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Review

The place of adsorption and biochromatography in extracorporeal liver support systems☆

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Abstract

Artificial and bioartificial liver devices aim at replacing some or all liver functions in the cases of end stage or fulminant disorders. Among all of its function, liver plays a key role in detoxification of substances that are hydrosoluble or bound to albumin. In this paper, the authors first reviewed the requirements for temporary liver support, then the adsorption-based systems that can be found on the market and finally propose new applications of biochromatography using perfusion-based bioartificial systems.

Keywords: Liver; Bioreactor; Adsorption columns; Encapsulation; Fluidized bed

Contents

1. Introduction

The liver performs many important metabolic functions and is the only internal organ that has the capacity to regenerate itself with new healthy tissues. Loss of liver cell functions may result in the disruption of many essential metabolic functions, which could lead to death. At present, liver transplantation is the only efficient treatment for patients suffering from acute or fulminant organ failure [1]. The shortage in specific organ donors has resulted in a high death rate among the potential patients waiting for a graft. Since 20 years, the expanding gap between the number of patients on waiting list and the number of liver transplants has highlighted the requirement for a temporary liver support. Such an artificial organ could be employed either as a bridge to transplantation or as a means for the patient to recover native liver function [2].

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As liver performs multiple and complex functions (detoxification, but also transformation and synthesis), artificial organ or bioartificial organ exploiting a synthetic cartridge to host biological components such as cells (hepatocytes in the case of a bioartificial liver) have been investigated. Among all of these potentialities, we only focus here on systems adapting some chromatography principles. Therefore, membrane-based bioartificial livers (BAL) will not be described, but could be found in other reviews [3–6].

One of the major liver functions is detoxification of substances carried by blood, which are perfused through the cellular network in the organ. To replace these functions, three different physical principles are available in extracorporeal devices under different forms. They are presented in Fig. 1 and will be discussed along this paper. It should be noticed that the term "convection", commonly used, represents indeed molecule transfer due to fluid motion, most properly named "solvent drag" transport.

2. Requirements for artificial liver support: what are the functions to be replaced?

The adult human liver normally weighs between 1.7 kg and 3.0 kg. It is both the second largest organ and the largest gland within the human body. The portal vein brings it venous blood from the spleen, pancreas, small intestine and large intestine, so that the liver can process the nutrients and by-products of food digestion. The liver is among the few internal human organs able of natural regeneration: as little as 25% of remaining liver can regenerate into a whole liver again. The various functions of the liver are carried out by the liver cells and more specifically by hepatocytes. These functions are numerous and partially depicted in Fig. 2.

As examples, the liver produces and excretes bile required for emulsifying fats, performs several roles in carbohydrate metabolism such as gluconeogenesis (synthesis of glucose from certain amino acids, lactate or glycerol), glycogenolysis (breakdown of glycogen into glucose) and glycogenesis (formation of glycogen from glucose). It is responsible for the mainstay of protein metabolism and performs several roles in lipid metabolism. The liver produces albumin, coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.

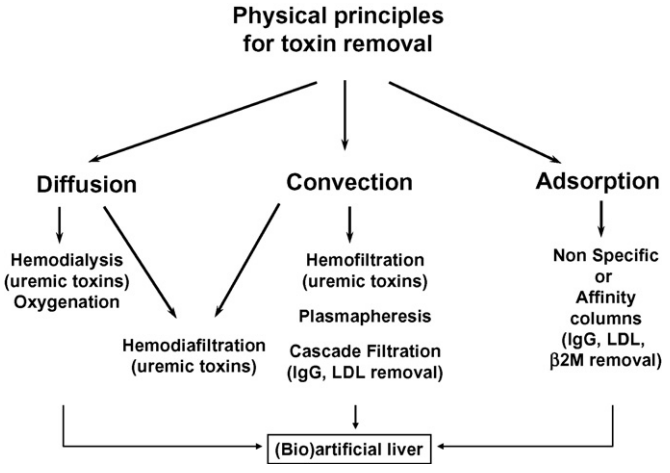

Fig. 1. Classification of the toxin removal methods available in the field of artificial organs.

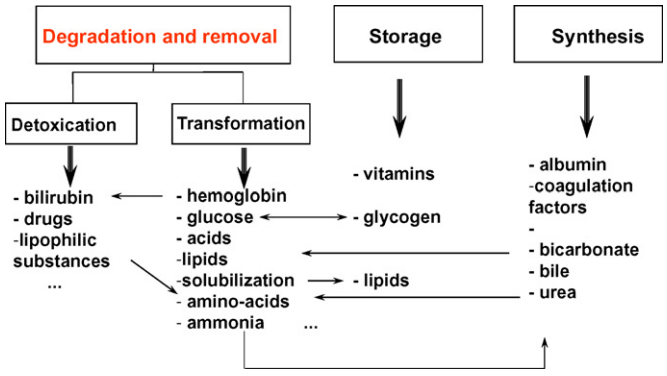

Fig. 2. Classification of major liver functions.

