T *et al*. Bioartificial endocrine pancreas (Bio-AEP) for treatment of diabetes: effect of implantation of Bio-AEP on the pancreas, Cell Transplantation 1999; 8: 399-404.

[38] Lee SH, Hao E, Savinov AY, Geron I, Strongin AY, Itkin-Ansari P, Human betacell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies, Transplantation 2009; 87: 983-991. http://dx.doi.org/10.1097/TP.0b013e31819c86ea

[39] Maki T, Ubhi CS, Sanchez-Farpon H, Sullivan SJ, Borland K, Muller TE *et al*. Successful treatment of diabetes with the biohybrid artificial pancreas in dogs, Transplantation 1991; 51: 43-51. http://dx.doi.org/10.1097/00007890-199101000-00006

[40] Risbud MV, Bhonde RR. Suitability of cellulose molecular dialysis membrane for bioartificial pancreas: *In vitro* biocompatibility studies, J Biomedical Mat Res 2001; 54: 436-444. http://dx.doi.org/10.1002/1097-4636(20010305)54:3<436::AID-JBM180>3.0.CO;2-8

[41] Ikeda H, Kobayashi N, Tanaka Y, Nakaji S, Yong C, Okitsu T *et al*. A newly developed bioartificial pancreas successfully controls blood glucose in totally pancreatectomized diabetic pigs, Tissue Eng 2006; 12: 1799-1809. http://dx.doi.org/10.1089/ten.2006.12.1799

[42] Dulong JL, Legallais C. A Theoretical study of oxygen transfer including cell necrosis for the design of a bioartificial pancreas, Biotech Bioeng 2007; 96: 990-998 http://dx.doi.org/10.1002/bit.21140

[43] Jain KK. Chapter 1: Drug delivery systems - An overview. In: Jain KK (ed) Methods in molecular biology, Vol. 437: Drug Delivery Systems, Humana Press, Totowa NJ, 2008; 1-50.

[44] Fan Q, Sirkar KK, Michniak B. Iontophoretic transdermal drug delivery system using a conducting polymeric membrane. J Membr Sci 2008; 321: 240-249 http://dx.doi.org/10.1016/j.memsci.2008.04.061

[45] Zhang Y, Chan HF, Leong KW. Advanced materials and processing for drug delivery: The past and the future, Ad. Drug Delivery Rev 2013; 65: 104-120. http://dx.doi.org/10.1016/j.addr.2012.10.003

[46] Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids, J Mol Biol 1965; 13: 238-252. http://dx.doi.org/10.1016/S0022-2836(65)80093-6

[47] Batzri S, Korn ED. Single bilayer liposomes prepared without sonication, Biochem Biophys Acta 1973; 298: 1015-1019. http://dx.doi.org/10.1016/0005-2736(73)90408-2

[48] Lasic DD. Mechanisms of liposome formation, J Liposome Res 1995; 5: 431-441. http://dx.doi.org/10.3109/08982109509010233

[49] Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential, Int J Nanomed 2006; 1: 297-315.

[50] Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement, Int J Pharm 1989; 55: R1-R4. http://dx.doi.org/10.1016/0378-5173(89)90281-0

Received on 04-04-2016 Accepted on 03-05-2016 Published on 31-05-2016

http://dx.doi.org/10.15379/2410-1869.2016.03.01.03