Finally, the liver breaks down hemoglobin, toxic substances and most medicinal products in a process called drug metabolism and converts ammonia to urea.

3. The role of adsorption in detoxication

Artificial liver support systems aim at the extracorporeal removal of water-soluble and protein-bound toxins (albumin being the preferential binding protein) associated with hepatic failure. Albumin contains reversible binding sites for substances such as fatty acids, hormones, enzymes, dyes, trace metals and drugs [7] and therefore, helps for the later kidney clearance of substances that are toxic in the unbound state. These substances that accumulate in liver failure are implicated in the development of hepato-renal syndrome, hepatic encephalopathy, hemodynamic instability, ongoing liver injury and inhibition of liver cell regeneration. It has been proposed that albumin binding sites for these putative toxins become saturated in patients with liver failure, consequent on decreased hepatic clearance, leading to an accumulation of unbound toxic substances and the development of organ dysfunction.

The design of affinity columns is not mandatory for liver support as it could be for other protein-specific diseases (such as familial hypercholesterolemia or immune disorders) [8]. Indeed, the range of substances to be removed is broad and not completely identified. Clinical studies showed that the critical issue of the clinical syndrome in liver failure is the accumulation of toxins not cleared by the failing liver. Based on this hypothesis, the removal of lipophilic, albumin-bound substances, such as bilirubin, bile acids, metabolites of aromatic amino acids, medium-chain fatty acids and cytokines, should be beneficial to the clinical course of a patient in liver failure.

For this purpose, the removal procedures are mainly based on non-specific adsorption on ion-exchangers and activated charcoal. Blood should not perfuse directly such components, due to bioincompatibity aspects. Therefore, several processes have been proposed to correctly handle toxins carried by plasma. They

are described in the following chapters. All of them need a physical barrier between the blood cells and the adsorption system. This physical sieve is always a membrane with adequate properties, through which toxins can be transferred by diffusion or convection.

Two systems are based on classical chromatography processes, i.e. perfusion of a column hosting adsorbents and three others are based on moving adsorbent phase.

4. Convection + adsorption systems

The following processes can be described as selective therapeutic plasmapheresis. In a first step, blood is withdrawn from the patient and separated by cross flow filtration in a hollow fiber membrane cartridge: water and some plasma solutes are transferred through a semi-permeable membrane under a convection process. The transmembrane pressure applied from blood to filtrate compartment ensures flow and mass transfers. Then, the filtrate perfuses the adsorption columns where toxins are retained and is finally mixed with blood cells and other plasma components before returning to the patient (Fig. 3).

4.1. ASAHI KASEI Medical (Tokyo, Japan)

The plasmapheresis step is here performed by a microporous membrane (Plasmaflo™) made of a copolymer of ethylene and vinyl alcohol (PEVA), with a maximum pore size of 0.3 μm. The extracted plasma flows through an activated charcoal column Hemosorba™ and an anion exchange column (copolymer of styrenedivinyl benzene) Plasorba™ that binds bilirubin and bile acids [9]. Each column contains 350 mL of adsorbent. After a partial hepatectomy (surgery removing the diseased part of the liver), this system is recommended as a support for future regeneration.

4.2. FPSA-Prometheus® from Fresenius Medical Care (Bad Homburg, Germany)

Here, the first step consists of blood fractionation by means of a capillary albumin filter (AlbuFlow AF01) presenting a molecular weight cut-off of 300 000 Da. The large pores of the membrane allow albumin-rich plasma to enter the secondary plasma circuit. Albumin-bound toxins are separated from the plasma by binding to the adsorber beads. Both columns host 350 mL of neutral resin styrenedivinyl benzene copolymer beads. The average particle diameter is approximately 600 μm. The inner porous structure is sponge-like and easily accessible for protein-bound liver toxins. Prometh 01 adsorbs water insoluble compounds, such as bile acids, phenols and aromatic amino acids. Prometh 02 has anion-exchanger properties because it contains positively charged sites and is able to remove negatively charged liver toxins, such as bilirubin. The cleared filtrate returns thus to the blood main stream [10].

To remove water-soluble toxins, an additional classical dialysis step is then placed downstream. The high flux dialysis membrane is used for the diffusive transfer of toxins from the blood to the dialysate side.

The whole extracorporeal circuit is adapted from a Fresenius 4008 dialysis machine. All the clinical results presented up to now are encouraging and prove the capacity of Prometheus to treat certain categories of patients [11].

5. Diffusion + adsorption systems

In all the systems below, substances carried by blood are removed in the "dialysate" phase, which is separated from blood by a semi-permeable membrane. This means toxins have first to cross this barrier by diffusion, before being treated (Fig. 4). Toxins that bind to albumin in the bloodstream and are associated with progressing liver failure have proven refractory to be removed by conventional hemodialysis. Such toxins can, however, be removed by adding a binder to the dialysate that serves to capture the toxin as it is dialyzed across the membrane.

5.1. Liver Dialysis Unit™ by Hemocleanse Technologies (Lafayette, USA)

This system previously named Biologic-DT was first marketed by the company HemoTherapies. This original method combined hemodialysis and adsorption, the adsorbents (powdered activated charcoal and cation-exchangers) being located in

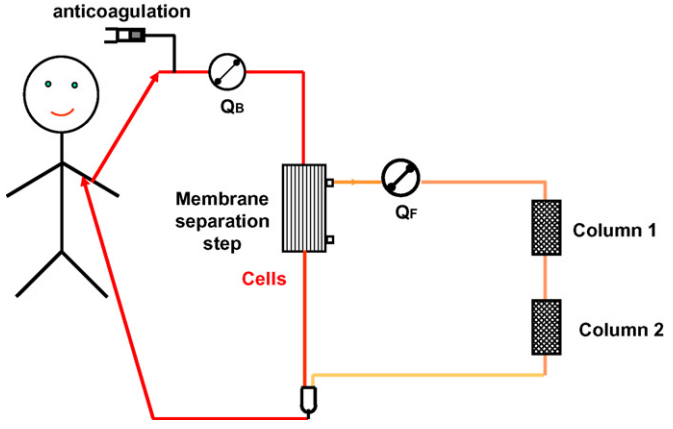

Fig. 3. Generic representation of combined filtration and adsorption columns systems for artificial liver support.

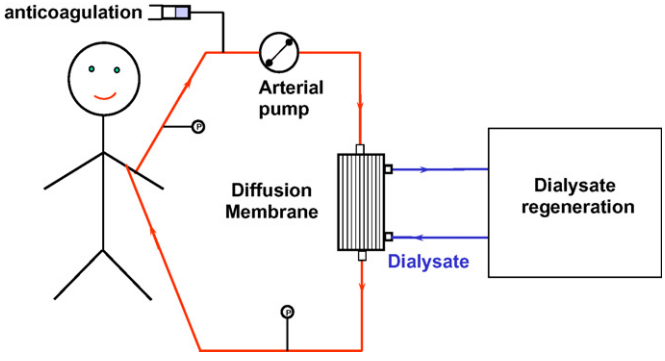

Fig. 4. Generic representation of combined diffusion and adsorption columns systems for artificial liver support.

the dialysate moving phase [12]. The motion is bidirectional and makes the available adsorption area huge: 1.5 L of bead suspension corresponds to 300 000 m^2. Dialysate content is adjusted so as to prevent unexpected removal of substances such as calcium, sodium due to diffusive effects. This treatment was shown to be efficient in cirrhotic patients [13].

5.2. MARS® from Teraklin (owned by Gambro, Lund, Sweden)

MARS primary circuit is based on a specific dialysis using a specially designed membrane named MARS Flux dialyser. The secondary circuit consists in 600 mL of 20% human albumin solution instead of physiological buffer used in classical hemodialysis. Due to the membrane properties, protein-bound toxins and water-soluble substances from the blood side diffuse into the albumin solution [14]. The MARS Flux dialyser presents a surface area of 2.1 m^2, a membrane thickness of 100 nm and a molecular cut-off of about 50 kDa. The specific membrane surface provides pseudo-binding sites for albumin when the secondary circuit is primed with albumin solution. The albumin molecules on the "dialysate" side of the membrane are in very close proximity to the surface of the membrane in contact with patient's blood. It is assumed that albumin-bound toxins move by physicochemical interactions between the plasma, albumin molecules bound to the dialysis side of the membrane and the circulating albumin solution. This solution is then dialyzed against a standard buffered dialysis solution to remove water-soluble substances by diffusion. The removal of the albumin-bound toxins is achieved by passage through an activated carbon adsorber and an anion-exchanger. The concentration gradient is maintained at the first dialysis step by circulation of the on-line regenerated albumin. A specific monitor has been designed by Teraklin company to handle the secondary circuit and is now commercially available with Gambro dialysis machine.

MARS therapy has been shown to result in a relative clearance of aromatic amino acids and in the substantial removal of albumin-bound toxins such as fatty acids, bile acids, tryptophan and bilirubin [15]. Physiologically important proteins (such as albumin, α1-glycoprotein, α1-antitrypsin, α2-macroglobulin, transferrin and thyroxin-binding globulin) and hormones (such as thyroxine and thyroid-stimulating hormone) are not significantly removed.

5.3. Albumin dialysis SPAD

Single-pass albumin dialysis (SPAD) is a non-commercial simple method of albumin dialysis using standard renal replacement therapy machines without an additional perfusion pump system. The patient's blood flows through a circuit with a high-flux hollow fiber hemodiafilter, identical to that used in the MARS system. The other side of this membrane is perfused with a buffered albumin solution in counterdirectional flow, which is, instead of being regenerated as in the MARS concept, discarded after passing the filter [16].

6. Bioartificial systems

As seen above, the artificial systems are only able to supply detoxication functions of the liver. In some cases, this might not be enough to save patients. An alternative is the design of bioartificial liver. A simplistic approach consists in considering such a device as a bioreactor based on synthetic elements able to offer an adequate environment to the liver cells. This environment would in turn lead to the maintenance of efficient functions of the cells aiming at liver supply, when placed in a bioreactor located in an extracorporeal circuit. The mandatory requirements for acceptable cell viability and functions in a bioartificial liver (BAL) are tentatively listed below, according to a biotechnological point of view:

1. Anchorage to a support or a matrix
2. Effective exchanges with blood or plasma
3. Protection from host immunological response

In addition, the synthetic components of the bioreactors should themselves be biocompatible. Several bioreactor designs have been proposed to fulfill most of the above conditions. They can be classified into three categories [4]: membrane-based devices, direct perfusion systems with cells immobilized on various supports and bead entrapment-based systems. The polymer bead matrix offered anchorage facilities to hepatocytes and its porous structure could act as an immunological barrier.

The beads containing hepatocytes were first developed by Tompkins et al. [17] and Dixit [18] for their direct implantation. Hepatocytes viability was found to be maintained in such a tridimensional structure [19], even after cryopreservation [20]. The beads might even protect the cells from shear stress damage in an extracorporeal bioreactor.

Since cell encapsulation is a widely used tool in biotechnology, several materials have been investigated to fulfill the requirements of a bioartificial liver. Several teams tested the properties of HEMA-MMA copolymer [21], chitosan-dextrose [22]. Calcium alginate is up to now the most popular material [23–25] because of its porosity, its mechanical properties and its biocompatibility. The bead diameter ranged from 400 μm to 1 mm, allowing for sufficient mass transfer and oxygenation of all hepatocytes.

The inclusion of hepatocytes within a semi-permeable spherical structure usually called "bead" or "capsule" makes this approach close to chromatography. Indeed, a key issue relies on the process itself: how can the plasma correctly perfuse a huge number of beads?

Most of the bioreactors designed for beads perfusion are based on fixed bed configuration, where the beads are densely packed into a column. Reactors designed for small animal trials operated properly [24]. Their major limitation for scaling-up is the perfusion velocity profile into the column: the formation of preferential channels resulted in poor perfusion for a large amount of beads and consequently limited mass transport outside the beads. In addition, high shear stresses on the effectively perfused beads could lead to possible damage on the bead

structure and as an effect to alginate and cells release to the bloodstream.

However, the hepatocytes entrapment into alginate beads still appeared promising since all the other criteria fixed for an operational BAL seemed to be fulfilled. For these reasons, we proposed to exploit the potentiality of hepatocytes entrapped in alginate beads in a more efficient bioreactor.

The use of fluidized bed or expanded bed reactors is widely spread in chemical engineering or in biochromatography when a diphasic mixture is present [26]. In combination with cells entrapped into beads, it has also found several biotechnological applications. Hence, we suggested applying this technology to a large scale extracorporeal BAL as an extent of the previous work of Fremond et al. [23] with a small scale bioreactor in fixed bed configuration, leading to the conception of the fluidized bed bioartificial liver (FBBAL) (Fig. 5) [27]. In vitro, we showed that encapsulation of an immortalized human cell line C3A followed by culture medium perfusion in a FBBAL maintained efficient cell functions for at least 6 h [28]. We now extend this study up to 48 h, which is much longer than a potential single treatment. For ex vivo or in vivo applications, the bioreactor behaviour may be unstable due to the low density difference between the liquid phase (plasma) and the solid phase (beads hosting hepatocytes). Indeed, the porosity of the fluidized bed (ε) depends on the superficial perfusion velocity (u) and on the terminal velocity (u_t) according to Richardson and Zaki semi-empirical formulation [26]:

$$\varepsilon = \left(\frac{u}{u_t}\right)^{1/n}, \quad u_t = \frac{(\rho_f - \rho_s) g d_p^2}{18\mu_f},$$

where n is a constant depending on the operating conditions, ρ_f the fluid density (kg m^{-3}), ρ_s the solid (bead) density (kg m^{-3}), μ_f the fluid viscosity (Pa s), d_p the bead diameter (m) and g is the gravity constant (m s^{-2}).

To circumvent this potential risk of beads' escape for the fluidized bed column, we propose to make them denser by the inclusion of glass microspheres (diameter from 10 μm to 50 μm). Mixing an adequate number of glass beads per milliliter

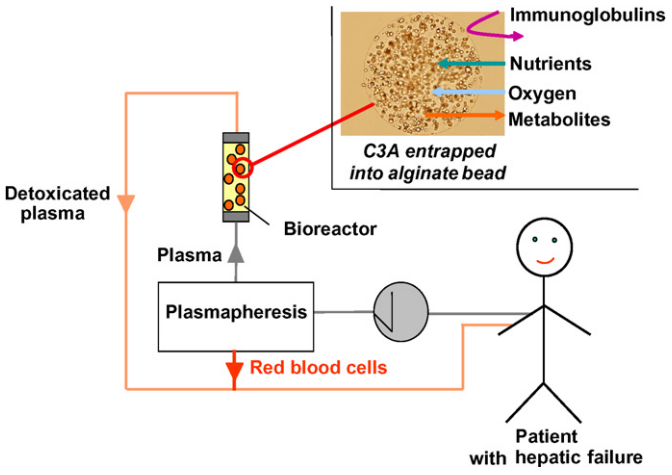

Fig. 5. Schematic description of the whole set up for the fluidized bed bioartificial liver.

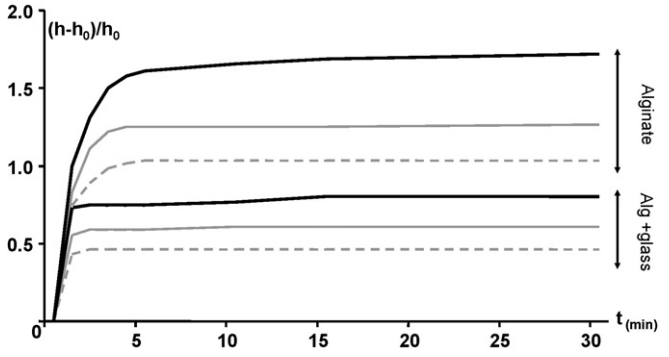

Fig. 6. Expansion of the fluidized bed when perfused with plasma either with empty beads (three top lines) or with beads made denser by inclusion of glass microspheres (three bottom lines) under three perfusion velocities: 0.74 mm s^{-1} (dotted line), 0.90 mm s^{-1} (grey line) and 1.06 mm s^{-1} (dark line).

of alginate allows for the reduction of the bed expansion, as shown on Fig. 6.

Mass transfers between the perfusion fluid and the beads were thus significantly increased, as compared to a static configuration [29]. Each bead followed a motion from the bottom to the top of the bioreactor and then fell down. Preferential flow was thus avoided and convective mass transfer was achieved due to the relative motion of the beads into the perfusion fluid.

In vitro, the results obtained with this type of bioreactor and alginate beads hosting hepatoblastoma C3A cells are very encouraging. The in vivo application requires up to 1 L of alginate beads (diameter 1 mm) to supply liver functions. The perfusion plasma flow rate could range 100–400 mL/min. with an expansion ratio of 2. The system developed is now applied by three groups in Europe. For instance, the same technology was adapted for Selden's group in London, with smaller diameter alginate beads. Ex vivo results with pathological plasma seem very encouraging (unpublished data).

7. Conclusions

Up to now, none of the presented system can claim its ability to fully replace all liver functions in an extracorporeal circuit. On the one hand, purely artificial techniques can only cover some detoxification aspects, which is already crucial in many clinical cases to save patients. On the other hand, bioartificial livers did not prove their full efficiency yet, mainly because both regulatory and logistic aspects limit for the moment the inclusion of significant numbers of patients to draw statistically relevant conclusions.

It appears nevertheless clear that the application of adsorbent techniques, perhaps in combination with bioartificial systems, present a potential supply to help the patient wait for a graft or even for tissue regeneration. In the biomedical field, the extension of techniques previously developed for other topics, such as biochromatography for instance, has always proved to be promising. This could be hopefully the case for artificial liver support.

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